PHARMACOGNOSTIC STUDY AND SOME BIOLOGICAL ACTIVITIES OF *ACACIA ALBIDA* DEL. (FABACEAE) ROOT BARK

BY

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JULY, 2012

DECLARATION

I declare that the work in this thesis entitled "PHARMACONOSTIC STUDY AND SOME BIOLOGICAL ACTIVITIES OF *ACACIA ALBIDA* DEL. (FABACEAE) ROOT BARK" has been performed by me in the Department of Pharmacognosy and Drug Development under the supervision of Dr. Abdulkarim Agunu and Prof. Hajara Ibrahim. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any University.

Name	Signature	Date

CERTIFICATION

This thesis titled "PHARMACONOSTIC STUDY AND SOME BIOLOGICAL ACTIVITIES OF *ACACIA ALBIDA* DEL. (FABACEAE) ROOT BARK" by Lawal Alaba Rachel meets the regulations governing the award of the degree of Master of Science (Pharmacognosy) of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this work to God Almighty, the Alpha and Omega. I ascribe all the glory of this work to him.

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ABSTRACT

Acacia albida Del (Fabaceae) is one of the largest thorn trees, reaching 30m in height, with spreading branches and a rounded crown. The bark is grey and fissure when old. It belongs to the family Fabaceae. The tree has medicinal value for treatment of respiratory infections and malaria. The bark contains a lot of tannin and has been employed in industries. Phytochemical screening on the root bark extract reveal the presence of Tannins, Flavonoids, Saponins and Alkaloids and were quantitatively estimated to be 12.18, 7.93, 1.90, 0.43 w/w respectively. Thin layer chromatography of the various constituents gave various spots with distinct R_f values. Antioxidant activity of the extract by 2,2- diphenyl-1-picric hydrazyl (DPPH) radical scavenging ability revealed that with increase in concentration of extract radical scavenging activity increases. The standard synthetic antioxidant: ascorbic acid has higher (99.08%) radical scavenging activity than the plant extract at 0.5mg/ml, however the plant extract at the same concentration of 0.5mg/ml exhibited significant ($P<0.01$) radical scavenging activity of 91.48%. It thus shows a radical scavenging activity as close as that of the standard.

Evaluation of the extract for glucose lowering effect revealed a significant ($P< 0.05$) reduction of high blood glucose level in alloxan induced diabetic rats from 335.33±3.3 on first day treatment to 151±14.42 on 4^{th} day treatment at a dose level of 200mg/kg body weight orally. These findings show that the root bark has antioxidant properties and hypoglycemic effects on alloxan induced diabetic rats. These may support the traditional use of the plant for diabetic management.

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LISTS OF ABBREVIATIONS

ACES- Vitamins A, B, C, and Selenium

AGEs - advanced glycation end products

BH_4 – tetrahydrobiopterin

BHA - butylated hydroxyl anisole

BHT - butylated hydroxytoluene

Cu-SOD -copper superoxide dismutase

DNA – dioxyribose nucleic acid

DPPH - 2, 2-diphenyl-1-picryl hydrazyl

eNOS - endothelial nitric oxide synthase

FAD - flavin adenine dinucleotide

FMN - flavin mononucleotide

GDM - Gestational Diabetes mellitus

GSH – glutathione

GSH-Px - glutathione peroxidase

H_2O_2 - hydrogen peroxide

HNO_2 - nitrous oxide

HOCl - hydrochlorous acid

HRO_2^- -hydroperoxyl

LDL -low-density lipoprotein

MEST-maximal electroshock test

Mn-SOD manganese superoxide dismutase

NAD(P)H –

NO -nitric oxide

NO_2^- - nitrogen dioxide

NOS - nitric oxide synthase

O_2^- -superoxide

OH hydroxyl

$ONOO^-$ -peroxynitrite

PG - propyl gallate

PTZ-Pentylenetetrazole

RNA- ribo nucleic acid

RNS – reactive nitrogen species

RO_2 . peroxyl

RONOO - alkyl peroxynitrates

ROS - reactive oxygen species

SODs -superoxide dismutases

STN- Strychnine

TBHQ - tertiary butylhydroquinone

UCP1 - overexpression of uncoupling protein-1

VSMC - vascular smooth muscle cells

WHO – World health organization

CHAPTER ONE

1.0 INTRODUCTION

A vast array of illnesses and medical complaints has always been and will continue to be a real part of the human condition. However, medicine, whether traditional folk medicine or mass-produced pharmaceutical drugs, have been beneficial in curing and treating all manner of diseases and illnesses throughout the centuries. Indeed, it is from the traditional folk medicine that great strides have been achieved in treating illnesses.The impact of plant derived medicine on human history has been remarkable; Opium, Digitalis and Chaulmoogra have all left their leaf prints on the human life (Abdulafatih, 1987).

Traditional medicine is a cultural gem of various communities around the world and encompasses all kinds of folk medicine, unconventional medicine and indeed any kind of therapeutical method that has been handed down by the tradition of a community or ethnic group (Adesina, 2009).

By the World Health Organization definition, traditional medicine is the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experiences and observations handed down from generations to generations whether verbally or in writing (WHO, 1976; 1991). With these descriptions, various forms of medicines and therapies such as herbal medicine, massage, homeopathy, mud bath, music therapy, reflexology, dance therapy, hydrotherapy, mind and spirit therapies, self-exercise therapies, aromatherapy, etc are a few element of traditional medicine (Adesina, 2009).

1.1 History of Herbal Medicine

Herbal medicine sometimes referred to as botanical medicine or herbalism is the use of herbs for their therapeutic or medicinal value. A herb is a plant or plant part used for its medicinal, aromatic or savory qualities. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs (Lai and Roy, 2004).

It hs been proposed that knowledge of medicinal plants was gained by accident. Early man could have gained some scientific knowledge by watching the effects produced by various plants eaten by domestic animals. Even today some herbalists try out remedies on domestic animals, especially when testing for toxicity, and on themselves or their relations (Akpata, 1979).

Undisputedly, the history of herbology is inextricably intertwined with that of modern medicine (Chong, 2003) as many drugs listed as conventional medications were originally derived from plants. Salicylic acid, a precursor of aspirin, was originally derived from *Sallix alba* (white willow bark) and the *Filipendula ulmaria* (meadowsweet plant). Cinchona bark is the source of malaria fighting quinine, Vincristine and Vinblastine used to treat certain types of cancer, come

from periwinkle (*Catharanthus roseus*). The opium poppy (*Papaver somniferum*) yields morphine, codeine. Even today, morphine the most important alkaloid of the opium poppy remains the standard against which new synthetic pain relievers is measured (Chong, 2003).

The use of plants as medicine is older than recorded history. As mute witness to this fact *Althaea offinalis* (marshmallow root), *Hyacinthus orientalis* (hyacinth), and *Achillea millefolium* (yarrow) have been carefully tucked around the bones of a stone aged man in Iraq. These three medicinal plants continue to be used today; marshmallow root is a demulcent herb, soothing to inflamed or irritated mucous membrane, such as sore throat or irritated digestive tract. Hyacinth is a diuretic that encourages tissues to give up excess water. *Achillea millefolium* (Yarrow) is a time-honoured cold and fever remedy that may once have been used as aspirin today.

One of the earliest records of the use of herbal medicine is that of Chaulmoogra oil from species of *Hydnocarpus* Gaertn.which was known to be effective in the treatment of leprosy. Such as was recorded in the pharmacopoeia of the Emperor Shen Nung of China between 2730 and 3000B.C. (Le Strange, 1977). Similarly, the seed of the opium poppy (*Papaver somniferum*) and castor oil seed (*Ricinus communis*) were excavated from some ancient Egyptian tombs, which indicated their use in that part of Africa as far back as 1500 B.C. The records available in such papyri as the Ebers papyrus also confirm that medicinal plants were used at that time in Egypt (Sofowora, 2006).

In the scriptural book of Ezekiel which dates from sixth century B.C is an admonition regarding plant life and the fruit thereof shall be for meat, and leaf thereof for medicine (Chong, 2003). By the Seventeenth century, the knowledge of herbal medicine was widely disseminated throughout Europe. As western medicine evolved from an art to a science in the nineteenth century,

information that had at one time been widely available became the domain of comparatively few. Once scientific methods were developed to extract and synthesize the active ingredients in plants, pharmaceuticals laboratories took over from provider of medicinal herbs as the producers of drugs. The use of herbs, which for most of history had been mainstream medical practice, began to be considered unscientific or at least unconventional and fall into relative obscurity (Chong, 2003).

1.2 Herbal Medicine Today

Herbal medicines are still in use today. In some respects they have gained a new momentum in the medical fields, and as many people seek alternative treatments and begin to check out traditional and eastern medicine, herbs are becoming more popular (Nolan, 2008). As physicians seek new treatments for many common illnesses they are beginning to revisit the traditional remedies using herbal medicines. Pharmaceutical medications, with their potentials for harmful side effects and addiction, are becoming less popular (Nolan, 2008). Herbal medicine has enjoyed a long, and colourful, history. From the early Chinese empires to modern physician's offices, herbal medicines have continued to be a part of the medical field. Research and clinical trials have helped to shape the field of medicine, and the future for herbal medicine looks bright. The world health organization estimates that 4 billion people, 80% of the world population, presently use herbal medicines for some aspect of their primary health care (Akerele, 1992).

Herbal medicine is a major component in all indigenous people's traditional medicine and a common element in Ayurvedic, Homeopathic, and Naturopathic traditional oriental and native

America Indian medicine. WHO notes that of 119 plant-derived pharmaceutical medicines, about 74% are used in modern ways that correlate directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forest and other places for their potential medicinal values (Chong, 2003).

1.3 Advantages of Herbal Medicine

Herbal medicines have numerous advantages over orthodox medicine. Herbal medicine is cheaper than orthodox medicine. The cost of the latter is increased by modern health technology which in many cases is in appropriate or irrelevant to the immediate needs of people in developing countries (Sofowora, 2006). Also the cost of modern health care has increased due to higher cost of basic commodities. Herbal medicine enjoys a wider acceptability because it blends readily into the socio-cultural life of the people in whose culture it is deeply rooted (Sofowora, 2006). Herbal medicine is a potential source of new drug, a source of cheap starting product for the synthesis of known drugs, or a cheap source of known drugs such as Reserpin*e* from *Rauwolfia* species, Vinblastine from *Catharanthus roseus* or recent discovery of a contraceptive in zoapatle (*Montanoa tomentosa* cerv; Hahn *et al.*, 1981). Herbal remedies do not have negative side effects. If any they are softer than allopathic medicine (Sofowora, 2006).

1.4 *Acacia albida*

Acacia albida (Del.) syn. *Faidherbia albida* Del. A. Chev is a monotypic genus in the legume subfamily Mimosoideae. Normally a deciduous tree with a height of up to 15m and which can

reach 25m or more. It has a large rounded crown and spreading branches, and trunk diameters of 1m or more. It is distinguished by its phenology, whitish twigs and paired thorns, blue green bipinnate leaves lacking a petiolar gland, but with glands between nearly all its 2-12 pinnate pairs. The inverted phenology does not occur in seedlings until their tap roots are well into the water table. Flower buds appear soon after leaves on current season's growth. About 100 creamy white flowers occur on spikes up to 16 cm long, but most abort and normally 5 or less mature into pods 3-4 months later (Zen-Nlo and Joly in Van Den Beldt, 1992). Pods (11-30 cm long x 1.4-6.7 cm wide) are orange to reddish brown, often coiled or twisted, and contain up to 30 seeds. Seeds are dispersed by herbivores eating the indehiscent pods or by the pods floating down rivers. It is a diploid species (2n = 26) over most of its range; a poly ploid (2n = 52) has been recorded from Israel (Halevy 1971). It grows in a wide range of climates and habitats, either scattered or gregarious, in closed canopy woodland or open savanna and in cultivated lands. It is usually a pioneer on alluvial flats but can form part of fire climax vegetation in the West African savannas, where optimal conditions are between 500-800 mm annual rainfalls.

The natural distribution range of *A. albida* extends through northern Africa, from Senegal to Ethiopia, and southwards through East Africa to the Transvaal and Lesotho. The largest concentration of the species occurs on the sandy alluvial soils of the Jebel Mara drainage system in the Sudan, where pure stands are found in belts often a mile wide (Wickens 1969). Outside Africa, *A. albida* occurs in Yemen, Israel, Lebanon and Jordan. It has been introduced to Cyprus and Pakistan (Brenan, 1983).

.A. albida occurs naturally in the Nigerian savanna, which is characterized by a short rainy season alternating with a long dry and hot season. Savanna vegetation dominated by acacias

covers about 75% of Nigeria's total landmass (Sanusi *et al.,* 1992). *A. albida* is distributed from Nigeria's border with Niger Republic (14"N) south to the edge of the Guinea Savanna zone (19"N), or within the 400-1000 mm isohyets. Itoccurs in a wide range of habitats from riparian vegetation to open savanna and cultivated land and on all soil types from alluvium to sandy-clays.

Although the species withstands drought as well as moderate amount of flooding, its best developmen t is on well-drained, coarse-textured soils with 650-700 mm annual rainfall. Where groundwater is easily accessible, *A. albida* is independent of rainfall as in the northeast corner of Nigeria near Lake Chad which has less than 400 mm mean annual rainfall. The species is seldom found in natural woodland or unexploited climax vegetation because it is intolerant of shade while young. It often occurs in association with *Acacia nilotica* in Sokoto, Katsina, and Kano (Kura *et al.,* 1990). The wood is used for fuel, it is lighter (specific gravity 0.6-0.7) and less suitable than manyAfrican acacia species. Cooked seeds are eaten as a human famine food both in Ghana, Nambia, Zambia and Zimbabwe. Ovambo Namibians use its bark for toothbrushes and is reputed to contain Fluorine. Thorny branches are used for fencing, barks and roots are used in local medicinal preparation.

1.5 Antioxidants

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the

chain reaction occurs in a cell, it can cause damage or death. When the chain reaction occurs in a purified monomer, it produces a polymer resin, such as a plastic, a synthetic fiber, or an oil paint film. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Sies, 1997). Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants or inhibition of the antioxidant enzymes cause oxidative stress and may damage or kill cells (Sies, 1997).

Antioxidants are widely used as ingredients in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation is harmful (Bjelakovic *et al.,* 2007). In addition to these uses of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline (Linster *et al*., 2007).

1.5.1 Sources of Antioxidants

1.5.2 Non Enzymatic Sources of Antioxidants

- **Uric Acid**

The antioxidant in highest concentration in human blood is uric acid, (El-Shohemy *et al.,* 2002) which provides about half of the total antioxidant capacity of human serum (Wu, 1989). Uric acid is an oxypurine produced from xanthine by the enzyme xanthine oxidase, and is a waste product of purine metabolism in primates, birds, and reptiles. An overabundance of this chemical in the body causes gout.

- **Ascorbic Acid**

Ascorbic acid or "vitamin C" is a monosaccharide oxidation-reduction (redox) catalyst found in both animals and plants. As one of the enzymes needed to make ascorbic acid has been lost by mutation during primate evolution, humans must obtain it from the diet; it is therefore a vitamin (Smirnoff, 2001). Most other animals are able to produce this compound in their bodies and do not require it in their diets (Linster *et al.,* 2007). Ascorbic acid is a redox catalyst which can reduce, and thereby neutralize, reactive oxygen species such as hydrogen peroxide. In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the redox enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants (Shigeoka *et al.,*

2002). Ascorbic acid is present at high levels in all parts of plants and can reach concentrations of 20 millimolar in chloroplasts (Smirnoff and Wheeler, 2000).

- **Glutathione**

Glutathione is a cysteine-containing peptide found in most forms of aerobic life (Amorini, 2009). Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. Due to its high concentration and its central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants (Meister and Anderson, 1983).

- **Melatonin**

Melatonin is a powerful antioxidant and, unlike conventional antioxidants such as vitamins C and E and glutathione, it is both produced in the human body and is acquired in the diet (fruits, vegetables, cereals and herbs etc., contain melatonin (Campion, 1987). Melatonin easily crosses cell membranes and the blood-brain barrier.

1.5.3 Enzymatic Sources of Antioxidants

- **Superoxide dismutases** (SODs) are a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide (Zelko *et al.,* 2002; Bannister *et al.,* 1987). SOD enzymes are present in almost all aerobic cells and in extracellular fluids (Johnson and Giulivi, 2005). They contain metal ion cofactors that, depending on the isozyme, can be copper, zinc, manganese or iron. In humans, the copper/zinc SOD is present in the cytosol, while manganese SOD is present in the mitochondrion (Bannister *et al.,* 1987). There also exists a third form of SOD in

extracellular fluids, which contains copper and zinc in its active sites (Nozik-Grayck, *et al.,* 2005). The mitochondrial isozyme seems to be the most biologically important of these three. In plants, SOD isozymes are present in the cytosol and mitochondria, with an iron SOD found in chloroplasts that is absent from vertebrates and yeast (Van Camp *et al.,* 1997).

- **Catalases** are enzymes that catalyze the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor. (Chelikani, *et al.,* 2004; Zamocky and Koller, 1999) This protein is localized to peroxisomes in most eukaryotic cells (del Rio *et al.,* 1992). Catalase is an unusual enzyme since, although hydrogen peroxide is its only substrate, it follows a ping-pong mechanism. Here, its cofactor is oxidized by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate (Hiner *et al.,* 2002). Despite its apparent importance in hydrogen peroxide removal, humans with genetic deficiency of catalase — "acatalasemia" — or mice genetically engineered to lack catalase completely, suffer few ill effects (Mueller *et al.,* 1997; Ogata, 1991).
- **Peroxiredoxins** are peroxidases that catalyze the reduction of hydrogen peroxide, organic hydroperoxides, as well as peroxynitrite (Rhee *et al.,* 2005). Peroxiredoxins seem to be important in antioxidant metabolism.
- **Thioredoxin and Glutathione Systems**

The thioredoxin system contains the 12-kDa protein thioredoxin and its companion thioredoxin reductase (Nordberg and Arner, 2001). Proteins related to thioredoxin are present in all sequenced organisms with plants, such as *Arabidopsis thaliana,* having a

particularly great diversity of isoforms (Viera, 2006). In its active state, thioredoxin acts as an efficient reducing agent, scavenging reactive oxygen species and maintaining other proteins in their reduced state (Arner, 2000) after being oxidized, the active thioredoxin is regenerated by the action of thioredoxin reductase, using NADPH as an electron donor (Mustarcich *et al.,* 2000).

