

**PHYTOCHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF
SOME SPECIES OF MISTLETOE ON FIVE HOST TREES IN ABU CAMPUS,
ZARIA, NIGERIA**

BY

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**DEPARTMENT OF BIOLOGICAL SCIENCES,
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DECLARATION

I declare that this dissertation entitled “PHYTOCHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF SOME SPECIES OF MISTLETOE ON FIVE HOST TREES IN ABU CAMPUS, ZARIA, NIGERIA” has been carried out by me in the Department of Biological Sciences. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this project report was previously presented for another degree or diploma at this or any Institution.

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CERTIFICATION

This dissertation entitled “PHYTOCHEMICAL COMPOSITIONS AND ANTIBACTERIAL ACTIVITY OF SOME SPECIES OF MISTLETOE ON FIVE HOST TREES IN ABU CAMPUS, ZARIA, NIGERIA” by TariDlama TIZHE meets the regulations governing the award of the degree of Master of Science in Botany of the 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 my late father, MrTizheDlama. May the Almighty God grant him eternal rest.

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ABSTRACT

This study focused on the identification of mistletoe species parasitic on *Terminaliamantaly*, *Terminaliacatappa*, *Citrus grandis*, *Khayasenegalensis* and *Albizzialebbeck* as well as the determination of the qualitative and quantitative phytochemical constituents and antibacterial activities of the leaf extracts of the host trees and their associated mistletoes. Fresh leaves of the mistletoes and their host trees were collected from four sampling areas within ABU campus, Samaru, Zaria, Nigeria. The leaves were air dried and pulverized. Maceration method was used for the extraction of the plant constituents. The susceptibility test of the samples on *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi* was carried out using agar well diffusion method. The study revealed that, *Tapinanthus globiferus*, *Globimetula braunii* and *Globimetula oreophila* were found parasitic on most of the host plant species except *G. oreophila* on *Terminaliacatappa*; *Englerinalecardii* was found parasitic only on *Albizzialebbeck* and *Citrus grandis*; *Tapinanthus dodoneifolius* was parasitic on *T. catappa* and *A. lebbeck* and *Tapinanthus belvisii* was found parasitic on *A. lebbeck*, *T. catappa* and *Terminaliamantaly* respectively. The qualitative phytochemical screening revealed the presence of tannins, saponins, flavonoids, cardiac glycosides and carbohydrates in both the ethanolic and aqueous leaf extracts of most of the host plants and the mistletoes on them. Steroid was found present only in the aqueous extract of *G. braunii* from *C. grandis* and *A. lebbeck*, but was found in most of the extracts of *Khayasenegalensis* and the mistletoe species on it. The quantitative phytochemical screening revealed that cyanogenic glycosides and flavonoids had the highest (31.00 %, in *T. globiferus* and 22.40 %, in *K. senegalensis* respectively) concentrations than all the other compounds in both the host plants and attached mistletoes while phenols and tannins had the lowest

(0.41 mg/ml, in *C. grandis* and 0.08 %, in *G. braunii* respectively) concentrations than all the other compounds. The antibacterial activity of the aqueous leaf extracts of the host trees and associated mistletoes revealed that *T. mantaly*, *C. grandis* and *A. lebbeck* were reactive against most of the test organisms (*S. aureus*, *B. subtilis*, *E. coli* and *S. typhi*.) at the concentration ranging from 25 to 200 mg/ml except *T. catappa* extract, which was only active against *S. aureus*. Similarly, the extracts of mistletoes obtained from the host plants except the ones from *T. mantaly* and *A. lebbeck* were also active against virtually all the test organisms. The control (ciprofloxacin), however, was the most active against all the test organisms compared to the plant extracts. In conclusion, this study showed that *A. lebbeck* was the most parasitized host tree in the study area compared to other hosts. All the aqueous leaf extracts of the host trees with the mistletoe species sourced from them except the mistletoes from *T. mantaly* and *A. lebbeck* respectively had an antibacterial activity at concentration ranging from 25 to 200 mg/ml on *S. aureus*, *B. subtilis*, *E. coli* and *S. typhi*.

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CHAPTER ONE

1.0

INTRODUCTION

1.1 Preamble

Mistletoe consists of about 1400 species around the world and belongs to the kingdom Plantae, subkingdom Tracheobionta, superdivision Spermatophyte, division Magnoliophyta, class Magnoliopsida, subclass Rosidae, order Santales (Judd *et al.*, 2002). Recent phylogenetic works confirm that mistletoes belong to five distinct families: Misodendronaceae, Eremolepidaceae, Santalaceae, Viscaceae and Loranthaceae (Der and Nickrent, 2008; Malecot and Nickrent, 2008; Vidal-Russell and Nickrent, 2008). The largest family of this mistletoe is Loranthaceae which has 75 genera and over 900 species (Judd *et al.*, 2002). Six major genera are found in Nigeria namely: *Tapinanthus*, *Agelanthus*, *Loranthus*, *Globimetula*, *Phragmanthera* and *Englerina*. *Tapinanthus* is far more widespread in the Nigeria savanna (Johri and Bhatnagar, 1972; Omolaja and Gamaye, 1998). In Nigeria, mistletoe is called ‘afomo’ in Yoruba, ‘apari’ in Igbo, it is ‘kauci’ in Hausa and ‘children’s matches’ in Eastern Cameroon presumably due to the match-like shape of the flower (Oluwole *et al.*, 2013). Mistletoes plants grow on a wide range of host trees, it affects their growth and can kill them with heavy infestation.

All mistletoes are hemi-parasites, bearing evergreen leaves that photosynthesize but depend on their host mainly for water and mineral nutrients (Milius, 2000). Most mistletoe seeds are spread by birds that eat the fruits (Cowles, 1964). The mistletoe seed germinates on the branch of a host tree or shrub and in its early stages of development it is independent of its host. Later, it forms a haustorium that penetrates the host tissue and takes water and nutrients from the host plant (Milius, 2000).

Various studies carried on the phytochemical constituents of mistletoes indicated the presence of glycosides, alkaloids, viscotoxins, phenylpropanoids, tannins and sugars (Jurin, 1993). Inuwa *et al.* (2012) reported that the phytochemical screening of *Globimetula braunii* indicated the presence of alkaloids, tannins, flavonoids and steroids. Mistletoes have also been reported to be used for the treatment of cancer, hypertension, diabetes, arthritis, heart problems, insomnia, infertility and pneumonia (Obatomi *et al.*, 1994; Osadebe and Ukwueze, 2004; Adodo, 2006; Uzochukwu and Osadebe, 2007; Osadebe and Omeje, 2009; Jadhav *et al.*, 2010). Kafaru (1994) further ascertained that they are very potent in curing circulatory problems and also as anticancer agents. According to Blumenthal *et al.* (1998), the extracts from the leaves of *Globimetula braunii* stimulate insulin secretion from pancreatic cells and help in the treatment of arthritis. Mistletoe plants have also been shown to have broad spectrum of antimicrobial properties against certain drug resistant bacteria and fungal organisms of farm animals (Deen and Sadiq, 2002).

The growth of mistletoes on different kind of plants, is of disease curing specificity, for example, mistletoes grown on guava, kolanuts and citrus are specific for curing diseases like cancer, hypertension, nervousness and insomnia, while those grown on cocoa are best used for curing diabetes (Ekhaize *et al.*, 2010). Although, without any literature supporting the claim, inhabitants of Michika local government area of Adamawa State, Nigeria, have indicated that mistletoe plants parasitizing lemon trees are more effective for treating people suffering from typhoid, pneumonia and hypertension than any other mistletoe (though not being specific of the mistletoe species type) found on other trees species. This could be due to the differences in host tree species composition as indicated by Scheer *et al.* (1992), Obatomi *et al.* (1994), Wagner *et al.* (1996) as well as Osadebe and Ukwueze (2004) who reported that, the composition and activities of

mistletoes are host tree and season dependent. However, in order to prove or disprove the claim that mistletoes growing on lemon trees are more effective against typhoid, pneumonia and hypertension than the ones parasitic on other trees, the need for this study is necessitated.

Ndamitso *et al.* (2013) in *in vitro* assay of the extracts of *Tapinanthus dodoneifolius* using agar plate-hole and nutrient broth dilution techniques, revealed a wide spectrum of antimicrobial activities against certain multiple drug resistant bacterial isolates with *Salmonella typhi* and *Staphylococcus aureus* being the most susceptible while *Bacillus subtilis* was the least. Inuwa *et al.* (2012) reported in their research on the phytochemical and antimicrobial activities of *Globimetula braunii* that, the ethanolic and aqueous extracts inhibited the growth of *Klebsiella aerogenes*, *Proteus* spp., *Escherichia coli* and *Pseudomonas aeruginosa*. Some herbalists in Samaru claimed to have been using mistletoe parasitic on locust beans (*Parkia biglobosa*) for cleansing of *Sorghum bicolor* (guinea cornseed) so as to clear it of fungi responsible for blacksmut disease of guinea corn. The mistletoes are also used by them for curing of pile and fever (Personal communication).

1.2 Statement of the Research Problem

Most of the currently used antibacterials are associated with adverse effects such as blood cancer, upper gastrointestinal complications, organ damages, toxicity, hypersensitivity, immunosuppression and tissue residues, thus posing public health hazard (Calixto, 2000). Also, these synthetic broad spectrum antibiotics are cost prohibitive and are not within the reach of the poor. And moreover, pathogens are now developing resistance to most of these synthetic drugs (Calixto, 2000).

There is a controversy among some groups of people in Michika local government area of Adamawa State, Nigeria on the efficacy of the mistletoes found on lemon trees in

contrast with that on other trees species on the causal agents of typhoid, pneumonia (*Salmonella typhi* and *Staphylococcus aureus*) and as a remedy to hypertension than any other mistletoes found on other trees species.

1.3 Justification for the Research

Many plant constituents have proven effective as remedy for some diseases and accounted for about seven thousand pharmaceutically important compounds in western pharmacopeia and a number of important drugs, like taxol and artemisinin (Aderogba *et al.*, 2004). Moreso, the use of plant extracts and phytochemicals, with known antimicrobial properties, can be of great significance in therapeutic treatments (Prusti *et al.*, 2008).

During recent years, considerable work has been done to investigate the pharmacological importance of mistletoes on scientific lines, but not much work has been reported so far on comparative pharmacological importance of the plant growing on different host trees (Ilesanmi and Olawoye, 2010; Yusuf *et al.*, 2013). Therefore, it is imperative to compare the phytochemical composition and antibacterial activity of different species of mistletoe obtained from the same and different host trees that are known to have antimicrobial effect.

The results obtained from this study will help determine mistletoe-host relationship and its efficacy on the causal agents of typhoid and pneumonia (*Salmonella typhi* and *Staphylococcus aureus*) and will also provide a reliable explanation to variations in the antimicrobial activity of mistletoe species in relation to their hosts.

1.4 Aim of the Research

The aim of the research was to study the phytochemical composition and antibacterial activity of some species of mistletoe and five host trees in ABU Campus, Zaria, Nigeria.

1.5 Objectives of the Study

The objectives of this study were to:

- a) Determine the species of mistletoe parasitic on *Terminalia catappa*, *Citrus grandis*, *Terminalia mantaly*, *Khaya senegalensis* and *Albizia lebbek*.
- b) Determine the qualitative and quantitative phytochemical composition of the mistletoes' host plants.
- c) Determine the qualitative and quantitative phytochemical composition of different species of mistletoe obtained from the same and different host trees.
- d) Determine the antibacterial activities of the extracts of the mistletoes' hosts: *Terminalia catappa*, *Citrus grandis*, *Terminaliamantaly*, *Khaya senegalensis* and *Albizia lebbek*.
- e) Determine the antibacterial activities of the extracts of different species of mistletoe obtained from the same and different host trees such as *Terminalia catappa*, *Citrus grandis*, *Terminaliamantaly*, *Khaya senegalensis* and *Albizia lebbek*.

1.6 Hypotheses

- a) There is no significant difference in the species of mistletoe parasitic on *Terminalia catappa*, *Citrus grandis*, *Terminalia mantaly*, *Khaya seneglensis* and *Albizia lebbek*.
- b) There is no significant difference in the qualitative and quantitative phytochemical compositions of the mistletoes' hosts.
- c) There is no significant difference in the qualitative and quantitative phytochemical compositions of different species of mistletoe obtained from the same and different hosts.

- d) There is no significant difference in the antibacterial activities of the extracts of the mistletoes' hosts: *Terminalia catappa*, *Citrus grandis*, *Terminaliamantaly*, *Khaya senegalensis* and *Albizzia lebbeck*.
- e) There is no significant difference in the antibacterial activities of the extracts of different species of mistletoe obtained from the same and different host trees such as *Terminalia catappa*, *Citrus sp.*, *Terminaliamantaly*, *Khaya senegalensis* and *Albizzia lebbeck*.

CHAPTER TWO

2.0

LITERATURE REVIEW

2.1 Phytochemical Constituents of Plants

Phytochemicals are compounds that occur naturally in plants. They contribute to the color, flavour and smell of plants. In addition, they form part of a plant's natural defense mechanism against diseases (Okwu, 2004). The term is generally used to refer to those chemicals that may have biological significance, for example carotenoids or flavonoids, but are not established as essential nutrients. There may be as many as 4,000 different phytochemicals having potential to affect diseases such as cancer, stroke or metabolic syndrome (Pauling, 2014). Some of these phytochemicals are:

2.1.1 Saponins

Saponins are glycosides with a distinctive foaming characteristic. They are found in many plants, but get their name from the soapwort plant (*Saponaria*), the root of which was used historically as a soap (Latin *sapo* ---> soap). They consist of a polycyclic aglycone that is either a choline steroid or triterpenoid attached via C3 and an ether bond to a sugar side chain. The aglycone is referred to as the sapogenin and steroid saponins are called saraponins. The ability of a saponin to foam is caused by the combination of the nonpolar sapogenin and the water soluble side chain (Augustin *et al.*, 2011).

2.1.1.1 Health benefits of saponin

Saponins have many health benefits. Some of these benefits according to Rao and Rao (1995) are:

a) *Cholesterol reduction*

Saponins bind with bile salt and cholesterol in the intestinal tract. Bile salts form small micelles with cholesterol facilitating its absorption. Saponins cause a reduction of blood cholesterol by preventing its re-absorption.

b) *Reduce cancer risk*

Saponins have anti-tumor and anti-mutagenic activities and can lower the risk of human cancers, by preventing cancer cells from growing. Saponins seem to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability.

c) *Immunity booster*

Plants produce saponins to fight infections by parasites. When ingested by humans, saponins also seem to help our immune system and protect against viruses and bacteria.

d) *Anti-oxidant*

The non-sugar part of saponins has also a direct anti-oxidant activity, which may result in other benefits such as reduced risk of cancer and heart diseases.

2.1.2 Tannins

Tannins are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins. The astringency from the tannins is that which causes the dry and puckery feeling in the mouth following the consumption of red wine, strong tea, or an unripened fruit (McGee, 2004). The term tannin refers to the use of tannins in tanning animal hides into leather; however, the term is widely applied to any large polyphenolic compounds containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. Tannins have molecular weights ranging from 500 to over 3000 (Ashok and Upadhyaya, 2012). Tannins may be classified chemically into two main groups, hydrolyzable and condensed. Hydrolyzable tannins (decomposable in water, with which they react to form other substances), yield

various water-soluble products, such as [gallic acid](#) and protocatechuic acid and sugars. Gallotannin, or common tannic acid, is the best known of the hydrolyzable tannins. Condensed tannins, the larger group, form insoluble precipitates called tanner's reds, or phlobaphenes. Among the important condensed tannins are the extracts from the wood or bark of quebracho, mangrove and wattle (Encyclopaedia Britannica, 2014).

2.1.3 Alkaloids

Alkaloids are any of a class of naturally occurring organic nitrogen-containing bases. Well-known alkaloids include [morphine](#), [strychnine](#), [quinine](#), [ephedrine](#) and [nicotine](#). Alkaloids are found primarily in plants and are especially common in certain families of flowering plants. More than 3,000 different types of alkaloids have been identified in a total of more than 4,000 [plant](#) species. Some certain families of plants that are particularly rich in alkaloids; for example, all plants of the [Poppy](#) family (Papaveraceae) are thought to contain them. The Ranunculaceae (buttercups), Solanaceae (nightshades), and Amaryllidaceae (amaryllis) are other prominent alkaloid-containing families (Encyclopaedia Britannica, 2014). A few alkaloids have been found in [animal](#) species, such as the New World beaver (*Castor canadensis*) and poison-dart frogs (*Phyllobates*). Ergot and a few other fungi also produce them. The function of alkaloids in plants is not yet understood. It has been suggested that they are simply waste products of plants' metabolic processes, but evidence suggests that they may serve specific biological functions. In some plants, the concentration of alkaloids increases just prior to seed formation and then drops off when the seed is ripe, suggesting that alkaloids may play a role in this process (Encyclopaedia Britannica, 2014). Alkaloids may also protect some plants from destruction by certain insect species. The chemical structures of alkaloids are extremely variable. Generally, an alkaloid contains at least one nitrogen atom in an [amine-type](#) structure—i.e., one derived from ammonia by

replacing hydrogen atoms with hydrogen-carbon groups called hydrocarbons. This or another nitrogen atom can be active as a [base](#) in acid-base reactions. The name alkaloid (“alkali-like”) was originally applied to the substances because, like the inorganic alkalis, they react with acids to form salts. Most alkaloids have one or more of their nitrogen atoms as part of a ring of atoms, frequently called a cyclic system. Alkaloid names generally end in the suffix -ine, a reference to their chemical classification as amines. In their pure form most alkaloids are colourless, nonvolatile, crystalline solids. They also tend to have a bitter taste (Encyclopaedia Britannica, 2014).

2.1.4 Steroids

Plant steroids are types of natural organic compounds found in plants. It contains a characteristic arrangement of four [cycloalkane](#) rings joined to one another. Many types of plant steroids exist and play important roles in the biological processes of plants, such as growth and development, cell division and resistance to damage from environmental stresses like cold weather (Hanson, 2010). Some plant steroids are also useful for their effects when consumed by human beings because their presence decreases the amount of [cholesterol](#) in the bloodstream (Hanson, 2010). Plant steroids should not be confused with [anabolic steroids](#) used to increase muscle mass, which are a synthetic substance that imitates the effects of human androgenizing hormones such as testosterone. The most biologically prominent plant steroid is brassinolide ($C_{28}H_{48}O_6$), which is important to the development of plant cells and promoting the plant's growth (Hanson, 2010). It is part of a larger class of plant steroids called brassinosteroids. Brassinolide is synthesized from [campesterol](#) ($C_{28}H_{48}O$), another plant steroid that is part of a group of similar steroid compounds called [phytosterols](#). Other examples of phytosterols, also commonly called [plant sterols](#), include [beta-sitosterol](#) ($C_{29}H_{50}O$) and brassicasterol ($C_{28}H_{46}O$) (Hanson, 2010).

2.1.5 Glycosides

Glycosides are a class of molecules in which, a sugar molecule is bonded to a "non-sugar" molecule. Glycosides can be classified by the aglycone, glycone or glycosidic bond. If the glycone portion of a glycoside is [glucose](#), then we refer to the molecule as a glucoside. If the glycone portion of the glycoside is [fructose](#), then we refer to the molecule as fructoside(www.wisegeek.com, retrieved 23rd July, 2014). If the glycone portion of the glycoside is [glururonic acid](#) then we refer to the glycoside as a glucuronide. Glycosides can be classified as alpha-glycosides or beta-glycosides as well. This classification depends on whether the glycosidic bond lies "above" or "below" the plane of the sugar part of the glycoside(www.wisegeek.com, retrieved 23rd July, 2014). Glycosides play important roles in our lives. Many plants store medicinally important chemicals in the form of inactive glycosides. The non-sugar portion contains the biochemically active properties of medical interest. Once the glycoside is split into its two components (sugar and non-sugar parts), the non-sugar component is now free to exert its chemical effects on the body. For example, digitalis is a glycoside that when ingested, causes the heart to contract (pump) more forcefully. This is useful in medicine, where heart failure is present (www.wisegeek.com, retrieved 23rd July, 2014).

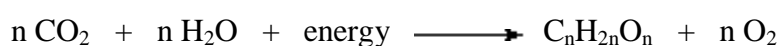
2.1.6 Terpenoids

Terpenes are a large class of **organic hydrocarbons** produced by a wide variety of plants, and are referred to as terpenoids when denatured by oxidation (drying and curing the flowers). These terpenoids form a group of naturally occurring compounds majority of which occur in plants, a few of them have also been obtained from other sources. Terpenoids are volatile substances, which give plants and flowers their fragrance. They occur widely in the leaves and fruits of higher plants, conifers, *Citrus* and *Eucalyptus*

(Bano, 2007). They are the main building blocks of any plant resin or “essential oils” and contribute to the scent, flavour and colours. They (terpenes) are the main class of aromatic compounds found in cannabis and have even been proven to interact synergistically with cannabinoids to provide for a range of different effects. **Terpenes** have been found to be essential building blocks of complex plant hormones and molecules, pigments, sterols and even cannabinoids in cannabis (Reichard, 2013). Terpenes also play an incredibly important role by providing the plant with natural protection from bacteria and fungi, insects and other environmental stresses. More noticeably, terpenes are responsible for the pleasant, or not so pleasant, aromas and flavours of cannabis. Although, over 200 terpenes have been reported in the plant, only a small minority has actually been studied for their pharmacological effects. Other terpenes such as limonene have relaxing effects and are found in anything with a *Citrus* smell such as oranges, lemons, rosemary and juniper. Limonene is known to have anti-bacterial, anti-depressant and anti-carcinogenic properties as well. It is thought to quickly penetrate cell membranes causing other terpenes to be absorbed more rapidly and effectively (Reichard, 2013). Because of limonene’s potent anti-carcinogenic and anti-fungal properties, it is thought to be the component protecting marijuana smokers from *Aspergillus* fungi and carcinogens found in cannabis smoke (Reichard, 2013).

2.1.7 Carbohydrates

Carbohydrate is an organic compound, it comprises of only oxygen, carbon and hydrogen. Carbohydrates are the most abundant class of organic compounds found in living organisms. They originate as products of photosynthesis, an endothermic reductive condensation of carbon dioxide requiring light energy and the pigment chlorophyll.



The carbohydrates are a major source of metabolic energy, both for plants and animals that depend on plants for food. Aside from the sugars and starches that meet this vital nutritional role, carbohydrates also serve as a structural material (cellulose), a component of the energy transport compound [ATP \(Adenosine Tri-Phosphate\)](#), recognition sites on cell surfaces, and one of three essential components of DNA (Deoxyribonucleic acid) and Ribonucleic acid (RNA)(Maber and Vivida, 2013). They are widely distributed molecules in plant and animal tissues. In plants, and arthropods, carbohydrates form the skeletal structures, they also serve as food reserves in plants and animals. They are important energy source required for various metabolic activities, the energy is derived by oxidation. Plants are richer in carbohydrates than animals(Maber and Vivida, 2013).

2.1.7.1 Classification of carbohydrates

Carbohydrates are classified into three groups: monosaccharides, oligosaccharides and polysaccharides.

Monosaccharides: Monosaccharides are often called simple sugars, these are compounds, which possess a free aldehyde or ketone group. They are the simplest sugars and cannot be hydrolyzed. The general formula is $C_n(H_2O)_n$ or $C_nH_{2n}O_n$. The monosaccharides are subdivided into trioses, tetrose, pentoses, hexoses, heptoses etc., and also as aldoses or ketoses depending upon whether they contain aldehyde or ketone group. Examples of monosaccharides are fructose, erythrulose, ribulose, etc (Maber and Vivida, 2013).

