QUALITATIVE AND QUANTITATIVE ANALYSIS OF THE PHYTOCHEMICALS IN SOME SELECTED SPECIES OF THE GENUS *PHYLLANTHUS* IN ZARIA, NIGERIA

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BY

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AUGUST, 2015

DECLARATION

I declare that this research entitled "Qualitative and Quantitative analysis of the Phytochemicals in some selected species of the Genus *Phyllanthus*" was carried out by me in the Department of Biological Sciences, Ahmadu Bello University, Zaria, under the supervision of Prof. S. P. Bako and Prof. S. O. Alonge. The information gotten from literatures has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at any institution.

Mercy Tope AJIBUA …… ……..
Signature Date

CERTIFICATION

This dissertation entitled "QUALITATIVE AND QUANTITAVE ANALYSIS OF THE PHYTOCHEMICALS IN SOME SELECTED SPECIES OF THE GENUS *PHYLLANTHUS*" by Mercy Tope AJIBUA meets the regulations governing the award of the degree, Masters in Botany of the Ahmadu Bello University, Zaria and it is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to God the Father, My Lord Jesus Christ and the Holy Spirit, and to the memory of my late parents, Mr and Mrs R.J Ajibua.

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ABSTRACT

Phyllanthus species are pan tropical plants originating from Western India. The genus *Phyllanthus* belongs to the family Euphorbiaceae. It is a large genus comprising of about 750 species in the tropical and subtropical regions. The aim of this research work was to compare the qualitative and quantitative analysis of the phytochemicals in the methanol and aqueous extracts of the roots, stem and leaves of the three selected species of the genus *Phyllanthus* i.e. *Phyllanthus discoideus, Phyllanthus amarus* and *Phyllanthus muellerianus*. The three species for this study were collected from the Main Campus of Ahmadu Bello University, Zaria, and also from the outskirts of Zaria, Nigeria. The plant parts were shade dried, pulverized and the secondary metabolites were extracted using two different solvents (methanol and aqueous (water)). The crude extracts of the plant parts (roots, stem and leaves) of the three selected *Phyllanthus* species from the methanol and aqueous solvents were subjected to Qualitative Phytochemical screening and Quantitative determination of the secondary metabolites, using standard procedures. Preliminary screening (qualitatively and quantitatively) of Phytochemicals is a useful and valuable step in the detection of bioactive principles and subsequently may lead to drug discovery and development. The data obtained revealed the presence of medicinally important bioactive compounds such as alkaloids, flavonoids, tannins, saponins, phenols, glycosides, anthraquinones, triterpenes and sterols, although at varying levels/intensities, from the methanol and aqueous extracts of the roots, stem and leaves of the three selected *Phyllanthus* species. The presence of these medicinally active secondary metabolites justifies the use of the three *Phyllanthus* species as herbal treatment of various diseases. These secondary metabolites are known to exhibit medicinal activity as well as

physiological activity. The Quantity of the secondary metabolites varied in the different parts of the three selected *Phyllanthus* species and in the different solvent extracts. In the combined ANOVA of the methanol and aqueous solvents, the highest phytochemical extracted from the three *Phyllanthus* plant parts was flavonoids (1.40g), followed by alkaloids (1.12g), then tannins (0.67g), and saponins (0.46g), the lowest was phenols (0.16g). The combined ANOVA for the three selected *Phyllanthus* species showed that the highest content of the phytochemicals was extracted from their leaves (1.34g), followed by the stem (1.26g), the lowest was from the roots (1.23g). The combined ANOVA for the plant parts of the *Phyllanthus* species under the two solvents showed that the highest content of phytochemicals was extracted from *Phyllanthus muellarianus* (1.38g), followed by *Phyllanthus discoideus* (1.29g) and the lowest was extracted from *Phyllanthus amarus* (1.14g). Among the two solvents used in this study (methanol and water), methanol extracted the highest contents of the Phytochemicals. Their qualitative analysis revealed their appearance, while their quantitative determination gave appropriate idea for the quantity, and this establishes the fact that the three selected plants are not the same and are not likely to have the same medicinal potential. It is noteworthy that while all these Phytochemicals are present in the different *Phyllanthus* species connoting taxonomic affinity, the differences in their levels/intensities uniquely confers individualism on each of the species and thus support their being treated as taxonomic species.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Introduction to Medicinal Plants

Medicinal plants are the nature's gift to man to make disease free healthy life. Humans since creation have been dependent on plants for good health, food, drink, shelter and clothing (Sofowora, 1986).