The glutathione system includes glutathione, glutathione reductase, glutathione peroxidases and glutathione *S*-transferases (Meister, 1988). This system is found in animals, plants and microorganisms (Meister, 1988; Creissen *et al.,* 1996). Glutathione peroxidase is an enzyme containing four selenium-cofactors that catalyzes the breakdown of hydrogen peroxide and organic hydroperoxides.

1.6 Antioxidant Action of Phenols

Antioxidant action of phenolic compounds is due to their high tendency to chelate metals. Phenolics possess hydroxyl and carboxyl groups, able to bind particularly iron and copper (Jung *et al.,* 2003). According to Morgan *et al.,* (1991) the chelating ability of phenolic compounds is probably related to the high nucleophilic character of the aromatic rings rather than to specific chelating groups within the molecule. Another mechanism underlying their antioxidant ability is that of metal ions. Metal ions decompose lipid hydroperoxide (LOOH) by the hemolytic cleavage of the O-O bond and give lipid alkoxyl radicals, which initiate free radical chain oxidation. Phenolic antioxidants inhibit lipid peroxidation by trapping the lipid alkoxyl radical. This activity depends on the structure of the molecules, and the number and position of the hydroxyl group in the molecules (Millic *et al.,* 1998). Phenolics (especially flavonoids) are able

to alter peroxidation kinetics by modifying the lipid packing order (Arora *et al.,* 2000). They stabilize membranes by decreasing membrane fluidity (in a concentration-dependent manner) and hinder the diffusion of free radicals and restrict peroxidative reaction (Blokhina *et al.,* 2003; Arora *et al.,* 2000). In addition to known protein-binding capacity of flavanols and procyanidins, they can interact with membrane phospholipids through hydrogen bonding to the polar head groups of phospholipids. As a consequence, these compounds can be accumulated at the membranes' surface, both outside and inside the cells. Through this kind of interaction, as they suggest, selected flavonoids help maintain membranes' integrity by preventing the access of deleterious molecules to the hydrophobic region of the bilayer, including those that can affect membrane rheology and those that induce oxidative damage to the membrane components.

1.7 Antioxidant action of Flavonoids

Flavonoids are low molecular weight (Fernandez *et al.,* 2006; Heim *et al.,* 2002) bioactive polyphenols which play a vital role in photosynthesising cells (Cushnie *et al*., 2005).The original "flavonoid" research apparently began in 1936, when Hungarian scientist Albert Szent-Gyorgi was uncovering a synergy between pure vitamin C and as yet unidentified co-factors from the peels of lemons, which he first called "citrin," and, later, "vitamin P". Flavonoids are secondary metabolites characterized by flavan nucleus (Heim, 2002) and C_6-C_3-C_6 carbon-skeleton (Peterson and Dwyer, 1998; Tsuchiya, 2010). These are group of structurally related compounds with a chromane-type skeleton having phenyl substituent in C_2-C_3 position (Rijke *et al.,* 2006). The basic structural feature of flavonoid is 2-phenyl-benzo-γ-pyrane nucleus consisting of two benzene rings (A and B) linked through a heterocyclic pyran ring (C) (Cushnie and Lamb, 2005).

Flavonoids differ in their arrangement of hydroxyl, methoxy and glycosidic side groups and in the conjunction between A and B rings (Heim *et al.,* 2002). A variation in C ring provides division of subclasses (Tsuchiya, *et al*., 2010). According to their molecular structure, they are divided into various classes: Flavones, Isoflavone, Flavonol, Flavanonol, Aurones, Chalcones, Flavanone, Isoflavanones, Dihydrochalcone, Flavan-3,4-diol, Anthocyanidin,Catechin.

In plants, flavonoids are often present as O-glycosides or C-glycosides. The O-glycosides possess sugar substituent bound to –OH of aglycone, usually at position 3 or 7, whereas, C-glycosides possess sugar groups bound to Carbon of aglycone usually 6-C or 8-C. The flavonoids are often hydroxylated in positions 3, 5, 7, 3', 4', and 5' which are frequently methylated, acetylated, or sulphated. Flavonoids are widely distributed among the plant kingdom (Cushnie and Lamb, 2005). Flavonoids are found in vegetables, fruits, nuts, seeds, stem, flowers, tea, wine etc. These are an integral part of our daily diet (Cook and Samman, 1996).

Flavonoids are powerful antioxidants against free radicals and are described as free-radical scavengers (Pal, *et al.,* 2009). This activity is attributed to their hydrogen-donating ability. Indeed, the phenolic groups of flavonoids serve as a source of a readily available ''H" atoms such that the subsequent radicals produced can be delocalized over the flavonoid structure (Tripoli *et al.,* 2007). Free radical scavenging capacity is primarily attributed to high reactivities of hydroxyl substituents that participate in the reaction (Heim *et al.,* 2002).



Fig 1.1: Scavenging capacity of free radical (R.)

Flavonoids inhibit lipid peroxidation in vitro at an early stage by acting as scavengers of superoxide anion and hydroxyl radicals. They terminate chain radical reaction by donating hydrogen atom to a peroxy radical as in fig 1.2; thus, forming flavonoids radical, which, further reacts with free radicals thus terminating propagating chain (Cook and Samman, 1996; Ferreira, 2010).

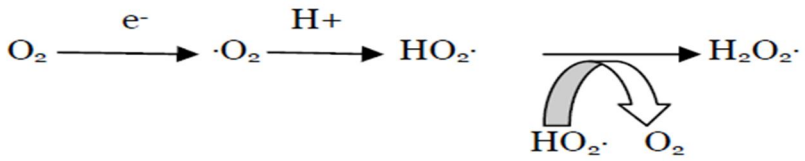


Fig 1.2: Formation of peroxy radical

Table 1.1: Reactive oxygen species that can be scavenge or their formation can be inhibited by flavonoids

S/No.	Reactive species	Mechanism
1	O_2 (Superoxide anion)	One-electron reduction product of O_2. Produced by phagocytes, formed in autoxidation reaction and generated by oxidases (heme proteins)
2	HO_2	Protonated form of O_2
3	H_2O_2 (Hydrogen peroxide)	Two electron reduction product of formed from O_2 by dismutation or directly from O_2. Reactivity of O_2 and H_2O_2 is amplified in presence of heme proteins
4	OH (Hydroxy radical)	Three electrons reduction products of O_2 generated by Fenton's reaction, transition metal (iron, copper)-catalysed Haber-Weiss reaction; also formed by decomposition of peroxynitrite produced by reaction of O_2 with nitric oxide radical.
5	RO. (Alkoxy radical), ROO. (Peroxyl radical)	Lipid peroxy radical (LOO.) produced from organic hydroperoxide, ROOH by hydrogen abstraction.
6	O_2	Singlet Oxygen

As described by Bors *et al.,* 1990 there are three structural features that are important determinants for the antioxidant potential of flavonoids: the ortho-dihydroxy (catechol) structure in the B-ring, which confers greater stability to aroxyl radicals, possibly through hydrogen bonding, and which participates in electron dislocation, the 2,3-double bond, in conjugation with a 4-oxo function, responsible for electron dislocation from the B-ring, the presence of both 3-(a)- and 5-(b)-hydroxyl groups . Among them the 3-OH group is the most significant determinant of electron-donating activity. The glycosilated flavonoids lose their activity in comparison with aglycones (Rene *et al.,* 2003; Rang *et al.,* 2007).

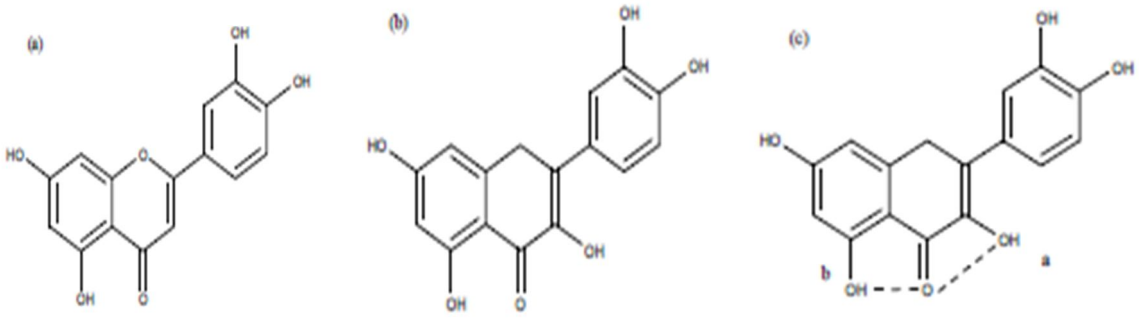


Fig 1.3: Structural groups responsible for antioxidant activity (Tripoli *et al.*, 2007)

3',4'-catechol structure in B-ring strongly enhances lipid peroxide inhibition and this arrangement is an important characteristic of most potent scavengers of peroxyl, superoxide and peroxynitrite radicals (Heim *et al.,* 2002) and its absence decreases antioxidant activity. The absence of the hydroxyl group at position 3 in flavanones and flavones decreases their antioxidant ability (Tripoli, *et al.,* 2007). Ghasemzadeh *et al.,* 2010 reported that high level of total phenolic and flavonoid in *Halia bara* variety possessses potent antioxidant activities.

Bitis *et al.,* (2003) isolated diosmetin, kaempherol, quercetin, kaempherol 3-glucoside (astragalin), quercetin 3-rhamnoside (quercitrin), quercetin 3-xyloside and quercetin 3-galactoside (hyperoside) from *Rosa agrestis* leaves and reported it to possess antioxidant activity (Bitis *et al.,* 2003). Shariffer *et al.,* (2009) reported antioxidant activity of methanolic extract of *Teucrium polium* and rutin and apigenin were found to be potent inhibitors of lipid peroxidation and oxidation of beta-carotene. Braca *et al.,* isolated several flavonoids from the leaves of *Licania licaniaeflora* and reported quercetin derivatives to possess strongest antioxidant activity and flavonone 8-hydroxy-naringen and kaempferol 3-O-α-rhamnoside possesses lowest antioxidant activity (Braca *et al.,* 2002).

1.8 Oxidative Stress

Oxidative stress is a general term used to describe the steady state level of oxidative damage in a cell, tissue, or organ caused by excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Turko *et al.,* 2001 and Maritim *et al.,* 2003). Free radicals and other "reactive oxygen species" are formed by a variety of normal processes within the body (including respiration and immune and inflammatory response) as well as by elements outside the body, such as air pollutants, sunlight, and radiation. ROS include free radicals such as superoxide ($\bullet O_2^-$), hydroxyl (•OH), peroxyl ($\bullet RO_2$), hydroperoxyl ($\bullet HRO_2^-$) as well as non-radical species such as hydrogen peroxide (H_2O_2) and hydrochlorous acid (HOCl) (Turko *et al.,* 2001; Evans *et al.*, 2002). RNS include free radicals like nitric oxide (•NO) and nitrogen dioxide ($\bullet NO_2^-$), as well as non-radicals such as peroxynitrite ($ONOO^-$), nitrous oxide (HNO_2) and alkyl peroxynitrates (RONOO) (Turko *et al.,*

200; Evans *et al.*, 2002). Of these reactive molecules, $\bullet O_2^-$, •NO and $ONOO^-$ plays important roles in the diabetic cardiovascular complications.

•NO is normally produced from L-arginine by endothelial nitric oxide synthase (eNOS) in the vasculature (Turko, *et al.*, 2001). •NO mediates endothelium-dependent vasorelaxation by its action on guanylate cyclase in vascular smooth muscle cells (VSMC), initiating a cascade that leads to vasorelaxation. •NO also displays antiproliferative properties and inhibits platelet and leukocyte adhesion to vascular endothelium (Turko, *et al.*, 2001). Therefore, •NO is considered a vasculoprotective molecule. However, •NO easily reacts with superoxide, generating the highly reactive molecule $ONOO^-$, and triggering a cascade of harmful events (Turko *et al*., 2001 and Vega-Lopez, 2004). First, $ONOO^-$ alters function of biomolecules by protein nitration as well as causing lipid peroxidation (Turko *et al*., 2001). As reported by Turko *et al*., 2001 increase levels of nitrotyrosine are associated with apoptosis of myocytes, endothelial cells and fibroblasts in diabetes. Second, ONOO- causes single-strand DNA breakage which in turn activates nuclear enzyme poly (ADP-ribose) polymerase (PARP) (Soriano *et al.*, 2001). Third, it decreases •NO bioavailability causing impaired relaxation and inhibition of the antiproliferative effects of •NO (Maritim *et al*., 2003). Furthermore, $ONOO^-$ oxidizes tetrahydrobiopterin (BH_4), an important cofactor for NOS, and causes uncoupling of NOS, which produces $\bullet O_2^-$ instead of •NO (Maritim *et al.*, 2003). ROS-induced peroxidation of membrane lipids alters the structure and the fluidity of biological membranes, which ultimately affects function (Guiglian *et al.*, 1997 and Beckman and Koppenol, 1996). All these pathological modifications contribute to the pathogenesis of vascular dysfunction.

Production of one ROS or RNS may lead to the production of others through radical chain reactions. O_2^- is produced by one electron reduction of oxygen by several different oxidases including NAD(P)H oxidase, xanthine oxidase, cyclooxygenase and even eNOS under certain conditions as well as by the mitochondrial electron transport chain during the course of normal oxidative phosphorylation, which is essential for generating ATP (Evans, 2003). Under normal conditions, $\bullet O_2^-$ is quickly eliminated by antioxidant defense mechanisms. $\bullet O_2^-$ is dismutated to H_2O_2 by manganese superoxide dismutase (Mn-SOD) in the mitochondria and by copper (Cu)-SOD in the cytosol (Evans, 2003). H_2O_2 is converted to H_2O and O_2 by glutathione peroxidase (GSH-Px) or catalase in the mitochondria and lysosomes, respectively. H_2O_2 can also be converted to the highly reactive •OH radical in the presence of transition elements like iron and copper. ROS can stimulate oxidation of low-density lipoprotein (LDL), and ox-LDL, which is not recognized by the LDL receptor, can be taken up by scavenger receptors in macrophages leading to foam cell formation and atherosclerotic plaques (Boullier *et al.*, 2001). $\bullet O_2^-$ can activate several damaging pathways in diabetes including accelerated formation of advanced glycation end products (AGE), polyol pathway, hexosamine pathway and PKC, all of which have been proven to be involved in micro- and macrovascular complications.

1.8.1 Oxidative Stress in Diseases

Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's disease, (Rhee *et al.,* 2005 and Wood *et al.,* 2003) Parkinson's disease, (Claiborne *et al.,* 1999) the pathologies caused by diabetes, (Jonsson and Lowther, 2007) rheumatoid arthritis, (Lee *et al.,* 2003) and neurodegeneration in motor neuron diseases. Oxidative damage in

DNA can cause cancer. However, several antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase etc. protect DNA from oxidative stress. It has been proposed that polymorphisms in these enzymes are associated with DNA damage and subsequently the individual's risk of cancer susceptibility (Khan *et al.,*2010).

1.8.2 Sources of Oxidative Stress in Diabetes

There are multiple sources of oxidative stress in diabetes including non-enzymatic, enzymatic and mitochondrial pathways.

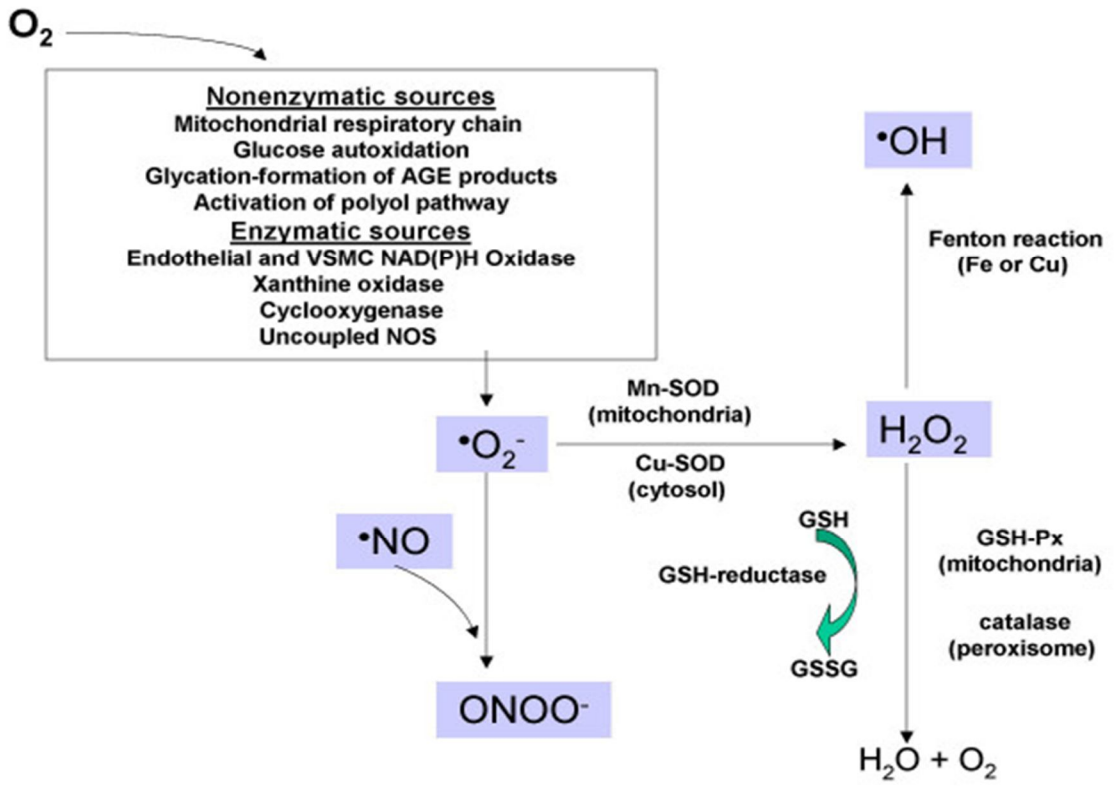


Fig 1.4: Generation of Reactive Species in Diabetes (Johansen *et al.*, 2005).

Highlighted in gray are some of the most important ROS and RNS in vascular cells. Oxygen is converted to $\bullet O_2^-$ via the activation of enzymatic and non-enzymatic pathways, which is then dismutated to H_2O_2 by SOD. H_2O_2 can be converted to H_2O by catalase or glutathione peroxidase (GSH-Px) or to •OH after reaction with Cu or Fe. Glutathione reductase regenerates glutathione (GSH). In addition, $\bullet O_2^-$ reacts rapidly with •NO to form $ONOO^-$.