Oligosaccharides: Oligosaccharides are compound sugars that yield 2 to 10 molecules of the same or different monosaccharides on hydrolysis. Oligosaccharides yielding 2 molecules of monosaccharides on hydrolysis is known as a disaccharide, and the ones yielding 3 or 4 monosaccharides are known as trisaccharides and tetrasaccharides

respectively and so on. The general formula of disaccharides is $C_n(H_2O)_{n-1}$ and that of trisaccharides is $C_n(H_2O)_{n-2}$ and so on. Examples include the sucrose, lactose, maltose, etc (Maber and Vivida, 2013).

Polysaccharides: Polysaccharides are compound sugars and yield more than 10 molecules of monosaccharides on hydrolysis. They are further classified depending on the type of molecules produced as a result of hydrolysis. They may be homopolysaccharides i.e, monosaccharides of the same type or heteropolysaccharides i.e., monosaccharides of different types. The general formula is $(C_6H_{10}O_5)_x$. Examples of homopolysaccharides are starch, glycogen, cellulose, pectin, etc (Maber and Vivida, 2013).

2.1.8 Flavonoids

According to Higdon and Frei (2003), flavonoids are a group of anti-oxidant compounds found primarily in plants. They are primarily known as pigments for flower colouration, producing yellow or red/blue pigmentation in the flower petals. There are several thousand types, which are broken down into six subgroups (Higdon and Frei, 2003). These are:

2.1.8.1 *Anthocyanidins*

This group is commonly found in red and blue coloured foods, such as berries, grapes and red wine. They may help to keep blood vessels healthy.

2.1.8.2 *Flavanols*

This group can be further broken down into three classes: catechins, theaflavin and proanthocyanidins. Catechins and theaflavins are found in teas. Green tea is higher in catechin while black teas are richer in theaflavins (Higdon and Frei, 2003). One

compound in particular, epicatechin, has been shown to improve blood flow and may be good for cardiac health. Cocoa, the main ingredient of dark chocolate, contains high amounts of epicatechin. Proanthocyanidins are found in chocolate, apples, berries, red grapes and red wine. These nutrients can increase vitamin C inside cells and may inhibit the destruction of collagen, the most abundant protein in the body (Higdon and Frei, 2003).

2.1.8.3 *Flavanones*

This group is found in *Citrus*fruits and juices such as oranges, grapefruits and lemons.

2.1.8.4 *Flavonols*

The most abundant and commonly known flavonoid in this group is quercetin, found in yellow onions, scallions, kale, broccoli, apples, berries and teas. Red wine is also high in quercetin (Higdon and Frei, 2003).

2.1.8.5 *Flavones*

This group is primarily found in the skin of *Citrus*fruits. Its intake is relatively low. This group is also found in foods such as parsley, thyme, celery and hot peppers (Higdon and Frei, 2003).

2.1.8.6 *Isoflavones*

This group is the most bioavailable of all flavonoids, and contains the soy flavonoid genistein. Some studies (Higdon and Frei, 2003) have found that soy flavonoids can reduce blood cholesterol, prevent osteoporosis, and may ease menopausal symptoms (Higdon and Frei, 2003).

2.1.8.7 *Potential health benefits of flavonoids*

Research on the potential health benefits of these compounds is mostly focused on the way they interact with other substances, especially in terms of their antioxidant activity. Anti-oxidant substances are able to change or neutralize the effects of reactive

substances called free radicals that can damage cells, leading to disease. Many of the studies on flavonoids have been done on materials in test tubes or animals, so it's not entirely clear how effective they are in humans, but they may lower the risk of a variety of health problems, including cardiovascular disease, age-related degenerative diseases, and cancers(<http://www.wisegeek.org/what-are-flavonoids.htm>,retrieved 23rd July, 2014).They may also help prevent tooth decay and reduce the occurrence of common illnesses, like the [flu](#). Flavonoids also appear to have a symbiotic relationship with vitamin C, and are thought to change the way cells associated with inflammation act, preventing or reducing it. Several studies show that they can disrupt the function of certain viruses and bacteria, including those associated with Human Immunodeficiency Virus (HIV) and certain types of herpes(<http://www.wisegeek.org/what-are-flavonoids.htm>, retrieved 23rd July, 2014). They may also improve symptoms related to psychological disorders, including mood instability, memory problems, and depression (<http://www.wisegeek.org/what-are-flavonoids.htm>, retrieved 23rd July, 2014).

Role of flavonoids in plants: In plants, these substances provide pigmentation and help filter UV rays. They also help protect the plants from microbe, fungus, and insect attacks; and help plants survive frost and droughts. Additionally, they help promote or inhibit the growth of certain plant parts, including seeds and pollen tubes, and are used as chemical signals to tell plant cells when to stop or start doing things (<http://www.wisegeek.org/what-are-flavonoids.htm>, retrieved 23rd July, 2014).

2.1.9 Phlobatannins

Phlobatannins are reddish, alcohol soluble and water insoluble phenolic substances. They can be extracted from plants, or be the result from treatment of tannin extracts with [mineral acids](#). The name *phlobaphen* (phlobatannins) come from the [Greek words](#),

phloios, meaning [bark](#) and *baphe*, meaning [dye](#) (Rompp and Verlag, 2006; Hemingway *et al.*, 1992; Foo and Karchesy, 1989).

2.2 Antimicrobial Effect of Some Mistletoe Species Extracts

Different research teams have worked on various species of the plant (mistletoe) and demonstrated some pharmacological properties, which supported the claimed ethnomedicinal uses (Dalziel, 1955; Bolksman *et al.*, 1982; Kuttan *et al.*, 1990; Obatomi *et al.*, 1994; Obatomi *et al.*, 1996; Osadebe and Ukwueze, 2004; Osadebe *et al.*, 2006). Orji *et al.* (2013) reported that the leaf extract of *Loranthus micranthus* inhibited the growth of *Candida albicans*, *Aspergillus* species and *Penicillium* species, which are causative agents of infectious diseases such as candidiasis, respiratory mycosis, vaginosis and pelvic inflammatory disease. The methanol extracts of both *Phragmanthera capitata* and *Globimetula oreophila* were reported by Oluwole *et al.* (2013) to have displayed a significant activity against both the Gram-positive and Gram-negative bacterial isolates used in their study. The results of the antimicrobial studies by Waly *et al.* (2012) indicated that the crude methanolic extracts of the six Loranthaceae species showed different degrees of inhibition, depending on bacterial strains and used concentration. At a concentration of 30 µg /20 µl of the tested extracts, no inhibition was noticed against all the tested microorganisms. On the other hand, increasing the concentration to 500 µg/20 µl (disc load) showed an inhibitory effect, with all the tested extracts, against the two Gram positive organisms (*Staphylococcus aureus* and *Bacillus subtilis*) where the inhibition zone diameter ranged from 9 –13 mm. They further reported that upon increasing the concentration of the tested extract to 1000 µg/20 µl, the effect of the tested extracts increased to include most of the tested Gram negative organisms and a significant increase in the activity of all the tested extracts was observed against the tested Gram positive bacteria. *Tapinanthus dodoneifolius*,

which belonging to the family Loranthaceae, revealed the presence of medicinally active constituents such as: alkanoids, flavonoids, anthraquinone, saponin, tannins, carbohydrates and glycosides in the methanol, chloroform and water fractions (Ndamitso *et al.*, 2013). In *In vitro* assaying of the extracts of the *T. dodoneifolius* using agar plate-hole and nutrient broth dilution techniques, a wide spectrum of anti-microbial activities against certain multiple drug resistant bacteria isolates was observed with *Salmonella typhi* and *Staphylococcus aureus* being the most susceptible while *Bacillus subtilis* the least (Ndamitso *et al.*, 2013). The minimum inhibitory concentration (MIC) of the extracts ranged from 6.25 to 15.6mg/ml while the minimum bactericidal concentration (MBC) ranged from 25.0 to 62.5mg/ml (Ndamitso *et al.*, 2013). Efuntoye *et al.* (2010) further attested the pharmacological activities of mistletoes in their research as they reported that, the methanolic extracts of *Tapinanthus bangwensis* obtained from different host plants showed activity against *Shigella dysenteriae* and *Salmonella typhimurium*. Inuwa *et al.* (2012) reported in their research on the phytochemical and antimicrobial activities of *Globimetula braunii* that the presence of alkaloids, carbohydrates, tannins and flavonoids were indicated and that the ethanolic and aqueous extracts inhibited the growth of *Klebsiella aerogenes*, *Proteus* spp., *Escherichia coli* and *Pseudomonas aeruginosa*. The *in-vitro* and *in-vivo* studies by Okpuzor *et al.* (2009) affirms that, *Globimetula braunii* has great potentials as a lipid-lowering agent based on the proven ability of the extract to lower cholesterol, triacylglycerol and **lipid peroxidation** in rats. Similarly, Okpuzor *et al.* (2009) observed significant increases in packed cell volume (PCV) and haemoglobin (Hb) in the rats treated with chloroform extract, ethyl acetate (EtOAc) and water (H₂O) fractions of *Globimetula braunii*. The red blood cell (RBC) count increased after administration of CHCl₃ and ethyl acetate fractions while white blood cell (WBC) count increased in the

crude and all its fractions except butanol. Bassey (2012) reported *Tapinanthus globiferus* as one of the mistletoes commonly consumed by the people of Akwa Ibom State as a herbal cure for ailments such as hypertension, diabetes, ulcer and heart diseases.

2.3 Host Range/ Trees Species Associated with Mistletoe Species

Mistletoes are a highly specialized and successful group of flowering plants that exploit and parasitize a wide range of host plants. They occur over a broad range of habitats all over the world (Polhill and Wiens, 1998). The occurrence and spread of mistletoe are believed to be determined by host specificity, environmental conditions, host plant characteristics (DelRio *et al.*, 1996) and the movement patterns of dispersal agents (Aukema and Martinez del Rio, 2002). For example, a total of ten mistletoe species (*Macrosolen cochinchinensis*, *Taxillus vestitus*, *Loranthus odoratus*, *Helixanthera ligustrina*, *Scurrula gracilifolia*, *Scurrula parasitica*, *Scurrula pulverulenta*, *Scurrula elata*, *Viscum album* and *Viscum articulatum*), eight belonging to five genera in the family Loranthaceae (*Macrosolen*, *Taxillus*, *Helixanthera*, *Scurrula* and *Loranthus*) and two belonging to one genus in the family Viscaceae (*Viscum*) were recorded in the study area (Devkota *et al.*, 2010). They further reported that, these ten mistletoe species were parasitizing 34 host plants such as *Quercus lanata*, *Quercus glauca*, *Rhus javanica*, *Ficus bengalensis*, *Juglan regia*, *Pinus wallichiana*, *Prunus domestica*, etc belonging to 28 genera of 21 unrelated host families. Zaroug *et al.* (2014) also reported that, a species of mistletoe (*Tapinanthus globiferus*) parasitized 22 plant species belonging to 14 families. Some of the hosts are fruit tree crops, others are useful hedges around orchards in the same habitat. The severely infected host plants according to them included the *Citrus* species, guava, *Ziziphus* species and *Moringa* species with *Albizzia lebbek* as one of the host plants lowly infested. They however, reported that, mango

trees were not infected despite the presence of mistletoe seeds deposited on their branches. This according to them suggested the tolerance of mango to mistletoe (*Tapinanthus globiferus*). The following are the trees species from which the mistletoes species used in this study were collected:

2.3.1 *Terminalia catappa*

Terminalia catappa is a large tree measuring up to 25-40m or 82-130 ft tall and grows in sub-tropical climates. It is often associated with coastal vegetation, growing at the edges of mangrove swamps or on rocky shores. Its main products are the nuts and timber. It is widely planted throughout the tropics as an ornamental tree for shade and for the edible nuts (Untwal and Kondawar, 2006; Inbaraj and Sulochana, 2006; Muhammad and Oloyede, 2004). The nut kernel can be eaten raw. The tree loses its leaves twice a year in most places with the colour of the leaves turning red to yellow before leaf shedding. The fruit is large (2-3 inches), edible, fleshy, green (unripe) and yellow or red (when ripe) (Untwal and Kondawar, 2006; Inbaraj and Sulochana, 2006 and Muhammad and Oloyede, 2004). *Terminalia catappa* is known by common names such as: country almond, Indian almond, Malabar almond, sea almond and tropical almond (Oudhia and Paull, 2008).

2.3.1.1 *Chemical constituents of the leaf extracts of Terminalia catappa*

In the research carried out by Shikha *et al.* (2013), they reported that the phytochemical analysis of the leaf extracts of all the four solvent extracts were screened for the presence of various bioactive phytochemical compounds. The analysis revealed the presence of proteins, steroids, glycosides, tannins and phenolic compounds, flavonoids, saponin and amino acids in the methanol extracts. Alkaloids were present in less amount. Starch and carbohydrates were absent. In ethanol extract, steroids, glycosides, flavonoid and saponin were found present. Tannins and phenolic compounds were

present in small amounts. Carbohydrates, amino acids, protein, starch and alkaloids were absent (Shikha *et al.*, 2013). In the petroleum ether extract only saponins were found to be present. In acetone extract, tannins and phenolic compounds, flavonoid, alkaloids and saponins were present. Carbohydrates, protein, glycosides, amino acid and starch were absent (Shikha *et al.*, 2013).

2.3.1.2 *Species of mistletoe associated with Terminalia catappa*

The following species of mistletoe were found associated with *Terminalia catappa* as reported by Rahmad *et al.* (2014): *Dendrophthoe pentandra*, *Scurrula ferruginia*, *Macrosolen cochinchinensis*, all of Loranthaceae family, *Viscum ovalifolium* and *Viscum articulatum*, both are of Santalaceae family.

2.3.1.3 *Medicinal uses/ antimicrobial activity of Terminalia catappa*

Ethnopharmacological surveys conducted around the world have revealed that among the plant species belonging to Combretaceae family, *Terminalia catappa* is the most requested medicinal plant (N'Guessan, 2008). It is traditionally used for its anti-parasitic, anti-infectious, anti-diarrheal, anti-hypertensive and anti-diabetic properties. It is also used in the treatment of asthma, skin diseases, gastrointestinal diseases, respiratory, cardiovascular and rheumatic diseases, scabies, leprosy, prurigo, sores, headache disorders, gonorrhoea and leucorrhoea (Ackah *et al.*, 2008; Nair and Sumitra, 2008; Annegowda *et al.*, 2010). Mbengui *et al.* (2013) reported the anti-bacterial potential of the bark and leaves of *T. catappa* against multi-resistant bacterial strains. Comparatively, the barks have shown good activities than the leaves and the methanolic extracts of the barks have shown better activities with bactericidal effects upon all the tested strains (Mbengui *et al.*, 2013).

2.3.2 *Terminalia mantaly*

Terminalia mantaly grows up to 10-20 m with an erect stem and neat, conspicuously layered branches. Bark pale grey, smooth and rather mottled. Leaves smooth, bright green when young, in terminal rosettes of 4-9 unequal leaves on short, thickened stems; length up to 7 cm, apex broadly rounded, base very tapered, margin wavy (Orwa *et al.*, 2009). Flowers small, greenish, in erect spikes to 5 cm long. Fruit small oval; seeds, about 1.5 cm long with no obvious wings. The generic name comes from the Latin 'terminalis' (ending), and refers to the habit of the leaves being crowded at the ends of the shoots (Orwa *et al.*, 2009).

2.3.2.1 *Species of mistletoe associated with Terminalia mantaly*

Didier *et al.* (2008) from their findings reported that of all the Loranthaceae drawn up into inventories, *Phragmanthera capitata* is the most abundant with 485 parasited host individuals of which *Terminalia mantaly* was inclusive.

2.3.2.2 *Medicinal uses/ antimicrobial activity of Terminalia mantaly*

In recent decades, traditional healers in southern region of Côte d'Ivoire prefer to use the bark of *Terminalia mantaly* instead of those of *T. catappa* (Zirihi, 1991). According to Coulibaly (2006), roots of *T. catappa* and leaves of *Terminalia mantaly* are used against the loss of voice. Kokora *et al.* (2013) reported the strongest inhibitory activity of aqueous and ethanolic extracts of *T. mantaly* on two strains of *Staphylococcus aureus* and *Escherichia coli*. And Zirihi *et al.* (2012) reported that the *T. mantaly* water extract was 64 times more active than the water extract of *T. catappa* and its hydroalcoholic extract was 2 times more active than that of the *T. catappa* on *Aspergillus fumigatus*.

2.3.3 *Khaya senegalensis*

Khaya senegalensis is a deciduous evergreen tree, 15-30 m high, up to 1m in diameter, with a clean bole to 8-16 m, buttresses not prominent or absent; bark dark grey, with

small, thin, reddish-tinged scales; slash dark pink to bright crimson, exuding a red sap. Leaves alternate, compound, stipules absent; petiole and rachis 13-33 cm long; leaflets 3-4 (max. 7) usually opposite pairs, oblong to narrowly oblong-elliptic, 4-12 x 2-5cm, apex acute to shortly acuminate, base rounded, margins entire, pale green, lateral nerves 8-16, petioles about 3.5 cm long (Orwa *et al.*, 2009). Inflorescence a lax, much-branched axillary panicle up to 17cm long; flowers tetramerous, monoecious but with well-developed vestiges of those of the opposite sex with very little external differences between sexes (Orwa *et al.*, 2009). Calyx pale green, lobed almost to the base, lobes subcircular, about 1 x 1 mm, imbricate; petals cream, free, oblong-ovate, 4 x 2.5 mm, contorted in bud; orange disk around the ovary. Fruit an upright, almost spherical, woody capsule, 4-6cm in diameter, opening by 4 valves from the apex (a distinction from *K. ivorensis*, which is closely related but has 5 valves). Seeds brown, 6 or more per cell, broadly transversely ellipsoid to flat, about 25 x 18 mm, margins narrowly winged. The specific name means 'of Senegal', which is where the type specimen was collected (Orwa *et al.*, 2009).

2.3.3.1 Chemical constituents of the leaf extracts of *Khaya senegalensis*

The phytochemical screening of the leaf extract of *Khaya senegalensis* by Sani *et al.* (2012), using petroleum ether, chloroform, acetone and ethanol revealed the presence of flavonoids, carbohydrates, glycosides, saponins, tannins, alkaloids and anthraquinones. Saponins and cardiac glycosides were present in all the extracts; flavonoids were present in chloroform, acetone and ethanol extracts. Carbohydrates were present in ethanolic, chloroform and acetone extracts. Tannins were present in petroleum ether and ethanolic extracts while alkaloids were present in petroleum ether, ethanolic and acetone extracts. There were no phlobatannins in any of the extracts with only the ethanolic extract containing anthraquinones. Kubmarawa *et al.* (2008) reported the presence of saponins,

tannins and phenols from both the aqueous and ethanol extracts of the root, stem-bark and leaves of the plant.

2.3.3.2 Medicinal uses/ antimicrobial activity of *Khaya senegalensis*

Khaya senegalensis is highly reported for its numerous medicinal uses (Arbonnier, 2004), it has been known to be used ethnomedicinally as a remedy for several human and animal ailments (Deen and Sadiq, 2002). It was active *in-vitro* against *Trypanosoma brucei brucei* (Atawodi, 2005), *Trypanosoma congolense* (Atawodi *et al.*, 2003; Atawodi, 2005) and helminthiasis (Fajimi and Taiwo, 2005), showing moderate to high efficacy against *Haemonchus*, *Cooperia*, *Oesophagostomum* and *Trichostrongylus* spp. The aqueous extract is taken against diarrhoea, gynaecological disturbances, digestive disorders and nervous confusions (Kubmarawa *et al.*, 2008). The water fractions of the stem-bark and the ethanol fractions of the roots, stem-bark and leaves were active on *Staphylococcus aureus*, *Streptococcus* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp. and *Bacillus subtilis* (Kubmarawa *et al.*, 2008). The stem-bark and leaves have been used in the forms of decoction and concoctions for the cure of vermifuge, abortifacient, antipyretic and malarial fever (Arbonnier, 2004). The dried stem-bark is used externally for the treatment of leprosy, dermatomes, sores and ulcers in adults (Le Grand, 1989).

2.3.4 *Albizzia lebbek*

Albizzia lebbek can attain a height of 30m and a diameter of 1 m; more often it is 15-20m tall with a diameter of 50 cm; bark grey-violet with rusty brown breathing pores, rough and fissured. It has compound leaves which is bipinnate, glabrous or slightly hairy on the axis; pinnae in 2-4 pairs, each with 2-11 pairs of obliquely oblong leaflets 15-45 x 8-22mm, shortly stalked; glabrous glands are raised, elliptic to circular, on the upper side of the stalk close to the base and between most pairs of leaflets (Orwa *et al.*,

2009). Flowers appear shortly after new leaves, are white, heavily scented, with the stamens free above the corolla, in heads 18-36 mm across excluding the stamens, on a stout stalk 5-7.5cm long, appearing singly or in small clusters in the leaf axils and in terminal panicles; stamens 30-40, yellowish-green on top side, white underside, up to 5cm long; flowerstalks up to 5mm long; corolla tube, 1 cm long (Orwa *et al.*, 2009). Pods pale straw to light brown at maturity, narrow-oblong, 15-26 x 3-5cm, papery, leathery, flat and not raised or constricted between seeds; seeds brown, flat, orbicular or elliptic, 8-10 x 6-7 mm; transversely placed with 6-12 in each pod. The genus is named after Filippo del Albizzi, a Florentine nobleman who in 1749, introduced *A. julibrissin* into cultivation. The species name is from the Arabic name for this plant, 'laebach'. When agitated by wind, the pods and enclosed seeds are said to produce an incessant rattle likened to women's chatter, hence the name 'woman's tongue' (Orwa *et al.*, 2009).

2.3.4.1 Chemical constituents of the leaf extracts of *Albizzia lebeck*

In the phytochemical screening of the leaf extracts of *Albizzia lebeck* by Rahul *et al.* (2010), ethyl acetate extract showed the presence of glycosides, tannins, saponins, flavonoids, carbohydrates, proteins and amino acids. Methanolic extract showed presence of alkaloids, tannins, saponins, flavonoids and carbohydrates. Water extract showed the presence of tannin, saponins, flavonoids and carbohydrates. Faisal *et al.* (2012) also in their findings reported that *A. lebeck* contained alkaloids, flavonoids, tannins and saponins.

2.3.4.2 Species of mistletoe associated with *Albizzia lebeck*

Zaroug *et al.* (2014) reported *Albizzia lebeck* among other host trees species as one of the trees parasitized by *Tapinanthus globiferus*.

2.3.4.3 Medicinal uses/antimicrobial activity of *Albizzia lebeck*

The barks of *A. lebeck* are used in toothache, piles, diarrhea and diseases of the gum. Decoctions of the leaves and barks are protective against bronchial asthma and other allergic disorders (Mishra *et al.*, 2010). The antimicrobial activity of *A. lebeck* as reported by Mohammed *et al.* (2012) showed to have inhibitory effect against *Bacillus subtilis*, *E. coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *S. typhi* and *S. aureus*.