Plants have been used for centuries as remedy for human diseases because they contain components of therapeutic values. They are natural sources of antimicrobial agents primarily because of the large biodiversity of the organisms and the relatively large quantity of metabolites that can be extracted from them (Nostro *et al*. 2000).

The use of plants for medicinal purposes is ordained by God and has always been part of human culture (Kafaru, 1996). The use of plant as medicinal substitute for synthetic drugs for treatment of various ailments of humans and livestock has increased over the past years. In India, over 8,000 species of medicinal plants have been used by households as alternative medicine for several thousands of years (Mehta *et al*. 2013).

According to World Health Organization (1998), the use of medicinal herbs has become widely popular even in the industrialized countries as a complementary way to cure and prevent diseases. It is also known that about 80% of the world's population does not have access to conventional drugs. Over the last decade, interest in drugs of plant origin and their uses in various diseases management have increased in many developed countries since plants used in traditional medicine are more likely to yield pharmacologically active compounds than developing new drugs synthetically (WHO,1998) .

Abugassa *et al.* (2008), stated that the growing attractiveness and acceptance of herbal medicines by many people as true and innocuous, in contrast to synthetic drugs, and

coupled with the idea that what is natural can only be good. It was also believed that herbal medicines are naturally superior to synthetic drugs.

Herbal prescriptions and natural remedies are commonly employed in developing countries for the treatment of various diseases. This practice is an alternative way to compensate for some perceived deficiencies in orthodox pharmacotherapy (Sofowora, 1993; Zhu *et al.* 2002)

For thousands of years, Africans particularly Nigerians have relied on medicinal plants and its knowledge to treat ailments (Van Wyk *et al.,* 1997). The use of herbs for diseases management in Africa and Nigeria in particular could be traced to early man who probably acquired the skill of healing through deliberate or accidental selection of plants and their parts (Sofowora, 1982).

Medicinal plants, according to Sofowora (1982), are those ones which one or more of its organs contain substances that can be used for therapeutic purposes. It may be in form of vegetable drug, which may either be organized (material which possess a cellular structure e.g. leaf, bark, petal, root etc.). Sofowora (1982), stated that medicinal plants may either be prepared in cold water or by bringing it to boil and then allowing it to cool, or tisane (which is tea made by either decoction or infusion).

Akueshi (1992), defined herbal medicine as the total combination of the knowledge and practice used in diagnosing, preventing or eliminating a physical, physiological or mental diseases. There has been a remarkable increase in the use of herbs lately in Nigeria unlike what the case used to be few years ago, and this may be due to the increasing failure rate of orthodox drugs (Akueshi, 1992).

The use of herbal medicine has been viewed by the pharmaceutical industry as sources of "qualified leads" in the identification of bioactive agents for use in production of synthetic

drugs. World Health Organization (2000), estimated that between 25-50% of modern drugs are derived from plants.

Sanchez-lamar *et al.* (1999), noticed that even though western medicine is well known and in use, but at the same time it has created problems due to some of its side effects such as carcinogenicity caused by synthetic drugs. This has entranced the interest in search of natural products with medicinal property e.g. natural occurring anti-oxidant and antibiotic for use in foods and medicine. Therefore, herbal medicine has been considered as an alternative to eradicate the side effects associated with synthetic drugs.

According to Farnsworth (1990), information on the chemical constituents of medicinal plants does not only aid in discovering new therapeutic drugs, but such information can also help in disclosing new source of economic products such as tannins, oils, gums, that are precursors for the synthesis of complex chemical substances.

The medicinal value of plants lies in some chemical substances or group of compounds that produces a definite physiological action in the human body. These chemical substances are called phytochemicals (Hill, 1974). These chemical substances can also be referred to as secondary metabolites. The most important of these bioactive groups are the alkaloids, terpenoids, steroids, flavonoids, tannins, saponins, glycosides and phenol compounds (Edeoga *et al.* 2006).

According to Adebanjo and Adewumi (1983), these physiological active substances, over the years are technically referred to as “drugs”, and the “drug” has been exploited in herbal medicine for the treatment of various ailments affecting man. The chemical substance in the plants varies as the plants from which they are obtained.