Non-enzymatic sources of oxidative stress originate from the oxidative biochemistry of glucose. Hyperglycemia can directly cause increased ROS generation. Glucose can undergo autoxidation and generate •OH radicals which will involve oxidative stress. This free radical destroys pancreatic beta cells and leads to diabetic complications (Turko *et al.*, 2001). In addition, glucose reacts with proteins in a non-enzymatic manner leading to the development of Amadori products followed by formation of (advanced glycation end products) AGEs. ROS is generated at multiple steps during this process. In hyperglycemia, there is enhanced metabolism of glucose through the polyol (sorbitol) pathway, which also results in enhanced production of $\bullet O_2^-$ (Turko *et al.,* 2001).

Enzymatic sources of augmented generation of reactive species in diabetes include NOS, NAD(P)H oxidase and xanthine oxidase (Guzik *et al.*, 2000, Guzik *et* al., 2002 and Aliciguzel, 2003). All isoforms of NOS require five cofactors/prosthetic groups such as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), heme, BH_4 and Ca_2+ -calmodulin. If NOS lacks its substrate L-arginine or one of its cofactors, NOS may produce $\bullet O_2^-$ instead of •NO and this is referred to as the uncoupled state of NOS (Maritim *et al.*, 2003, Guzik *et al.*, 2000, Guzik *et al.*, 2002 and Aliciguzel, 2003). NAD(P)H oxidase is a membrane associated enzyme that consists of five subunits and is a major source of $\bullet O_2^-$ production (Guzik *et al*, 2000,Guzik *et al.*,

2002, Kitada *et al*., 2003 and Etoh *et al*., 2007). Guzik *et al*., 2000 investigated $\bullet O_2^-$ levels in vascular specimens from diabetic patients and probed sources of $\bullet O_2^-$ using inhibitors of NOS, NAD(P)H oxidase, xanthine oxidase and mitochondrial electron transport chain. This study demonstrated that there is enhanced production of $\bullet O_2^-$ in diabetes and this is predominantly mediated by NAD(P)H oxidase. Furthermore, the NOS-mediated component is greater in patients with diabetes than in patients who do not have diabetes.

The mitochondrial respiratory chain is another source of non-enzymatic generation of reactive species. During the oxidative phosphorylation process, electrons are transferred from electron carriers NADH and $FADH_2$, through four complexes in the inner mitochondrial membrane, to oxygen, generating ATP in the process. Under normal conditions, $\bullet O_2^-$ is immediately eliminated by natural defense mechanisms. A research report indicated that hyperglycemia-induced generation of $\bullet O_2^-$ at the mitochondrial level is the initial trigger of vicious cycle of oxidative stress in diabetes (Miller, 1997). When endothelial cells are exposed to hyperglycemia at the levels relevant to clinical diabetes, there is increased generation of ROS and especially $\bullet O_2^-$, which precedes the activation of four major pathways involved in the development of diabetic complications. Nishikawa *et al.,* (2000) elegantly demonstrated that generation of excess pyruvate via accelerated glycolysis under hyperglycemic conditions floods the mitochondria and causes $\bullet O_2^-$ generation at the level of Complex II in the respiratory chain. What is more important is that blockade of $\bullet O_2^-$ radicals by three different approaches using either a small molecule uncoupler of mitochondrial oxidative phosphorylation , overexpression of uncoupling protein-1 (UCP1) or over expression of Mn-SOD, prevented changes in NF-κB as well as polyol pathway, AGE formation and PKC activity. Based on this information, it has been postulated by several groups that mitochondrial $\bullet O_2^-$ is the initiating snowball that turns oxidative stress into an

avalanche in diabetes by stimulating more ROS and RNS production via downstream activation of NF-κB-mediated cytokine production, PKC and NAD(P)H oxidase. Thus, inhibition of intracellular free radical formation would provide a causal therapy approach in the prevention of oxidative stress and related vascular complications in diabetes (Johansen *et al.,* 2005).

1.8.3 Diabetes

The term diabetes mellitus referred to simply as diabetes describes a metabolic disorder of multiple etiology characterized by high blood sugar levels with disturbances of carbohydrate, fat and protein resulting from defect in insulin secretion, insulin action, or both. Insulin is a hormone that is secreted or produced by the groups of cells (beta cell) within the pancreas called islet of langerghans. The pancreas is an organ that sits behind the stomach and has many functions in addition to insulin production. Insulin in addition to helping glucose enter the cells also helps in regulating the level of glucose in the blood. After a meal, blood glucose level rises. In response to this increase in blood glucose level, the pancreas releases more insulin into the blood stream to help glucose enter the cells and lowers blood glucose level.

1.8.4 Classification of Diabetes

1.8.5 Type 1 Diabetes

Type 1 Diabetes is an auto immune disease. This form of diabetes, previously encompassed by the terms insulin-dependent diabetes, type 1 diabetes or juvenile-onset diabetes, results from autoimmune mediated destruction of the beta cells of the pancreas. The rate of destruction is quite variable, being rapid in some individuals and slow in others (Zimmet *et al*., 1994). The

rapidly progressive form is commonly observed in children, but also may occur in adults (Humphrey *et al.,* 1998). The slowly progressive form generally occurs in adults and is sometimes referred to as latent autoimmune diabetes in adults. Children and adolescents may present ketoacidosis as the first manifestation of the disease while others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress. Others, particularly adults, may retain residual beta-cell function for many years (Zimmet *et al.,* 1994). Individuals with this form of type I diabetes often become dependent on the insulin for survival eventually and at risk for ketoacidosis (Willis *et al.,* 1996). At this stage of the disease, there is little or no insulin secretion as manifested by low or undetectable levels of plasma c-peptide (Hother-Nielsen *et al.,* 1988).

Idiopathy

There are some forms of type 1 diabetes which have no known etiology. Some of the patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity. This form of diabetes is more common among individuals of African and Asia origin. In another form found in Africans, an absolute requirement for insulin replacement therapy in affected patients may come and go, and patients periodically develop ketoacidosis. (Ahren *et al.,* 1984).

Prevalence of Type 1 diabetes

Type I diabetes mellitus has wide geographic variation in incidence and prevalence. Annual incidence varies from 0.6 cases per 100,000 populations in China to 41.4 cases per 100,000 in Finland. The incidence of type 1 diabetes mellitus varies from 4 to 10/100.000 among the 0-19yr

old population in Africa (Kadiki and Moawad, 1994). Substantial variations are observed between nearby countries with differing lifestyle, such as Estonia and Finland and between genetically similar population such as Iceland and Norway. Most countries report that incidence rate have at least doubled or more in the last 20 years. Incidence appears to increase with distance from the equator (Alberti *et al*., 1998).

1.8.6 Type 2 Diabetes

Diabetes of this type previously encompassed non-insulin-dependent diabetes, or adult-onset diabetes. It is a term used for individuals who have relative (rather than absolute). Insulin deficiency. People with this type of diabetes frequently are resistant to the action of insulin (Defronzo *et al.,* 1997; Lilioja *et al.,* 1993). Initially, and often throughout their lifetime these individuals do not need insulin treatment to survive. This form of diabetes is frequently undiagnosed for many years because the hyperglycemia is often not severe enough to provoke noticeable symptoms of diabetes. (Mooy *et al.,* 1995; Harris, 1993). The patients are however at increased risk of developing macro and micro vascular complications (Mooy *et al.,* 1993). The majority of patients with this form of diabetes are obese and obesity itself causes or aggravates insulin resistance (Campbell *et al.,* 1993; Bogardus *et al.,* 1985). Many of those who are not obese by traditional weight criteria may have an increase percentage of body fat distributed predominately in the abdominal region (Kissebah *et al.,* 1982). Patients with this form of diabetes may have insulin levels that appear normal or elevated, the high blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their beta-cell function been normal (Polonsky *et al.,* 1996). Thus insulin secretion is defective and

insufficient to compensate for the insulin resistance. On the other hand, some individuals have essentially normal insulin action, but markedly impaired insulin secretion. The risk of developing type 2 diabetes increases with age, obesity, and lack of physical activity (Zimmet, 1991; Harris *et al.,* 1995). It occurs more frequently in women with prior GDM and individuals with hypertension and dyslipidaemia (Alberti, 1998).

Prevalence of Type 2 diabetes

Type 2 diabetes is less common in non-Western countries where the diet contains fewer calories and calories expenditure on a daily basis is higher. However, as people in these countries adopt western lifestyles, weight gain and type 2 diabetes mellitus are becoming virtually epidemic.

1.8.7 Gestational Diabetes

Gestational Diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2%-5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable but requires careful medical supervision throughout the pregnancy. About 20-50% of affected women develop type 2 diabetes later in life. Even though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital cardiac and central nervous system anomalies, and skeletal muscle malformation. Increased fetal insulin may inhibit fetal surfactant production and cause respiratory distress syndrome (Lawrence *et al.,* 1989).

1.8.8 Pathophysiology of Diabetes

Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells. Therefore deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus. Most of the carbohydrates in food are converted within a few hours to monosaccharide glucose, the principal carbohydrate found in blood and used by the body as fuel. Insulin is released into the blood by beta cells (b-cell) found in the islets of langerhans in the pancreas, in response to rising levels of blood glucose. Typically, after eating glucose is used by two thirds of the body's cells for use as fuel for conversion to other needed molecules or for storage. Lowered glucose levels result in the reduced release of insulin from beta cells and in the reverse conversion of glycogen to glucose when glucose levels fall. This is mainly controlled by the hormone glucagon which acts in an opposite manner to insulin. Glucose thus recovered by the liver re-enters the blood stream; muscle cells lack the necessary export mechanism. If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or resistance) or if the insulin is defective, then glucose will not be associated properly by those stored appropriately in the liver and muscle. The net effect is persistent synthesis, and other metabolic derangements, such as acidosis.

1.8.9 Epidemiology of Diabetes

In 2000, according to the World Health Organization at least 171 million people worldwide suffer from diabetes or 2.5% of the population (Wild *et al.,* 2004). Its incidence is increasing rapidly, and it's estimated that by the year 2030, this number will almost double (Wild *et al.,*

2004). Diabetes mellitus occurs throughout the world, but is more common especially type 2 in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030 (Wild *et al.,* 2004). The increase in incidence of diabetes in developing countries follows the trend of urbanization and lifestyles changes, perhaps most importantly a Western life style-diets.

1.9 Effect of Flavonoids on Diabetes Mellitus

Flavonoids in treatment of diabetes mellitus: All flavonoids cannot cure diabetes mellitus because most types of this disease are basically genetic and no single drug can correct an inborn error. However, flavonoids can ameliorate some of the consequences of diabetes mellitus (Havsteen, 2002). Flavonoids have been identified to be good inhibitors of aldose reductase (Patra *et al.,* 2010). The fig (1.5) depicts the mechanism of flavonoids in diabetes mellitus.

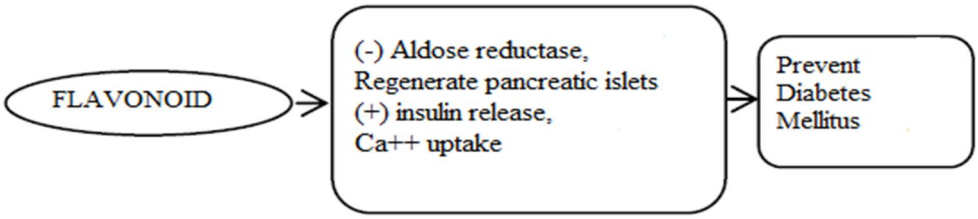


Fig 1.5: Effects of flavonoid on Diabetes mellitus

It has been reported by several researchers that quercetin possess antidiabetic activity and it has been found that it brings about regeneration of pancreatic islets and increases insulin release in streptozotocin-induced diabetes. Also, it has been reported to stimulate Ca^{2+} uptake from isolated islet cells thus suggesting it to be effective even in non-insulin dependent diabetes (Tapas *et al.,*

2008). Li *et al.,* (2009) indicated that flavonoids in *Ipmoea batalas* leaf possess antidiabetic activity against alloxan-induced diabetes at a dose of 100 mg/kg. Sriram *et al.,* (2011) reported fisetin to be a therapeutic agent for treatment of diabetes mellitus at a dose of 10 mg/kg.

1.10 Research Statement

Oxidative stress, a result of imbalance between the antioxidant defense system and the formation of reactive oxygen species, may induce damage to cellular biomolecules such as DNA, RNA, proteins, enzymes, carbohydrates, and lipids through oxidative modification and contribute to the pathogenesis of human diseases. As a result, reactive oxygen species have been implicated in many diseases, including cardiovascular disease, diabetes, rheumatic joint inflammation, cataracts, Parkinson's and Alzheimer's disease.

1.11 Justification

Antioxidants through their scavenging power are useful for the management of reactive species mediated diseases. However, synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT), tertiary butylated hydroxy quinine and gallic acid esters have been suspected to show low solubility and are also carcinogenic. Hence, strong limitations have been placed on their use and there is a trend to replace them with naturally occurring antioxidants

considering the very long traditional use of herbal medicine and the large body of evidences supporting their effectiveness.

1.12 Aims and Objectives

To evaluate the pharmacognostic characters of the root bark of *Acacia albida*

To determine phytochemical constituents of *Acacia albida* root bark

To evaluate the antioxidant and antidiabetic activity of *Acacia albida* root bark

To determine the acute toxicity of *Acacia albida* root bark.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of the Family Fabaceae/Leguminosae

Fabaceae or (Leguminosae) is a large and economically important family of flowering plants, which is commonly known as the legume family, pea family, bean family or pulse family. The name 'Fabaceae' comes from the defunct genus Faba, now included into Vicia. Leguminosae is an older name still considered valid, and refers to the typical fruit of these plants, which are called legumes. Fabaceae is the third largest family of flowering plants, behind Orchidaceae and Asteraceae, with 730 genera and over 19,400 species, according to the Royal Botanical Gardens. The largest genera are *Astragalus* with more than 2,000 species, *Acacia* with more than 900 species, and Indigofera with around 700 species. Other large genera include *Crotalaria* with 600 species and Mimosa with 500 species. The species of this family are found throughout the world, growing in many different environments and climates. A number are important agricultural plants, including: *Glycine max* (soybean), *Phaseolus* (beans), *Pisum sativum* (pea), *Cicer arietinum* (chickpeas), *Medicago sativa* (alfalfa), *Arachis hypogaea* (peanut), *Ceratonia siliqua* (carob), and *Glycyrrhiza glabra* (licorice), which are among the best known members of Fabaceae. The Fabaceae comprises of three subfamilies with distribution and some representative species (Hutchinson, 1964).

- **Mimosoideae**: 80 genera and 3,200 species. Mostly tropical and warm temperate Asia and America.Typically, *Mimosa*, *Acacia*.

- **Caesalpinioideae**: 170 genera and 2,000 species, cosmopolitan. Typically,*Caesalpinia*, *Senna*, *Bauhinia*, *Amherstia*.
- **Papilionoideae**: 470 genera and 14,000 species, cosmopolitan. Typically, *Astragalus*, *Lupinus*.

In the Caesalpinioideae, the flowers are often zygomorphic, as in *Cercis*, or nearly symmetrical with five equal petals in *Bauhinia*. The upper petal is the innermost one, unlike in the Faboideae. Some species, like some in the genus *Senna*, have asymmetric flowers, with one of the lower petals larger than the opposing one, and the style bent to one side. The calyx, corolla, or stamens can be showy in this group.

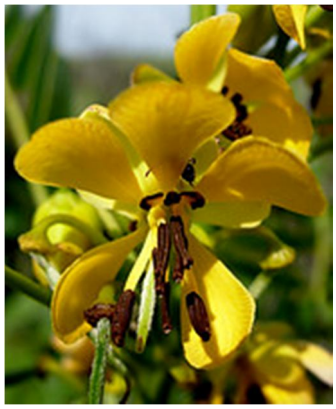


Fig 2.1: Structure of a Typical Ceasalpinoid Flower

Floral formulae · | ·, ♂, K5, $C_{5,}$ $A_{10,}$ $\underline{G_1}$

In the Mimosoideae the aestivation of petals and sepals have been of fundamental importance in distinguishing the three subfamilies within Fabaceae (Polhill and Raven, 1981), with the Mimosoideae characterized as having valvate (non-overlapping), rather than imbricate (as in the

Caesalpinioideae and Papilionoideae), aestivation of the petals and typically the sepals (Luckow et al., 2003). Most Mimosoids have small (< 1-2 cm), regular or actinomorphic (radially symmetrical) flowers aggregated into spicate or capitate heads, with stamens usually 3 to 10 but sometimes many (>100). The filaments of the stamens are often long and brightly coloured (orange to red), making for attractive inflorescences. Root nodules are generally present suggesting most if not all mimosoids exhibit symbiotic nitrogen fixation (Sprent, 2001). Leaves in this subfamily are mostly bipinnate (i.e., twice pinnately compound) and often have specialized glands. In many of the endemic Australian species of *Acacia* the leaves are reduced to simple "phyllodes".



Fig 2.2: Structure of a Typical Mimosoid Flower

Floral formulae Ø, ♂, K $(_5)$, C_5 or 4, Aα, $\underline{G_1}$

Papilionoideae is characterized by pentamerous (floral parts in fives or multiples thereof), the flowers are zygomorphic, and have a specialized structure. The upper petal, called the banner, is large and envelops the rest of the petals in bud, often reflexing when the flower blooms. The two adjacent petals, the wings, surround the two bottom petals. The two bottom petals are fused together at the apex (remaining free at the base), forming a boat-like structure called the keel.

The stamens are always ten in number, and their filaments can be fused in various configurations, often in a group of nine stamens plus one separate stamen. Although this floral architecture is ubiquitous in the subfamily, exceptions do occur, with nearly actinomorphic flowers (e.g., *Cadia* in tribe Sophoreae; *Inocarpus* in tribe Dalbergieae) and flowers lacking 1 or more of the five petals known from several groups (e.g., tribe Amorpheae). Unlike the other subfamilies, many papilionoids have the ability to synthesize quinolizidine alkaloids, isoflavones, and non-protein amino acids such as canavanine, while bipinnate leaves, complex leaf glands and compound pollen grains are lacking (Polhill, 1994).