2.3.5 *Citrus* species

Citrus belongs to the genus *Citrus* L., sub-tribe Citrinae, sub-family Aurantioideae, family Rutaceae, order Sapindales, superorder Rosidae and sub-class dityledoneae. They yield pulpy fruits covered with fairly thick skins. Plants in this group include the [lemon](#) (*C. limon*), [lime](#) (*C. aurantifolia*), sweet [orange](#) (*C. sinensis*), sour orange (*C. aurantium*), [tangerine](#) (*C. reticulata*), [grapefruit](#) (*C. paradisi*), [citron](#) (*C. medica*), and [shaddock](#) (*C. maxima*, or *C. grandis*; pomelo) (www.encyclopaediabritannica.com/citrus 2013). *Citrus* trees are evergreen trees that produce fruits of different forms and sizes (from round to oblong), which are full of fragrance, flavour and juice. It has a rough, robust and bright colour from green to yellow skin or rind known as epicarp or flavedo, which covers the fruits and protects from damages. The glands contain the essential oils that give the fruit its typical *Citrus* fragrance. It consists of a white, thick and spongy mesocarp or albedo which together with the epicarp forms the pericarp or peel of the fruit. The internal part constitutes the pulp which is divided into separate segments or juice sacs (with or without seeds, according to varieties) by a thick radical film or endocarp. This part is rich in soluble sugars, ascorbic acid, pectin, fibers, different organic acids and potassium salt that gives the fruit its characteristic citrine flavour (Rogger, 1999; Rogger, 2002).

2.3.5.1 Chemical constituents of the leaf extracts of *Citrus* species

Gangai and Nirmala (2013) in the phytochemical screening of *Citrus sinensis* reported the presence of alkaloids, terpenoids, tannins and flavonoids. The phytochemical studies of five varieties of *Citrus* species: sweet orange (*Citrus sinensis*), tangerine (*Citrus reticulata*), lemon (*Citrus limonum*), lime (*Citrus aurantifolia*) and grape (*Citrus grandis*) revealed the presence of bioactive compounds comprising alkaloids (0.22-1.60%), saponin (0.30-0.98%), flavonoids (0.30-0.89), phenols (0.02-0.64%) and tannins (0.23-1.45%) (Okwu *et al.*, 2007). Pathan *et al.* (2012) reported that the phytochemical screening of the leaf extracts of *Citrus aurantifolia* revealed the presence of carbohydrates, alkaloids, steroids, tannins and flavonoids.

2.3.5.2 Species of mistletoe associated with *Citrus* species

Oyetunji and Edagbo (2013) reported the use of *Tapinanthus bangwensis* parasitizing *Citrus sinensis* for their studies in which they said that, mistletoe thrives on its hosts relative to the available nutrients, water content and to a slight extent on the host photosynthate; while the extent to which mistletoe can affect the host is dependent on how much of the resource is diverted by the parasite and also the overall supply available to the host. Six species of Loranthaceae (*Globimetula braunii*, *Globimetula opaca*, *Tapinanthus ogowensis*, *Helixanthera mannii*, *Phragmanthera capitata* and *Tapinanthus globiferus*) were reported to have parasitized *Citrus* species by Didier *et al.* (2008).

2.3.5.3 Medicinal uses/antimicrobial activity of *Citrus* species

Okwu *et al.* (2007) reported the antifungal activity of some *Citrus* species such as *C. sinensis*, *C. reticulata*, *C. aurantifolia*, *C. grandis* and *C. limonum* on the growth

of *Fusarium oxysporum*, which causes damping off disease of okra. The peel extracts of *C. reticulata*, *C. aurantifolia* and *C. sinensis* showed the strongest inhibition against the fungi. Antibacterial effect of *Citrus* species had been reported by Hindi *et al.* (2014) who confirmed the antibacterial effect of aqueous extracts of the peel, juice and leaves of *C. aurantium*, *C. sinensis*, *C. grandis* and *C. reticulata* against *S. aureus*, *S. pyogenes*, *E. faecalis*, *P. aeruginosa*, *K. pneumoniae*, *E. coli*, *S. typhi*, *Proteus spp.* and *M. catarrhalis*.

2.4 Test Organisms

2.4.1 *Bacillus subtilis*

Bacillus subtilis is a Gram-positive, rod-shaped bacterium, commonly found in soil. It was originally named “*Vibrio subtilis*” when it was discovered in 1835 by Christian Gottfried Ehrenberg. It was renamed “*Bacillus subtilis*” in 1872 by Ferdinand Cohn(<http://www.britannica.com/EBchecked/topic/47965/bacillus>, retrieved 23rd, July, 2014). This bacterium is also known by the names hay bacillus, grass bacillus, or *Bacillus globigii*. *Bacillus subtilis* is an endospore-forming bacterium, and the endospore that it forms allows it to withstand extreme temperatures as well as dry environments. *Bacillus subtilis* is considered an obligate aerobe, but can also function anaerobically when in the presence of nitrates or glucose (<http://www.britannica.com/EBchecked/topic/47965/bacillus> retrieved 23rd, July, 2014). *Bacillus subtilis* is not considered pathogenic or toxic and is not a disease causing agent. *Bacillus subtilis* has a flagellum which makes motility faster. *Bacillus subtilis* is a ubiquitous naturally occurring saprophytic bacterium that is commonly recovered from soil, water, air and decomposing plant material. Under most conditions, however, it is not biologically active and is present in the spore form. Different strains of *Bacillus subtilis* can be used as biological control agents under different situations

(<http://www.britannica.com/EBchecked/topic/47965/bacillus>, retrieved 23rd July, 2014). There are two general categories of *B. subtilis* strains; those that are applied to the foliage of a plant, and those applied to the soil or trans-plant mix when seeding. The term *Bacillus* has been applied in a general sense to all cylindrical or rodlike bacteria. The largest [species](#) are about 2 µm (micrometres; 1 µm = 10⁻⁶ m) across by 7 µm long and frequently occur in chains. In 1877, German botanist, [Ferdinand Cohn](#), described two different forms of hay bacillus (now known as *Bacillus subtilis*): one that could be killed upon exposure to heat and one that was resistant to heat. He called the heat-resistant forms “spores” ([endospores](#)) and discovered that these dormant forms could be converted to a vegetative, or actively growing state (<http://www.britannica.com/EBchecked/topic/47965/bacillus>, retrieved 23rd July, 2014). Today, it is known that all *Bacillus* species can form dormant spores under adverse environmental conditions. These endospores may remain viable for long periods of time. Endospores are resistant to heat, chemicals and sunlight and are widely distributed in nature, primarily in soil, from which they invade dust particles (<http://www.britannica.com/EBchecked/topic/47965/bacillus>, retrieved 23rd July, 2014).

2.4.1.1 Diseases caused by *Bacillus subtilis*

Bacillus subtilis is considered a benign organism as it does not possess traits that cause disease. It is not considered pathogenic or toxigenic to humans, animals or plants. *Bacillus subtilis* is only known to cause disease in severely immunocompromised patients, and can conversely be used as a [probiotic](#) in healthy individuals (Oggioni *et al.*, 1998).

2.4.2 *Escherichia coli*

Escherichia coli (commonly referred to as *E. coli*) is a species of bacteria commonly found in the intestines of humans and other warm-blooded animals. There are many different types of *E. coli*, and while some live in the intestine quite harmlessly, others may cause a variety of diseases. The bacterium is found in faeces and can survive in the environment (Parry, 2002). *Escherichiacoli* was first discovered in 1885 by Theodor Escherich, a German bacteriologist. *Escherichia coli* has since been commonly used for biological laboratory experiment and research. *Escherichia coli* is an aerobic, Gram-negative, rod-shaped bacterium that can be commonly found in animal feces, lower intestines of mammals, and even on the edge of hot springs (Parry, 2002). However, *E. coli* prefers to live at a higher temperature rather than the cooler temperatures. *Escherichia coli* is a Gram-negative organism that does not sporulate. Therefore, it is easy to eradicate by simple boiling or basic sterilization. *Escherichiacoli* can also be classified into hundreds of strains on the basis of different serotypes. *Escherichia coli* O157:H7, for example, is a well-studied strain of the bacterium *E. coli*, which produces Shiga-like toxins, causing severe illness by eating cheese and contaminated meat (Atlanta, 2007). Furthermore, enteric *E. coli* can be classified into six categories based on its virulence properties, such as enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteroadherent aggregative *E. coli* (EAaggEC) and verotoxigenic *E. coli* (VTEC) (Parry, 2002). These enteric *E. coli* can cause several intestinal and extra-intestinal infections such as urinary tract infections and mastitis. However, *E. coli* are not always harmful to human bodies or other animals. Most *E. coli* live in our intestines, where they help our body breakdown the food we eat as well as assist with waste processing, vitamin K production and food absorption (Parry, 2002).

Different strains of *E. coli* can be found in different type of animals, so we can determine the source (from human or from other animals) of the stools by examining which strain of *E. coli* is presenting in the stools. *Escherichia coli* can also be found in environments at higher temperature, such as on the edge of hot springs (Parry, 2002).

2.4.2.1 Diseases caused by *Escherichia coli*

The commonest infection caused by *E. coli* is infection of the urinary tract, the organism normally spreads from the gut to the urinary tract. *Escherichia coli* is also the commonest cause of cystitis (infection of the bladder), and in a minority of patients the infection may spread up to the urinary tract to the kidneys, causing pyelonephritis (Champman *et al.*, 2002). Otherwise, healthy patients in the community may develop cystitis, and patients in hospital who have catheters, or tubes, placed in the urethra and bladder are also at risk. *Escherichia coli* is also among the bacteria that cause intra-abdominal infections following leakage from the gut into the abdomen, as for example with a ruptured appendix or following traumatic injury to the abdomen. *Escherichia coli* may also cause infections in the intestine (Champman *et al.*, 2002). Diarrhoeal infections (intestinal) are caused by a group of *E. coli* known as 'enterovirulent' (harmful to the intestines). Overspill from the primary infection sites to the bloodstream may cause blood poisoning (*E. coli* bacteraemia). In rare instances, *E. coli* may cause meningitis in very young children (Champman *et al.*, 2002). *Escherichia coli* O157:H7 is one of the most infective strains that can cause food poisoning. It belongs to enterohemorrhagic strain of the *E. coli* and can lead to bloody diarrhea and kidney failure when one gets infected by contaminated ground beef, unpasteurized milk or contaminated water. The toxin that *E. coli* O157:H7 produces is a Shiga-like toxin, which is a regulated toxin that catalytically inactivate 60S ribosomal subunits of most eukaryotic cells, blocking mRNA translation and thus causing cell death (Champman *et*

al., 2002). Some important symptoms are diarrhea that is acute and severe, either bloody or not bloody, stomach cramping, vomiting, loss of appetite, abdominal pain, and fever. The causes can usually clear up on their own in 1-3 days with no treatment required. However, patients should avoid dairy products because these products may induce temporary lactose intolerance, and therefore make the diarrhea worse (Champman *et al.*, 2002).

2.4.3 *Salmonella typhi*

Salmonella typhi is an obligate parasite that has no known natural reservoir outside of humans. *Salmonella typhi* (more commonly known as the bacteria responsible for typhoid fever) can be very dangerous if not taken care of properly. This Gram-negative enteric bacillus belongs to the family *Enterobacteriaceae*. It is a motile, facultative anaerobe that is susceptible to various antibiotics. Currently, 107 strains of this organism have been isolated, many containing varying metabolic characteristics, levels of virulence and multi-drug resistance genes that complicate treatment in areas that resistance is prevalent (WHO, 2010). This type of bacteria can only live in the bloodstream or intestinal tract of humans, but is also found in sewage. Even though most people either die or use antibiotics to stop the growth of these bacteria, a very small percentage of the people who get typhoid fever have certain antibodies that are able to restrict the growth of *Salmonella typhi* and therefore are able to live (WHO, 2010). These people plus the people that are cured through antibiotics are called carriers because even though they will have no more symptoms of typhoid fever, they will still have the bacteria inside them. Since *Salmonella typhi* is passed through body fluids, you can contract it by eating some food or a drink handled by a carrier. You can also contract these bacteria by having food or water that has been contaminated with sewage containing *Salmonella typhi* (WHO, 2010).

2.4.3.1 *Epidemiology of Salmonella typhi*

The encounter of humans to *S. typhi* is made via faecal-oral route from infected individuals to healthy ones. Poor hygiene of patients shedding the organism can lead to secondary infection as well as consumption of shellfish from polluted bodies of water (WHO, 2010). The most common source of infection, however, is drinking water tainted by urine and feces of infected individuals. The estimated inoculum size necessary for infection is 100,000 bacteria. Typhoid fever also represents the second most commonly reported laboratory infection (WHO, 2010). The entry of this bacterial species into the human body is most commonly achieved by ingestion, with the importance of aerosol transmission unknown. Once ingested, the organisms multiply in the small intestine over the period of 1-3 weeks, breach the intestinal wall, and spread to other organ systems and tissues. The innate host defenses do little to prevent infection due to the inhibition of oxidative lysis and the ability to grow intracellularly after uptake. About 2-5% of previously infected individuals become chronic carriers who show no signs of the disease, but actively shed viable organisms capable of infecting others. A famous example is “Typhoid” Mary Mallon, who was a food handler responsible for infecting at least 78 people, killing 5 in the United States (WHO, 2010). These highly infectious carriers pose a great risk to public health due to their lack of disease-related symptoms (WHO, 2010).

2.4.3.2 *Prevention of typhoid fever*

The key to avoiding infection by *S. typhi* is prevention of faecal contamination in drinking water and food supplies. Since the only source of this agent is infected humans, it is possible to control transmission by proper hygiene, waste management, water purification and treatment of the sick (WHO, 2010). These measures are attained in developed societies, attributing to the low incidence. The United States has an

average of around 400 infections annually, almost exclusively among people who have recently traveled to developing countries (WHO, 2010). Prevention can also be aided by vaccination against the bacteria, however, the effectiveness of this has been questionable. In addition, it is shown that large inoculum sizes can overwhelm the developed immunity and result in disease (WHO, 2010).

2.4.4 *Staphylococcus aureus*

Staphylococci are Gram-positive spherical bacteria that occur in microscopic clusters resembling grapes. Taxonomically, the genus *Staphylococcus* is in the bacterial family called Staphylococcaceae, which includes three lesser known genera: *Gamella*, *Macrococcus* and *Salinicoccus* (Kluytmans *et al.*, 1997). *Staphylococcus* was first identified in 1880 in [Aberdeen, United Kingdom](#), by the surgeon, Sir [Alexander Ogston](#), in [pus](#) from a surgical abscess in a knee joint (Cole *et al.*, 2001). This name was later appended to *Staphylococcus aureus* by Rosenbach who was credited by the official system of nomenclature at the time (Kluytmans *et al.*, 1997). It is estimated that 20% of the human population are long-term carriers of *S. aureus*, which can be found as part of the normal [skin flora](#) and in anterior nares of the nasal passages (Kluytmans *et al.*, 1997; Cole *et al.*, 2001). The best-known of its nearby phylogenetic relatives are the members of the genus *Bacillus* in the family Bacillaceae, which is on the same level as the family Staphylococcaceae. The Listeriaceae are also a nearby family. *Staphylococcus aureus* forms a fairly large yellow colony on rich medium and it is often haemolytic on blood agar (Gill *et al.*, 2005).

2.4.4.1 Pathology of *Staphylococcus aureus*

Staphylococcus aureus is the most common cause of *staph* infections and is responsible for various diseases including: mild skin infections (impetigo, folliculitis, etc.), invasive diseases (wound infections, osteomyelitis, bacteremia with metastatic complications,

etc.) and toxin-mediated diseases (food poisoning, toxic shock syndrome(TSS), scaled skin syndrome, etc.)(Gill *et al.*, 2005). Infections are preceded by colonization. Common superficial infections include carbuncles, impetigo, cellulitis, folliculitis. Community-acquired infections include bacteremia, endocarditis, osteomyelitis, pneumonia and wound infections are less common(Gill *et al.*, 2005). *Staphylococcus aureus* also causes economically important mastitis in cows, sheep and goats (Gill *et al.*, 2005). *Staphylococcus aureus* infections can spread through contact with pus from an infected wound, skin-to-skin contact with an infected person by producing hyaluronidase that destroys tissues, and contact with objects such as towels, sheets, clothing, or athletic equipment used by an infected person (Cimolai, 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted at Samaru, Zaria, Kaduna State, Nigeria, which falls within the Guinea Savannah zone. It also falls between Longitude 7°37'22"E to 7°40'36"E and Latitude 11° 9'14"N to 11°10'9"N (Mortimore, 1970). (Figure 1).

3.2 Collection and Identification of Plants Materials

The fresh leaves of the mistletoe species growing on *Terminalia catappa* (umbrella tree), *Citrus grandis* (lemon tree), *Terminalia mantaly*, *Khaya sengalensis* and *Albizzia lebbek* alongside with the leaves of the host tree species within Samaru, Zaria Local Government Area of Kaduna State, Nigeria were collected. These were then taken to the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria for identification.

3.3 Preparation of Plant Materials

The leaves of the mistletoes and host tree species were collected and air dried in laboratory in the Department of Biological Sciences, Ahmadu Bello University, Zaria. The leaves were pulverized (grinded) into fine powder using wooden pestle and mortar after drying.

3.4 Phytochemical Screening

The qualitative phytochemical analysis was carried out in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, while the quantitative analysis was carried out at the National Research Institute for Chemical Technology (NARICT), Zaria, Nigeria.

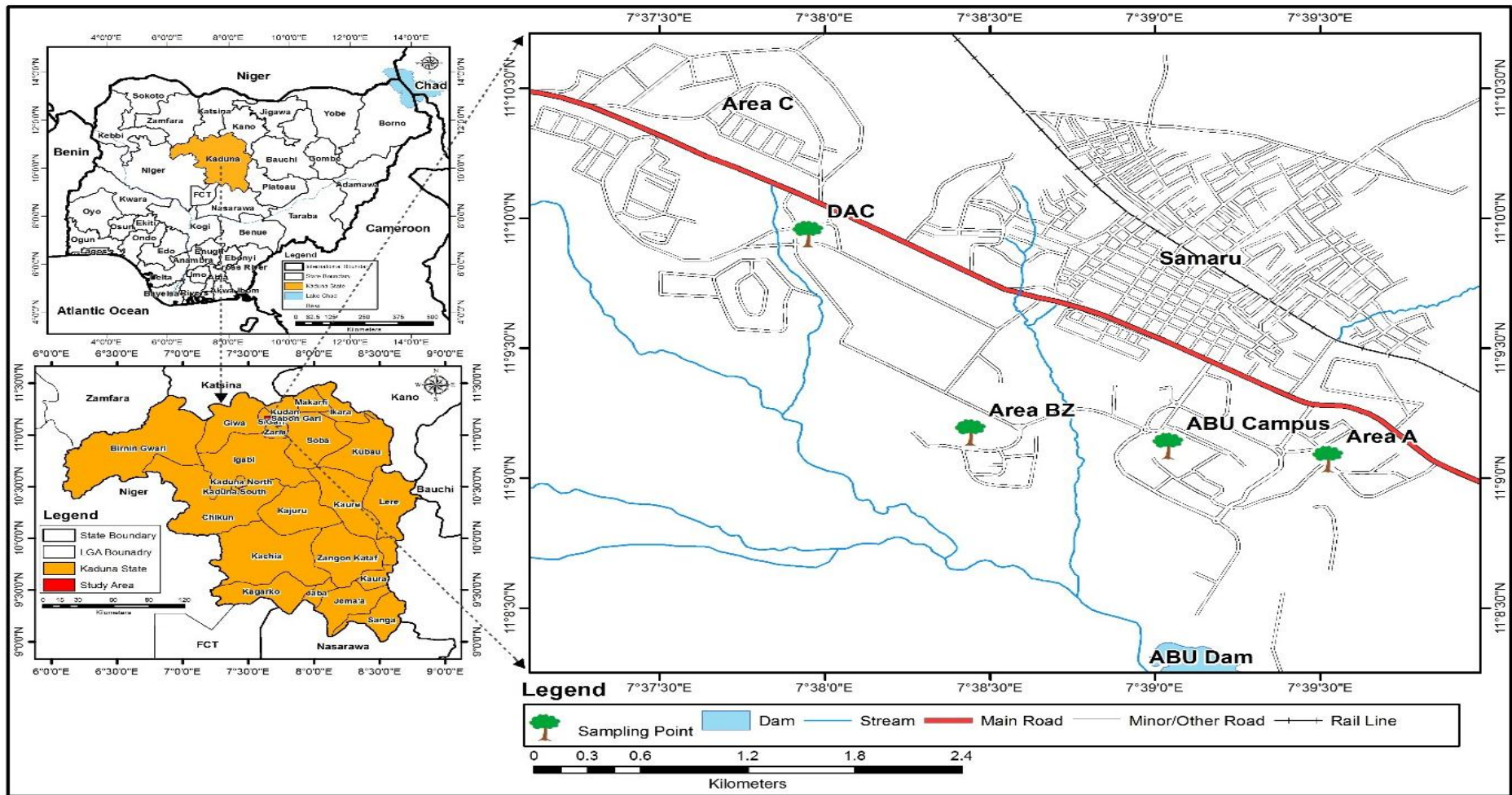


Figure 1: Map of Samaru showing sampling areas

Source: Adopted and modified from the administrative map of Kaduna State (Field work, 2014)

3.5 Extraction of the Plant Material

Maceration method of extraction according to Nagappan (2012), using water and ethanol as solvents was used. Powdered plant material (40g) was weighed into a 250ml conical flask and 200ml of 96 % ethanol was added. It was then stirred several times with sterile glass rod after which the mouth of the conical flask was covered with aluminium foil and kept for two days at room temperature, shaking it occasionally. After the period of two days, the mixture was filtered through muslin cloth and finally by Whatman number 1 filter paper. The solvent was then removed from the extract by putting the extract in evaporating dish placed on water bath set at temperature of 50⁰C. Finally, the residue was then collected and kept in a refrigerator at 4⁰C in a specimen bottle pending analysis. The same procedure was used for the aqueous extract.

3.6 Qualitative Analysis of Phytochemical Constituents

3.6.1 Test for tannins

The extract prepared (2 ml) was stirred with 2ml of distilled water and few drops of Iron (III) chloride (FeCl₃) solution (5%w/v) was added. The formation of a green precipitate indicated the presence of tannins (Sofowora, 1993).

3.6.2 Test for saponins

The extract prepared (5 ml) was shaken vigorously with 5ml of distilled water in a test tube. The formation of stable foam, honey comb in shapes, indicated the presence of saponins (Sofowora, 1993).

3.6.3 Test for steroids

A red colour produced in the lower chloroform layer when 2ml of the extract was mixed with 2ml of chloroform and 2ml of concentrate sulphuric acid added in a test tube indicated the presence of steroids (Ogbuwu, 2008).

3.6.4 Test for alkaloids

The extract prepared (3 ml) was stirred with 3ml of 1% hydrochloric acid (HCl) on a steam bath. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was an evidence for the presence of alkaloids (Harborne, 1973).

3.6.5 Test for terpenes

The extract (5 ml) was diluted with distilled water and 1ml of acetic anhydride was added after which 1ml of concentrated tetraoxosulphate(VI) acid (H_2SO_4) was added down the walls of the test tube to form a layer underneath. The observance of the reddish-violet colour indicated the presence of terpenes (Trease and Evan, 1983)

3.6.6 Test for cardiac glycosides

Salkowski's test was employed. The extract (2 ml) was dissolved in 2ml of chloroform, then 2ml of sulphuric acid was added carefully and shaken gently. A reddish brown ring colour at the interface signified the presence of a steroidal ring (i.e., a glycone portion of glycoside) (Trease and Evan, 1983).

3.6.7 Test for carbohydrates

Molisch's test was used for this. The extract (3 ml) was added to 2ml of Molisch's reagent and the resulting mixture shaken properly, then 2ml of concentrated H_2SO_4 was poured carefully down the side of the test tube. A violet ring at the interface indicated the presence of carbohydrate (Sofowora, 1993).

3.6.8 Test for flavonoids

To 1ml of the extract, was added 1ml of 10% lead acetate solution. The formation of a yellow precipitates was taken as a positive test for flavonoids (Sofowora, 1993).