Phytochemicals are chemical compounds that occur naturally in plants. ‘Phyto’ means plant in Greek. Some are responsible for colour and other organoleptic properties, such as the

deep purple of blueberries and the smell of garlic. The term is generally used to refer to those chemicals that may have biological significance, for example anti-oxidants but are not established as essential nutrients. Scientists estimate that there may be as many as 10,000 different phytochemicals having the potential to affect diseases such as cancer, stroke or metabolic syndrome. Salicin having anti-inflammatory and pain relieving properties was originally extracted from the bark of the white willow tree and later synthetically produced became the staple over counter drug, asprin.

Phytochemicals are non nutritive chemicals that have protective or disease preventive properties. The most important of the phytochemicals are alkaloids, flavonoids, tannins, saponins and phenol compounds (Hill, 1974). They are known to have beneficial effect on cardio vascular system, and they as well play a role of prevention of neuro-degenerative disease and diabetes. Phytochemicals are naturally occurring chemical compounds in plant based foods. They protect plants against bacteria, viruses and fungi, and also provide plants with colour, odour and flavor (Keen *et al*. 2005).

Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity (Zheng and Wang, 2003). Studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti-carcinogenic, anti-bacterial, and anti-viral activities (Sala *et al*., 2002).The ingestion of natural anti-oxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing. In recent years, there has been a worldwide trend towards the use of the natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables (Kitts *et al.*, 2000).

The action of phytochemicals varies by colour and type of the food. They may act as anti-oxidants or nutrient or protection or to prevent carcinogens (cancer causing agents) from forming. Some phytochemicals with physiological properties may be elements rather than complex organic molecules. For example selennin which is abundant in many fruits and vegetables is involved with major metabolic pathways, including thyroid hormone metabolism and immune function. There are currently many phytochemicals in trials for a variety of diseases. Lycopene has been tested in human studies for cardiovascular diseases and prostrate cancer. The contributions of Polyphenols to the prevention of cardio-vascular diseases, cancer, osteoporosis and anti-oxidant character with potential health benefits have been reported by Art and Hollman (2005) and Lambert *et al.* (2005).

The plant *Phyllanthus*, of the family Euphorbiaceae is widely distributed in most tropical areas of Africa. *Phyllanthus* is employed as an effective cure in treating several diseases, ranging from diabetes, Malaria, laxative, dysentery, flu, kidney stone elimination e.t.c. (Unander *et al*. 1990)

1.2 Statement of Research Problem

In developing countries like Nigeria, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects (Shariff, 2001).

Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by the extraction of their constituents (Shariff, 2001).

The increasing accumulation of toxins in the human system due to the use of orthodox drugs is causing a lot of degenerating effects on man, such as kidney problems, rapid aging, and development of sickle cells e.t.c (Mann *et al.*, 1978).

The extraction of bioactive agents from plants is one of the most intensive areas of natural product research today, yet the field is far from being exhausted (Sofowora, 2006). However, Sandberg and Bruhn (1979) reported that only around ten percent of all plants that further investigation is worthwhile.

Furthermore, the carcinogenicity of the orthodox drugs has become a fast way of developing cancerous cells by humans. In addition to these, the high cost of getting access to orthodox drugs and medicine has become a problem to the larger population in under developed and developing countries of the world.

Some diseases causal micro-organisms have developed resistance to many orthodox drugs. There is therefore an upsurge of the interest in herbal remedies in several parts of the world with many herbal remedies being incorporated into orthodox medical practice (Montefiore *et al*., 1989).

Phyllanthus plant naturally occurs in the wild with great ethno medicinal antibiotics for healthcare. However, the benefit has not been maximally exploited, because the phytochemical abilities of the plant have not been reported fully.

Although, countries like China, India, America, Brazil and Germany are into packaging and selling of *Phyllanthus* plants, not much is known about the comparative analysis of the qualitative and quantitative properties of the phytochemicals of the plant. .

There is little or no documented literature on the research of the qualitative and quantitative comparative analysis of the phytochemical composition in *Phyllanthus discoideus, Phyllanthus amarus* and *Phyllanthus muellerianus.*

1.3 Justification

The use of *Phyllanthus* species as a medicinal plant has increased over the years, providing modern medicine with numerous plants derived therapeutic agents with low or no side

effects (Evans, 2002). However, ascertaining the degree of the quality and quantity of the phytochemicals of *Phyllanthus discoideus, Phyllanthus amarus* and *Phyllanthus muellerianus* will go a long way in providing the right application to combating different diseases.

This research work focused at comparing the quality and quantity of the Phytochemicals in some selected species of *Phyllanthus.* The ability to ascertain the quality and the quantity of the chemical composition of the plants will give us an insight on how to have a maximum exploitation of these species of *Phyllanthus* for ethno-medicinal and pharmaceutical purposes in order to save lives.