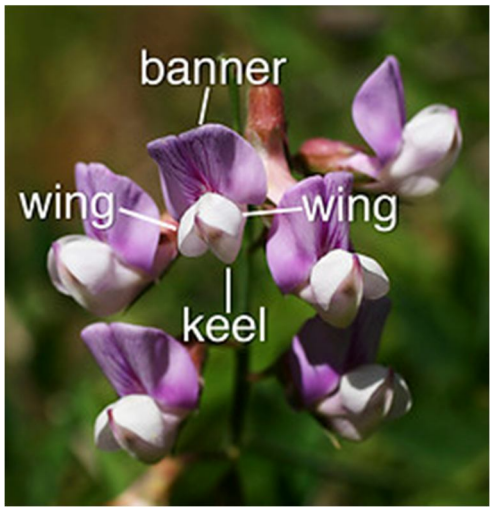


Fig 2.3: Structure of a Typical Papilionoid Flower.

Floral formulae · | ·, ♂, K $(_5)$, C_{1+2+} $(_2)$, A $(_4)$, $\underline{G_1}$

Leaves

Fabaceae is characterized by leaves simple to compound (pinnate, rarely palmate, or bipinnate), unifoliate, trifoliate (*Medicago, Trifolium*), sometimes phyllodic (many species of *Acacia*), or reduced to a tendril (as in *Lathyrus*), spirally arranged, with stipules present that are sometimes

large and leaf-like (*Pisum*) or developed into spines (*Prosopis, Robinia*) (Wojciechowski, *et al.,* 2006) .

Flowers

Flowers are usually regular or irregular (i.e., actinomorphic to zygomorphic in symmetry, respectively), bisexual, with a single superior carpel (hypogynous to perigynous), pentamerous, arranged singly or in racemes, spikes, or heads. The principal unifying feature of the family is the fruit, the legume (Polhill, 1994).

Uses

Legumes have demonstrated agricultural importance for thousands of years, beginning with the domestication of lentils (*Lens esculenta*) in Iran dating to 9,500 to 8,000 years ago, their use as a food source during the prehistory of North and South America (beans, more than 3,000 years ago), and their use by the Roman Empire as a food source and for soil improvement (Graham and Vance, 2003). Today legumes are an increasingly invaluable food source not just for humans, accounting for 27% of the world's primary crop production, but also for farm animals (Graham and Vance, 2003). Legumes were grown on more than 13% of the total arable land under cultivation in the world in 2004 (Gepts *et al.,* 2005). Grain legumes alone contribute 33% of the dietary protein nitrogen needs of humans, while soybeans (*Glycine max*) and peanut (*Arachis hypogeae*) provide more than 35% of the world's processed vegetable oil and a rich source of dietary protein for the poultry and pork industries (Graham and Vance, 2003).

While they produce nitrogen-containing protein in abundance, legumes are deficient in sulfur containing amino acids and other nutrients needed by people and animals. For this reason, legumes and cereal crops are often raised together to account for the amino acids and other elements they are each deficient in (Gepts *et al.,* 2005). Some legume species perform hydraulic lift, which makes them ideal for intercropping (Sprent, 2009).

2.2 Flavonoids of the Leguminosae

The family is extremely rich in flavonoids, both common and unusual types, and is alone among the families of the dicotyledons in containing representatives of all classes of flavonod (Bate-Smith, 1962). This may be partly due to size (the third largest of all flowering plants) but must also be because the leguminosae occupy a central position in the Archilamydeae and the Celastraceae. The three sub-families tend to have certain characteristic flavonoids or related phenolics. The Mimosoideae are rich in catechins, leucoanthocyanins and other tannins. The Ceasalpinioideae have ellagitannins (*in caesalpinia*) and the rare heartwood pigments haematoxylin and brazilin (Harborne, 1967). The Papilionate are characterized by having isoflavones and rotenoids but are also rich in anthocyanins and flavonol glycosides. Unusual flavonoids which appear in all three sub-families are 6-hydroxylated flavonols quercetagetin derivatives) and flavonoids lacking a 5-hydroxyl group (fisetin, robinetin etc).

Flavonoids have been reported to exert wide range of biological activities. These includes: anti-inflammatory, antibacterial, antiviral, antiallergic (Cushnie and Lamb, 2005; Cook and Samman, 1996), cytotoxic antitumour, treatment of neurodegenerative diseases, vasodilatory action

(Williams *et al.,* 2004; Tsuchiya *et al*., 2010; Chebil *et al.,* 2006). In addition flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyclo-oxygenase and lipoxygenase enzyme activities. They exert these effects as antioxidants, free radical scavengers, chelators of divalent cation (Cook and Samman, 1996; Chebil *et al.,* 2006; Middleton *et al.,* 2000). These are also reported to inhibit variety of enzymes like hydrolases, hyalouronidase, alkaline phosphatise, arylsulphatase, cAMP phosphodiesterase, lipase, α-glucosidase, kinase (Narayana *et al.,* 2001).

2.3 Alkaloids of the Leguminosae

Many legumes synthesize the lupin alkaloids (Smith, 1976). The lupin quinolizidine alkaloids seems to be especially characteristic and of the tribes Sophoraeae, Genisteae and Podalyriceae of subfamily Lotoideae of the leguminosae. Some are very restricted, but the lupine, cytosine and sparteine types are so widespread that the tribes producing lupin alkaloids may have originated from a common ancestral stock (Cranmer and Turner, 1967). Variants of the lupin characterize smaller groups within the tribes.

The naturally occurring ℓ-Lupinine form is obtained from the seeds and herbs of *Lupinus luteus* and other lupines species belonging to the natural order Leguminosae; and also found in *Anabais aphylla* L. (Chenopodiaceae) (Kar, 2003).

The differences in alkaloid content in bitter and fodder types of *Lupinus albus* have been studied and have been found to contain sparteine, lupainine, and 13-hydroxylupaine, but they differ in regard to other alkaloid present (Szymanska, 1970). The bitter type contains angustifoline

(Scibor-Marchocka, 1970), multifoline, and probably 13-epi-hydroxylupanine, although this last is never found in any variety. The fodder type contains possibly a ketosparteine and three other components. New Lupinus species that have been investigated and it include *Lupinus paniculatus* Desr. Which contain sparteine and lupanine (Mammarella and Comin, 1971). Mollov *et al.,* 1971 embarked on a survey of Bulgarian leguminosae. *Cytisus hirsutus* L. collected from the slopes of the Lozen mountains in May at thebegining of the blossoming period, contains (-)- sparteine, (+)-lupanine, 13—hydroxylupanine and a new alkaloid, 7-hydroxy-sparteine.

2.4 Description of Genus *Acacia*

Acacia is a genus of shrubs and trees belonging to the subfamily mimosoideae of the family Fabaceae, first described in Africa by the Swedish botanist Carl Linnaeus in 1773. The plants tend to be thorny and pod bearing, with sap and leaves typically bearing large amounts of tannins. The generic name derived from “akakia” the name given by early Greek botanist-physician Pedanius Discorides (CA 40-900) to the medicinal tree *Acacia nilotica* in his book *material medica*. Acacias are known as thorn trees, whistling thorns or wattles.

The genus *Acacia* previously contained roughly 1300 species, about 960 of them native to Australia, with the remainder spread around the tropical to warm-temperate regions of both hemispheres, including Europe, Africa, southern Asia, and the Americas. However, in 2005 the genus was divided into five separate genera under the tribe "Acacieae." The genus *Acacia* was retained for the majority of the Australian species and a few in tropical Asia, Madagascar and

Pacific Islands. Most of the species outside Australia, and a small number of Australian species, were reclassified into *Vachellia* and *Senegalia*. The two final genera, *Acaciella* and *Mariosousa*, each contain about a dozen species from the Americas.

The leaves of *Acacias* are compound pinnate in general. In some however, more especially in the Australian and Pacific Islands species, the leaflets are suppressed and the leaf stalks (petioles) become vertically flattened and serve the purpose of leaves (Singh, 2004). The small flowers have 5 very small petals, almost hidden by the long stamens, and are arranged in dense globular or cylindrical clusters. *Acacia* flowers can be distinguished from those of a large related genus *Albizia*, by their stamens which are joined at the base (Singh, 2004). The plants often bear spines, especially those species growing in arid regions. These sometimes represent branches which have become short, hard and pungent, or sometimes leaf-stipules. Acacia seeds can be difficult to germinate.

2.4.1 Phytochemistry of Acacia

Acacias contain a number of organic compounds that defend them from pests and grazing animals. Many of these compounds are psychoactive in humans. The alkaloids found in Acacias include dimethyltryptamine (DMT), 5-methoxy-dimethyltryptamine (5-MeO-DMT) and N-methyltryptamine (NMT).

Nineteen different species of Acacia in the Americas contain cyanogenic glycosides, which, if exposed to an enzyme which specifically splits glycosides, can release hydrogen cyanide (HCN) in the acacia "leaves" (This sometimes results in the poisoning death of livestock).

In Asia, there are a number of reports of HCN poisoning of livestock when fed on the pods and leaves of *A. leucophloea* (Gupta and Naurival, 1966; Prasad *et al.*, 1977; Vihan and Panwar, 1987). In South Africa, Steyn and Rimington (1935) reported the presence of cyanogenic glycosides in *A. haematoxilon*, *A. lasiopetala*, *A. tortilis* subsp. *heteracantha*, *A. robusta* and *A. hebeclada* subsp. *hebeclada*. They found higher concentrations of HCN from leaves and immature pods than from mature pods. In *A. robusta*, the glycoside was present without the corresponding hydrolytic enzyme. They isolated the cyanogenic compound acacipetalin from *A. lasiopetala* and *A. hebeclada*. Siegler *et al.*, (1975) isolated a further compound, dihydroacipetalin, from the leaves and shoots of *A. sieberiana* var. *woodii*.

There are numerous reports of the presence of polyphenolic compounds in the various components of plants of the genus *Acacia*. From the standpoint of nutrition, the most important of these are the tannins, compounds with molecular weights in excess of 500, which are able to form complexes with, and to precipitate proteins (McLeod, 1974; Mangan, 1988). Hydrolyzable tannins are readily broken down by acids, bases and enzymes to form sugars and phenolic carboxylic acid and are therefore easily degraded in the gut. In contrast, condensed tannins are not degraded and so are available to form complexes with proteins, resulting in reduced feed intake and digestibility.

Acacic acid-type saponins (AATS) from leguminosae, is a class of very complex glycosides possessing a common aglycon unit of the oleanane-type (acacic acid = 3β, 16α, 21β trihydroxy-olean-12-en-28 oic acid), having various oligosaccharide moieties at C-3 and C-28 and an acyl group at C-21. About sixty molecules of this type have been actively explored in recent years from Leguminosae family, from a chemical point of view and some fifty were reported to

possess cancer related activities. These include cytotoxic/antitumor, immunomodulatory, antimutagenic, and apoptosis inducing properties and appear to depend on the acylation and esterification by different moieties at C-21 and C-28 of the acacic acid-type aglycone.

Some AATS can be useful in the search for new potential antitumor agents from Fabaceae. Furthermore, the sequence 28-*O*-{Glc-(1→3)-[Ara$_f$-(1→4)]-Rha-(1→2)-Glc-Acacic acid}, often encountered in the genera *Acacia* may represent a chemotaxonomic marker of the Mimosoideae subfamily.

2.4.2 Uses of *Acacia*

The *Acacia* trees provide a valuable browse for game and livestock as well as being a valuable source of highly nutritious pods which can be stored as a dry season supplement for livestock (Hines and Eckman 1993). Tannins are present in most vascular plants and in a number of *Acacia* species they occur in sufficient concentrations for use in tanning leather. Tannins also act as an anti-nutritional factor due to their ability to precipitate proteins from an aqueous solution. Condensed tannins are known to be present in *A. nilotica* pods and have been shown to lower growth rate in sheep due to their ability to reduce nitrogen and neutral detergent fibre digestibility; a similar effect is also produced by the hydrolysable tannins in *A. sieberiana* pods (Kumar, 1992).

The polysaccharide exudate (gum) produced by a number of *Acacia* species are used for a number of domestic purposes, including adhesives, a constituent of ink, in crafts, as a cosmetic, in confectionery and as a food. Industrially, gum arabic is applied as an adhesive, as a protective

colloid and safeguarding agent for inks, sensitizer for lithographic plates, coatings for special papers, sizing agent to give body to certain fabrics, and anti-corrosive coating for metals; it is also used in the manufacture of matches and ceramic pottery (Cossalter, 1991).

The astringent activity of tannins causes a contraction of mucous surfaces, coagulates proteins and is useful in stopping bleeding of small wounds and other discharges (Brown, 1977). Hagos *et al.,* (1987) have demonstrated that the stem bark of *A. tortilis* subsp. raddiana, which is used in Somalia against asthma, contains pharmaceutically active compounds that inhibit muscular contractions of the lower small intestine of the guinea pig.

The species is not regarded as a fuelwood source (Cline-Cole *et al.,* 1990); however, given its availability it has been used more and more as other resources have been steadily depleted.

2.5 *Acacia albida* Scientific Classification

Kingdom Plantae
Division Magnoliophyta
Class Magnoliopsida
Order Fabales
Family Fabaceae
Sub-family Mimosoideae
Genus Acacia
Species Albida (Delile A. Chev)
Common Names
Hausa Gawo

2.5.1 Description of *Acacia albida*

Acacia albida is one of the largest thorn trees, reaching 30m in height, with spreading branches and a rounded crown. It is the fastest growing savanna tree in Africa (National Academy of Sciences, 1975). It is a long-lived species, with an average life span of 70-90 years in the Sudan and known to live more than 150 years in Zambia. *Acacia albida* has an extensive taproot system which develops rapidly to reach an adequate moisture layer. This characteristic makes the species relatively drought-resistant. On coarse alluvial sands of Sudan, a six month-old seedling with 8cm of a real growth had a taproot of 70cm, a three-year-old seedling excavated in northern Nigeria produced a taproot in excess of 9.9m (Weber, 1978).

The most unusual phenological characteristic of the species is retention of the leaves during the dry season and shedding of leaves at the onset of the wet season. No other African savanna species is known to possess this reverse deciduous cycle. It prefers a well drained sandy soil with a permanent water table and thrives in climates characterized by long summers, or a dry season with long days. It tolerates seasonal waterlogging and salinity but cannot withstand heavy clayey soils. Typically it occurs on bush-fallow of cultivated fields or land grazed by livestock, and rarely occurs in natural woodlands that have not been exploited by man (Weber, 1978).

Young trees have inverted cone-shaped crown, while older trees have a hemispherical large canopy. Young branches and twigs are cream coloured to whitish, stipular spines whitish, straight in axillary pairs, somewhat swollen at the base, up to 5 cm long, with a brown tip (The basal thickening is a characteristic distinguishing this species from the acacias with long thorns like *A. tortilis* ssp. raddiana, *A. nilotica* and *A. seyal*). Bark is grey, rough, deeply fissured and scaly with age. Leaves are bipinnate with 3-12 pairs of pinnae carrying, 6-23 pairs of leaflets up

to 12 mm long x 5 mm wide. Contrary to all other native "acacias" albida sheds its leaves in the rainy season and keeps them throughout the dry season, which is made possible by the fact that the species behaves as a phreatophyte (Weber, 1978).

Other characteristics are the lack of glands on the petioles, but present between each pair of pinnae. Flowers are slender, creamy white spikes, 4-14 cm long. Calyx 1-1.7 mm long, glabrous to pubescent with 5 sepals, Stamen filaments are shortly connate (united) at the base, Anthers large, eglandular (without glands). Corolla 3-3.5mm long with 5 free petals. Fruit is an unusual pod, bright orange to reddish-brown, thick, indehiscent, characteristically and conspicuously curled and twisted; large, up to 25 x 5 cm. Each pod contains 10-29 dark brown, ovoid, shiny seeds each measuring 10 x 6.0 mm and separated by thin septum. The seed coat is tough, leathery and waterproof. The specific epithet albida means somewhat whitish, referring to the colour of the stem (Vogt, 1995).

2.5.2 Ethnomedicinal Uses of *Acacia albida*

Acacia albida has been employed to treat conditions as varied as malaria, fever, toothaches, vomiting, diarrhea and other digestive disorders. *Acacia albida* has also been used to treat cough, pneumonia, kidney disorders, opthalmia, rheumatism, heart weakness, hemorrhage and postpartum complications when conventional medicine is not available. The bark is used to clean teeth, as it is believed to contain fluorine; an extract is used for toothache in humans and eye infections in livestock. The extremely high concentration of tannins (20-25%) found in the bark of the tree makes this plant a natural choice for the treatment of diarrhea, bleeding and

hemorrhage due to the tissue-drying and astringent properties of these molecules (Booth and Wickens, 1988).

The ubiquity of the medicinal use of *Acacia albida* in communities where it is found naturally is likely due to the tree's longstanding association with agriculture and human settlement. Many archeologists believe that the use of these trees helped to extend the reach of agriculture by improving soil fertility in parts of North Africa and the Middle East not affected by soil-enriching seasonal floods. These same trees, with their high tolerance to drought and nutrient rich if unpalatable seeds, may also have helped communities survive periodic famines. The close proximity of this useful plant to early agricultural communities would have likely inspired these peoples to be able to test its efficacy for a broad spectrum of ailments. It has played an important role in the development of centuries-old agrarian civilizations in large parts of the African continent (Weber, 1978).

The multiple uses of *Acacia albida* indicates that the species would be of interest to the agronomist for increasing crop protection without the use of expensive fertilizers; the watershed manager for improvement in soil water-holding capacity and decrease in erosion; the forester for timber uses; and the farmer an improvement in his living standards without a change in cultural traditions (Kirmse and Norton, 1984).



Fig 2.4: *Acacia albida* Tree in its Natural Habitat during the Dry Season



Fig 2.5: ***Acacia albida*** **Tree in its Natural Habitat during the Wet Season**



(a) ***Acacia albida*** **Branch**



(b) ***Acacia albida*** **Flower**





(c) *Acacia albida* Pods **(d) *Acacia albida* Roots**

Figure 2.6 (a-d): Some Morphological groups of *Acacia albida*

2.6 Reported Biological/Pharmacological Effects of *Acacia albida*

Activity of Plant Extracts Used in Northern Nigerian Traditional Medicine against Methicillin-Resistant *Staphylococcus Aureus* (Mrsa) was repoted by Aliyu *et al., (*2008). It was observed that

Methicillin-resistance *Staphylococcus aureus* (MRSA) has been emerging world wide as one of the most important hospital and community acquired pathogens (Kim *et al*., 2005). In Nigeria, Kenya and Cameroon, the rate of MRSA spread was about 21-30% in 2003 (Kesah *et al*., 2003), however, prevalence rate of about 69% of MRSA isolates was obtained in a study among healthy women in Zaria, Nigeria (Onanuga , *et al*., 2005). However, many indigenous plants are widely used in the treatment of various infectious diseases; such plants include *Acacia albida*. The results of the antibacterial activity by these workers revealed that only eight of the twelve plants extracts they worked on have demonstrated activity against the 25 hospital isolates of Methcillin-Resistant Staphylococcus Aureus used. The activity of methanol extract of *Acacia albida* (30mm) was significant against the MRSA. The MIC and MBC values obtained showed that methanol extract of *Acacia albida* was most potent against the MRSA isolates (MIC, 3 mgmL-1 and MBC, 4mgmL-1). The Methcillin-Resistant Staphylococcus Aureus activity of the plant according to the workers may be attributed to the rich tannins contents of bark of the plant.