3.7 Quantitative Analysis of the Phytochemical Constituents

3.7.1 Preparation of fat free sample

The sample (2 g) was weighed and defatted with 100ml of diethyl ether using a Soxhlet apparatus for 2 hours (Padamanabhan *et al.*, 2014).

3.7.2 Determination of total phenols

This was carried out by Spectrophotometric method. The fat free sample was boiled with 50ml of petroleum ether for the extraction of the phenolic component for 15 minutes. The extract (5 ml) was then pipetted into a 50ml flask, and 10ml of distilled water was added. Ammonium hydroxide solution (2 ml) and 5ml of concentrated amylalcohol were added also. The sample was made up to mark with distilled water and left to react for about 30 minutes for colour development. This was measured at 505nm using spectrophotometer (Padamanabhan *et al.*, 2014)..

3.7.3 Determination of tannins

This was carried out using Pearson (1976) method. A measured weight of each sample (1g) was dispersed in 10ml distilled water and agitated for 30 minutes at room temperature being shaken every 5 minutes. At the end of the 30 minutes, it was centrifuged and the extract gotten. Then 2.5ml of the supernatant (extract) was dispersed into a 50ml volumetric flask. Similarly 2.5ml of standard tannic acid solution was dispersed into a separate 50ml flask. A 1ml folin Denis reagent (which is a mixture of phosphomolybdate and phosphotunstate) was measured into each flask followed by 2.5ml of saturated sodium trioxocarbonate (Na_2CO_3) solution. The mixture was diluted to mark with distilled water in the flask (50ml) and incubated for 90 minutes at room temperature. The absorbance was measured at 725nm using spectrophotometer. The tannin content was calculated as follows:

$$\% \text{ Tannin} = A_n/A_s \times C \times 100/W \times V_f/V_a$$

Where: A_n = Absorbance of test sample

A_s = Absorbance of standard solution

C = Concentration of standard solution

W = Weight of sample used

V_f = Total volume of extract

V_a = Initial volume of extract

3.7.4 Determination of flavonoid

This was carried out by the method of Boham and Kocipai- Abyazan (1994). 10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was then filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

3.7.5 Determination of saponin

The method of Obadoni and Ochuko (2001) was used. Out of the grinded samples, 20g of each was put into a conical flask and 100ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90°C. The concentrate was transferred into a 250ml separatory 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 and 60ml of N-butanol was added. The combined N-butanol extracts were washed twice with 10ml 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; the saponin content was then calculated as percentage.

3.7.6 Determination of alkaloid

This was carried out using Harborne (1973) method. 5g of the sample was weighed into a 250ml beaker and 80ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

3.7.7 Determination of cyanogenic glycosides

This was carried out using Onwuka (2005) method. The sample (5g) was dissolved with 50ml of distilled water. The extraction was allowed to continue overnight. The extract was filtered and the filtrate was used for cyanide determination. To 1ml of the sample filtrate, 4ml of alkaline picrate was added and the absorbance was recorded at 550nm using spectrophotometer and cyanide content was extrapolated from a cyanide standard curve. The cyanide content was calculated as indicated below.

$$\text{Cyanide (mg/g)} = \text{Absorbance} \times \text{GF} \times \text{DF}$$

Where:

GF = Gradient factor

DF = Dilution factor

3.8 Antimicrobial Activity Testing

3.8.1 Collection of the bacterial isolates

This was carried out in the Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria.

The test organisms (microorganisms) were isolates of bacteria which were collected from the Department of Microbiology, Ahmadu Bello University, Zaria. These isolates include:

- a) *Salmonella typhi*
- b) *Staphylococcus aureus*
- c) *Bacillus subtilis* and
- d) *Escherichia coli*

3.8.2 Preparation of different concentrations of the leaf extracts

The extract of each of the samples was prepared into four (4) different concentrations ranging from 25 to 200 mg/ml (i.e 25, 50, 100 and 200mg/ml) and each with two (2) replications. The extract concentrations were prepared by weighing 2g of the extract into 10ml sterile distilled water (200mg/ml). A doubling dilution of the diluted extract (200mg/ml) was carried out into three (3) different labeled bottles to obtain concentrations of 100, 50and 25mg/ml respectively.

3.8.3 Standardization of the inocula

The test organisms (inocula) were prepared by streaking the organisms on the freshly prepared nutrient agar plates to obtain discrete bacterial colonies. A colony was then picked and subcultured unto sterile nutrient broth and incubated at 37⁰C for 24hours. After the incubation period, a loopful of broth culture was transferred into bottles containing sterile distilled water to obtain a bacterial cell density of 1.5×10⁸cfu/ml as determined by Mcfarland turbidity standard (Scale number one).

3.8.4 Susceptibility testing of the extracts

This was carried out using agar well diffusion method. The standardized organisms were uniformly streaked unto freshly-prepared Mueller Hinton Agar with the aid of a sterile swab stick (cotton swabs). Four wells were punched on the inoculated agar plates

using a sterile cork borer of 6 mm. The wells were properly labeled according to the different concentrations of the extracts prepared for the various samples. The punched wells were then filled with 0.2ml of the extracts. The plates were allowed to stay on the bench for 1 hour for the extract to diffuse into the agar after which they were incubated at 37⁰C for 24 hours. After the incubation period, the plates were observed for any evidence of inhibition, which appeared as clear zones that were completely devoid of growth around the wells. The diameter of the clear zones was measured with a transparent ruler calibrated in millimeter (mm).

3.8.5 Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the extract was determined using tube dilution method. Doubling (two fold) dilution of the extract was carried out in properly-labelled test tubes using Mueller Hinton Broth (MHB) as a diluent. The lowest concentration inhibitory to each organism when the extract was tested during sensitivity test was serially diluted in test tubes containing Mueller Hinton Broth. Each test tube containing the broth and the extract was inoculated with the standardized organisms. A tube containing sterile Mueller Hinton Broth without any organism was used as a control. The tubes were then incubated at 37⁰C for 24 hours. After the incubation period, the tubes were observed for the presence or absence of growth using turbidity as a criterion. The lowest concentration (dilution) in the series without visible signs of growth was considered to be the minimum inhibitory concentration (MIC).

3.8.6 Determination of minimum bacteriocidal concentration (MBC)

The results from the minimum inhibitory concentration (MIC) were used to determine the minimum bacteriocidal concentration (MBC). A sterile wire loop was dipped into all the tubes that did not show turbidity in the MIC test, it was then streaked onto a freshly prepared nutrient agar plates. The plates were then incubated at 37⁰C for 24 hours. After

the incubation period, the plates were then examined for the presence or absence of growth. This was done so as to determine whether the antimicrobial effect of the extract was bacteriocidal or bacteriostatic.

3.9 Statistical Analysis

All data obtained were subjected to one way analysis of variance (ANOVA) and where there was significance difference, Duncan multiple range test (DMRT) was used to separate the means. In determining the relationship between the infected and uninfected host tree species, chi-square was used.

CHAPTER FOUR

4.0 RESULTS

4.1 Infestation of Trees by Mistletoes

The results of the study revealed that all the selected tree species were infected in virtually all the sampling areas and there was significant ($P < 0.05$) relationship between the infected and uninfected tree species in all areas except *T. catappa*, which had insignificant ($P < 0.05$) relationship between its infected and uninfected trees in all the sampling areas (Table 4.1).

Albizia lebeck in sampling Area A was the highest in occurrence compared to other trees in other areas. However, the *A. lebeck* in Area BZ had the highest percentage (77.27 %) infection by mistletoes whereas the ones in ABU main campus had the least (45.80 %) (Table 4.1).

Khaya senegalensis and *C. grandis* in Area A were the highest in occurrence, however, it had the least percentage (29.48 and 56.41 % respectively) of infection by mistletoes while the *K. senegalensis* and *C. grandis* in sampling Area BZ had the highest percentage infection (57.71 and 80.00 % respectively) compared to those in other areas (Table 4.1).

ABU main campus had the highest number of *T. mantaly* as well as the percentage of those infected by mistletoes (61.33 %) whereas in area BZ, none of the *T. mantaly* was infected (Table 4.1).

In addition, ABU main campus had the highest number of *T. catapa* observed, while area A had the least percentage (25.00 %) of those infected by mistletoes whereas those in area BZ had the highest percentage (66.67 %) of those also infected by mistletoes (Table 4.1).

Table 4.1: The Selected Host Tree Species in each Sampling Areas Indicating hosts Infected and Uninfected by Mistletoes

Sampling area	Host tree	Number Observed	Infected (%)	Uninfected (%)	X ²	Df	P- value	Level of infestation of each Tree sp from all areas (%)
Area A	<i>A. lebeck</i>	837	456 (54.48)	381 (45.52)	48.24	3	0.00	58.26 ^a
ABU main campus		262	120 (45.80)	142 (54.20)				
Area BZ		198	153 (77.27)	45 (22.73)				
DAC		92	51 (55.43)	41 (44.57)				
Total		1389	780 (56.16)	609 (43.84)				
Area A	<i>K. senegalensis</i>	631	186 (29.48)	445 (70.52)	105.36	3	0.0	48.20 ^a
ABU main campus		457	242 (52.95)	215 (47.05)				
Area BZ		454	262 (57.71)	192 (42.29)				
DAC		134	70 (52.24)	64 (47.76)				
Total		1676	760 (45.35)	916 (54.65)				
Area A	<i>C. grandis</i>	39	22 (56.41)	17 (43.59)	8.09	3	0.04	49.10 ^a
ABU main campus		3	0 (0.00)	3 (100.00)				
Area BZ		20	16 (80.00)	4 (20.00)				
DAC		10	6 (60.00)	4 (40.00)				
Total		72	44 (61.11)	28 (38.89)				
Area A	<i>T. mantaly</i>	13	4 (30.77)	9 (69.23)	34.13	3	0.00	28.86 ^a
ABU main campus		150	92 (61.33)	58 (38.67)				
Area BZ		16	0 (0.00)	16 (100.00)				
DAC		30	7 (23.33)	23 (76.67)				
Total		209	103 (49.28)	106 (50.72)				
Area A	<i>T. catappa</i>	8	2 (25.00)	6 (75.00)	4.06	3	0.26	38.24 ^a
ABU main campus		267	86 (32.21)	181 (67.79)				
Area BZ		6	4 (66.67)	2 (33.33)				
DAC		65	18 (27.69)	47 (72.31)				
Total		346	110 (31.79)	236 (68.21)				

NB: X²: Chi-square; **Df:** Degree of freedom; **%:** Percentage; **sp:** species; **DAC:** Division of Agricultural College; **ABU:** Ahmadu Bello University. Means with the same superscript along the column are not significantly different (P<0.05), using DMRT.

All the tree species, except *T. catappa* had significant ($P < 0.05$) relationship between the infected and uninfected in each of the sampling areas (Table 4.1).

4.2 Rate of infestation of tree species by mistletoe

In comparison of the host tree species from all the sampling Areas, *A. lebeck* had the highest (58.26%) while *T. mantaly* had the lowest (28.86 %) rate of infestation by mistletoes than other tree species. However, this difference in the rate of infestation was not significantly ($P < 0.05$) different (Table 4.1).

4.3 Species of Mistletoe Identified on the selected Host Trees in Area A

Globimetula oreophila was the most common species of mistletoe identified on four of the selected host trees while *Tapinanthus dodoneifolius* was the least in occurrence in Area A (Table 4.2).

The four different species of mistletoe identified on *Albizia lebeck* included the: *G. oreophila*, *G. braunii*, *T. globiferus* and *T. dodoneifolius*. However, on *Citrus grandis*, *Khaya senegalensis* and *Terminalia mantaly*, only one species of mistletoe, *G. braunii*, *T. globiferus* and *G. oreophila* was found respectively. *Terminalia catappa* was not found in Area A (Table 4.2).

4.4 Species of Mistletoe Identified on the selected Host Trees in Area BZ

Globimetula oreophila was the most common species of mistletoe identified on *C. grandis* and *K. senegalensis*. *Tapinanthus belvisii* and *T. dodoneifolius* were the least in abundance (Table 4.2).

Two species of mistletoe identified on *A. lebeck* were *T. belvisii* and *T. dodoneifolius* while on *C. grandis* and *K. senegalensis*, only *G. oreophila* was identified on each. However, on *Terminalia catappa*, only *G. braunii* was identified. *Terminalia mantaly* was not found in Area BZ (Table 4.2).

4.5 Mistletoe Species Identified on the selected Host Trees in DAC

At the Division of Agricultural College, mistletoe species of: *G. oreophila*, *E. lecardii*, *T. globiferus* and *T. belvisii* were found parasitic on more than one selected host tree. However, *T. dodoneifolius* was found parasitic only on *A. lebbeck* (Table 4.2).

From this sampling Area, the three species of mistletoe identified on *A. lebbeck* were *E. lecardii*, *T. dodoneifolius* and *G. oreophila*. *Citrus grandis* and *T. mantaly* had two species of mistletoe (*T. globiferus*, *E. lecardii* and *T. belvisii*, *T. globiferus* respectively). *Terminalia catappa* and *K. senegalensis* had one species of mistletoe each identified on them (*T. belvisii* and *G. oreophila* respectively) (Table 4.2).

4.6 Mistletoe Species Identified on the Selected Host Trees in ABU Main Campus

Tapinanthus globiferus and *G. braunii* were species of mistletoe found common on three of the host trees (*T. catappa*, *T. mantaly* and *K. senegalensis*). *Globimetula oreophila*, *T. dodoneifolius* and *T. belvisii* were only found parasitic on *A. lebbeck* (Table 4.2).

In this sampling area, three species of mistletoe (*G. oreophila*, *T. dodoneifolius* and *T. belvisii*) were found parasitic on *A. lebbeck*. On *T. catappa* and *K. senegalensis*, two species of mistletoe (*G. braunii* and *T. globiferus*) were found on each of them. *Citrus grandis* was not found in the area (Table 4.2).

The summary of the observations from all the sampling sites showed that *G. braunii* and *T. globiferus* were the most common mistletoe species found parasitic on all the selected host trees (*T. catappa*, *T. mantaly*, *K. senegalensis*, *A. lebbeck* and *C. grandis*). *Globimetula oreophila* was found parasitic on four of the host trees (*A. lebbeck*, *K. senegalensis*, *T. mantaly* and *C. grandis*) and *T. belvisii* on three host trees (*A. lebbeck*, *T. mantaly* and *T. catappa*). *Englerina lecardii* was found parasitic only on two hosts

Table 4.2: Mistletoe Species Identified on the Selected Host Trees in each of the Sampling Areas

S/N	Host Tree	MISTLETOE SPECIES SAMPLING AREAS			
		Area A	Area BZ	ABU Main Campus	DAC
1.	<i>Albizzia lebeck</i>	<i>Globimetula oreophila</i> <i>Globimetula braunii</i> <i>Tapinanthus globiferus</i> <i>Tapinanthus dodoneifolius</i>	<i>Tapinanthus belvisii</i> <i>Tapinanthus dodoneifolius</i>	<i>Globimetula oreophila</i> <i>Tapinanthus dodoneifolius</i> <i>Tapinanthus belvisii</i>	<i>Englerina lecardii</i> <i>Tapinanthus dodoneifolius</i> <i>Globimetula oreophila</i>
2.	<i>Citrus grandis</i>	<i>Globimetula braunii</i>	<i>Globimetula oreophila</i>	-	<i>Tapinanthus globiferus</i> <i>Englerina lecardii</i>
3.	<i>Khaya senegalensis</i>	<i>Tapinanthus globiferus</i>	<i>Globimetula oreophila</i>	<i>Globimetula braunii</i> <i>Tapinanthus globiferus</i>	<i>Globimetula oreophila</i>
4.	<i>Terminalia mantaly</i>	<i>Globimetula oreophila</i>	-	<i>Globimetula braunii</i>	<i>Tapinanthus belvisii</i> <i>Tapinanthus globiferus</i>
5.	<i>Terminalia catappa</i>	<i>Tapinanthus dodoneifolius</i> <i>Globimetula braunii</i>	<i>Globimetula braunii</i>	<i>Tapinanthus globiferus</i> <i>Globimetula braunii</i>	<i>Tapinanthus belvisii</i>

NB: - No mistletoe found on the host tree; DAC: Division of Agricultural College; ABU: Ahmadu Bello University

(*A. lebeck* and *C. grandis*) and *T. dodoneifolius* was found parasitic only on *A. lebeck* (Table 4.3).

Albizia lebeck recorded the highest number (6) of mistletoe species (*T. dodoneifolius*, *T. globiferus*, *G. braunii*, *G. oreophila*, *E. lecardii* and *T. belvisii*) found parasitic on it. *Citrus grandis*, *T. catappa* and *T. mantaly* each had four different species found parasitic on them whereas *Khaya senegalensis* had three different mistletoes species parasitic on it (Table 4.3).

4.7 Qualitative Phytochemical Composition of *Terminalia catappa* and Associated Mistletoe Species (*Tapinanthus globiferus* and *Globimetula braunii*)

The results revealed that tannins, saponins, flavonoids and carbohydrates were present in both the ethanolic and aqueous extracts of *T. catappa* and its associated mistletoe species (*T. globiferus* and *G. braunii*) (Table 4.4).

Cardiac glycosides were present in all the extracts (aqueous and ethanolic) of the host tree and its parasitic plants, except in the aqueous extract of *T. globiferus*. Triterpenes were present only in aqueous extracts of *T. globiferus* and *T. catappa* and ethanolic and aqueous extracts of *G. braunii*. Steroid was present only in the aqueous extract of *G. braunii* but not detected in the ethanol and aqueous extracts of the other plants. Alkaloid was present in ethanolic extracts of both *T. globiferus* and *G. braunii* and in both extracts of *T. catappa* (Table 4.4).

4.8 Qualitative Phytochemical Composition of *Terminalia mantaly* and Associated Mistletoe Species (*T. globiferus* and *G. braunii*).

The results revealed that tannins, saponins, flavonoids and carbohydrates were present in all the ethanolic and aqueous extracts of *T. mantaly*, *T. globiferus* and *G. braunii* (Table 4.5). Triterpenes were present in all the extracts of *T. globiferus* and *G. braunii* but not detected in the ethanolic extracts of *T. mantaly*. Furthermore, cardiac glycosides were present in all the

Table 4.3: Mistletoe Species Identified on each of the Selected Host Trees from all the Sampling Areas

S/N	HOST TREES	MISTLETOE SPECIES
1.	<i>Albizzia lebeck</i>	<i>Tapinanthus globiferus*</i> <i>Globimetula braunii*</i> <i>Tapinanthus dodoneifolius</i> <i>Globimetula oreophila</i> <i>Englerina lecardii</i> <i>Tapinanthus belvisii</i>
2.	<i>Khaya senegalensis</i>	<i>Tapinanthus globiferus*</i> <i>Globimetula braunii*</i> <i>Globimetula oreophila</i>
3.	<i>Citrus grandis</i>	<i>Tapinanthus globiferus*</i> <i>Globimetula braunii*</i> <i>Globimetula oreophila</i> <i>Englerina lecardii</i>
4.	<i>Terminalia catappa</i>	<i>Tapinanthus globiferus*</i> <i>Globimetula braunii*</i> <i>Tapinanthus belvisii</i> <i>Tapinanthus dodoneifolius</i>
5.	<i>Terminalia mantaly</i>	<i>Tapinanthus globiferus*</i> <i>Globimetula braunii*</i> <i>Globimetula oreophila</i> <i>Tapinanthus belvisii</i>

* Mistletoe species found common on all the selected host trees

Table 4.4: Qualitative Phytochemical Composition of *Terminalia catappa* and Associated Mistletoe Species (*Tapinanthus globiferus* and *Globimetulabraunii*)

PHYTOCHEMICALS	MISTLETOE SPECIES				HOST TREE	
	<i>T. globiferus</i>		<i>G. braunii</i>		<i>T. catappa</i>	
	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous
Tannins	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Steroids	-	-	-	+	-	-
Triterpenes	-	+	+	+	-	+
Flavonoids	+	+	+	+	+	+
Cardiac glycosides	+	-	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Alkaloids	+	-	+	-	+	+

Key: + Present, - Not detected

Table 4.5: Qualitative Phytochemical Composition of *Terminalia mantaly* and Associated Mistletoe Species (*Tapinanthus globiferus* and *Globimetula braunii*)

PHYTOCHEMICALS	MISTLETOE SPECIES				HOST TREE	
	<i>T. globiferus</i>		<i>G. braunii</i>		<i>T. mantaly</i>	
	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous
Tannins	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Steroids	-	-	-	+	-	-
Triterpenes	+	+	+	+	-	+
Flavonoids	+	+	+	+	+	+
Cardiac glycosides	+	-	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Alkaloids	+	-	+	+	-	-

Key: + Present, - Not detected

extracts except the aqueous extracts of *T. globiferus*. Steroid was detected only in the aqueous extract of *G. braunii*. Alkaloid was present only in the ethanolic extract of *T. globiferus* and in both the ethanolic and aqueous extracts of *G. braunii* but not detected in the other plants extracts (Table 4.5).

4.9 Qualitative Phytochemical Composition of *Albizzia lebeck* and Associated Mistletoe Species (*Tapinanthus globiferus* and *Globimetula braunii*).

Triterpenes, flavonoids and carbohydrates were present in the extracts of *A. lebeck* and its two mistletoes species (*T. globiferus* and *G. braunii*). Other compounds were present only in some of the plant extracts (Table 4.6).

Cardiac glycosides were present in all the plant extracts except the aqueous extracts of *T. globiferus*. Alkaloids were detected in all the extracts except ethanolic extract of *T. globiferus*. Steroids were detected in the aqueous extract of *T. globiferus* and *A. lebeck*. Saponins were present in *T. globiferus*, *G. braunii* and *A. lebeck* extracts except the ethanolic extracts of *G. braunii* and *A. lebeck*. Tannins, however, were not detected in all the ethanolic extracts of *A. lebeck* and its parasitic mistletoes, but were present in all the aqueous extracts (Table 4.6).

4.10 Qualitative Phytochemical Composition of *Khaya senegalensis* and Associated Mistletoe Species (*Tapinanthus globiferus* and *Globimetula braunii*).

The results revealed that tannins, triterpenes, cardiac glycosides and carbohydrates were present in all the extracts of *K. senegalensis* and its two parasitic mistletoe plants (*T. globiferus* and *G. braunii*). Other compounds were present only in some of the other plants extracts (Table 4.7).

Saponins and steroids were present in all the extracts except in the ethanolic extract of *T. globiferus*. Flavonoid was not detected only in the ethanolic extract of *K. senegalensis* but was present in its aqueous extract and in all the extracts of *Tapinanthus*

Table 4.6: Qualitative Phytochemical Composition of *Albizia lebeck* and Associated Mistletoe Species Extracts (*Tapinanthus globiferus* and *Globimetula braunii*)

PHYTOCHEMICALS	MISTLETOE SPECIES				HOST TREE	
	<i>T. globiferus</i>		<i>G. braunii</i>		<i>A. lebeck</i>	
	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous
Tannins	-	+	-	+	-	+
Saponins	+	+	-	+	-	+
Steroids	-	-	-	+	-	+
Triterpenes	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Cardiac glycosides	+	-	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Alkaloids	-	+	+	+	+	+

Key: + Present, - Not detected

Table 4.7:Qualitative Phytochemical Compositions of *Khaya senegalensis* and Associated Mistletoe Species Extracts(*Tapinanthus globiferus*and *Globimetula braunii*).