A large proportion of the world population however depends on herbal medicine because of its effectiveness and the fact that plant-based remedies have been highlighted due to their fewer side effects in comparison to synthetic drugs (Mann *et al*., 1978).

Secondary metabolites are Phytochemicals produced as bi- products of primary metabolism (Bako and Aguh, 2007). The qualitative and quantitative analysis of the phytochemicals in the three species of *Phyllanthus* will be used to verify the presence or absence of the classes of Phytochemicals as well as help to ascertain their relatedness or not.

Preliminary screening of phytochemicals is a useful and valuable step in the detection of the bioactive principles present in a medicinal plant and subsequently may lead to drug discovery and development (Manjulika *et al.*, 2014).

Since there is little or no documented literature in research on qualitative and quantitative analysis of the phytochemicals in *Phyllanthus discoideus, Phyllanthus amarus* and *Phyllanthus muellerianus,* this research was aimed at narrowing that gap, and also to provide adequate information on the appropriate application of the species for disease treatment, in the society at large.

The maximum knowledge of the qualitative and quantitative composition of the phytochemicals in *Phyllanthus discoideus, Phyllanthus amarus* and *Phyllanthus muellerianus* will help us in knowing how to make use of these plants in the treatment of diseases and ailments.

Sandberg and Bruhn (1979), reported that only around 10% of all plants had been investigated in detail for bioactive agents. For this reason alone it can be argued that further investigation is worthwhile.

Another justification for screening plants for bioactive agents is that by isolating such an agent it is possible to demonstrate that the reported physiological activity of the plant is real. The fact that this activity has been shown to be due to a particular chemical compound makes detailed pharmacological and other academic studies possible (Soforowa, 2006).

Academically, the phytochemical screening of bioactive constituents in plants can help to provide chemotaxanomic evidence for the classification of genera or species, especially those whose classification is based on morphological grounds alone (Sofowora, 2006).

1.4 Aim

The aim of this research work was to compare the qualitative and quantitative analysis of the phytochemicals in the methanol and aqueous extracts of the roots, stem and leaves of the three selected species of the genus *Phyllanthus* i.e. *Phyllanthus discoideus, Phyllanthus amarus* and *Phyllanthus muellerianus.*

1.5 Objectives

The objectives of this study were to:

1. To detect and compare the quality of the Phytochemicals present in the methanol extracts of the roots, stem and leaves of *Phyllanthus discoideus, Phyllanthus amarus* and *Phyllanthus muellerianus*

2. To detect and compare the quality of the Phytochemicals present in the aqueous extracts of the roots, stem and leaves of *Phyllanthus discoideus, Phyllanthus amarus* and *Phyllanthus muellerianus.*
3. To determine and compare the quantity of the Phytochemicals present in methanol extracts of the roots, stem and leaves of *Phyllanthus discoideus, Phyllanthus amarus* and *Phyllanthus muellerianus.*
4. To determine and compare the quantity of the Phytochemicals present in aqueous extracts of the roots, stem and leaves of *Phyllanthus discoideus, Phyllanthus amarus* and *Phyllanthus muellerianus.*
5. To compare the quality and quantity of the Phytochemicals extracted by the methanol solvent and aqueous solvent from the roots, stem and leaves of *Phyllanthus discoideus, Phyllanthus amarus* and *Phyllanthus muellerianus*

1.6 Hypotheses

- There is no difference in the quality of the Phytochemicals in the methanol crude extracts of the roots, stem and leaves of *Phyllanthus discoideus, Phyllanthus amarus,* and *Phyllanthus muellerianus*
- There is no difference in the quality of the Phytochemicals in the aqueous crude extracts of the roots, stem and leaves of *Phyllanthus discoideus, Phyllanthus amarus,* and *Phyllanthus muellerianus* .
- There is no significant difference in the quantity of the Phytochemicals in the methanol crude extracts of the roots, stem and leaves of *Phyllanthus discoideus, Phyllanthus amarus,* and *Phyllanthus muellerianus.*

- There is no significant difference in the quantity of the Phytochemicals in the aqueous crude extracts of the roots, stem and leaves of *Phyllanthus discoideus, Phyllanthus amarus,* and *Phyllanthus muellerianus.*
- There is no significant difference in the quantity of the Phytochemicals extracted by methanol solvent and aqueous solvent from each of the roots, stem and leaves of *Phyllanthus discoideus, Phyllanthus amarus,* and *Phyllanthus muellarienus.*

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Natural Plant Products and Medicine

The early medicines of man were obtained from natural sources; empiricism, superstition and traditional medicinal folklore have guided man to find healing in herbs and barks, fruits, leaves, roots, seeds, and stems of plants under different climates in different parts of the world. Lichens and Fungi have also had their share as contributive sources of drugs for centuries (Sofowora, 1989).