Tijani *et al.,* 2008 studied the crude aqueous extract of *Faidherbia albida* for its anti-pyretic, anti-inflammatory and anti-diarrhoeal effects using yeast-induced pyrexia, kaolin-induced oedema and castor oil-induced diarrhoeal models in rats.

Anti-pyretic activity-The administration of 500 mg extract/kg body weight of the extract significantly ($p < 0.05$) reduced the body temperature of the rats from post yeast temperature of 40.1 ± 0.68 to 37.8 ± 0.13 and 37.2 ± 0.14 in the 3rd and 4th h respectively compared to control. Aspirin also reduced the rectal temperature of the yeast treated rats in the 1st, 2nd, 3rd and 4th h of the study. This reduction was found to be significantly ($P < 0.05$) different from both control and the extract treated rats. The anti-pyretic action of *F. albida* was found by the workers to probably dependent on its inhibition of PGE1 synthesis.

Anti-inflammatory activity-The extract showed inhibition of the kaolin-induced rat paw oedema in a dose-dependent manner throughout the duration of study. At 500 mg extract/kg body weight, the extract significantly ($P < 0.05$) inhibited formation of oedema in rat paw than aspirin throughout the duration of the study. In the study, injection of commonly used NSAIDs were observed to significantly reduce inflammatory indices while histamine and 5- hydeoxytryptamine (5-HT) were not effective.

Anti-diarrhoeal activity-Doses of 250 and 500 mg extract/kg body weight significantly ($p < 0.05$) protected the rats against castor oil-induced diarrhoea throughout the period of study dose-dependently. Aspirin at 16 mg/kg was also observed to protect the rats against castor oil-induced diarrhea in the1st and 2nd h of the study significantly.

The workers concluded that *F. albida* possesses anti-pyretic, anti-inflammatory and antidiarrhoeal effects, which may be due to the presence of its phytochemical constituents (tannins and alkaloids) and its inhibitory effects on prostaglandin synthesis. The study also provides empirical evidence for its use in folkloric medicine.

In another report by Tijani *et al.,* (2009) on the effect of *faidherbia albida* (*Acacia albida*) on some biochemical parameters of rats infected with *trypanosoma brucei brucei* reported the effect of hydro-alcoholic extract of the stem bark of *Faidherbia albida* on some biochemical parameters of rats infected with *Trypanosoma brucei brucei.* The extract significantly ($P<0.01$) reduce the Parasitaemia level time dependently in all the treated groups when compared with the negative control throughout the duration of observation.

There was a highly significant ($p<0.01$) time dependent increase in total protein, albumin and globulin levels in all the treated groups on days 5 and 10 post treatment of the study when compared with the infected untreated control.

The extract elevated the albumin level in the treated groups which may suggest the ability of the extract to prevent hepatocellular damage caused by the trypanosome infection. *F. albida* does possess trypanostatic effect as evident in the reduced rate of proliferation of parasitaemia in all the extract treated groups. In addition it prolonged the life of trypanosome-infected rats by reducing the accumulation of free fatty acids in circulation implicated in lysis of red blood cell leading to anaemia a leading cause of eventual death from Trypanosomiasis. It is therefore effective in the management of Trypanosomiasis related anemia thus providing scientific evidence for its folkloric medicinal use in the management of Trypanosomiasis.

The PCV value increased significantly ($p<0.01$) in the treated groups and the positive control on days 5 and 10 post treatment when compared to the negative control. The significant increase in packed cell volume (PCV) values of all infected and treated rats irrespective of time of treatment were suggested to be partly due to the ability of the extract to reduce the level of circulating surface active agents such as the free fatty acids especially linoleic acid that has been implicated in lysis of red blood cells.

The free fatty acid levels decreased significantly ($p<0.01$) in all the treated groups on days 5 and 10 post treatment of the study when compared with the infected untreated control. The results obtained from the study on the anti-trypanosomiasis effect of stem bark extract of *F. albida*

showed that the extract possess trypanostatic effects, since it slowed down the rate of proliferation of the trypanosome parasite and suggested may be due to the ability of the extract to inhibit prostaglandin biosynthesis as has been reported by Tijani *et al*. (2008).

According to Oseni *et al.,* (2012), in a work titled comparison of in vitro growth inhibitory activities of aqueous extracts of selected individual plants with the multi-component concoction, he observed that multiple-plant herbal concoctions (multi-component herbal concoctions) are used as remedies in many cultures around the world which is due to the advantages of synergism and low dosage of active principles compared with single plant herbal remedies. An aqueous concoction obtained from a combination of leaves of *Psidium guajava, Acacia albidia and Ficus exasperate* called choru liri (C.L.) is used for the treatment of bacterial infections amongst many tribes in northern Ghana. In the study, aqueous extracts *of P. guajava, A. albidia and F. exasperata* were screened separately for phytochemicals and their bacterial growth inhibitory activity evaluated in vitro using the agar diffusion method with *Escherichia coli* and *Salmonella typhi* as test organisms. The result was compared with that of an aqueous concoction obtained from a combination of the three plants (multi-component concoction) to determine any indication of synergism or additive effect. Ciprofloxacin was used as control. Phytochemical screening of the extracts revealed the presence of phytochemicals with reported antimicrobial activities. All the extracts exhibited antibacterial activity with the multi-component extract demonstrating a superior antibacterial property against the test organisms at concentrations ranging between 1.0-5.0 mg/ml. The antibacterial activity of the multi-component concoction was statistically not significantly different from that of Ciporfloxacin ($P = 0.87$). There was no indication of

synergism or additive effect in the multi-component concoction. This result justifies the use of the concoction in the treatment of bacterial infections in the northern part of Ghana.

In another report by Danjuma *et al.,* 2010 the methanol stem bark extract of *Acacia albida* was studied for its anticonvulsant effects in mice and chicks. The test systems selected were the maximal electroshock test (MEST) in chicks, Pentylenetetrazole (PTZ) and Strychnine (STN) induced seizure tests in mice. The effect of the extract on diazepam induced sleep in mice was conducted. The extract (50, 100, 200 mg/kg) significantly ($p< 0.001$) shortened onset of sleep compared to normal saline (control) from 4.8±0.2 to 2.2±0.3, 2.0±0.0 and 2.0±0.3, respectively. The methanol stem bark extract of *Acacia albida* also increased total sleeping time from 58.2±14.0 to 201.0±23.9, 111.4±16.1 and 89.6±22.5 at 50, 100 and 200 mg/kg, respectively. The increase was significant ($p< 0.001$) at 50 mg/kg. *Acacia albida* stem bark extract at 200 mg/kg protected 50% of the mice against STN induced seizure with 63.3% survival rate. There was no protection against STN induced seizure at 50 and 100 mg/kg of the extract but a 16.3% and 33.3% protection against mortality was observed respectively. The extract was also able to delay, though insignificantly the onset of seizure at all the doses tested. In the PTZ induced seizure test, the extract did not protect the mice against seizure nor mortality but there was a significant ($p< 0.05$) delay in onset of seizure at 100 and 200 mg/kg. Similarly the extract of *Acacia albida* did not protect chicks against MEST. However there was a non significant dose dependent shortening of recovery time at the doses tested.

A study of the anti-malarial activity of ethanolic stem bark extract of *Faidherbia albida (*Del) a. Chev (Mimosoidae) in Mice was conducted by Salawu *et al*., (2010).

Test on early malarial infection (4-day suppressive test) of the anti-plasmodial effect of the extract produced a significant ($P \leq 0.05$) and dose- dependent decrease in parasite counts. the average percentage chemo suppression produced was 24, 72.93 and 89.50% at 100, 200 and 400 mg extract/kg body weight respectively while, 5mg Chloroquine /kg body weight produced 98.34% chemo suppression.

Test on established infection (Rane test) of the anti-plasmodial effect of the extract produced significant ($P \leq 0.05$) and dose- dependent decrease in parasite counts. The mean percentage suppression of parasitaemia was 41, 86.67 and 88.33 at the doses of 100, 200 and 400 mg extract/kg body weight respectively. At 5 mg/kg, Chloroquine produced 99.44% chemo suppression. The extract produced significant ($P<0.05$, 0.01) dose-dependently prolonged the survival time of mice while, chloroquine at 5mg/kg body weight significantly ($P<0.001$) prolonged the survival time of treated mice.

Test on residual infection (repository test) of the anti-plasmodial effect of the extract produced significant ($P \leq 0.05$) and dose dependent decrease in parasite counts. The mean percentage chemo-suppression produced were 25, 32.14 and 51.78 at 100, 200 and 400mg extract/kg body weight respectively. At 5mg/kg, Chloroquine produced 71.42% chemo-suppression. The extract at the doses used and chloroquine at 5 mg/kg body weight respectively significantly ($P<0.05$) increased the body weight of mice when compared to the control.

The results obtained from the study showed that the ethanolic stem bark extract of *Faidherbia albida* possess significant suppressive effect against early infection, curative effect against

established infection and prophylactic effect against residual infection of the parasite at safe doses. The study therefore showed that the extract possess greater potential to be explored for development of antimalarial phytomedicine and further established scientifically the basis for its continuous use in folk medicine for the management of malaria.

Acute and sub-acute toxicity profiles of ethanolic stem bark extract of *F. albida* were evaluated in wistar albino rats by Salawu *et al* 2010. The acute toxicity was studied using the method of Lorke (1983). The sub-acute toxicity study was carried out using standard operating procedure on toxicity. The effects of the extract on clinical signs, feed and water intake, body weight changes, haematology, plasma biochemical parameters, relative organ weight (ROW) were evaluated. The oral LD_{50} of the extract was estimated to be greater than 5000 mg/ kg body weight.

Effect of the extract on haematological parameters in rats -There was highly significant ($p < 0.01$) increase in the white blood cell count in the groups treated 250 mg extract/kg and a highly significant ($p<0.01$) reduction in the lymphocyte levels in the 125 and 250 mg extract/ kg groups. There was a highly significant ($p<0.01$) increase in platelet count in all the treated groups when compared with the control. There was a highly significant ($p < 0.01$) increase in the neutrophil count in the 125 mg group and a significant ($p < 0.05$) increase at 250 mg extract/kg. There were no significant changes for all the treated groups in red blood cells, packed cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, monocytes and eosinophils.

Effects of the extract on relative organ weight (Row) of rats-There was a significant ($P < 0.05$) reduction in the row in the testes at 250 and 500 mg extract/kg body weight when compared with the control.

Effects of the extract on liver enzyme levels in rats produced a highly significant ($p < 0.01$) increase in the levels of aspartate and alanine transaminases at all dose levels and in the level of alkaline phosphatase at 125 and 500 mg extract/kg but there was no significant change in the levels of the total and direct bilirubin in all the treated groups.

Effects of the extract on liver proteins in rats-Significant ($p < 0.05$) increase in protein concentration was produced in rats given 500 mg extract/kg while there was no significant difference in albumin and globulin in all the treated groups.

Effect of the extract on renal indices of rats-No significant change was observed in urea, potassium, bicarbonate, sodium and chloride ions for all the treated groups. However, there was a highly significant ($P < 0.01$) reduction in the concentration of creatinine in all the extract treated groups.

Effect of the extract on haematological parameters in rats- There was highly significant ($p < 0.01$) increase in the white blood cell count in the groups treated 250 mg extract/kg and a highly significant ($p<0.01$) reduction in the lymphocyte levels in the 125 and 250 mg extract/ kg groups. There was a highly significant ($p<0.01$) increase in platelet count in all the treated groups when compared with the control. There was a highly significant ($p < 0.01$) increase in the neutrophil

count in the 125 mg group and a significant ($p < 0.05$) increase at 250 mg extract/kg. There were no significant changes for all the treated groups in red blood cells, packed cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, monocytes and eosinophils.

The extract produced slight increase in body weight of rats given 125 mg extract/kg body weight. However, dose-dependent highly significant ($P < 0.01$) decrease in body weight was observed at 250 and 500 mg/ kg-treated rats in weeks 2 and 3 of the study.

Feed and water intake was not affected by the treatment. The relative organ body weight ratio (ROW) - for all organs was not affected by the treatment except significant ($P < 0.05$) increase in the testes of rats treated with 250 and 500 mg extract/kg body weight. Although the treatment elicited highly significant ($P < 0.01$) changes in the levels of the hepatic and some of the haematological parameters, they were within the normal reference range for rats. The study revealed that the stem bark of the plant may be considered relatively safe when used sub-acutely. A study by Tanner *et al*., (1990) reported significant differences in the growth rates of sheep supplemented with fruits from four different *Acacia* spp. (*A. tortilis, F. albida, A. nilotica* and *A. sieberiana*) even though there were no significant differences in total nitrogen intake. The tannin-rich fruits, particularly of *A. sieberiana* and *A. nilotica*, reduced feed intake and the efficiency of nitrogen utilization by forming complexes with proteins which resulted in a shift of nitrogen excretion from urine to faeces. The growth rates of sheep fed the diets low in polyphenolics (*F. albida* and *A. tortilis*), were not significantly different from sheep

supplemented with noug meal (*Guizotia abyssinica*), indicating their suitability as dietary supplements.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 List of materials

Cappilary tubes

Chromatographic tank

Digital glucometer/ Strips (Accu-chek active®)

Mortar and Pestle

Test tubes

Thin layer chromatographic plates

UV Spectrophotometer

Water bath

Chemicals/ Reagents

2,2, diphenyl-1-picric hydrazyl (DPPH)

Acetic anhydride

Alloxan monohydrate

Aluminium chloride

Ascorbic acid

Benzene

Chloroform

Dinitrobenzoic acid

Dragendorff's reagent

Ethanol

Ether-ethyl

Fehling's solution A and B

Ferric Chloride

Folin-Ciocalteu reagent

Gallic acid

Glibenclamide

Hydrochloric acid

Mayer's reagent

Metallic magnesium

Methanol

Million's reagent

Molisch reagents

N/50 Iodine

N-butanol

Normal saline

Phlorogucinol

Potassium acetate

Potassium acetate

Potassium ferrocyanide

Quercetin

Sodium chloride

Sodium hydroxide

Sudan III & IV

Sulphuric acid

Wagner's reagent

Water

Laboratory Animals

Albino Wister rats

Swiss albino mice

3.1.1 Collection and Identification of Plant Material

Acacia albida Del. (Fabaceae) was collected at the University farm of Ahmadu Bello University, Zaria, Kaduna and identified by Late Dr. F. Tiseer of the Department of Biological Science, Ahmadu Bello University, Zaria. It was then confirmed and authenticated by U.S Gallah of the same department by comparism with a voucher number 900334.

3.2 Preparation of Plant Material

The plant root bark was removed, air dried and grinded with a milling machine, then stored at room temperature for subsequent use in study. Then 1.5kg of the plant material was extracted by maceration with aqueous methanol 70% (v/v). The filtrate obtained was evaporated to dryness on water bath which gave a yield of 240.7g and a percentage yield of 16.05 w/w. Thereafter, 100g of the extract was further subjected to partitioning by dissolving in water and partitioned with chloroform. Aqueous fraction yield was 34.85g and chloroform fraction yield was 0.7g. These were concentrated to dryness. The aqueous fraction was stored carefully for further studies.

3.3 Pharmacognostic Studies on the Root Bark of *Acacia albida*

3.3.1 Morphological Examination of the Root Bark of *Acacia albida*

The crude plant material was evaluated morphologically based on conclusions drawn from impressions on the sense organs (Kokate *et al.,* 2002).

3.3.2 Microscopical Examination of the Root Bark of *Acacia albida*

The fresh and powdered root bark of *Acacia albida* was used for this study. A thin anatomical section of the fresh plant sample and small quantity of the powder were separately put in clean test-tube containing chloral hydrate and heated on a water bath for clearing. Over heating was avoided as this caused crystallization leading to false observation. When the tissue were sufficiently cleared, they were carefully mounted on a glass slide with dilute glycerol and

observed with a compound microscope to identify microscopic diagnostic features of the plant (Kokate, 1994).

3.3.3 Chemo-microscopical examination of the Root Bark of *Acacia albida*

The powdered root bark was treated separately with various detecting chemical reagents on microscope and observed for the presence of chemical substances such as cellulose, hemicelluloses, lignin, calcium oxalate crystals, calcium carbonate crystals, starch, mucilage, tannins, fixed oils and fats using standard methods as stated below.

A. Identification of Cell Inclusions

i. Test for Starch

The powdered plant sample was stained with N/50 iodine on a glass slide. One drop of glycerol was added and the sample observed under the microscope. Appearance of a blue-black colouration will indicate presence of starch grains (Evans, 1996).

ii. Test for Tannins

The powdered plant sample was mounted on a glass slide in 5% Ferric chloride solution; one drop of glycerol was then added. The sample was observed under the microscope. Appearance of bluish or greenish colouration will indicate the presence of tannins (Evans, 1996).

iii. Test for Fixed Oils and Fats

The powdered plant material was placed on a slide and a drop of Sudan IV reagent was added. Appearance of pinkish colour indicates the presence of oils.

iv. Test for Calcium Oxalate/ Calcium Carbonate

The powdered plant material was cleared with chloral hydrate after which it was mounted in glycerol. Then some bright crystals were observed, two drops of 80% sulphuric acid was then added to the sample. Disappearance of the crystals on addition of 80% sulphuric acid without effervescence indicates calcium oxalate crystals while disappearance of the crystals on addition of the 80% sulphuric acid with effervescence indicate calcium carbonates (Evans, 1996).

v. Test for Proteins

The powdered plant sample was mounted in million' reagent, a red stain with the reagent will indicate the presence of proteins (Evans, 1996).

B. Identification of Cell Wall Materials

i Test for Cellulose

The plant sample was placed on a glass slide and a drop of N/50 iodine was added followed by a drop of 80% sulphuric acid. Appearance of bluish colouration indicates the presence of cellulose on the cell wall (Kokate, 1994).

ii. Test for Hemicellulose

The plant sample was treated with N/50 iodine solution. Appearance of blue colour on the cell wall indicates hemicelluloses (Kokate, 1994).

iii. Test for Lignin

The plant sample was placed on a slide and a drop of Phloroglucinol was added followed by a drop of concentrated hydrochloric acid. Appearance of bright red stain on sclerencymatous tissue indicates lignin (Kokate, 1994).

iv. Test for Suberin

The powdered root bark material was treated with Sudan III, mounted in glycerin and warmed slightly. Appearance of red stains on cork cells indicates the presence of suberin (Kokate, 1994).