PHYTOCHEMICALS	MISTLETOE SPECIES				HOST TREE	
	<i>T. globiferus</i>		<i>G. braunii</i>		<i>K. senegalensis</i>	
	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous
Tannins	+	+	+	+	+	+
Saponins	-	+	+	+	+	+
Steroids	-	+	+	+	+	+
Triterpenes	+	+	+	+	+	+
Flavonoids	+	+	+	+	-	+
Cardiac glycosides	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Alkaloids	+	-	-	-	+	+

Key: + Present, - Not detected

globiferus and *G. braunii*. Alkaloid was present in the ethanolic extract of *T. globiferus* and in ethanolic and aqueous extracts of *K. senegalensis* (Table 4.7).

4.11 Qualitative Phytochemical Composition of *Citrus grandis* and Associated Mistletoe Species (*Tapinanthus globiferus* and *Globimetula braunii*).

The results revealed that, tannins, saponins, cardiac glycosides and carbohydrates were present in the ethanolic and aqueous extracts of *C. grandis*, *T. globiferus* and *G. braunii* (Table 4.8)

Triterpenes and flavonoids were present in all the extracts of the plants except the ethanolic extract of *C. grandis*. Alkaloid was also present in all the plant extract except in the aqueous extract of *T. globiferus*. Steroids were only detected in the aqueous extracts of both *G. braunii* and *C. grandis* (Table 4.8).

4.12 Quantitative Phytochemical Composition of Different Host Plants and Attached Mistletoe Species.

The results of the quantitative phytochemical screening of the plants and attached mistletoes (Table 4.9) revealed that, saponins and flavonoids were of the higher concentrations (31.00 % and 22.40 % respectively) than other compounds in both the host plants and attached mistletoe species, while phenols and tannins had the lowest concentrations (0.28 mg/ml and 0.08 % respectively).

4.12.1 *Albizia lebbek* and associated mistletoe species

Tapinanthus globiferus had the highest concentrations of alkaloids (6.10 %), cyanogenic glycosides (3.94 mg/g) and flavonoids (11.85 %) while *A. lebbek* showed the highest concentrations of saponins (14.10 %), tannins 1.29 %) and phenols (4.99 mg/ml). Alkaloids concentration in *Globimetula braunii* was the lowest (1.00 %) and it was only significantly ($P < 0.05$) lower than the highest (6.10 %) in *Tapinanthus globiferus*. Saponins content in *A. lebbek* was only significantly ($P < 0.05$) higher than

Table 4.8: Qualitative Phytochemical Composition of *Citrus grandis* and Associated Mistletoe Species (*Tapinanthus globiferus* and *Globimetula braunii*).

PHYTOCHEMICALS	MISTLETOE SPECIES				HOST TREE	
	<i>T. globiferus</i>		<i>G. braunii</i>		<i>C. grandis</i>	
	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous
Tannins	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Steroids	-	-	-	+	-	+
Triterpenes	+	+	+	+	-	+
Flavonoids	+	+	+	+	-	+
Cardiac glycosides	+	+	+	+	+	+
Carbohydrates	+	+	+	+	+	+
Alkaloids	+	-	+	+	+	+

Key: + Present, - Not detected

Table 4.9: Quantitative Phytochemical Compositions of Different Host Plants and Attached Mistletoes Species

Host/Mistletoes	Phytochemical compositions					
	ALK (%)	SAP (%)	CYA (mg/g)	FLA (%)	TAN (%)	PHE (mg/ml)
<i>Albizia lebbbeck</i>						
<i>A. lebbbeck</i>	2.00 ^b	14.10 ^a	3.50 ^a	7.95 ^b	1.29 ^a	4.99 ^a
<i>T. globiferus</i>	6.10 ^a	10.44 ^b	3.94 ^a	11.85 ^a	0.09 ^b	0.35 ^b
<i>G. braunii</i>	1.00 ^b	14.00 ^a	2.37 ^b	10.20 ^a	0.11 ^b	0.36 ^b
SE±0.330.27	0.23	0.490.18	0.21			
<i>Terminalia catappa</i>						
<i>T. catappa</i>	3.20 ^a	11.66 ^a	7.84 ^b	10.49 ^b	1.24 ^a	0.80 ^b
<i>T. globiferus</i>	1.76 ^b	12.12 ^a	12.00 ^a	13.70 ^a	1.32 ^a	2.48 ^a
<i>G. braunii</i>	0.01 ^c	14.50 ^a	2.99 ^c	10.00 ^b	1.22 ^a	0.48 ^b
SE±	0.32	0.83	0.45	0.59	0.06	0.08
<i>Terminalia mantaly</i>						
<i>T. mantaly</i>	3.94 ^a	14.00 ^a	6.95 ^a	10.13 ^b	0.72 ^b	0.58 ^b
<i>T. globiferus</i>	0.40 ^b	10.00 ^b	3.20 ^c	10.13 ^b	1.28 ^a	2.69 ^a
<i>G. braunii</i>	0.28 ^b	16.00 ^a	3.80 ^b	15.00 ^a	0.08 ^c	0.50 ^b
SE±	0.03	0.65	0.09	0.58	0.08	0.09
<i>Citrus grandis</i>						
<i>C. grandis</i>	0.22 ^c	12.00 ^b	3.58 ^b	10.48 ^b	0.72 ^a	0.41 ^c
<i>T. globiferus</i>	1.90 ^b	31.00 ^a	7.07 ^a	7.40 ^b	0.37 ^b	0.80 ^a
<i>G. braunii</i>	2.90 ^a	6.00 ^c	5.32 ^{ab}	21.10 ^a	0.64 ^a	0.54 ^b
SE±	0.12	0.75	0.49	0.93	0.03	0.03
<i>Khaya senegalensis</i>						
<i>K. senegalensis</i>	1.44 ^a	14.96 ^b	15.00 ^a	13.91 ^b	0.38 ^a	0.28 ^b
<i>T. globiferus</i>	0.04 ^b	14.00 ^b	3.13 ^b	22.40 ^a	0.18 ^a	0.68 ^a
<i>G. braunii</i>	1.54 ^a	22.00 ^a	3.35 ^b	8.55 ^c	0.36 ^a	0.50 ^{ab}
SE±	0.25	0.57	0.40	1.09	0.05	0.07

NB: Means with the same superscript along the column of each of the host plant are not significantly different at P<0.05, using DMRT.

ALK= alkaloid, **SAP**= saponin, **CYA**= cyanogenic glycosides, **FLA**= flavonoid, **TAN**= tannin, **PHE**= phenol, **SE±**= Standard Error

the lowest (10.44 %) in *T. globiferus*. The highest cyanogenic glycosides (3.94 mg/g) in *T. globiferus* was only significantly ($P < 0.05$) higher than the lowest (2.37 mg/g) in *G. braunii*. The highest flavonoid (11.85 %) in *T. globiferus* was only significantly ($P < 0.05$) higher than the lowest (7.95 %) in *A. lebeck*. On the other hand, the lowest tannin (0.09 %) and total phenols (0.35 mg/ml) contents of *T. globiferus* was only significantly ($P < 0.05$) lower than the highest in (1.29 % and 4.99 mg/ml respectively) *A. lebeck* (Table 4.9).

4.12.2 *Terminalia catappa* and associated mistletoe species

The highest alkaloid content in *T. catappa* (3.20 %) was significantly ($P < 0.05$) higher than that observed in the mistletoe plants while the lowest in *Globimetula braunii* (0.01 %) was significantly ($P < 0.05$) lower than that in *T. globiferus* and *G. braunii*. The highest saponin in *G. braunii* (14.50 %) was significantly ($P < 0.05$) similar to the lowest in *T. catappa* (11.66). The highest cyanogenic glycosides (12.00 mg/g), flavonoid (13.70 %), tannin (1.32 %) and total phenols (2.48 mg/ml) contents occurred in *T. globiferus* were significantly ($P < 0.05$) higher than those observed in *G. braunii* and *T. catappa*. However, the highest tannin (1.32 %) content in *T. globiferus* was not significantly ($P < 0.05$) different from that observed in *G. braunii* (Table 4.9).

4.12.3 *Terminalia mantaly* and associated mistletoe species

The lowest alkaloid content in *G. braunii* (0.28 %) was only significantly ($P < 0.05$) lower than the highest in *T. mantaly* (3.94 %). On the other hand, the highest saponin in *G. braunii* (16.00 %) was only significantly ($P < 0.05$) higher than the lowest in *T. globiferus* (10.00 %). *Terminalia mantaly* had the highest cyanogenic glycosides (6.95 mg/g) content which was significantly ($P < 0.05$) higher than that of the two mistletoes. The lowest cyanogenic glycosides in *T. globiferus* (3.20 mg/g) was significantly ($P < 0.05$) lower than that of the other plants. Flavonoid contents of *T. mantaly* (10.13 %)

and *T. globiferus*(10.13 %) were significantly ($P<0.05$) similar but lower than the highest in *G. braunii*(15.00 %). Tannin (1.28 %) and total phenols (2.69 mg/ml) contents of *T. globiferus* were the highest and were significantly ($P<0.05$) higher than that in *G. braunii* and *T. mantaly* (Table 4.9).

4.12.4 *Citrus grandis* and associated mistletoe species

The *T. globiferus* and *G. braunii* showed higher concentrations of most phytochemicals than *C. grandis*. The highest alkaloid content in *G. braunii*(2.90 %) was significantly ($P<0.05$) higher than that of *T. globiferus*(1.90 %) and *C. grandis*(0.22 %). Saponins content in *T. globiferus*(31.00 %) was significantly ($P<0.05$) higher than that in *C. grandis*(12.00 %) and *G. braunii*(6.00 %). The lowest cyanogenic glycosides in *C. grandis*(3.58 mg/g) was only significantly ($P<0.05$) lower than the highest in *T. globiferus*(7.07 mg/g). The lowest flavonoid in *T. globiferus*(7.40 %) was only significantly ($P<0.05$) lower than the highest in *G. braunii*(21.10 %). *Citrus grandis* had the highest tannin content (0.72 %) which was only significantly ($P<0.05$) higher than the lowest in *T. globiferus*(0.37 %). On the other hand, the lowest phenol content in *C. grandis*(0.41 mg/ml) was significantly ($P<0.05$) lower than that of *T. globiferus*(0.80 mg/ml) and *G. braunii*(0.54 mg/ml) (Table 4.9).

4.12.5 *Khaya senegalensis* and associated mistletoe species

The highest alkaloid contents in *G. braunii*(1.54 %) was only significantly ($P<0.05$) higher than the lowest in *T. globiferus*(0.04 %) while the lowest saponins content in *T. globiferus*(14.00 %) was only significantly ($P<0.05$) lower than the highest in *G. braunii*(22.00 %). The lowest cyanogenic glycosides in *T. globiferus*(3.13 mg/g) was only significantly ($P<0.05$) lower than the highest in *K. senegalensis*(15.00 mg/g). On the other hand, the highest flavonoid in *T. globiferus*(22.40 %) was significantly ($P<0.05$) higher than that of *G. braunii*(8.55 %) and *K. senegalensis*(13.91 %).

The highest tannin content in *K. senegalensis* (0.38 %) was similar to that observed in *T. globiferus* (0.18 %). The total phenols content of *T. globiferus* (0.68 mg/ml) was only significantly ($P < 0.05$) higher than that in *K. senegalensis* (0.28 mg/ml) (Table 4.9).

4.13 Comparison of the Quantitative Phytochemical Compositions of the Five Different Host Plants

The comparison of the quantitative phytochemical compositions revealed that *Albizia lebbek* had the highest concentrations of tannins (1.29 %) and total phenols (4.99 mg/ml). *Terminalia mantaly* had the highest concentration of alkaloids (3.94 %) and *K. senegalensis* had the highest concentrations of saponins (14.96 %), cyanogenic glycosides (15.00 mg/g) and flavonoids (13.91 %). However, *A. lebbek* had the lowest concentrations of cyanogenic glycosides (3.50 mg/g) and flavonoids (7.95 %); *C. grandis* and *T. catappa* had the lowest concentrations of alkaloids (0.22 %) and saponins (11.66 %) respectively while *K. senegalensis* had the lowest concentration of tannins (0.38 %) and total phenols (0.28 %) (Table 4.10).

The highest alkaloids in *T. mantaly* (3.94 %) was only similar to that in *T. catappa* (3.20 %). The lowest alkaloids in *C. grandis* (0.22 %) was significantly ($P < 0.05$) lower than that of other plants. The lowest saponins in *T. catappa* (11.66 %) was only lower than the highest in *K. senegalensis* (14.96 %). The highest cyanogenic glycosides (15.00 mg/g) and flavonoids (13.91 %) contents in *K. senegalensis* were significantly ($P < 0.05$) higher than that in the other plants. The lowest cyanogenic glycosides in *C. grandis* (3.58 mg/g) was significantly ($P < 0.05$) similar to that in *A. lebbek* (3.50 mg/g), while the lowest flavonoids in *A. lebbek* (7.95 %) was significantly ($P < 0.05$) lower than that of other host plants. The lowest tannins content of *K. senegalensis* (0.38 %) was lower than that in *A. lebbek* (1.29 %) and *T. catappa* (1.24 %). The lowest phenols (0.28 mg/ml) in *K. senegalensis* was only significantly ($P < 0.05$) lower than that in *A. lebbek*

Table 4.10: Comparison of the Quantitative Phytochemical Compositions of the Five Different Host Host Plants

Host Plant	Phytochemical Compositions					
	ALK (%)	SAP (%)	CYA (mg/g)	FLA (%)	TAN (%)	PHE (mg/ml)
<i>A. lebbbeck</i>	2.00 ^b	14.10 ^{ab}	3.50 ^c	7.95 ^c	1.29 ^a	4.99 ^a
<i>T. catappa</i>	3.20 ^a	11.66 ^b	7.84 ^b	10.49 ^b	1.24 ^a	0.80 ^b
<i>T. mantaly</i>	3.94 ^a	14.00 ^b	6.95 ^b	10.13 ^b	0.72 ^b	0.58 ^b
<i>C. grandis</i>	0.22 ^c	12.00 ^{ab}	3.58 ^c	10.48 ^b	0.72 ^b	0.41 ^b
<i>K. senegalensis</i>	1.44 ^b	14.96 ^a	15.00 ^a	13.91 ^a	0.38 ^b	0.28 ^b
SE±	0.29	0.91	0.39	0.52	0.13	0.15

NB: Means with the same superscript along the column are not significantly different at P<0.05

ALK= alkaloid, **SAP**= saponin, **CYA**= cyanogenic glycosides, **FLA**= flavonoid, **TAN**= tannin, **PHE**= phenol, **SE±**= Standard Error

(4.99 mg/ml) (Table 4.10).

4.14 Comparison of the Quantitative Phytochemical Compositions of *Tapinanthus globiferus* Obtained from all the Host Plants

The comparison of the quantitative phytochemical compositions revealed that the highest alkaloids content of *T. globiferus* (6.10 %) parasitic on *A. lebbeck* was significantly ($P < 0.05$) higher than the lowest (0.04 %) in *T. globiferus* that was obtained from *K. senegalensis*. The highest saponins content of *T. globiferus* (31.00 %) obtained from *C. grandis* was significantly ($P < 0.05$) higher than the lowest (10.00 %) in *T. globiferus* from *T. mantaly*. The lowest cyanogenic glycosides (3.13 mg/g) in *T. globiferus* obtained from *K. senegalensis* was only significantly ($P < 0.05$) lower than that in *T. globiferus* (7.07 mg/g) from *C. grandis* and the highest (12.00 mg/g) in the one obtained from *T. catappa*. The lowest flavonoids (7.40 %) in *T. globiferus* obtained from *C. grandis* was significantly ($P < 0.05$) lower than the highest (22.40 %) in *T. globiferus* obtained from *K. senegalensis*. The highest tannins content of *T. globiferus* (1.32 %) parasitic on *T. catappa* was significantly ($P < 0.05$) higher than the lowest (0.09 %) in *T. globiferus* parasitic on *A. lebbeck*. On the other hand, the lowest phenols content of *T. globiferus* (0.35 mg/ml) obtained from *A. lebbeck* was significantly ($P < 0.05$) lower than the highest in *T. globiferus* (2.68 mg/ml) obtained from *T. mantaly* (Table 4.11).

4.15 Comparison of the Quantitative Phytochemical Compositions of *Globimetula braunii* Obtained from all the Host Plants.

The comparison of the phytochemical compositions revealed that *Globimetula braunii* parasitic on *C. grandis* had four compounds of the highest concentrations (alkaloids, cyanogenic glycosides, flavonoids and total phenols); *G. braunii* parasitic on *K. senegalensis* and *T. catappa* had the highest concentrations of saponins

(22.00 mg/g) and tannins (1.22 %) respectively than *G. braunii* parasitic on other host plants. *Globimetula braunii* parasitic on *A. lebbeck* had two compounds of the lowest concentration (cyanogenic glycosides and phenols) and *G. braunii* parasitic on *T. catappa*, *C. grandis*, *K. senegalensis* and *T. mantaly* had the lowest concentrations of alkaloids (0.01 %), saponins (6.00 %), flavonoids (8.55 %) and tannins (0.08 %) respectively than *G. braunii* parasitic on other host plants (Table 4.11).

The lowest alkaloids of *G. braunii* parasitic on *T. catappa* (0.01 %) was significantly ($P < 0.05$) lower than the highest (2.90 %) in *G. braunii* parasitic on *C. grandis* whereas the highest saponins content of *G. braunii* (22.00 %) obtained from *K. senegalensis* was significantly ($P < 0.05$) higher than the lowest (6.00 %) in *G. braunii* obtained from *C. grandis*. The highest cyanogenic glycosides of *G. braunii* (5.32 mg/g) obtained from *C. grandis* was significantly ($P < 0.05$) higher than the lowest (2.37 mg/g) in the one obtained from *A. lebbeck*. The highest flavonoids in *G. braunii* (21.10 %) obtained from *C. grandis* was significantly ($P < 0.05$) higher than the lowest (10.00 %) in the one obtained from *T. catappa* while the highest tannins content of *G. braunii* (1.22 %) obtained from *T. catappa* was significantly ($P < 0.05$) higher than the lowest in *G. braunii* (0.36 %) from *A. lebbeck*. On the other hand, the highest phenols content of *G. braunii* (0.54 mg/ml) obtained from *C. grandis* was significantly ($P < 0.05$) similar to the lowest in *G. braunii* (0.36 mg/ml) obtained from *A. lebbeck* (Table 4.11).

4.16 Comparison of the Quantitative Phytochemical Compositions of the Host Plant and Attached Mistletoe Species.

Generally, with the exception of one host plant and attached mistletoe species, saponins, cyanogenic glycosides, flavonoids and phenols were comparable among the host plants (Table 4.12). The comparison revealed that *A. lebbeck*, *T. catappa* and *K. senegalensis* and their two associated mistletoe species (*G. braunii* and *T. globiferus*) had two

Table 4.11: Comparison of the Quantitative Phytochemical Compositions of *T. globiferus* and *G. braunii* Obtained from all the Host Plants

Mistletoe species	Host Plant	Phytochemical Compositions					
		ALK (%)	SAP (%)	CYA (mg/g)	FLA (%)	TAN (%)	PHE (mg/ml)
<i>T. globiferus</i>	<i>A. lebeck</i>	6.10 ^a	10.44 ^{cd}	3.94 ^c	11.85 ^b	0.09 ^c	0.35 ^c
	<i>T. catappa</i>	1.76 ^b	12.12 ^{bc}	12.00 ^a	13.70 ^b	1.32 ^a	2.48 ^a
	<i>T. mantaly</i>	0.40 ^c	10.00 ^d	3.20 ^c	10.13 ^b	1.28 ^a	2.69 ^a
	<i>C. grandis</i>	1.90 ^b	31.00 ^a	7.07 ^b	7.40 ^c	0.37 ^b	0.80 ^b
	<i>K. senegalensis</i>	0.04 ^c	14.00 ^b	3.13 ^c	22.40 ^a	0.18 ^{bc}	0.68 ^b
<i>G. braunii</i>	<i>A. lebeck</i>	1.00 ^c	14.00 ^b	2.37 ^c	10.20 ^c	0.11 ^d	0.36 ^a
	<i>T. catappa</i>	0.01 ^d	14.50 ^b	2.99 ^{bc}	10.00 ^c	1.22 ^a	0.48 ^a
	<i>T. mantaly</i>	0.28 ^d	16.00 ^b	3.80 ^b	15.00 ^b	0.08 ^d	0.50 ^a
	<i>C. grandis</i>	2.90 ^a	6.00 ^c	5.32 ^a	21.10 ^a	0.64 ^b	0.54 ^a
	<i>K. senegalensis</i>	1.54 ^b	22.00 ^a	3.35 ^{bc}	8.55 ^c	0.36 ^c	0.50 ^a
	SE±	0.28	0.58	0.40	1.18	0.06	0.10

NB: Means with the same superscript along the column are not significantly different at P<0.05

ALK= alkaloid, **SAP**= saponin, **CYA**= cyanogenic glycosides, **FLA**= flavonoid, **TAN**= tannin, **PHE**= phenol, **SE±**= Standard Error

compounds of the highest concentrations: alkaloids (3.03 %) and phenols (1.90 mg/ml), cyanogenic glycosides (7.61 mg/g) and tannins (1.26 %), saponins (16.99 %) and flavonoids (14.95 %) respectively compared to other host plants and their associated mistletoe species. *Khaya senegalensis* and associated mistletoes had the lowest concentrations of alkaloids (1.01 %), tannins (0.31 %) and phenols (0.49 mg/ml); *A. lebbbeck* and associated mistletoes had the lowest concentrations of cyanogenic glycosides (3.27 mg/g) and flavonoids (10.00 %) and *T. catappa* and associated mistletoes had the lowest concentrations of saponins (12.76 %) compared to other host plants and associated mistletoes (Table 4.12).

The highest alkaloids content (3.03 %) in *A. lebbbeck* and its two associated mistletoes was significantly ($P < 0.05$) higher than that of other host plants and their associated mistletoe species. The highest saponins content (16.99 %) in *K. senegalensis* and associated mistletoe species was similar to that of the other host plants and their associated mistletoe species. The highest cyanogenic glycosides (7.61 mg/g) in *T. catappa* and its mistletoes was only significantly ($P < 0.05$) higher than the lowest (3.27 mg/g) in *A. lebbbeck*. The lowest flavonoids content (10.00 %) in *A. lebbbeck* and its mistletoe species was only significantly ($P < 0.05$) lower than the highest (14.95 %) in *K. senegalensis* and its associated mistletoes (Table 4.12). On the other hand, the highest tannins (1.26 %) in *T. catappa* and its mistletoe species was significantly ($P < 0.05$) higher than that in the other host plants and their associated mistletoe species. The lowest phenols content (0.49 mg/ml) in *K. senegalensis* and its associated mistletoes was only significantly ($P < 0.05$) similar to that in *C. grandis* and its mistletoe while the phenols content (1.90 mg/ml) in *A. lebbbeck* and its mistletoe species was significantly ($P < 0.05$) higher than that in other host plants and their associated mistletoe species (Table 4.12).