3.4.1 Preliminary Phytochemical Screening of *Acacia albida* Root Bark

Basic phytochemical tests to detect the presence of alkaloids, tannins, saponins, anthraquinones, cardenolides, etc. were carried out on the aqueous root bark extract of *A. albida.*

3.4 Test for Carbohydrates

a. Molisch's Test

Few drops of Molisch's reagent was added to a little quantity of extract in a test tube and a small quantity of concentrated sulphuric acid was allowed to run down the side of the test tube to form

a lower layer. A purple to violet colour at the interface indicate the presence of carbohydrate (Evans, 1996).

b. Fehling's Test

To 2ml of extract, 5ml of a mixture of Fehling solutions A and B in the ratio 1:1 was added and the mixture boiled for few minutes. A brick-red precipitate indicates the presence of free reducing sugar (Evans, 1996).

3.4.1 Test for Glycosides

A. Test for Anthraquinone Glycosides

i. Borntrager's Test

Small portion of the extract was shaken with 10ml of benzene and filtered. Then 5ml of 10% ammonia solution was added to the filterate and stirred. The production of pink, red or violet colour in the ammonical (lower) phase indicates the presence of free hydroxyl-anthraquinones (Evans, 1986).

ii. Modified Borntrager's Test

Sample was boiled with 10ml aqueous sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of benzene, the benzene layer was separated and half its own volume of 10 per cent ammonia solution added. A pink, red, or violet colouration in the ammonia phase (lower layer) indicates the presence of anthraquinone derivatives in the extract (Evans, 1996).

B. Test for Cardiac Glycosides

a. Keller-killiani Test

The extract (0.5g) was dissolved in 2ml of glacial acetic containing one drop of ferric chloride solution. This was then underlayed with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring while, in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer (Sofowora, 2006).

b. Salkowski Test

The extract (0.5g) was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interface indicated the presence of a steroidal ring (i.e. aglycone portion of the cardiac glycoside) (Sofowora, 2006).

c. Legal Test

The extract was dissolved in pyridine and a few drop of 2 percent sodium nitroprusside together with a few drops of 20 percent NaOH were added. A deep red colour which faded to a brownish yellow indicated the presence of cardenolides (Sofowora, 2006).

3.4.3 Test for Saponins

a. Frothing Test

The plant extract (0.5g) was shaken with water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for the presence of saponins (Sofowora, 2006). In order to remove 'false-positive' results, blood haemolysis test was performed on the extract that frothed in water.

b. Haemolysis Test

About 2ml of 0.9% aqueous sodium chloride solution was taken into two test tubes. To one tube, 2ml of the extract was added while same volume of distilled water was added to the other tube. About 3 drops of human blood was added to each tube. The content was observed for 30 minute. Haemolysis in the tube containing the extract indicates the presence of saponins.

c. Liebermann-Burchards Test

Equal volume of acetic anhydride was added to the extract. About 1ml of concentrated sulphuric acid was added down the side of the tube. The colour change was observed immediately and later, red, pink or purple colour indicates the presence of triterpenes while blue or blue-green indicates steroids (Evans, 1996).

3.4.4 Test for Flavonoids

a. Shinoda's Test

The extract (0.5g) was dissolved in 1-2ml of 50% methanol. Metallic magnesium and four to five drops of concentrated hydrochloric acid were added. A red or orange colour indicates the presence of flavonoid aglycones.

b. Sodium Hydroxide Test

Few drops of aqueous Sodium hydroxide were added to 5ml of extract, a yellow colouration indicates the presence of flavonoid (Evans, 1996).

3.4.5 Test for Tannins

i. Lead Sub-acetate Test

About 3 drops of lead acetate solution was added to a solution of the extract. A coloured precipitate indicates tannins.

ii. Ferric Chloride Test

About 0.5ml of plant extract was stirred with 10ml of distilled water, and then filtered. Few drops of ferric chloride reagent added to the filtrate. A blue-black, green, or blue-green precipitate was taken as evidence for the presence of tannins (Evans, 1996).

3.4.6 Test for Alkaloids

About 0.5g of plant extract was stirred with 5ml of 1 percent aqueous hydrochloric acid on a steam bath; 1ml of the filterate was treated with a few drops of Mayer's, reagent and a second 1ml portion was treated similarly with Dragendorff's reagent . Turbidity or precipitation with either of these reagents was taken as preliminary evidence for the presence of alkaloids. Wagner's reagent was also used (Evans, 1996).

3.5.0 Quantitative Phytochemical Screening of *Acacia albida* Root Bark

3.5.1 Saponin Determination

To 20g of the plant powder in a conical flask, 100 ml of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4hr with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated after which 60 ml of n-butanol was added and re-extracted with n-butanol. The combined n-butanol fractions were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage of the original weight of plant sample (Obadoni and Ochuko, 2001).

3.5.2 Tannin Determination

To 0.5g of the plant powder in a 50 ml plastic bottle, 50 ml of distilled water was added and shaken for 1hr in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1M $FeCl_3$ in 0.IN HCl and 0.008M potassium ferrocyanide. The absorbance was measured with an ultraviolet spectrophotometer at 395nm wavelength within 10 min, using tannic acid as a standard solution (Van-Burden and Robinson, 1981).

3.5.3 Alkaloid Determination

To 10g of the plant powder was mixed 50ml of four volumes of ether and one volume of alcohol (95 per cent) shaken and allowed to stand for 10minutes followed by the addition of 1.5ml of dilute ammonia solution mixed with 2ml of water and shaken frequently for one hour. The mixture was transferred to a small percolator plugged with cotton wool and, when the liquid cease to flow, it was packed firmly and the percolation was continued with 25ml of ether-alcohol mixture and then with ether until the extraction of the alkaloid was completely effected. The volume of the percolate was reduced to 20ml by evaporation on a water bath and then transferred to a separator, washing the flask with thrice with 10ml of chloroform, and then added to the separator followed by 20ml of N/2 Sulphuric acid and shaken. It was allowed to separate and the lower layer collected. The extraction was continued with successive quantities, each with 10ml of a mixture of 3 volume of N/10Sulphuric acid and 1 volume of alcohol 95% until complete extraction of the alkaloid was effected. The mixed acid solution was washed with 10, 5, and 5ml

of chloroform and extracting each chloroform solution with the same 20ml of N/10 Sulphuric acid and discarding the chloroform (British Pharmacopoeia, 1968).

Futhermore, the combined acid solution was neutralized with dilute ammonia and 5ml in excess, shaken with successive quantities of each with 25ml of chloroform until extraction was completed. The chloroform solution was washed with the same 10ml of water and filtering into a flask through a plug of cotton wool previously moistened with chloroform. Most of the chloroform was distil and the remainder of the solution transferred to a shallow open dish. The remainder of the chloroform was evaporated; the residue was heated in an oven at 100 for 15min. The residue was dissolved in 2ml of chloroform, added 10ml of N/20 Sulphuric acid, warmed to remove the chloroform, cool and the excess acid was titrated with N/20 Sodium Hydroxide, using methyl red solution as indicator. Each ml of N/20 Sulphuric acid is equivalent to 0.01447g of alkaloids calculated as Hyoscyamine (British Pharmacopoeia, 1968).

3.5.4 Determination of Total Flavonoid Content

Aluminium chloride colorimetric technique was used for flavonoids estimation (Chang *et al.,* 2002). The extract (0.5ml of 1:10 g/ml) in methanol was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. It was left at room temperature for 30 min after which the absorbance of the reaction mixture was measured at 415nm with a double beam UV/Visible spectrophotometer (Shemadu, Japan). The calibration curve was plotted by preparing quercetin solutions at concentrations 12.5 to 100 g/ml in methanol.

3.5.5 Determination of Total Phenol Content

The total phenol of the extract was measured at 765nm by Folin Ciocalteu reagent (McDonald *et al.,* 2001). The dilute methanolic extract (0.5ml of 1:10 g/ml) or gallic acid (standard phenol ccompound) was mixed with Folin Ciocalteu reagent (5ml, 1:10 diluted with distilled water) and aqueous sodium carbonate (4ml, 1M). The mixture was allowed to stand for 15 min and the total phenols were determined by spectrophotometer at 765 nm. The standard curve was prepared using 0, 50,100, 150, 200, 250 mg/l solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol value was expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

3.6 Thin Layer Chromatographic Studies of Phytochemical Constituents of *A. albida*

3.6.1 Saponins

Two grams of powdered root bark of *A. albida* was extracted with 10ml 70% EtOH by refluxing for 10min. The filtrate was condensed, enriched with saturated n-BuOH, and thoroughly mixed. The butanol fraction was retained, condensed and used for chromatography. The saponins were separated using n-butanol, acetic acid, water (5:2:3) solvent mixture. The colour and Rf values of the spots were recorded by exposing the chromatogram to iodine vapour (Mallikharjuna *et al.,* 2007).

3.6.2 Phenols

The powdered root bark of *A. albida* was lixiviated in methanol on mechanical shaker for 24h. The condensed filtrate was used for chromatography. The Phenols were separated using n-butanol, acetic acid, water (5:2:3) solvent mixture. The colour and Rf values of the phenols were recorded under visible light after spraying the plate with Folin-Ciocalteu's reagent heating at 80^0C for10min (Mallikharjuna *et al*., 2007).

3.6.3 Flavonoids

The powdered root bark of *Acacia albida* was extracted with methanol. The extract was partitioned with chloroform and water. The condensed aqueous fraction was used for chromatography. The flavonoids were separated using n-butanol, acetic acid, water (5:2:3) solvent mixture. The colour and R_f values of the spots were recorded under visible light after spraying the plates with aluminium chloride.

3.6.4 Antioxidant Test on TLC

The antioxidant test used to evaluate the potential antioxidant activity by means of TLC is based on the method of Takao *et al.,* (1994). The detection of this activity is founded on the principle of capture of free radical provided by DPPH. When DPPH is reduced by a donor of hydrogen, its original purple colour turns to yellow-pale. The developed TLC plate was allowed to dry and

sprayed with 0.5% solution of DPPH in methanol. After optimal time of reaction (30min), the antioxidant activity will appear as yellow pale stains on purple background.

3.7 DPPH Radical Scavenging Activity

The antioxidant activity of the plant extract was assessed on the basis of the free radical scavenging effect of 2, 2-diphenyl-1-picryl hydrazyl (DPPH) free radical activity (Torkoglu *et al.,* 2006). 1ml of various concentration of extract (500, 250, 125, 62.5ug/ml) was added to 4ml of 0.004% methanol solution of DPPH. After 30min of incubation at room temperature, absorbance was read at 517nm against a blank. Ascorbic acid was used as standard. The results were expressed as percentage scavenging of radical.

$$\% \text{ scavenging of DPPH} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

3.8 Determination of Median Lethal Dose (LD_{50})

Phase 1: Three groups of three rats per group were given 10, 100, 1000mg/kg of the extract orally respectively. The treated rats were monitored for 24 hour for mortality and general behaviour.

Phase 2: After 24 hours, 3 groups of one rat each were given doses 1600, 2900, and 5000mg/kg based on the finding of phase 1. The rats were again monitored for 24 hours. The geometric mean of the least dose that killed rat and the highest dose that did not kill rat was taken as the median lethal dose (Lorke, 1983).

3.9 Evaluation of the Hypoglycemic Properties of *Acacia Albida*

Albino wistar rats (150-200g) were procured from the Animal House of Pharmacology Department, Ahmadu Bello University, Zaria and were acclimatized for 14 days before the commencement of the experiment. The acclimatized animals were fasted for 18 hours prior to the day of commencement of the experiment. However, the animals were allowed free access to water ad libtum prior to and throughout the duration of the experiment. Hyperglycemia was induced by intraperitoneal administration of 150 mg/kg of alloxan monohydrate in 0.9% normal saline. 5g of glucose was dissolved in 100ml of distilled water and given to the animals to help overcome the early hypoglycemic phase. After 72 hours, blood was withdrawn from the tail of the animals by tail tipping method. Rats with blood glucose level above 200mg/dl were considered as diabetic and included in the experiment. The animals were separated into 5 groups of 6 rats each and administered the extract (Resiraphaw *et al.,* 2002) at the following designated doses.

Experimental Design

Group 1 Control rats fed with normal saline according to body weight

Group 2 Diabetic rats treated with aqueous extract 100mg/kg

Group 3 Diabetic rats treated with aqueous extract 200mg/kg

Group 4 Diabetic rats treated with aqueous extract 300mg/kg

Group 5 Diabetic rats treated with Standard drug (Glibeclamide) 0.5mg/kg

Fasting blood samples were collected from the tail vein at different time intervals and estimated by a digital glucometer (Accu check active ®).

3.10 Statistical Analysis

Results were expressed as mean ± standard error of mean. Statistical analysis was carried out using student's *t-test* and $p<0.05$ was considered.

CHAPTER FOUR

4.0 RESULTS

4.1 Plant Material Identification

The plant was identified as *Acacia albida* in the herbarium section of the Department of Biological Sciences, Ahmadu Bello University Zaria with the voucher number 900334.

4.2 Morphological Characters of *Acacia albida*

Acacia albida bark occurs curved in shape. The outer surface is grey in colour and is well marked with fissures due to increase in the girth of the root and lack of elasticity in the dead tissues of the bark. Inner surface is pinkish-red and is provided with faint to well marked longitudinal striations. The bark breaks with a fibrous fracture and has a characteristic odour and taste.

4.2.1 Microscopical Characters of *Acacia albida*

A transverse section of the bark of *Acacia albida* revealed some aerating pores through which exchange of gases takes place. These represent the lenticels. Internally are a few layers of polygonal, thick-walled, radially arranged suberised cork cells (phellem) with absence of intercellular spaces. Beneath the cork is the cork-cambium (phellogen) and beneath this are a few layers of thin–walled cells formimg the phelloderm. The cortex is clearly visible containing parenchymatous cells. Powdered sample contains, numerous sclerencymatous fibres, fragments

of cork cells, abundant oval shaped starch grains, prismatic crystals of calcium oxalate were in abundant in the phloem tissue.

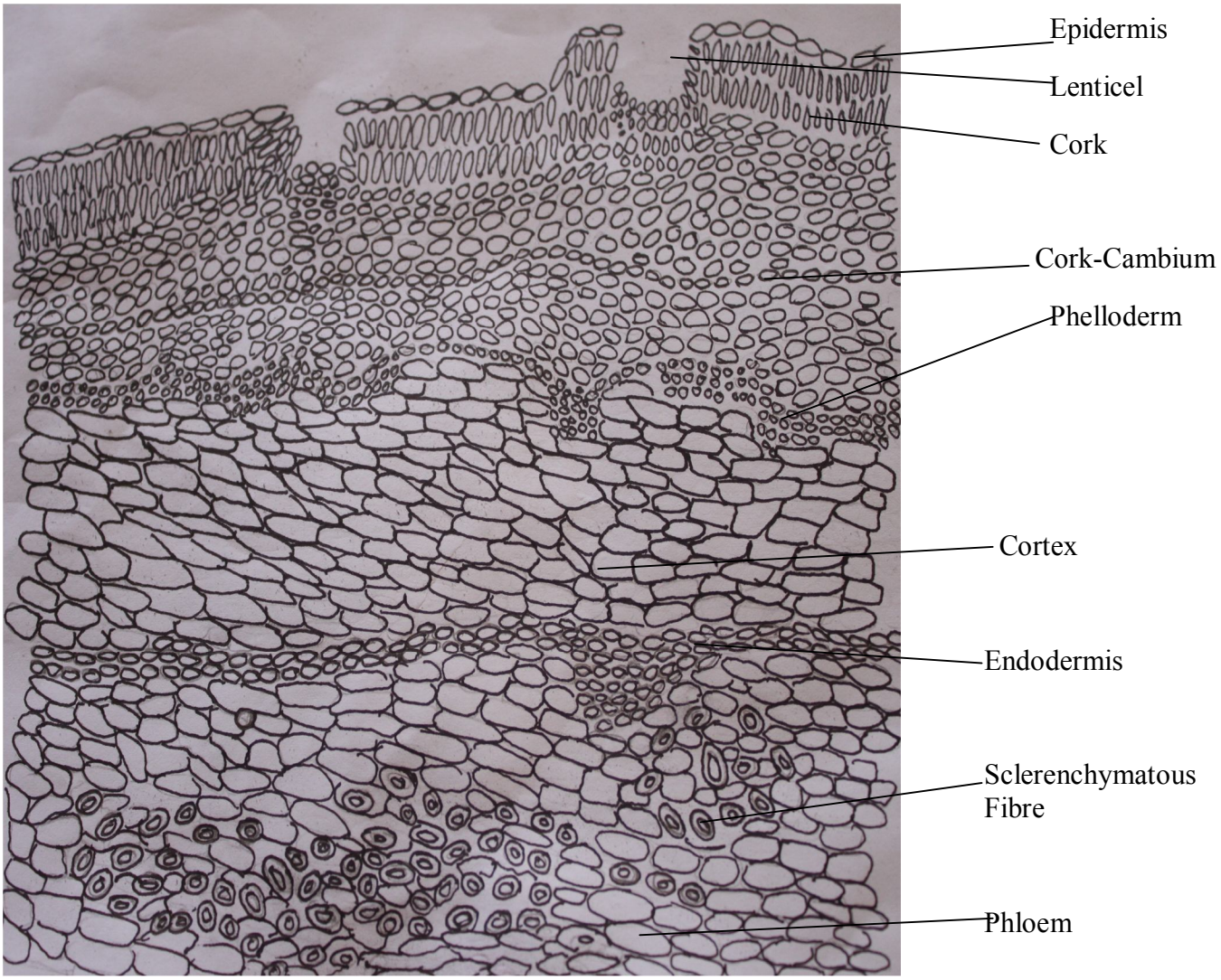
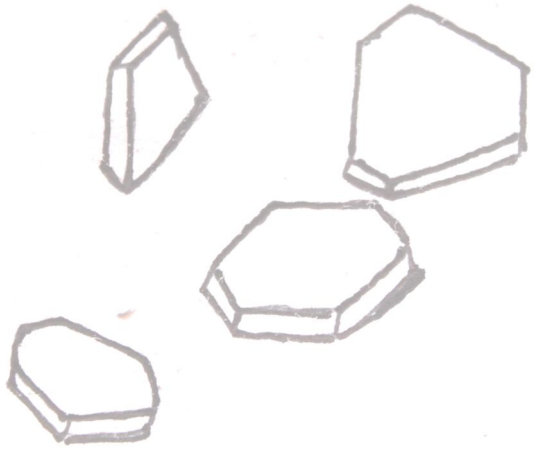