Table 4.12: Comparison of the Quantitative Phytochemical Compositions of the Host Plants and Attached Mistletoes Species

Host & mistletoe species	Phytochemical compositions					
	ALK (%)	SAP (%)	CYA (mg/g)	FLA (%)	TAN (%)	PHE (mg/ml)
<i>A.l, G.b., T.g</i>	3.03 ^a	12.85 ^a	3.27 ^b	10.00 ^b	0.49 ^c	1.90 ^a
<i>T.c., G.b., T.g</i>	1.66 ^b	12.76 ^a	7.61 ^a	11.39 ^{ab}	1.26 ^a	1.25 ^{ab}
<i>T.m., G.b., T.g</i>	1.54 ^b	13.33 ^a	4.65 ^{ab}	11.75 ^{ab}	0.69 ^b	1.26 ^{ab}
<i>C. g., G.b., T.g</i>	1.67 ^b	16.33 ^a	5.32 ^{ab}	12.99 ^{ab}	0.58 ^{bc}	0.58 ^b
<i>K. s., G.b., T.g</i>	1.01 ^c	16.99 ^a	7.16 ^a	14.95 ^a	0.31 ^d	0.49 ^b
SE±	0.15	1.93	1.16	1.48	0.05	0.42

NB: Means with the same superscript along the column are not significantly different at P<0.05

A.l = *Albizzia lebbek*, **G.b** = *Globimetula braunii*, **T.g** = *Tapinanthus globiferus*,
T.c = *Terminalia catappa*, **T.m** = *Terminalia mantaly*, **C.g** = *Citrus grandis*,
K.s = *Khaya senegalensis* **SE±** = Standard Error

4.17 The Antibacterial Activity of the Aqueous Leaf Extracts of *Terminalia mantaly* on Some Selected Species of Bacteria

Ciprofloxacin (control) showed the highest zones of inhibition on all the test organisms (with the highest [45.00 mm] on *S. typhi*) compared to all the *T. mantaly* aqueous leaf extract concentrations (Table 4.13). The aqueous leaf extract of *T. mantaly* showed an increase in activity with increase in concentration, thereby had the highest zone of inhibition (25.50 mm) at 200mg/ml and the lowest (10.00 mm) at 25mg/ml for all the test organisms. For most of the organisms, aqueous leaf extract of *T. mantaly* at 200 and 100mg/ml gave comparable diameters of inhibition, which in turn were higher than those of the other concentrations.

4.18 The Antibacterial Activity of the Aqueous Leaf Extracts of *T. catappa* on Some Selected Species of Bacteria

Ciprofloxacin (control) was observed to be more active against all the test organisms with the highest activity (45.00 mm) on *S. typhi* compared to all the *T. catappa* extract concentrations (Table 4.14). The aqueous leaf extract of *T. catappa* was only active against *S. aureus*, but inactive against the other test organisms with its activity increasing with an increase in concentration thereby having the highest zone of inhibition (24.00 mm) at 200mg/ml and the lowest (13.50 mm) at 25mg/ml (Table 4.14).

4.19 The Antibacterial Activity of the Aqueous Leaf Extracts of *Khaya senegalensis* on Some Selected Species of Bacteria

Ciprofloxacin (control) showed the highest zone of inhibition on all the test organisms compared to all the *K. senegalensis* extract concentrations (Table 4.15).

The aqueous leaf extract of *K. senegalensis* was observed to be inactive against *E. coli* and *B. subtilis* at all concentrations, but was active against *S. aureus* and *S. typhi*. This

Table 4.13: The Antibacterial Activity of the Aqueous Leaf Extracts of *Terminalia mantalyon* Some Selected Species of Bacteria

Extract Conc (mg/ml)	Diameter of zone of inhibition (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>
200	25.50 ^b	15.00 ^b	19.00 ^b	23.50 ^b
100	23.00 ^b	12.50 ^c	17.00 ^b	19.00 ^b
50	18.50 ^c	10.00 ^d	10.00 ^c	10.00 ^c
25	16.50 ^c	0.00 ^e	10.00 ^c	0.00 ^d
Ciprofloxacin	40.00 ^a	40.00 ^a	40.00 ^a	45.00 ^a
SE±	1.07	0.22	1.41	1.91

NB: Means with the same superscript along the column are not significantly different at P<0.05.

Table 4.14: The Antibacterial Activity of the Aqueous Leaf Extracts of *Terminalia catappa* on Some Selected Species of Bacteria

Extract Conc (mg/ml)	Diameter of zone of inhibition (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>
200	24.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
100	19.00 ^c	0.00 ^b	0.00 ^b	0.00 ^b
50	17.00 ^{cd}	0.00 ^b	0.00 ^b	0.00 ^b
25	13.50 ^d	0.00 ^b	0.00 ^b	0.00 ^b
Ciprofloxacin	40.00 ^a	40.00 ^a	40.00 ^a	45.00 ^a
SE±	1.28	0.00	0.00	0.00

NB: Means with the same superscript along the column are not significantly different at P<0.05.

Table 4.15: The Antibacterial Activity of the Aqueous Leaf Extracts of *Khaya senegalensis* on Some Selected Species of Bacteria

Extract Conc (mg/ml)	Diameter of zone of inhibition (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>
200	24.00 ^b	0.00 ^b	0.00 ^b	23.50 ^b
100	19.00 ^c	0.00 ^b	0.00 ^b	19.00 ^c
50	15.50 ^d	0.00 ^b	0.00 ^b	16.00 ^c
25	11.50 ^e	0.00 ^b	0.00 ^b	10.00 ^d
Ciprofloxacin	40.00 ^a	40.00 ^a	40.00 ^b	45.00 ^a
SE±	0.95	0.00	0.00	0.92

NB: Means with the same superscript along the column are not significantly different at P<0.05.

increased with increase in extract concentration and thereby had the highest zone of inhibition (24.00 mm) at 200mg/ml and the lowest (10.00 mm) at 25mg/ml (Table 4.15).

4.20 The Antibacterial Activity of the Aqueous Leaf Extracts of *Citrus grandis* on Some Selected Species of Bacteria

Ciprofloxacin (control) showed the highest zone of inhibition on all the test organisms compared to all the concentrations of *C. grandis* extracts (Table 4.16).

Escherichia coli and *S. typhi* were resistant to the aqueous leaf extract of *C. grandis* at all the concentrations, but *S. aureus* and *B. subtilis* were both susceptible to the aqueous leaf extracts of the *C. grandis* at all concentrations. The inhibition increased with increase in concentration thereby had the highest zone of inhibition (20.50 mm) at 200mg/ml and the lowest (11.00 mm) at 25mg/ml leaf extract. The diameter zone of inhibition of *C. grandis* extract on *S. aureus* showed to be significantly ($P < 0.05$) similar at all the concentrations (Table 4.16).

4.21 The Antibacterial Activity of the Aqueous Leaf Extracts of *Albizia lebbek* on Some Selected Species of Bacteria

Ciprofloxacin (control) gave the highest zones of inhibition on all the test organisms compared to all the concentrations of the aqueous leaf extract of *A. lebbek* (Table 4.17).

The aqueous leaf extract of *A. lebbek* was active against three of the test organisms and this increased with increase in extract concentration thereby had the highest activity (23.50 mm) at 200mg/ml and the lowest (5.00 mm) at 50 mg/ml for both *E. coli* and *S. typhi* and 100 mg/ml for only *B. subtilis* (Table 4.17). *Albizia lebbek* was inactive at all concentrations against *S. aureus*, at 25 mg/ml against *E. coli* and *S. typhi* and at 25 mg/ml and 50 mg/ml against *B. subtilis*.

Table 4.16: The Antibacterial Activity of the Aqueous Leaf Extracts of *Citrus grandison* Some Selected Species of Bacteria

Extract Conc (mg/ml)	Diameter of zone of inhibition (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>
200	20.50 ^b	0.00 ^b	16.50 ^b	0.00 ^b
100	17.50 ^b	0.00 ^b	13.50 ^{bc}	0.00 ^b
50	15.00 ^b	0.00 ^b	11.50 ^c	0.00 ^b
25	12.00 ^b	0.00 ^b	11.00 ^c	0.00 ^b
Ciprofloxacin	40.00 ^a	40.00 ^a	40.00 ^a	45.00 ^a
SE±	2.26	0.00	1.07	0.00

NB: Means with the same superscript along the column are not significantly different at P<0.05.

Table 4.17: The Antibacterial Activity of the Aqueous Leaf Extract of *Albizia lebbek* on Some Selected Species of Bacteria

Extract Conc (mg/ml)	Diameter of zone of inhibition (mm)			
	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>
200	0.00 ^b	17.50 ^b	23.50 ^b	18.00 ^b
100	0.00 ^b	15.00 ^{bc}	19.00 ^c	14.00 ^{bc}
50	0.00 ^b	10.00 ^c	0.00 ^d	5.00 ^{cd}
25	0.00 ^b	0.00 ^d	0.00 ^d	0.00 ^d
Ciprofloxacin	40.00 ^a	40.00 ^a	40.00 ^a	45.00 ^a
SE±	0.00	1.75	0.81	3.16

NB: Means with the same superscript along the column are not significantly different at P<0.05.

4.22 The Antibacterial Activity of the Aqueous Leaf Extract of *Tapinanthus globiferus* Parasitic on *Terminalia mantaly* on Some Selected Species of Bacteria

Ciprofloxacin (control) was active against all the test organisms whereas the aqueous leaf extract of *T. globiferus*, at all the concentrations, was not active against all the test organisms (Table 4.18).

4.23 The Antibacterial Activity of the Aqueous Leaf Extract of *Globimetula braunii* Parasitic on *Terminalia mantaly* on Some Selected Species of Bacteria

Ciprofloxacin (control) was active against all the test organisms, whereas the aqueous leaf extract of *G. braunii*, at all the concentrations, was not active against all test organisms (Table 4.18).

4.24 The Antibacterial Activity of the Aqueous Leaf Extract of *Tapinanthus globiferus* Parasitic on *Khaya senegalensis* on Some Selected Species of Bacteria

Ciprofloxacin (control) showed the highest zones of inhibition on all test organisms, compared to all the concentrations of the aqueous leaf extract of *T. globiferus* obtained from *K. senegalensis* (Table 4.19).

The aqueous leaf extracts of *T. globiferus* obtained from *K. senegalensis* was active against all test organisms. This inhibition of growth increased with increase in extract concentration. However, at the lowest concentration (25mg/ml) of the extract, no activity was observed. The highest zone of inhibition (28.00 mm) was at 200mg/ml and this was significantly ($P < 0.05$) higher than that (21.00 mm) at 100mg/ml, except for *S. aureus*. The lowest (17.50 mm) was at 50mg/ml for all the organisms. For *E. coli*, *S. aureus* and *S. typhi*, the aqueous leaf extract of *T. globiferus* obtained from *K. senegalensis* at 100mg/ml and 50mg/ml gave a comparable diameter zone of inhibition (Table 4.19).

Table 4.18: The Antibacterial Activity of the Aqueous Leaf Extracts of *T. globiferus* and *G. braunii* Obtained from *Terminalia mantalyon* on Some Selected Species of Bacteria

Diameter of zone of inhibition (mm)					
Test organisms					
Mistletoe species	Extract conc (mg/ml)	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>
<i>T. globiferus</i>	200	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	100	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	50	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	25	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	Ciprofloxacin	40.00 ^a	40.00 ^a	40.00 ^a	45.00 ^a
	SE±	0.00	0.00	0.00	0.00
<i>G. braunii</i>	200	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	100	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	50	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	25	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	Ciprofloxacin	40.00 ^a	40.00 ^a	40.00 ^a	45.00 ^a
	SE±	0.00	0.00	0.00	0.00

NB: Means with the same superscript along the column are not significantly different at P<0.05. SE± = Standard Error

Table 4.19: The Antibacterial Activity of the Aqueous Leaf Extracts of *T. globiferus* and *G. braunii* Obtained from *Khaya senegalensis* on Some Selected Species of Bacteria

Diameter of zone of inhibition (mm)					
Test organisms					
Mistletoe species	Extract conc (mg/ml)	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>
<i>T. globiferus</i>	200	26.50 ^b	28.00 ^b	28.00 ^b	28.00 ^b
	100	22.50 ^{bc}	21.00 ^c	20.00 ^c	20.50 ^c
	50	19.00 ^c	18.50 ^c	18.00 ^d	17.50 ^c
	25	0.00 ^d	0.00 ^d	0.00 ^e	0.00 ^d
	Ciprofloxacin	40.00 ^a	40.00 ^a	40.00 ^a	45.00 ^a
	SE±	1.97	0.81	1.07	1.92
<i>G. braunii</i>	200	16.50 ^b	30.00 ^b	27.00 ^b	21.00 ^b
	100	14.00 ^b	21.00 ^c	17.00 ^c	17.50 ^b
	50	14.00 ^b	17.00 ^d	16.00 ^d	16.50 ^b
	25	0.00 ^c	12.00 ^e	0.00 ^e	0.00 ^c
	Ciprofloxacin	40.00 ^a	40.00 ^a	40.00 ^a	45.00 ^a
	SE±	0.92	0.44	0.00	1.38

NB: Means with the same superscript along the column are not significantly different at P<0.05. SE±= Standard Error

4.25 The Antibacterial Activity of the Aqueous Leaf Extracts of *Globimetula braunii* Parasitic on *Khaya senegalensis* on Some Selected Species of Bacteria

Ciprofloxacin (control) gave in the highest zones of inhibition for all test organisms, compared to all concentrations of the aqueous leaf extract of *G. braunii* obtained from *K. senegalensis* (Table 4.19).

The aqueous leaf extract of *G. braunii* obtained from *K. senegalensis* inhibited all the test organisms and this increased with increase in concentration thereby had the highest zone of inhibition (30.00 mm) at 200mg/ml and the lowest (12.00 mm) at 25mg/ml for only *E. coli* and 50mg/ml for the other test organisms. For *S. aureus* and *S. typhi*, the aqueous leaf extract of *G. braunii* obtained from *K. senegalensis* at 200mg/ml, 100mg/ml and 50mg/ml gave significantly ($P < 0.005$) similar diameter zone of inhibition (Table 4.19). On the other hand, the zones of inhibition (30.00 mm 27.00 mm) at 200mg/ml was significantly ($P < 0.05$) higher than those due to 100mg/ml for *E. coli* (21.00 mm) and *B. subtilis* (17.00 mm) (Table 4.19).

4.26 The Antibacterial Activity of the Aqueous Leaf Extracts of *Tapinanthus globiferus* Parasitic on *Terminalia catappa* on Some Selected Species of Bacteria

Ciprofloxacin (control) gave in the highest zones of inhibition for all test organisms, compared to all concentrations of the aqueous leaf extract of *T. globiferus* obtained from *T. catappa* (Table 4.20).

The aqueous leaf extract of the *T. globiferus* obtained from *T. catappa* inhibited the growth of only two of the test organisms, at 200 and 100mg/ml for *S. typhi* and 200mg/ml for *E. coli* (Table 4.20).

Table 4.20: The Antibacterial Activity of the Aqueous Leaf Extracts of *T. globiferus* and *G. braunii* Obtained from *Terminalia catappa* on Some Selected Species of Bacteria

Diameter of zone of inhibition (mm)					
Test organisms					
Mistletoe species	Extract conc (mg/ml)	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>
<i>T. globiferus</i>	200	0.00 ^b	19.00 ^b	0.00 ^b	19.00 ^b
	100	0.00 ^b	0.00 ^b	0.00 ^b	13.50 ^b
	50	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^c
	25	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^c
	Ciprofloxacin	40.00 ^a	40.00 ^a	40.00 ^a	45.00 ^a
	SE±	0.00	0.44	0.00	0.81
<i>G. braunii</i>	200	0.00 ^b	0.00 ^b	0.00 ^b	11.00 ^b
	100	0.00 ^b	0.00 ^b	0.00 ^b	13.00 ^b
	50	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^c
	25	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^c
	Ciprofloxacin	40.00 ^a	40.00 ^a	40.00 ^a	45.00 ^a
	SE±	0.00	0.00	0.00	0.89

NB: Means with the same superscript along the column are not significantly different at P<0.05. **SE±** = Standard Error

4.27 The Antibacterial Activity of the Aqueous Leaf Extract of *Globimetula braunii* Parasitic on *Terminalia catappa* on Some Selected Species of Bacteria

Ciprofloxacin (control) gave the highest zones of inhibition on all test organisms, compared to all the concentrations of the aqueous leaf extract of *G. braunii* obtained from *T. catappa* (Table 4.20).

Most of the test organisms were resistant to the aqueous leaf extract of *G. braunii* obtained from *T. catappa* except *S. typhi*, which was susceptible to the plant extract only at the two higher concentrations (100 and 200mg/ml). The highest diameter zone of inhibition (13.00 mm on *S. typhi*) was at 100mg/ml and this was similar to that at 200mg/ml (11.00 mm) (Table 4.20).

4.28 The Antibacterial Activity of the Aqueous Leaf Extract of *Tapinanthus globiferus* Parasitic on *Citrus grandis* on Some Selected Species of Bacteria

Ciprofloxacin (control) had the highest zones of inhibition on all the test organisms, compared to the aqueous leaf extract of *T. globiferus* obtained from *C. grandis* (Table 4.21).

The aqueous leaf extract of *T. globiferus* obtained from *C. grandis* inhibited the growth of all the test organisms especially at three of the higher concentrations, thereby had the highest diameter zone of inhibition (26.00 mm on *E. coli*) at 200mg/ml and the lowest (13.00 mm) on *S. aureus* and *S. typhi* at 100mg/ml. *Bacillus subtilis* was resistant to the plant extract at virtually all its concentrations except at 200mg/ml where it was susceptible with 12.00 mm diameter zone of inhibition (Table 4.21).

Table 4.21: The Antibacterial Activity of the Aqueous Leaf Extracts of *T. globiferus* and *G. braunii* Obtained from *Citrus grandison* Some Selected Species of Bacteria

Diameter of zone of inhibition (mm)					
Test organisms					
Mistletoe species	Extract conc (mg/ml)	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>
<i>T. globiferus</i>	200	15.00 ^b	26.00 ^b	12.00 ^b	11.00 ^b
	100	13.00 ^c	23.00 ^c	0.00 ^c	13.00 ^c
	50	0.00 ^d	20.00 ^d	0.00 ^c	0.00 ^d
	25	0.00 ^d	0.00 ^e	0.00 ^c	0.00 ^e
	Ciprofloxacin	40.00 ^a	40.00 ^a	40.00 ^a	45.00 ^a
	SE±	0.44	0.63	0.00	0.92
<i>G. braunii</i>	200	17.50 ^b	0.00 ^b	17.50 ^b	20.00 ^b
	100	14.00 ^{bc}	0.00 ^b	0.00 ^c	13.00 ^c
	50	0.00 ^{cd}	0.00 ^b	0.00 ^c	0.00 ^d
	25	0.00 ^d	0.00 ^b	0.00 ^c	0.00 ^d
	Ciprofloxacin	40.00 ^a	40.00 ^a	40.00 ^a	45.00 ^a
	SE±	2.94	0.00	1.12	0.00

NB: Means with the same superscript along the column are not significantly different at P<0.05. **SE±** = Standard Error

4.29 The Antibacterial Activity of the Aqueous Leaf Extract of *Globimetula braunii* Parasitic on *Citrus grandis* on Some Selected Species of Bacteria

Ciprofloxacin (control) gave the highest zones of inhibition on all the organisms, compared to all concentrations of *G. braunii* extract obtained from *C. grandis* (Table 4.21).

The aqueous leaf extract of *G. braunii* obtained from *C. grandis* inhibited all the test organisms (except *E. coli*) especially at the highest concentration. It was not active against *E. coli* at all the concentrations and on all the organisms at 25 and 50mg/ml plant extract (Table 4.21).

4.30 The Antibacterial Activity of *Tapinanthus globiferus* Parasitic on *Albizzia lebeck* on Some Selected Species of Bacteria

Ciprofloxacin (control) showed inhibition of the growth of all the test organisms, compared to all the *T. globiferus* extracts obtained from *A. lebeck* (Table 4.22). The aqueous leaf extract of *T. globiferus* from *A. lebeck* at all concentrations was not active against all the organisms (Table 4.22).

4.31 The Antibacterial Activity of *Globimetula braunii* Parasitic on *Albizzia lebeck* on Some Selected Species of Bacteria

The control (ciprofloxacin) inhibited the growth of all the test organisms, compared to all the concentrations of *G. braunii* extract obtained from *A. lebeck* (Table 4.22). The aqueous leaf extract of *G. braunii* obtained from *A. lebeck* at all concentrations was not active against all the organisms (Table 4.22).

Table 4.22: The Antibacterial Activity of the Aqueous Leaf Extracts of *T. globiferus* and *G. braunii* Obtained from *Albizia lebeckon* Some Selected Species of Bacteria

Diameter of zone of inhibition (mm)					
Test organisms					
Mistletoe species	Extract conc (mg/ml)	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>
<i>T. globiferus</i>	200	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	100	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	50	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	25	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	Ciprofloxacin	40.00 ^a	40.00 ^a	40.00 ^a	45.00 ^a
	SE±				
<i>G. braunii</i>	200	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	100	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	50	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	25	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
	Ciprofloxacin	40.00 ^a	40.00 ^a	40.00 ^a	45.00 ^a
	SE±	0.00	0.00	0.00	0.00

NB: Means with the same superscript along the column are not significantly different at P<0.05. **SE±**= Standard Error

4.32 The Minimum Inhibitory Concentrations (MIC) of the Aqueous Leaf Extracts of five Host Plants on Some Selected Bacteria Species

The minimum inhibitory concentration (MIC) of the aqueous leaf extract of *T. catappa* against *S. aureus* was 3.125 mg/ml whereas it had no activity against *E. coli*, *B. subtilis* and *S. typhi* (Table 4.23). The MIC of the *T. mantaly* aqueous leaf extract was 3.125mg/ml for *S. aureus* and *S. typhi* whereas it was 12.50mg/ml for *E. coli* and *B. subtilis* (Table 4.23). *Khaya senegalensis* aqueous leaf extract had no activity on *E. coli* and *B. subtilis*, but its MIC was 3.125mg/ml against *S. aureus* and 25mg/ml against *S. typhi*. *Citrus grandis* also had no activity against *E. coli* and *S. typhi*, but gave a MIC of 25mg/ml against *S. aureus* and 50mg/ml against *B. subtilis*. *Albizia lebbek* aqueous leaf extract had no activity against *S. aureus*, but gave a minimum inhibitory concentration of 50mg/ml against *E. coli*, *B. subtilis* and *S. typhi* (Table 4.23).

4.33 The Minimum Bacteriocidal Concentrations (MBC) of the Aqueous Leaf Extracts of five Host Plants on Some Selected Bacteria Species

The minimum bacteriocidal concentration (MBC) of aqueous leaf extract of *T. catappa* on *S. aureus* was 6.25mg/ml (Table 4.24). The MBC of *T. mantaly* aqueous leaf extract was 25mg/ml for *E. coli* and *B. subtilis* whereas it was 6.25mg/ml for both *S. aureus* and *S. typhi* (Table 4.24). *Khaya senegalensis* aqueous leaf extract had no activity on *E. coli* and *B. subtilis* and so its MBC could not be determined, but on *S. aureus* and *S. typhi* where its activity was observed, it gave an MBC of 6.25mg/ml and 50mg/ml respectively (Table 4.24). The MBC of *C. grandis* aqueous leaf extract had no activity on *E. coli* and *S. typhi* hence its MBC could not be determined, but it gave an MBC of 50 mg/ml for *S. aureus* and 100 mg/ml for *B. subtilis* (Table 4.24). *Albizia lebbek* aqueous leaf extract had no activity against *S. aureus* and so its MBC could not be

Table 4.23: Minimum Inhibitory Concentrations of the Aqueous Leaf Extracts of Host Plants

Host plants	Minimum Inhibitory Concentrations (mg/ml)				
	<i>T. catappa</i>	<i>T. mantaly</i>	<i>K. senegalensis</i>	<i>C. grandis</i>	<i>A. lebbeck</i>
<i>S. aureus</i>	3.125	3.125	3.125	25.00	-
<i>E. coli</i>	-	12.50	-	-	50.00
<i>B. subtilis</i>	-	12.50	-	50.00	50.00
<i>S. typhi</i>	-	3.125	25.00	-	50.00

NB: - No activity

Table 4.24: Minimum Bacteriocidal Concentrations of the Aqueous Leaf Extracts of Host Plants

Host plants Test organisms	Minimum Inhibitory Concentrations (mg/ml)				
	<i>T. catappa</i>	<i>T. mantaly</i>	<i>K. senegalensis</i>	<i>C. grandis</i>	<i>A. lebeck</i>
<i>S. aureus</i>	6.25	6.25	6.25	50.00	-
<i>E. coli</i>	-	25.00	-	-	100.00
<i>B. subtilis</i>	-	25.00	-	100.00	100.00
<i>S. typhi</i>	-	6.25	50.00	-	100.00

NB: - No activity

but it gave an MBC of 100mg/ml against *E. coli*, *B. subtilis* and *S. typhi* (Table 4.24).

4.34 The Minimum Inhibitory Concentrations of the Aqueous Leaf Extracts of *T. globiferus* and *G. braunii* Obtained from five Host Tree Species on Some Selected Bacteria Species.

The aqueous leaf extract of *T. globiferus* obtained from *T. mantaly* had no activity against all test organisms and so its minimum inhibitory concentration (MIC) could not be determined (Table 4.25). Furthermore, MIC of the aqueous leaf extracts of *G. braunii* obtained from *T. mantaly*, *T. catappa* and *A. lebbeck* and *T. globiferus* obtained from *A. lebbeck* and *C. grandis* were not determined due to their lack of activity against the test organisms (Table 4.25). The aqueous leaf extract of *T. globiferus* obtained from *T. catappa* had no activity on *S. aureus*, *E. coli* and *B. subtilis*, but its MIC was 100mg/ml on *S. typhi*. The MIC of the aqueous leaf extract of *T. globiferus* sourced from *K. senegalensis* was 50mg/ml on *S. aureus*, but had no activity on *E. coli*, *B. subtilis* and *S. typhi*. Aqueous leaf extract of *G. braunii* obtained from *K. senegalensis* had no activity on *S. aureus*, *E. coli* and *S. typhi*, but gave an MIC of 50mg/ml on *B. subtilis*. In addition, the aqueous leaf extract of *G. braunii* sourced from *C. grandis* had an MIC of 100mg/ml on *S. aureus*, *B. subtilis* and *S. typhi*, but had no activity on *E. coli* (Table 4.25).

4.35 The Minimum Bacteriocidal Concentrations of the Aqueous Leaf Extract of *T. globiferus* and *G. braunii* obtained from five Host Tree Species on Some Selected Bacteria Species.

The minimum bacteriocidal concentrations (MBC) for the aqueous leaves extracts of *T. globiferus* and *G. braunii* was the same as observed for minimum inhibitory concentration (MIC) (Table 4.26).

Table 4.25: The Minimum Inhibitory Concentrations of the Mistletoe Species Leaf Extracts

Host plant	Mistletoe species	Minimum Inhibitory Concentrations (mg/ml)			
		<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>
<i>T. mantaly</i>	<i>T. globiferus</i>	-	-	-	-
	<i>G. braunii</i>	-	-	-	-
<i>T. catappa</i>	<i>T. globiferus</i>	-	-	-	100
	<i>G. braunii</i>	-	-	-	-
<i>K. senegalensis</i>	<i>T. globiferus</i>	50	-	-	-
	<i>G. braunii</i>	-	-	50	-
<i>A. lebeck</i>	<i>T. globiferus</i>	-	-	-	-
	<i>G. braunii</i>	-	-	-	-
<i>C. grandis</i>	<i>T. globiferus</i>	-	-	-	-
	<i>G. braunii</i>	100	-	100	100

NB: - No activity of extract

Table 4.26: The Minimum Bacteriocidal Concentrations of the Mistletoe Species Leaf Extracts

Host plant	Mistletoe species	Minimum Bacteriocidal Concentrations (mg/ml)			
		Organisms			
		<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>
<i>T. mantaly</i>	<i>T. globiferus</i>	-	-	-	-
	<i>G. braunii</i>	-	-	-	-
<i>T. catappa</i>	<i>T. globiferus</i>	-	-	-	100
	<i>G. braunii</i>	-	-	-	-
<i>K. senegalensis</i>	<i>T. globiferus</i>	50	-	-	-
	<i>G. braunii</i>	-	-	50	-
<i>A. lebeck</i>	<i>T. globiferus</i>	-	-	-	-
	<i>G. braunii</i>	-	-	-	-
<i>C. grandis</i>	<i>T. globiferus</i>	-	-	-	-
	<i>G. braunii</i>	100	-	100	100

NB: - No activity of extract

4.36 Comparison of the Antibacterial Activities of the Aqueous Leaf Extracts of *Terminalia mantaly* and its Associated Mistletoe Species (*Tapinanthus globiferus* and *Globimetula braunii*)

The aqueous leaf extract of *T. mantaly* significantly ($P < 0.05$) inhibited all the test organisms more than the aqueous leaf extracts of the two mistletoe species sourced from it. The inhibition zones of diameter of the aqueous leaf extracts of the two mistletoe species against the test organisms were comparable (Table 4.27).

4.37 Comparison of the Antibacterial Activities of the Aqueous Leaf Extracts of *Terminalia catappa* and its Associated Mistletoe Species (*T. globiferus* and *G. braunii*)

The aqueous leaf extract of *T. catappa* significantly ($P < 0.05$) inhibited *S. aureus*, *E. coli* and *S. typhi* more than the aqueous leaf extracts of the two mistletoe species obtained from it. However, there was no significant difference ($P < 0.05$) in the diameter of inhibition zones of *T. catappa* and the two mistletoe species on *B. subtilis* (Table 4.27).

Inhibition zone of *G. braunii* was significantly (at $P < 0.05$) lower than that of *T. globiferus* on *S. typhi* (Table 4.27).

4.38 Comparison of the Antibacterial Activities of Aqueous Leaf Extracts of *Khaya senegalensis* and its Associated Mistletoe Species (*T. globiferus* and *G. braunii*)

The activity of the aqueous leaf extracts of *T. globiferus* and *G. braunii* obtained from *K. senegalensis* was higher than that of their host plant (*K. senegalensis*) on *E. coli* and *B. subtilis*. On the other hand, the aqueous leaf extract of the host (*K. senegalensis*) significantly ($P < 0.05$) inhibited *S. aureus* and *S. typhi* more than the two mistletoe species (Table 4.27).

Table 4.27: Comparison of the Antibacterial Activities of the Aqueous Leaf Extracts of Five Host Plants and their Associated Mistletoe Species (*Tapinanthus globiferus* and *Globimetula braunii*)

Diameter of zone of inhibition (mm)				
Test organisms				
Host plant/Mistletoe species	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>
<i>Terminalia mantaly</i>	24.70 ^a	15.50 ^a	19.20 ^a	19.50 ^a
<i>T. globiferus</i>	8.00 ^b	8.00 ^b	8.00 ^b	9.00 ^b
<i>G. braunii</i>	8.00 ^b	8.00 ^b	8.00 ^b	9.00 ^b
SE±	0.36	0.07	0.47	0.64
<i>Terminalia catappa</i>	22.70 ^a	11.80 ^a	8.00 ^a	15.50 ^a
<i>T. globiferus</i>	8.00 ^b	8.00 ^b	8.00 ^a	13.80 ^b
<i>G. braunii</i>	8.00 ^b	8.00 ^b	8.00 ^a	9.00 ^c
SE±	0.43	0.15	0.00	0.40
<i>Khaya senegalensis</i>	22.00 ^a	8.00 ^c	8.00 ^c	22.70 ^a
<i>T. globiferus</i>	21.60 ^a	21.50 ^b	21.20 ^a	22.20 ^a
<i>G. braunii</i>	16.90 ^b	24.00 ^a	20.00 ^a	20.00 ^b
SE±	0.79	0.31	0.00	0.85
<i>Citrus grandis</i>	21.00 ^a	8.00 ^b	18.50 ^a	9.00 ^c
<i>T. globiferus</i>	15.50 ^b	21.80 ^a	10.40 ^b	21.20 ^a
<i>G. braunii</i>	13.60 ^b	8.00 ^b	11.50 ^b	15.60 ^b
SE±	1.24	0.21	0.52	0.00
<i>Albizzia lebeck</i>	8.00 ^a	16.50 ^a	16.50 ^a	16.40 ^a
<i>T. globiferus</i>	8.00 ^a	8.00 ^b	8.00 ^b	9.00 ^b
<i>G. braunii</i>	8.00 ^a	8.00 ^b	8.00 ^b	9.00 ^b
SE±	0.00	0.58	0.27	1.05

NB: Means with the same superscript along the column of each of the host plant are not significantly different at P<0.05. **SE±**= Standard Error.

4.39 Comparison of the Antibacterial Activities of the Aqueous Leaf Extracts of *Citrus grandis* and its Associated Mistletoe Species (*T. globiferus* and *G. braunii*)

The aqueous leaf extract of *C. grandis* gave higher inhibitions on *S. aureus* (21.00 mm) and *B. subtilis* (18.50 mm) compared to the aqueous leaf extracts of the two mistletoe species obtained from it. However, *T. globiferus* sourced from *C. grandis* gave higher inhibition on *E. coli* (21.80 mm) and *S. typhi* (21.20 mm). Comparably, the aqueous leaf extract of *T. globiferus* had the highest diameter zone of inhibition (21.80 mm) on *E. coli* and the lowest (8.00 mm) on *E. coli* due to the activity of the aqueous leaf extracts of *C. grandis* and *G. braunii* (Table 4.27).

4.40 Comparison of the Antibacterial Activities of Aqueous Leaf Extracts of *Albizia lebbek* and its Associated Mistletoe Species (*T. globiferus* and *G. braunii*)

The aqueous leaf extract of *A. lebbek* gave higher inhibition zones (16.50 mm) on *E. coli*, *B. subtilis* and *S. typhi* than the two mistletoe species obtained from it. The aqueous leaf extracts of the two mistletoes species and that of the *A. lebbek* from which they were sourced had similar activity on all test organisms (Table 4.27).

4.41 Comparison of the Antibacterial Activities of the Aqueous Leaf Extracts of the Five Host Plant Species

The aqueous leaf extract of *T. mantaly* was the most active on most of the test organisms compared to that of the other host plants. The aqueous leaf extract of *T. catappa* was the least active on most of the organisms compared to the aqueous leaf extracts of the other host plants. In comparison also, among the test organisms, *S. aureus* was the most susceptible to the aqueous leaf extracts of most of the host plants compared to other test organisms whereas *E. coli* was the least susceptible to the

aqueous leaf extracts of most of the host plants compared to other test organisms (Table 4.28).

4.42 Comparison of the Antibacterial Activities of the Aqueous Leaf Extracts of all the *Tapinanthus globiferus* Obtained from Five Host Plant Species

The aqueous leaf extract of *T. globiferus* obtained from *K. senegalensis* followed by that obtained from *C. grandis* gave higher zones of inhibition against all the test organisms compared to the aqueous leaf extracts of *T. globiferus* obtained from the other host plants. On the other hand, the aqueous leaf extracts of *T. globiferus* obtained from *A. lebbeck* and *T. mantaly* gave the least zones of inhibition against all test organisms compared to the rest (Table 4.29).

4.43 Comparison of the Antibacterial Activities of the Aqueous Leaf Extracts of *Globimetula braunii* Obtained from Five Different Species of Host Plants

The aqueous leaf extract of *G. braunii* sourced from *K. senegalensis* gave higher zones of inhibition against all test organisms. This was followed by that sourced from *C. grandis* compared to the aqueous leaf extracts of *G. braunii* obtained from the other host plants. However, the aqueous leaf extracts of *G. braunii* sourced from *T. mantaly* and *A. lebbeck* produced the least zones of inhibition (Table 4.29).

Table 4.28: Comparison of the Antibacterial Activities of the Aqueous Leaf Extracts of the Five Host Plants

Test organisms Host plants	Diameter zone of inhibition (mm)			
	<i>S.aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>
<i>Terminalia mantaly</i>	24.70 ^a	15.50 ^a	19.20 ^a	19.50 ^b
<i>Terminalia catappa</i>	22.70 ^b	8.00 ^b	8.00 ^c	9.00 ^d
<i>Khaya senegalensis</i>	22.00 ^b	8.00 ^b	8.00 ^c	22.70 ^a
<i>Citrus grandis</i>	21.00 ^b	8.00 ^b	18.50 ^a	9.00 ^d
<i>Albizia lebbek</i>	8.00 ^c	16.50 ^a	16.50 ^b	16.40 ^c
SE_±	0.59	0.35	0.39	0.76

NB: Means with the same superscript along the column are not significantly different at P<0.05. **SE_±**= Standard Error

Table 4.29: Comparison of the Antibacterial Activities of the Aqueous Leaf Extracts of *Tapinanthus globiferus* and *G. braunii* Obtained from Five Host Plants

Test organisms		Diameter zone of inhibition (mm)			
Mistletoe species	Host plant	<i>S. aureus</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>S. typhi</i>
<i>T. globiferus</i>	<i>T. mantaly</i>	8.00 ^c	8.00 ^c	8.00 ^c	9.00 ^c
	<i>T. catappa</i>	8.00 ^c	11.80 ^b	8.00 ^c	15.50 ^b
	<i>K. senegalensis</i>	21.60 ^a	21.50 ^a	21.20 ^a	22.20 ^a
	<i>C. grandis</i>	13.60 ^b	21.80 ^a	10.40 ^b	21.20 ^a
	<i>A. lebbeck</i>	8.00 ^c	8.00 ^c	8.00 ^c	9.00 ^c
	SE±	0.40	0.22	0.00	0.42
<i>G. braunii</i>	<i>T. mantaly</i>	8.00 ^b	8.00 ^b	8.00 ^c	9.00 ^d
	<i>T. catappa</i>	8.00 ^b	8.00 ^b	8.00 ^c	13.80 ^c
	<i>K. senegalensis</i>	16.90 ^a	24.00 ^a	20.00 ^a	20.00 ^a
	<i>C. grandis</i>	15.50 ^a	8.00 ^b	11.50 ^b	15.60 ^b
	<i>A. lebbeck</i>	8.00 ^c	8.00 ^c	8.00 ^c	9.00 ^c
	SE±	0.62	0.09	0.22	0.33

NB: Means with the same superscript along the column are not significantly different at P<0.05. SE±= Standard Error

CHAPTER FIVE

5.0

DISCUSSION

The growth of mistletoes on different plantspecies are of disease curing specificity, for example, mistletoe on cocoa was found to be best used for curing diabetes (Ekhaise *et al.*, 2010). Considerable works(Ilesanmi and Olawoye, 2010; Yusuf *et al.*, 2013) have been done to investigate the pharmacological importance of mistletoes. However, there is dearth of information on the comparative pharmacological importance of mistletoe plant growing on different host tree species.

In this study, four sampling areas were used within which the different mistletoe species were obtained from five host tree species: *Albizzia lebbeck*, *Terminalia catappa*, *Terminalia mantaly*, *Citrus grandis* and *Khaya senegalensis*. The collection revealed that *A. lebbeck* had the highest infestation of different species of mistletoes, followed by *C. grandis* and *T. mantaly* compared to the other host trees. This incidence could be attributed to the relative occurrence of *A. lebbeck* in the sampling areas more than the other host trees, host specificity of the mistletoes, the host plant characteristics and the movement patterns of dispersal agents. Similar report was published by Aukema and Martinez (2002) as well as Norton and Carpenter (1998) who reported that the relatively abundant citruses and guava in the study area influenced the host choice of mistletoe. Overton (1994) also reported that the characteristics such as branch size, age and height of a host plant can have a strong effect on mistletoe attachment resulting in size related mistletoe infection patterns. It was also observed that out of the several species of mistletoe obtained from these five different host trees, *T. globiferus* and *G. braunii* were the most common and found parasitic on all the five host trees. This could be due to their seeds being very sticky in nature compared to that of other mistletoes seeds, thus enhancing their distribution by birds and other animals (Del Rio *et al.*, 1996; Aukema,

2004). It can also be as a result of their being less host specific compared to the other mistletoe species (Boussim *et al.*, 2004).

The qualitative phytochemical analysis of the five host plants extracts (*A. lebbeck*, *T. catappa*, *T. mantaly*, *C. grandis* and *K. senegalensis*) and their associated mistletoe species (*T. globiferus* and *G. braunii*) revealed the presence of some compounds, which were known to exhibit medicinal as well as physiological activities (Sofowora, 1993). Some of these compounds (tannins, saponins, flavonoids, carbohydrates, cardiac glycosides and triterpenes) were found common to both the host plants and the mistletoe plants obtained from them. This was due to the fact that the composition and activities of mistletoes are host plant-dependent as reported by Bassey (2012), Osadebe and Ukwueze (2004), Wagner *et al.* (1996), Obatomi *et al.* (1994) and Scheer *et al.* (1992). Some compounds were present in both the aqueous and ethanolic extracts of the host plants (tannins and saponin in *T. catappa* and *T. mantaly*) or in either of the extracts of the host plants (triterpenes, tannins, saponins and steroids in *T. catappa*, *T. mantaly* and *A. lebbeck*) but were not detected in both the aqueous and ethanolic extracts of the two parasitic plants obtained from it (alkaloids and cardiac glycosides in *T. catappa* and *K. senegalensis*). This might be due to the compounds being in low concentrations thus making their qualitative identification difficult.

The qualitative analysis of the extracts showed that some compounds were not detected in both the aqueous and ethanolic extracts of the host plants, but were present in the extracts of the parasitic plants obtained from them. For example, steroids in aqueous extract of *G. braunii* obtained from *T. catappa* and *T. mantaly* and alkaloid in ethanolic extract of *T. globiferus* and in ethanolic and aqueous extracts of *G. braunii* both obtained from *T. mantaly*. This is contrary to the reports of Bassey (2012), Osadebe and Ukwueze (2004), Wagner *et al.* (1996), Obatomi *et al.* (1994) and Scheer *et al.* (1992).

They indicated that the contents of these compounds in the mistletoes dependent on that of the host plant. However, the fact that these compounds were not detected did not mean that the host plants were completely devoid of them as was proven by the results of the quantitative analysis of the plants extracts. It could be that the compounds were in very low concentrations and detecting them qualitatively was difficult. Considering the fact that mistletoes are hemi-parasites, having the ability to manufacture some part of their food requirements, the presence of these compounds, which were not detected in their host plants could be attributed to the compounds being in higher concentrations in the parasitic plants due to the parasites metabolic process. The variations observed in the compounds present in the extracts of the host plants and their associated mistletoe species could be linked to the differences in the ability of the two solvents (ethanol and water) to extract the phytochemical constituents, even though they are both polar protic solvents. Ethanol extracts compounds that could partially dissolve in water and other organic solvents while water extracts only compounds that could fully or partially dissolve in water. This was supported by Ndamitso *et al.* (2013) who reported that the differences in the phytochemical components of plant extracts largely depend on the type of solvent and probably the extraction method(s) used. The aqueous extracts contained most of the compounds detected compared to the ethanolic extracts. This might be due to the fact that those compounds were amphiphilic and that the polarity of water is higher than that of ethanol. The phytochemical constituents of *T. catappa* and *K. senegalensis* detected in this study in both the aqueous and ethanolic extracts were also confirmed present by Neelavathi *et al.* (2013) and Wakirwa *et al.* (2013) respectively. In *A. lebbek*, the compounds confirmed present in both of the extracts were also reported by Rahul *et al.* (2010) in the ethyl acetate, methanol and water extracts.

In the quantitative phytochemical analysis of the host plants extracts and their associated mistletoe species, it was observed that *A. lebbeck* had the highest concentrations of saponins, tannins and phenols compared to its two parasitic plants. *Terminalia catappa* had the highest concentration of alkaloids compared to its parasitic plants, *T. mantaly* and *K. senegalensis* had the highest concentrations of alkaloids and cyanogenic glycosides; tannins and cyanogenic glycosides respectively compared to their parasitic plants. This was due to the fact that these host plants supply their parasitic plants with the raw materials (water and mineral nutrients) needed by them (Milius, 2000), therefore, the composition of the parasitic plants partly depended on what was derived from their host plants (Bassey, 2012). So, it was possible that the host plants might have higher concentrations of some compounds more than their parasitic plants. However, the concentration of some of these compounds (alkaloids and phenols), present in the host plants especially in *C. grandis* were lower than those present in their parasitic plants. This contradicts the reports of researchers such as Wagner *et al.* (1996), Osadebe *et al.* (2008) and Bassey (2012) who indicated that the phytochemical constituents of mistletoes are dependent on that of their host plants, but factors like plants' reproductive period (Encyclopaedia Britannica, 2014), seasons and environmental factors (Osadebe *et al.*, 2008) all affect the availability and concentrations of plants' phytochemical constituents. For example, in some plants, the concentration of alkaloids increases just prior to seed formation and then drops when the seed is ripe (Encyclopaedia Britannica, 2014).

In the comparison of the phytochemical constituents of the five host plants, the concentrations of some compounds in some of the host plants (alkaloids in *T. mantaly*, saponins, cyanogenic glycosides and flavonoids in *K. senegalensis* and tannins and phenols in *A. lebbeck*) were higher compared to those of other host plants. This might

be attributed to the differences in their physiological activities. The differences in the phytochemical constituents of *T. globiferus* and *G. braunii* parasitic on the different host trees might be due to their differences in species and host plant, which is the source of most of their components.

The antibacterial activity of the five host plant extracts against two Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*E. coli* and *S. typhi*) bacteria used in this study was observed to be very effective against the test organisms. This is probably due to the presence of most of the compounds of medicinal importance detected in the plant extracts. The activities of the plant extracts differ according to the host plant which might be due to the differences in their reproductive periods (Encyclopedia Britannica, 2014), organism tested against and the concentration used. The bioactive components of the plant extracts that were detected might be responsible for the antibacterial activities of the plants. The activities of all the plant extracts was observed to increase with an increase in concentration as the highest activities were observed at the highest concentrations. This is probably due to the high concentration of bioactive components detected in them. Sebiomo *et al.* (2010) also reported an increase in the activities of some antibiotics and ginger extracts against two pathogenic bacteria as their concentrations increased. However, the activity of ciprofloxacin (control) against the four test organisms in this study was more than that of the host plant extracts and the mistletoes parasitic on them. The extract of *T. mantaly* was observed to be very active against the four organisms on which it was tested with the highest level of inhibition against *S. aureus*. The antimicrobial activity of *T. mantaly* aqueous extract was confirmed by Zirihi *et al.* (2012) when the antifungal activities of the aqueous extract were rated 64 times more active than that of *T. catappa* aqueous extract and its hydroalcoholic extract was 2 times more active than that of *T. catappa* on *Aspergillus*

fumigatus. The comparison of the *T. mantaly* antibacterial activity with that of its two parasitic plants showed that *T. mantaly* extract was far more active against the test organisms than that of its two parasitic plants. *Terminalia mantaly* extract was more active on the four test organisms compared to the extracts of the other four host plants. Similar observation was reported by Kokora *et al.* (2013) when the antibacterial activity of the aqueous and ethanolic extracts of *T. mantaly* and four other plants were tested against some bacteria with only *T. mantaly* extract showing activity against the test organisms.

The minimum concentration of *T. mantaly* extract that was inhibitory to the test organisms ranged from 3.13mg/ml to 12.50mg/ml whereas the minimum concentration of *T. mantaly* extract that was bacteriocidal (MBC) ranged from 6.25mg/ml to 25mg/ml. The activity of the plant might be due to its high alkaloids content which was known to have medicinal and physiological activities (Sofowora, 1993). The antibacterial activity test of *T. catappa* aqueous leaf extract showed that the extract was only effective against *S. aureus* which was contrary to the report of Muhammad and Mudi (2011) who reported that, the ethanolic and aqueous methanol fraction was active only against *S. typhi* but inactive against *S. aureus* and other organisms. This might be due to the differences in the solvents used as organic extracts perform better activity than aqueous extracts. This is probably due to differences in extractability of the organic constituents (Mbengui *et al.*, 2013).