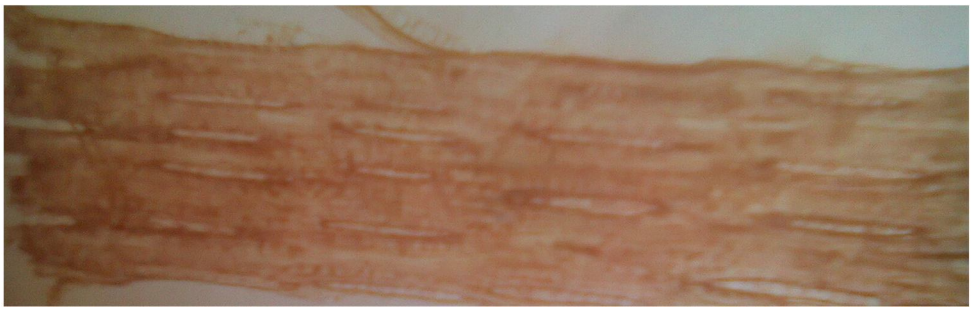
Fig 4.1: Transverse Section of *Acacia albida* Root Bark, X100



(a) Cork cells



(b) Prism form of Calcium oxalate crystals



(c) Sclerenchymatous fibres

Fig 4.2 (a-c) : Some Microscopic Features from *Acacia albida* Root Bark Powder, X100

Table 4.1 Chemomicroscopic Characters of *Acacia albida*

Test	**Observation**	**Inference**
Starch	Blue-black colour observed	Presence of starch
Tannins	Greenish colour observed	Tannin present
	Appearance of pinkish colour	Presence of oil
Calcium oxalate/carbonate	Crystals disappeared on addition of H_2SO_4	Calcium oxalate present
Protein	Reddish colour observed	Protein present
Cellulose	Bluishcolour observed	Cellulose present
Hemicellulose	Nocolour change	Hemicellulose absent
Lignin	Red stain observed on sclerenchymatous fibre	Lignin present
Suberin	Red colouration observed on the inner side of the primary walls of cork cells	Suberin present

Table 4.2 Preliminay Phytochemical Screening on the Root Bark of *Acacia albida*

Constituents	**Test**	**Observation**	**Inference**
Carbohydrates	Molisch's test	A purple colour at the interphase was observed.	This indicates the presence of carbohydrate.
	Fehling's test	Brick-red precipitates were observed.	This indicates the presence of reducing sugars.
Anthraquinones	Borntrager's test	No colour change observed.	This implied the absence of hydroxyl anthraquinone.
	Modified Borntrager's test	No colour change observed.	Absence of anthraquinone derivatives.
Cardiac glycosides	Keller-Killani	No ring was observed.	Absence of deoxy sugar characteristic of cardenolides.
	Legal test	No colour change observed.	Absence of cardenolides
	Salkwoski test	No colour change observed at the interphase.	Absence of steroidal ring.
Saponins	Frothing	Persistent Frothing observed.	Saponin is present.
	Haemolysis test	Haemolysis was observed.	Saponin confirmed.
Steriod/triterpenes	Liebermann-Burchard's test	Purple ring observed.	Presence of triterpene saponin but absence of steroidal saponin.
Flavonoids	Shinoda's test	A yellow colouration observed.	Presence of flavonoid aglycone
	NaOH test	A yellow colouration observed.	Presence of flavonoid.
Tannins	Lead sub-acetate test	Coloured precipitate observed.	Presence of tannin.
	Ferric Chloride test	Blue-green precipitate observed.	Condensed tannin present.
Alkaloids	Dragendorff's test	Precipitate observed	Alkaloid present.

	Mayer's test	Precipitate observed	Alkaloid present.
	Wagner's test	Precipitate observed	Alkaloid present.

4.3 Quantitative Pyhtochemical Estimation on the Extract of *Acacia albida* Root Bark

4.3.1 Total Flavonoid Content

The total flavonoid content of the plant extract spectrometrically was found to be 79.26mg quercetin equivalent /g of extract powder by reference to standard curve (y= 0.009x-0.031, r^2 = 0.769).

4.3.2 Total Phenol Content

The total phenolic content in the extract was determined spectrophotometrically according to the Folin-Ciocalteu method and calculated as Gallic acid equivalent. Accordingly, the total phenolic content of the extract is 686.72mg Gallic acid equivalent/g of powder extract by reference to standard curve y =(0.006x +0.072,r^2 =0.982).

Table 4.3: Quantitative estimation of some Phytochemical Constituents of *Acacia albida* Root Bark Extract

Constituents	Percentage Yield (% w/w)*
Alkaloids	0.43±0.60
Flavonoids	7.93±0.05
Saponins	1.90±0.75
Tannins	4.06±0.20

*Mean value of 3 counts ± SEM

4.4 Separation of Phytochemicals from the Extract of *Acacia albida* by Thin Layer Chromatography.

Table 4.4 Quanlitative Separation of Phenols on TLC from the Root Bark of *Acacia albida* Extract

S/No.	Colour of Spots	R_f value
a.	Grey	0.50
b.	Grey	0.60
c.	Grey	0.64
d.	Grey	0.66
e.	Grey	0.88

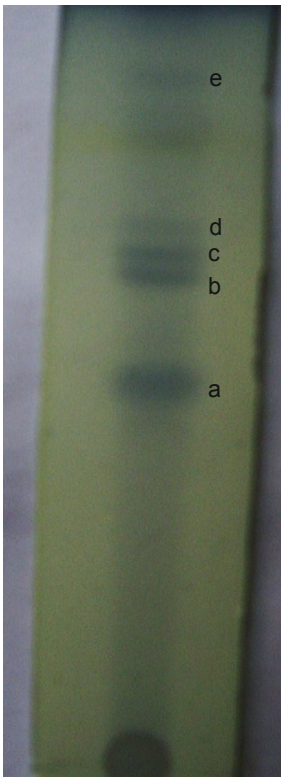


Fig 4.3 Thin Layer Chromatogram of the Phenolic Fraction from *Acacia albida* Root Bark Extract.

Note: The spots and the R_f values are a (0.50), b (0.60), c (0.64), d (0.66), e (0.88).

Table 4.5 Quanlitative Separation of Saponins on TLC from the Root Bark Extract of *Acacia albida*

S/No.	Colour of Spots	R_f value
a.	Yellow	0.46
b.	Yellow	0.62
c.	Yellow	0.67

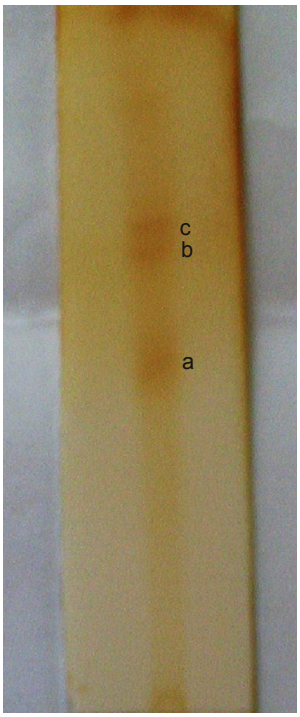


Fig 4.4 Thin Layer Chromatogram Separation of Saponin Fraction from *Acacia albida* Root Bark Extract.

Note: The spots and R_f values are a (0.46), b (0.62), c (0.67).

Table 4.6 Quanlitative Separation of Flavonoid on TLC from the Root Bark of *Acacia albida* Extract

S/No	Colour of Spots	R_f Value
a	Yellow	0.80
b	Yellow	0.64
c	Yellow	0.60

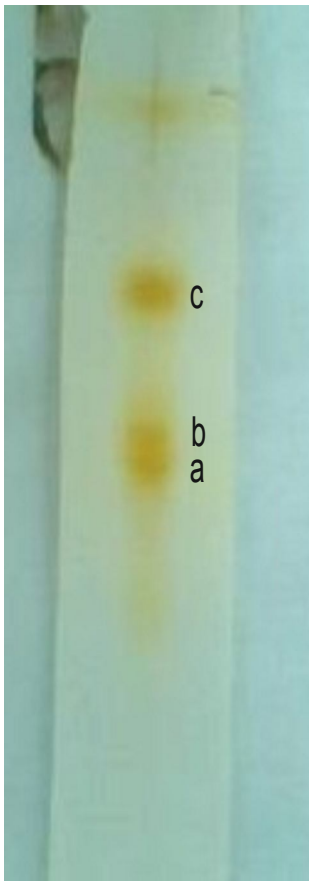


Fig 4.5 Thin Layer Chromatogram of Flavonoid Fraction from *Acacia albida* Root Bark Extract.

Note: The Spots and R_f Values are a (0.60), b (0.64), c (0.80)



Fig 4.6(a-b): Zones of Antioxidant Activities of *Acacia albida* Root Bark

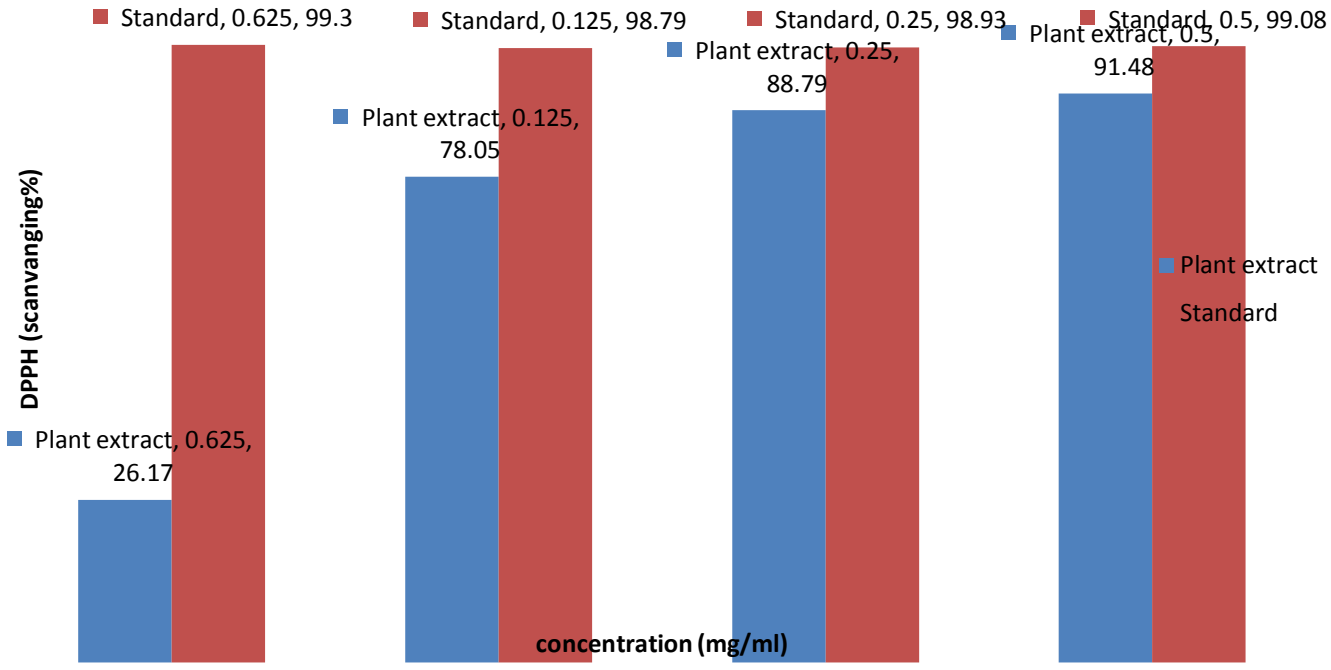


Fig 4.7 DPPH Scavenging Activity of *Acacia albida* and Ascorbic Acid (Standard) at Different Concentrations

4.5 Lethal Dose (LD_{50}) on the of *A. albida* Extract

The LD_{50} was calculated from the doses administered at the first and second phases according to Locke's method. The preliminary acute toxicity established the oral LD_{50} of the extract was found to be above 5000 mg/kg.

Dose (mg/kg)	Result of First Phase
10	0/3
100	0/3
1000	0/3

Dose (mg/kg)	Result of Second Phase
1600	0/3
2900	0/3
5000	0/3

LD_{50} = > 5000mg/kg

4.6 Effect of the Hypoglycemic Property of the Root Bark Extract of *Acacia albida*

Table 4.8 shows the effect of root bark extract of *Acacia albida* on alloxan-induced rats. The administration of the herbal extract at all doses caused decrease in blood glucose level. However, the decrease with significant difference ($p<0.05$) was observed with the aqueous extract at 200 mg/kg which reduced the glucose level from 335 ± 3.33 on 1st day treatment to 151 ± 14.52 on the 4th day treatment. Similarly, the standard drug reduced the blood glucose from 354.33 ± 15.95 on 1st day treatment to 253.83 ± 44.65 on the 4th day treatment (Table 4.8).

Table: 4.7 Effect of Aqueous Extract of *Acacia Albida* Root Bark on Blood Glucose Level of Alloxan-Induced Diabetic Rats

Group / Treatment	Dose	1st day	2nd day	4th day
I Control	Normal saline	304.33±8.49	241.67±57.52	131.5±35.81
II *Acacia albida* extract	100mg/Kg	424±52.61	305±53.01	118±26.49
III *Acacia albida* extract	200mg/Kg	335.33±3.33*	257.33±13.27	151±14.42*
IV *Acacia albida* extract	300mg/Kg	339.5±11.98	309.66±56	156±45.10
V Glibenclamide	0.5mg/Kg	354.33±15.95	307.5±53.29	253.83±44.65*

*P <0.05= significant; ± SEM: Standard Error of Mean; n = 6 (numbers of animal per group)

CHAPTER FIVE

5.0 DISCUSSION

Medicinal plants since time immemorial have been used in virtually all cultures as sources of medicine. The widespread use of herbal remedies and healthcare preparations as those described in various texts are obtained from commonly used traditional herbs and medicinal plants has been traced to the occurrence of natural products with medicinal properties (Hoareau *et al.,* 1999).

The macroscopical characters of the root bark contain starch grain, sclerenchyma fibre, and cork cell in surface view. These can serve as diagnostic parameters. According to World Health Organization, the macroscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.

The microscopical studies (fig 4.1) of the transverse section bark showed presence of lenticels which are aerating pores formed in the bark through which exchange of gases between the atmosphere and the plant takes place. Internally are a few layers of polygonal, thick-walled, radially arranged suberised cork cells (phellem) with absence of intercellular space. Cork cells whose walls are impregnated with suberin serve as a protective tissue that replaces the epidermis in woody plants. Beneath the cork is the cork-cambium (phellogen) and beneath this are a few layers of thin–walled cells forming the phelloderm. The cortex is clearly visible containing parenchymatous cells.

Macroscopic features are lost when plant material is reduced into powder; therefore for proper identification microscopic examination is imperative (Uwumarongie and Onwukaeme, 2011).
The Chemo-microscopic examination (table 4.1) shows the presence of food reserves such as starch grains which are of universal occurrence in plants (with exception of fungi); and is the most common and widely distributed carbohydrates.

Tannins which are phenolic substances are widely distributed in plants. Tannins are aseptic, i.e. free from attack of parasitic fungi and insects. The presence of tannins makes wood hard and durable. They are extensively used in tanning, i.e. converting hide into leather.

Proteins, Fat/Oil which are important food reserves were observed. Cellulose was present. It is a chemical substance which is universally present in the cell walls of all plant cells with the exception of fungi; it is the chief constituent of cell wall. In the primary wall, cellulose is associated with hemicelluloses and pectose, making the wall soft and elastic. Almost pure cellulose occurs in the secondary wall formed later, making the wall stiff but flexible. Lignin was also present on the fibres observed. It is a hard and chemically complex substance laid down during secondary growth and responsible for considerable thickening and strengthening. As it is laid down, it replaces the water as bulking agent leading to an increase in compression strength. Suberin impregnates the cork cells. Cork cells are hydrophilic substances consisting of oxidized and polymerized fatty acids and their esters which make cork cells impermeable to water, prevent hydration of cells and form good thermal insulation to water. Crystals of calcium oxalate were present, while calcium carbonate was absent.

Ghani (1990) stated that chemical composition of cell wall of various plant tissues often furnishes an important chemo-microscopical character which can be used in the identification of plant drug and adulteration, for quality control of raw material and also provide useful information for the preparation of monograph of the plant.

The preliminary phytochemical screening tests (table 4.2) of the plant extract revealed the presence of Alkaloids, Flavonoids, Saponins, and Tannins. Any of these secondary metabolites singly or in combination could be responsible for the antidiabetic activity of the extract, and several of such compounds are known to possess potent antioxidant activity (Lee *et al.,* 2004).
A phytochemical study reveals that plants in this family contain tannins (Tijani *et al.,* 2008). Tannins have shown potential antiviral, antibacterial and antiparasitic effects (Souza *et al.,* 2006). Tannins isolated from the stem bark of *Myracrodruon urundeuva* have anti-inflammatory and antiulcer potency on rodents, showing a strong antioxidant property for possible therapeutic applications (Souza, *et al.,* 2006). Foods rich in tannins can be used in the treatment of HFE hereditary hemochromatosis, a hereditary disease characterized by excessive absorption of dietary iron resulting in a pathological increase in total body iron stores.

Alkaloids are generated especially by higher plants – about 10 to 25% of those contain alkaloids. Preparations of plants containing alkaloids and their extracts, and later pure alkaloids have long

been used as psychoactive substances. Cocaine and cathinone are stimulants of the central nervous system. Mescaline and many of indole alkaloids (such as psilocybin, dimethyltryptamine and ibogaine) have hallucinogenic effect. Morphine and codeine are strong narcotic pain killers. Quinine from Chinchona species is a powerful antimalarial agent that was formerly the drug of choice for treating the disease.

Phytochemical screening of aqueous *A. albidia* stem bark extract by Oseni, (2012) revealed the presence of all the phytochemicals tested with the exception of steroids, this is found true in this present work as only triterpenoid saponin were found present.Saponins are phytochemicals which can be found in most vegetables, beans, and herbs. Saponins have health benefits. Studies have illustrated the beneficial effects on blood cholesterol levels, cancer, bone health and stimulation of the immune system. The non-sugar part of saponins has also a direct antioxidant activity which may result in other benefits such as reduced risk of cancer and heart disease.

Flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants. They are the most important pigments for flower colouration producing yellow or red/blue pigmentation in petals. They have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses, and carcinogens. They show anti-allergic, anti-inflammatory, and anti-microbial and anti-cancer activity. Aqil *et al.,* (2006) observed that majority of antioxidant activity is due to flavonoids.

Thin layer chromatography of the plant sample gave good resolution of various components present in each of the phytochemical constituents. Thin layer chromatography is an easy and fast method of separation of phytochemicals which substantiate the presence of such metabolites in the plant.

The TLC separation of Phenol revealed 5 spots (fig 4.3). Phenolic compounds are commonly found in plants, and they have been reported to have multiple biological effects, including antioxidant activity. Many studies have revealed that the phenolic content in plants can be correlated to their antioxidant activities. (Dragland *et al.,* 2003 Duh *et al*., 1999; Kujala, *et al.,* 2000; Wang 2003, Wu *et al*., 2004). Plants have the limitless ability to synthesize phenols and their derivatives. The presence of phenols in all types of tissue is a characteristic of plants.

The TLC separation of Saponins (fig 4.4) revealed the presence of three spots when the chromatogram was exposed to iodine vapour. The occurrence of saponins has been reported in over seventy families of higher plants.

The TLC separation of Flavonoid revealed 3 spots after spraying the chromatogram with aluminium chloride (fig 4.5). Flavonoids occur in plant as glycosides or aglycones. Flavonoids have been shown to possess significant antioxidant activity (van Acker., 1996). The family Leguminosae are extremely rich in Flavonoids, both common and unusual types, and are alone among the families of dicotyledons in containing representatives of all classes of Flavonoid (Bate-Smith, 1962).

Based upon the preliminary phytochemical test, quantitative determination of phytoconstituents were carried out on the powdered plant material by various standard methods and found that Alkaloids 0.43, Flavonoids 7.93, Saponins 1.90 and Tannins 4.06 %w/w were present in the root bark of this plant (table 4.3). The quantitative estimation values are useful to evaluate the quantities of specific constituent present in the plant extract. The quantity of the chemical constituents is in the following order Flavonoids>Tannins>Saponins>Alkaloids. Flavonoid and tannin which are phenolic compounds ranked highest in quantity. This may explain the rich antioxidant nature of the plant extract which has been attributed to phenolic constituents present in plants (Dragland, *et al.,* 2003).

Result of total flavonoid content stands at 79.26mg quercetin equivalent/g of extract powder with reference to standard curve (y =0.009x-0.031, r^2 = 0.769) Here there is a high correlation observed with the standard curve. Flavonoids are powerful antioxidants against free radicals and are described as free-radical scavengers (Pal, *et al.,* 2009). This activity is attributed to their hydrogen-donating ability. Indeed, the phenolic groups of flavonoids serve as a source of a readily available ‘‘H” atoms such that the subsequent radicals produced can be delocalized over the flavonoid structure (Tripoli, *et al.,* 2007). Free radical scavenging capacity is primarily attributed to high reactivities of hydroxyl substituents that participate in the reaction (Heim *et al.,* 2002). In other words, Flavonoids stabilize the reactive oxygen species by reacting with reactive compound of the radical. Because of the high reactivity of hydroxyl group of the flavonoids, radicals are made inactive.

The content of total phenolics in the extract was determined spectrophometrically according to the Folin-Ciocalteu method and calculated as Gallic acid equivalent. Accordingly, the total phenolic content of the extract is 686.72mg/g.

To date the potential antioxidant as well as the total phenolic content of *Acacia albida* has not been mentioned in literature. In this present study however, it was observed that the plant has considerable phenolic content with reference to the standard curve which gave a high correlation $y = (0.006x + 0.072, r^2 = 0.982)$. Phenolic compounds are commonly found in plants, and they have been reported to have multiple biological effects, including antioxidant activity. Many studies have revealed that the phenolic content in plants can be correlated to their antioxidant activity (Kujala, 2004; Lavelli, 2000). It is now widely accepted that dietary polyphenolics may play a role in protecting the body against chronic diseases such as cancer, cardiovascular diseases (Mojzisova *et al*., 1999; Karaca *et al.,* 2006), and diabetes mellitus (Knekt *et al.,* 2002).

Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS) which are formed under normal physiological conditions but become deleterious when not being quenched by the antioxidant systems (Fang, 2002). There are convincing experimental and clinical evidences that the generation of reactive oxygen species is increased in both types of diabetes and that the onset of diabetes is closely associated with oxidative stress (Johansen, 2005; Rosen, 2001). Free radicals are formed disproportionately in diabetes by glucose autoxidation, polyol pathway and non-enzymatic glycation of proteins (Obrosova, 2002. Abnormally high levels of free radicals and simultaneous decline of antioxidant defense systems

can lead to the damage of cellular organelles and enzymes, increased lipid peroxidation and development of complications of diabetes mellitus (Maritim, 2003).

Oxidative stress due to increased production of reactive oxygen species and abnormal antioxidant status may facilitate the progressive impairment of B-cell function in the pathogenesis of type 2 diabetes. However, the supplementation of antioxidants will lead to fight against free radicals and lower or inhibit their activity. These antioxidants therefore enable normal functioning of pancreas and lead to insulin secretion and avoiding diabetic complication (Song *et al.,* 2005).

The result of free radical scavenging potential of extract of *Acacia albida* and ascorbic acid at different concentrations were tested by DPPH method. The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Lee *et al*., 2003).

Antioxidant react with DPPH which is a nitrogen-centred radical with a characteristic absorption and convert it to 2,2-diphenyl-1-picryl hydrazine, due to its hydrogen donating ability at a very rapid rate (Jayaprakasha *et al.,* 2004). When the DPPH radical are scavenged, the colour of the reaction mixture changes from purple to yellow. The degree of discolouration indicates the scavenging potential of the antioxidant extract.

It is observed that the radical scavenging activity of the extract increased with increasing concentration. This result is comparable with that of Gwori *et al.,* (2011) who found the same for *Acacia nilotica.* . Sanchez-Moreno *et al.,* (1999) reported that DPPH radical scavenging ability is directly proportional to the concentration of phenolic components, as the concentration of the phenolic components increases DPPH scavenging activity increases. Furthermore, Siddhuraju (2003) reported that substantial DPPH radical scavenging capacity could be explained by the presence of phenolic contents of which the plant extract also contained. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts.

At 0.5 mg/ml *Acacia albida* and ascorbic acid displayed 91.48% and 99.08% radical scavenging activity respectively. The radical scavenging of the extract can be compared with the standard as their percentage inhibitions are very close with great significant difference at $P \leq 0.01$. Shimada *et al.,* (2004) reported that antioxidant activity of extract may be due to its hydrogen donating ability.

Futhermore, from the result of quantitative estimation of the bioactive constituents of the plant extract, percentage yield of flavonoid ranked highest and the total phenol content of the extract is 686.72mg/g i.e. showing a great amount of phenol in the extract. These results thus provide an explanation for the high antioxidant activity showed by the plant extract. It has being reported by various researchers that antioxidant activity of plant material is largely due to the flavonoid and phenolic content in them (van Acker, 1996; Dragland *et al.,* 2003).

Antioxidants may guard against reactive oxygen species (ROS) toxicities by the prevention of ROS construction, by the disruption of ROS attack, by scavenging reactive metabolites and converting them to less reactive molecules or by enhancing the resistance of sensitive biological target to ROS attack (Sen, 1995). Free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are associated with many pathological conditions such as Diabetes, atherosclerosis, arthritis, ischemia, reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen, 1999; Cook and Samman, 1996) however, antioxidants through their scavenging power are useful for the management of various radical mediated diseases.

The acute toxicity studies established the LD_{50} (median lethal dose) to be above 5000mg/kg. This finding probably suggests that the plant extract is relatively safe.

The administration of the plant extract significantly decreased blood glucose level in diabetic rats when compared to the diabetic control. Various reports revealed that *Acacia albida* possess plethora of benefits under various experimental conditions. The present study indicated that *Acacia albida* decreased blood glucose level in alloxan induced diabetic rats, with a significant decrease obtained with 200mg/kg (Table 4.7). According to Noor *et al.,* (2008) who observed blood glucose leveling in diabetic rats treated with Aloe Vera reported that, there are two possible explanations for this finding. The plant may exert its effect by preventing the death of beta cell and/or secondly, it may permit recovery of partially destroyed beta cells. Burcelain *et al.,* (1995) reported that the hypoglycemic action of the extract of herbal plants in diabetic rats may be possible through the insulinomimitic action or by other mechanism such as stimulation of glucose uptake by the peripheral tissue, inhibition of endogenous glucose production of

activation of gluconeogenesis in liver and muscles. The antidiabetic activity of *Acacia albida* may be possible through the mechanism as reported by Noor *et al.,* (2008) and Burcelain *et al.,* (1995) this is an interesting finding and suggests that *Acacia albida* may have antioxidant or free radical scavenging activity.

Flavonoids can ameliorate some of the consequences of diabetes mellitus (Havsteen *et al*., 2002). Numerous studies have been carried out to explore the potential role of flavonoid in the treatment of diabetes (Jung *et al.,* 2006; Matsui *et al.*, 2006; Qi *et al.,* 2010). A good number of studies have already demonstrated the hypoglycemic effects of flavonoids using different experimental models and treatments - the drug candidates have been shown to exert such beneficial effects against the disease manifestation, either through their capacity to avoid glucose absorption or to improve glucose tolerance. It has also been demonstrated that flavonoids can act *per se* as insulin secretagogues or insulin mimetics, probably by influencing the pleiotropic mechanisms, to attenuate the diabetic complications; besides, the drug candidates have been found to stimulate glucose uptake in peripheral tissues, and regulate the activity and/or expression of the rate-limiting enzymes involved in carbohydrate metabolism pathway. As a result, flavonoids are now-a-days regarded as promising and significantly attractive natural substances to enrich the current therapy options against diabetes (Brahmachari, 2011).

Futhermore, flavonoids have been identified to be good inhibitors of aldose reductase (Patra *et al*., 2010). It has been reported by several researchers that quercetin possesses antidiabetic activity and it has been found that it brings about regeneration of pancreatic islets and increases insulin release in streptozotocin-induced diabetes. Also, it has been reported to stimulate Ca2+

uptake from isolated islet cells thus suggesting it to be effective even in non-insulin dependent diabetes (Tapas *et al.,* 2008).

From the present study, with the possession of antioxidant activity by *Acacia albida* it may be suggested that it can serve as a source of antioxidant for ameliorating the menace of oxidative stress in diabetes and other diseases.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

The result of the macroscopical, microscopical, and chemomicroscopic characters of the root bark of *Acacia albida* can serve as diagnostic parameters which can be used in the identification of the plant drug and adulteration, for quality control and also provide useful information for the preparation of monograph of the plant.

The qualitative phytochemical screening revealed the present of various secondary metabolites present in the extract while quantiative estimation provide information as to the amount of the various metabolite.

Acacia albida has high antioxidant activity and also possess the potential to reduced high blood glucose level which may be due to its high phenol contents and it antioxidant activity. The experimental result obtained in the present laboratory study indicates that *Acacia albida* extract possesses antidiabetic property. Despite widespread use of *Acacia albida* in folklore medicines, literature contains few reports on its antioxidant activity. *Acacia albida* is recognized as having high scavenging potential of free radicals which may be due to the presence of hydroxyl groups existing in the phenolic and flavonoid compounds that can provide the essential constituents as a radical scavenger. It can be suggested that *Acacia albida* could be used as an easily accessible and cheap source of natural antioxidant, which can be used as supplement to aid the therapy of free radical mediated diseases such as cancer, diabetes, and inflammation. This finding provides a substantive scientific proof of the traditional use of *Acacia albida* for treating diabetes.

RECOMMENDATIONS

The extract merit further experiment *in vivo* and further studies are needed on the isolation and characterization of the antioxidant and antidiabetic components to elucidate their antioxidant and antioxidant activity mechanism.

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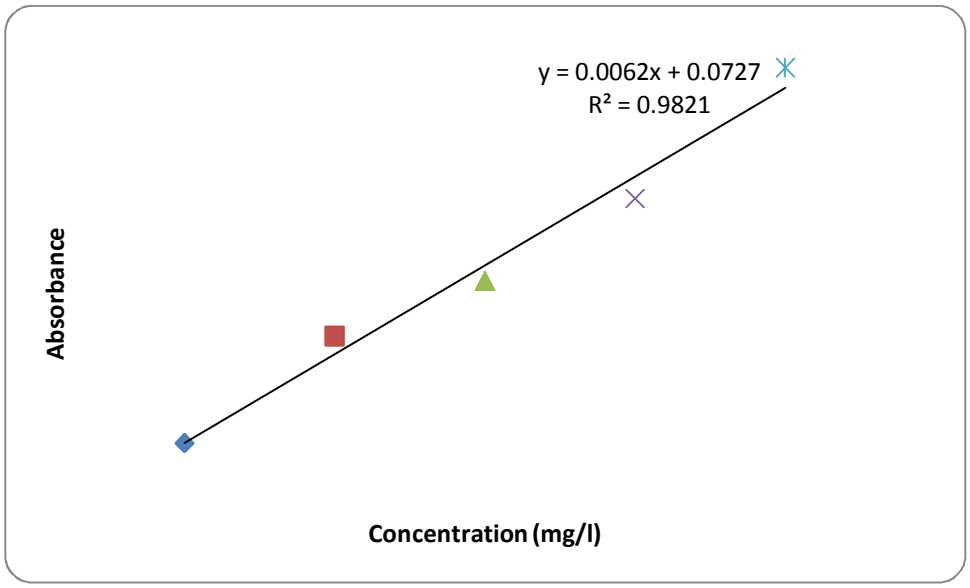
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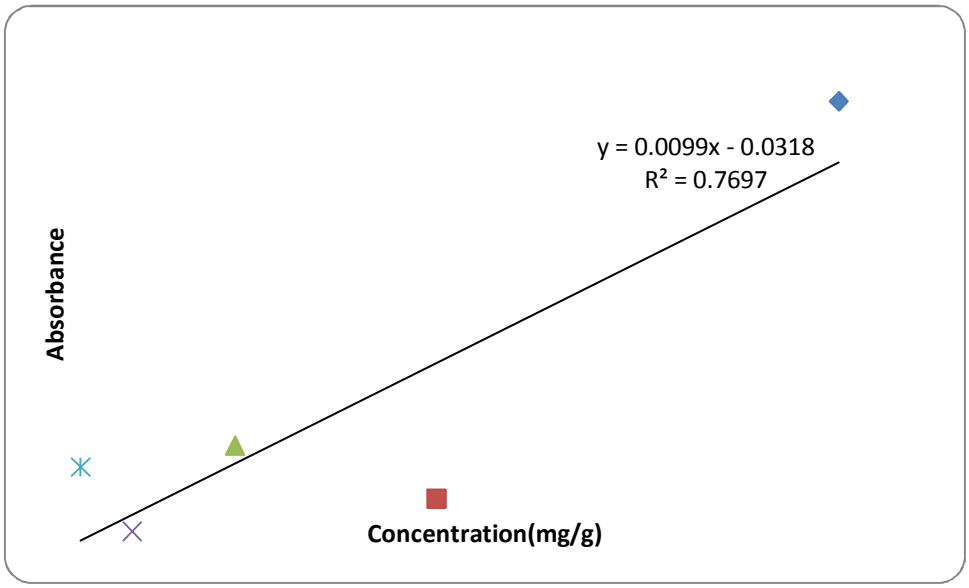
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APPENDICES

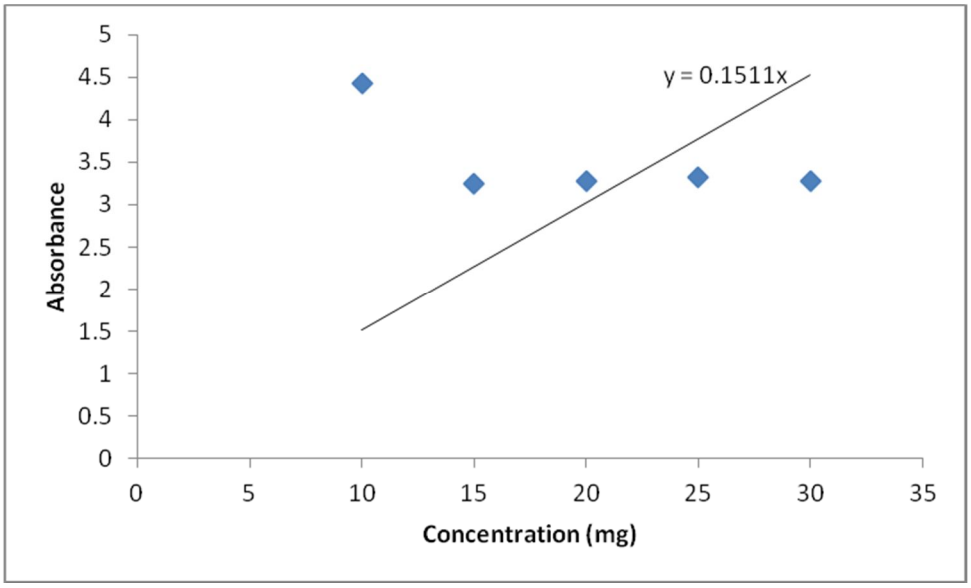
Appendix A: Determination of Total Phenolic Content from *Acacia albida* Root Bark Extract



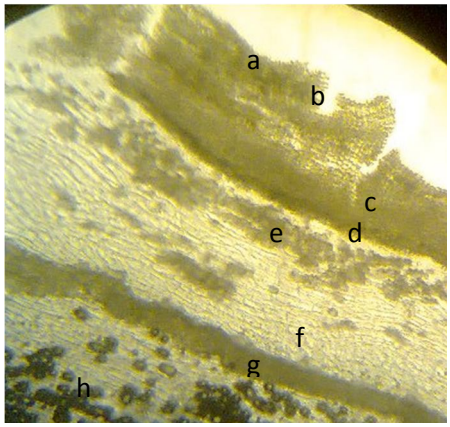
Appendix B: Determination of Total Flavonoid Content from Acacia albida Root Bark Extract



Appendix C: Determination of Tannic Acid for *Acacia albida* Root Bark Extract



Appendix D:Transverse section of *Acacia albida* Root Bark



Note: a **(**Epidermis), b (Lenticels), c (cork), d (Cork-cambium), e (Phelloderm), f (Cortex) g (Endodermis), h (Sclerenchymatous fibre)