In the comparison of the antibacterial activities of the aqueous leaf extracts of the five host plants, *T. catappa* was observed to be active on *S. aureus* as also reported by Mbengui *et al.* (2013), which was next to *T. mantaly* in its activity against the organism. Upon comparison with the activity of its parasitic plants, it was observed that *T. catappa* aqueous leaf extract was more active on three of the test organisms than the two

parasitic plants with one of the parasitic plant (*T. globiferus*) being next to it in its activity against *S. typhi*. This could possibly be as a result of the high alkaloid contents of the host (*T. catappa*) and high cyanogenic glycoside, flavonoid and phenol contents of the parasitic plant (*T. globiferus*).

The aqueous leaf extract of *K. senegalensis* was observed to be active against *S. aureus* and *S. typhi*, but not active against *E. coli* and *B. subtilis*. This result was similarly supported by the findings of Kubmarawa *et al.* (2008) who found that, the aqueous fraction of the leaf extract of *K. senegalensis* was not active against all the four organisms on which it was tested while the ethanolic fraction was active against all the test organisms. Wakirwa *et al.* (2013) reported that the ethanolic leaf extract of *K. senegalensis* recorded a pronounced inhibition on *S. aureus* at different concentrations of the extract. The plants used in study were harvested during rainy season and at different stages of their reproductive periods. Therefore, the differences in these results could be due to the differences in the solvents used and the morphogenetic and phenotogical variation of the plant harvested at the vegetative, floral budding, full flowering, fresh fruiting and mature fruiting stages (Cuneyt and Jolita, 2007).

It was observed that the aqueous leaf extract of *K. senegalensis* was more active on *S. typhi* compared to the other host plants. This is possibly due to its higher contents of saponin, cyanogenic glycoside and flavonoid. The concentrations of 3.13mg/ml and 25mg/ml were observed to be the minimum concentration of *K. senegalensis* that could be inhibitory to the growth of *S. aureus* and *S. typhi* respectively while concentrations of 6.25mg/ml and 50mg/ml were observed to be the minimum concentrations of *K. senegalensis* that could be bacteriocidal to the two bacterial species. There was higher antibacterial activity of *K. senegalensis* aqueous leaf extract compared to that of the two mistletoe species obtained from it when tested against *S. aureus* and *S. typhi*. The extract

of *K. senegalensis* was more active on these organisms probably due to its high tannin and cyanogenic glycoside contents. However, the two mistletoes extracts were more active against *E. coli* and *B. subtilis* than *K. senegalensis* leaf extract probably due to their high alkaloid, saponin and phenol contents.

Citrus grandis aqueous leaf extract was active only against *S. aureus* and *B. subtilis* but inactive against *E. coli* and *S. typhi* as was similarly reported by Hindi *et al.* (2014). However, they also reported that the juice of *Citrus reticulata* exhibited inhibitory activity against all the test organisms including *S. aureus*, *S. pyogenes*, *K. pneumonia*, *E. coli*, *S. typhi*, *Proteus* spp., and *M. catarrhalis*. This might be due to high bioactive components of the fruit juice than that of the leaf extract. In comparison, the extract of *C. grandis* was active against *S. aureus*, which was next to the activity of the extracts of *K. senegalensis*, *T. mantaly* and *T. catappa* respectively and *B. subtilis* which was next to the activity of *T. mantaly* extract in its level of inhibition compared to other plants extracts. This could be due to low concentrations of some bioactive compounds present in it compared to those plants. In comparison also, the four test organisms were observed to be more susceptible to the extracts of the two mistletoe species obtained from *C. grandis* than they were to *C. grandis* extract. This could be associated with the low concentrations of alkaloids, cyanogenic glycosides and phenols in *C. grandis* compared to those in the two mistletoe species obtained from it.

The aqueous leaf extract of *A. lebbek* was observed to be very active against *E. coli*, *B. subtilis* and *S. typhi* as similarly reported by Sahid and Firdous (2012). However, the extract was not active against *S. aureus*, which was contradicting to their report as their finding showed that *A. lebbek* was also active against *S. aureus*. This could be due to the different plant parts and the solvents used in both studies. In comparing the efficacy of *A. lebbek* with that of the other host plants, *A. lebbek* extract was more active

against *E. coli*. This could be attributed to its high tannin and phenol contents compared to those of the other plants.

The extracts of the two mistletoe species (*T. globiferus* and *G. braunii*) obtained from *T. mantaly* and *A. lebbeck* were all not active against the four test organisms on which it were tested. These results were not in conformity with the reports of Bassey (2012), Osadebe *et al.* (2004), Wagner *et al.* (1996), Obatomi *et al.* (1994) and Scheer *et al.* (1992) since their host plants were active against some of the organisms on which their extracts were tested. However, Cuneyt and Jolita (2007) reported that the morphogenetic and phenotogenical variation of plant harvested at the vegetative, floral budding, full flowering, fresh fruiting and mature fruiting stages could affect the microbial activity of plant.

The two mistletoe species obtained from *T. catappa* were unlike their host plant, being active against *S. typhi* at the higher concentrations of their extracts. *Tapinanthus globiferus* was also active against *E. coli* in addition to being active against *S. typhi*. This could be due to the higher concentrations of some compounds it had (saponins and flavonoids in *G. braunii* as well as tannins and phenols in *T. globiferus*) compared to the host plant.

The aqueous leaf extract of *T. globiferus* obtained from *C. grandis* was active against all the test organisms compared to the extract of *C. grandis*. Although, the aqueous extract of *C. grandis* was very active at all the concentrations on *S. aureus* and *B. subtilis*, the extract of *G. braunii* obtained from it was also active against *S. typhi* in addition to *S. aureus* and *B. subtilis*, but only at its higher concentrations. This could be linked to the higher concentrations of saponins, cyanogenic glycosides and phenols of the *T. globiferus* and alkaloids and flavonoids of the *G. braunii*. The activities at higher concentrations of the plants' extracts could be due to more effectiveness of some of the

compounds present in the extracts with increase in concentration and less actively with decrease in the concentration of the plant extract.

In comparing the antibacterial activities of all the *T. globiferus* and *G. braunii* obtained from the five different host plants, the aqueous leaf extracts of *T. globiferus* and *G. braunii* obtained from *K. senegalensis* were the most active against *S. aureus*, *B. subtilis* and *S. typhi* followed by the extracts of *T. globiferus* and *G. braunii* obtained from *C. grandis* which were the most active against *E. coli* compared to the *T. globiferus* and *G. braunii* parasitic on other host plants. This confirmed their ethnopharmacological importance. This outstanding antibacterial activity could be due to the higher concentrations of flavonoids in *T. globiferus* and higher concentrations of saponins in the *G. braunii* obtained from *K. senegalensis*, as flavonoids were known to disrupt the functions of viruses and bacteria as reported by Higdon and Frei (2003) while saponins destroy microbes (Rao and Rao, 1995); and the higher concentrations of saponins in *T. globiferus* and higher concentrations of cyanogenic glycosides, alkaloids, flavonoids and phenols of the *G. braunii* obtained from *C. grandis*.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The five host trees were parasitized by different mistletoe species. *Globimetula braunii* and *T. globiferus* were the predominant and common mistletoe species on the five tree species.

All the host plants and the mistletoe species contained compounds which are of medicinal values. Qualitatively, cardiac glycosides and carbohydrate were common to all the host plants while carbohydrates and flavonoids were common to all the two mistletoe species obtained from the five host plants.

Quantitatively, *K. senegalensis* had the highest contents of saponins (14.96%), cyanogenic glycosides (15.00 mg/g) and flavonoids (13.91%). *Terminalia mantaly* had the highest content of alkaloids (3.94%) and *A. lebbeck* had the highest contents of tannins (1.29%) and phenols (4.99mg/ml). *Tapinanthus globiferus* and *G. braunii* parasitic on *C. grandis* had the highest contents of saponins (31.00%) and alkaloids (2.90%), cyanogenic glycosides (5.32 mg/g) and phenols (0.54 mg/ml) respectively. The *T. globiferus* and *G. braunii* from *T. catappa* had the highest contents of cyanogenic glycosides (12.00 mg/g) and tannins (1.32%) and tannins (1.22%) respectively. The *T. globiferus* and *G. braunii* from *K. senegalensis* had the highest contents of flavonoids (22.40%) and saponins (22.00%), while the *T. globiferus* from *A. lebbeck* and *T. mantaly* had the highest content of alkaloids (6.10%) and phenols (2.69 mg/ml) respectively.

All the host plants had an antibacterial activity on the test organisms and *T. mantaly* was significantly the most active against most of the test organisms at concentrations: 100 and 200mg/ml.

The *T. globiferus* and *G. braunii* obtained from the five host trees all had an antibacterial activity except the ones sourced from *A. lebbeck* and *T. mantaly* with the two mistletoe species obtained from *K. senegalensis* having significantly the best antibacterial activity.

6.2 Recommendations

- a. Further work should be carried out using other species of mistletoe obtained from *K. senegalensis* and *C. grandis* in order to further evaluate the slight variation in the antibacterial activities of the two species of mistletoe obtained from the same host.
- b. Efficacy test of mistletoes obtained from *K. senegalensis* and *C. grandis* should be carried out on both human and other animal enteric bacteria of different species and strains so as to meet up with the quest of finding an alternative to the conventional antibiotics that are mostly harmful and resistant to pathogenic organisms.
- c. Mistletoe species parasitic on other *Citrus* species should be explored and compared for their antibacterial activities.
- d. The bioactive components of these plants responsible for the antibacterial activities against each of these organisms should be identified.
- e. Herbalists/pharmaceutical companies using mistletoes for medicinal purposes against the diseases caused by *E.coli*, *B. subtilis*, *S. typhi* and *S. aureus* should go for mistletoes parasitic on either *K. senegalensis* or *C. grandis* instead of those parasitic on *T. mantaly*, *T. catappa* and *A. lebbeck* as they are more active on these bacteria.

- f. The growth of mistletoes on trees within Ahmadu Bello University should regularly be checked by the University Management so as to avoid killing of the trees by heavy infestation by the mistletoes.
- g. The use of water for the extraction of phytochemicals is recommended as it is cheaper, extracts considerable amount of compounds which could fully or partially dissolve in water and possessed antibacterial activity.

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APPENDICES



Plate I: *Terminalia catappa*plant



Plate II: *Terminalia mantaly*plant



Plate III: *Albizzia lebeck* plant



Plate IV: *Khaya senegalensis* plant



Plate V: *Citrus grandis* plant



Plate VI: *Tapinanthus dodoneifolius* (mistletoe) plant



Plate VII: *Tapinanthus globiferus*(mistletoe) plant



Plate VIII: *Globimetula braunii*(mistletoe) plant



Plate IX: Susceptibility test of the aqueous leaf extract of *Globimetula braunii* obtained from *Citrus grandis* on *Staphylococcus aureus*
 1= 200mg/ml; 2= 100 mg/ml; 3= 50 mg/ml; 4= 25 mg/ml; a= zone of inhibition



Plate X: Susceptibility test of the aqueous leaf extract of *G. braunii* obtained from *Khaya senegalensis* on *S. aureus* and *E. coli*
 1= 200mg/ml; 2= 100 mg/ml; 3= 50 mg/ml; 4= 25 mg/ml; a= zone of inhibition

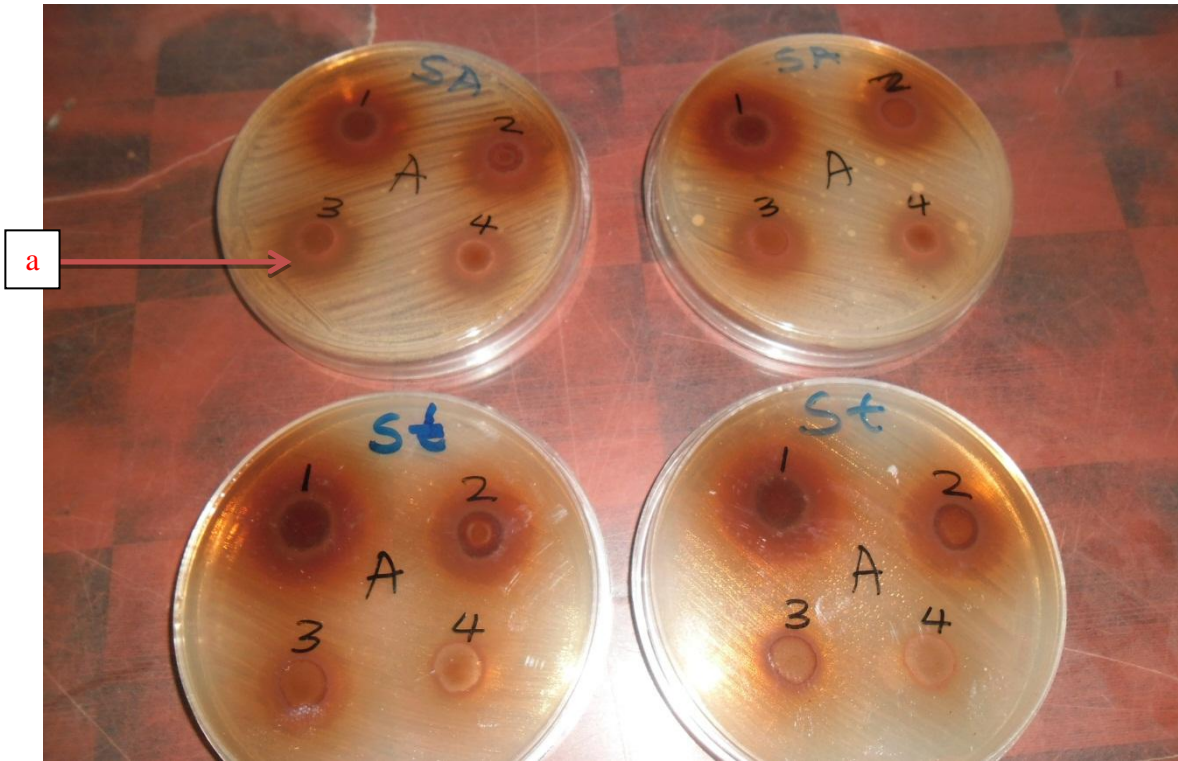


Plate XI: Susceptibility test of the aqueous leaf extract of *K. senegalensis* on *S. aureus* and *S. typhi*

1= 200mg/ml; 2= 100 mg/ml; 3= 50 mg/ml; 4= 25 mg/ml; a= zone of inhibition
St= *S. typhi*; SA= *S. aureus*

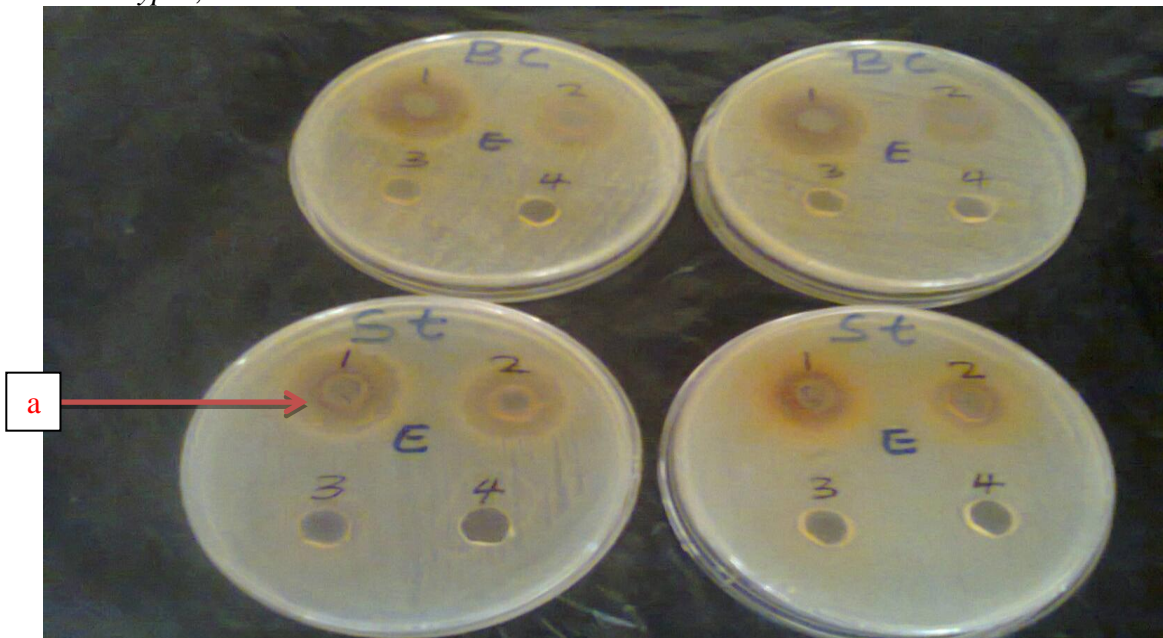


Plate XII: Susceptibility test of the aqueous leaf extract of *A. lebbeck* on *B. subtilis* and *S. typhi*

1= 200mg/ml; 2= 100 mg/ml; 3= 50 mg/ml; 4= 25 mg/ml; a= zone of inhibition

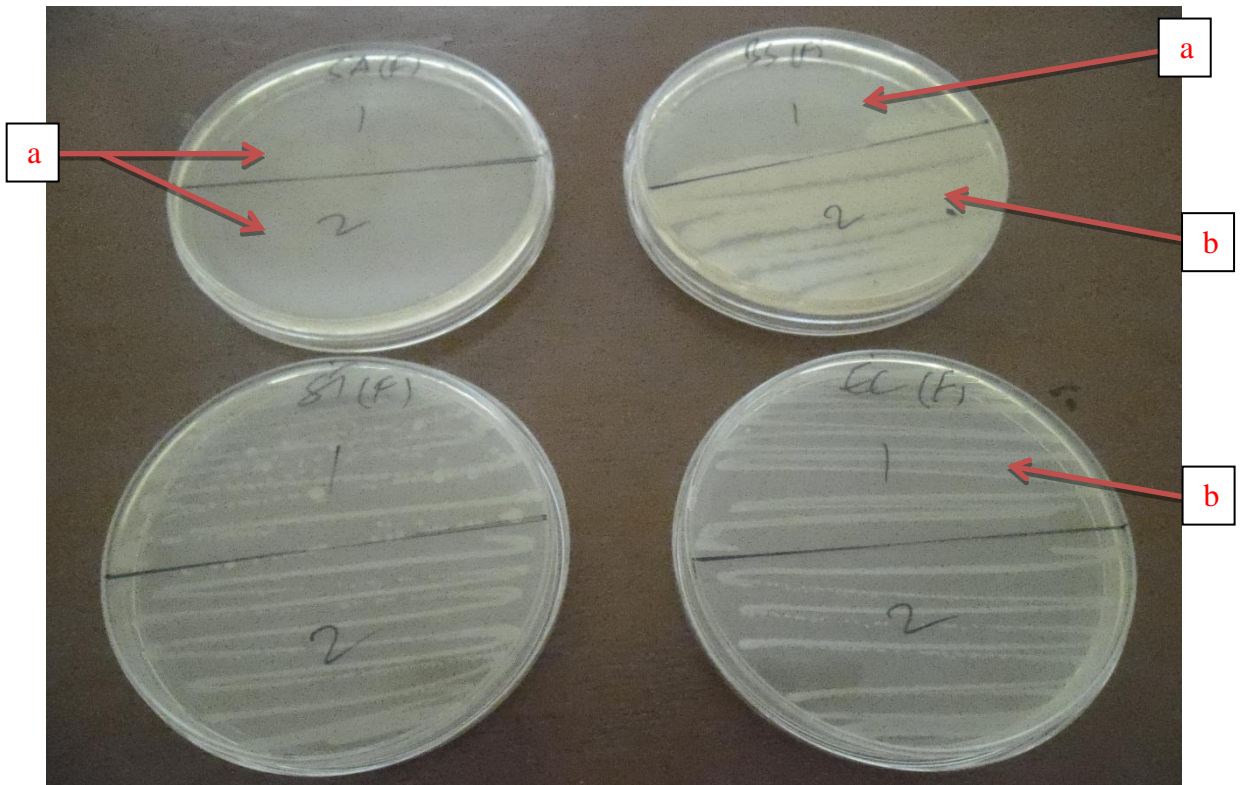


Plate XIII: Minimum Bacteriocidal Concentration (MBC) test of *G. braunii* aqueous leaf extract obtained from *K. senegalensis* on *S. typhi* and *E. coli*.

1= 50 mg/ml; 2= 25 mg/ml

a= no growth of organism (i.e effect of extract was bacteriocidal)

b= growth of organism (i.e effect of extract was bacteriostatic)

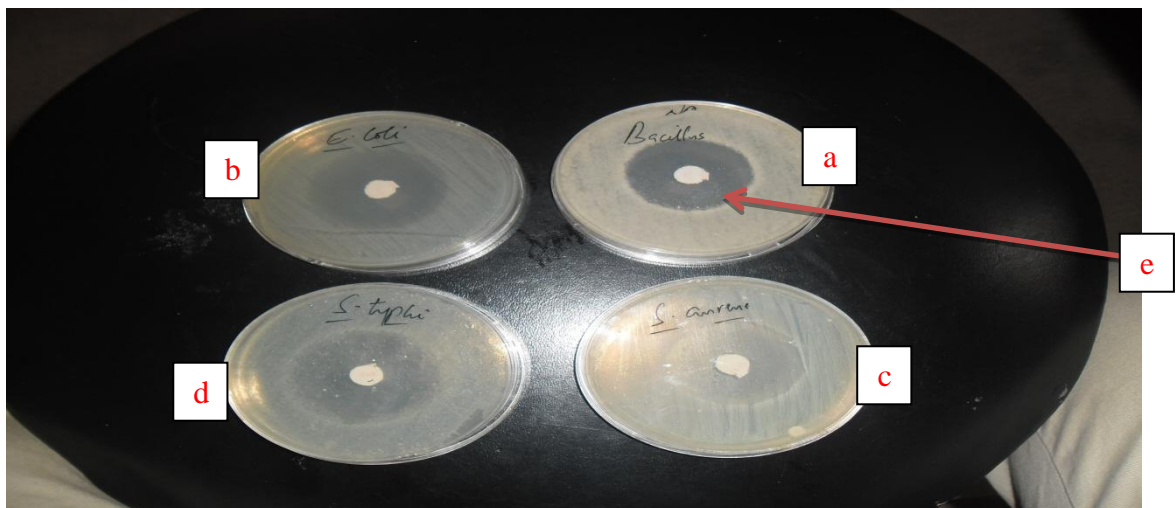


Plate XIV: Susceptibility test of Ciprofloxacin (control)

a= *B. subtilis*; b= *E. coli*; c= *S. aureus*; d= *S. typhi* e= zone of inhibition

Culture media Used

The culture media used for the analysis were Mueller Hinton Agar, MHA (containing 30.0 % beef infusion, 1.75 % casein hydrolysate, 0.15 % starch, 1.7 % agar, pH adjusted to neutral at 25⁰C), Nutrient Agar, NA (containing 0.5 % peptone, 0.3 % beef extract/yeast extract, 1.5 % agar, 0.5 % NaCl, distilled water, pH adjusted to neutral [6.8] at 25⁰C) and Mueller Hinton Broth, MHB (containing beef, dehydrated infusion from casein hydrolysate, starch, pH 7.3±0.1 at 25⁰C).