Although plants are unique in their activities, but it has also been found that a particular plant may be used by different tribes or countries for different ailments, this shows that plant possesses a very wide range of healing powers which is attributed to the natural plant products which constitute their chemical composition.

Plants are immensely complicated factories, which turn the relatively simple ingredients of air and water into enzymes, sugar, protein, solid cellulose, liquid oils, and scents to attract pollinating insects and poison to kill off predators, and also producing secondary metabolites to combat diseases (Mabey, 1977).

2.2 Medicinal Plants

Ethno-medicinal study is today recognized as the most viable method of identifying new medicinal plants or refocusing on those earlier reported for brochure constituents (Farnsworth, 1990). For thousands of years, Africans have relied on medicinal plants and its knowledge thereof, to treat ailments (Van Wyk *et al.*, 1997).

Medicinal plants are capable of synthesizing an overwhelming variety of low-molecular-weight compounds. Presently, 100,000 such compounds have been isolated from higher

plants (Verpoorte and Memelink, 2002). The biosynthesis of secondary metabolites varies among plants even in different organs of plants (Khan *et al*., 2010).

The use of medicinal plants predates the introduction of antibiotics and other modern drugs into the African continent. Africans have been able to cure a lot of diseases by using concoctions made from different plants, and these have been passed from generation to generation.

Plants have been used as sources of traditional drugs and pharmaceutical preparations for man and other animals. According to a survey by the Uanited Nations Commission for Trade and Development (UNCTAD, 1974), more than 33% of modern drugs and medicinal products are derived from plants (UNCTAD, 1974).

Medicinal plants have always played a key-role in the world health, since they are the sources of many important scientific drugs of modern world (UNCTAD, 1974). It is not a surprise that the earliest drugs in the history of medicine were all derived from plants e.g.

- Quinine derived from the bark of cinchona
- Morphine derived from seed capsules of opium poppy
- Penicillin derived from the mould *Penicillum notatum*
- Acetylsalicylic derived from the willow bark, used originally for headaches, and has both analgesic and *anti-pyretic* properties
- Colechine derived from the root of meadow saffron (Mabey, 1977).

Medicinal herbs have been used for healing as an alternative to medicine by all cultures for several thousands of years. About 80% of the world's population does not have access to conventional drugs and therefore rely on medicinal herbs (Abugassa *et al.,* 2008).

Western medicine is well known and in use, but at the same time it has created problems due to some side effects such as carcinogenicity caused by the synthetic drugs in Western Medicine. This has enhanced the interest in search for natural products with medicinal property e.g. naturally occurring anti-oxidants and anti-biotic for use in foods and medicine. Therefore ethno-medicine, using medicinal herbs has been considered an alternative, in order to eradicate side-effects associated with synthetic drugs (Sanchez-lamar *et al.,* 1999).

In Nigeria, the use of medicinal plants has become a common practice. This practice concentrates solely on the physical aspects of man's healing (Akueshi, 1992). The Federal Government of Nigeria has taken one bold step to explore the pharmaceutical potentials of medicinal plants. A National Institute for Pharmaceutical Research and Development was established at Abuja in 1997. This institution relies mainly on plants as sources of raw materials. NIPRISAN, NIPRIPAN and NIPRIFAN are plant medicinal products recently developed by the Institute for the treatment of sickle cell anemia, peptic ulcer and skin fungal infection respectively (personal communication).

The World Health Organisation (1978), defined traditional medicine as the sum total of all knowledge and practices, whether explicable or not, used in diagnosis, prevention, elimination of physical, mental or social imbalance and relying exclusively on practical experiences, and observation handed down from generation to generations whether verbally or in writing.

The toxic side effect of the drugs of modern medicine and the lack of medicines for many chronic ailments has led to the reemergence of the herbal medicine, with possible treatments for many health problems. Consequently, the use of plant-based medicine has increased all over the world (British Medical Association, 1993).

2.3 The Secondary Metabolites and their Medicinal Uses

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants is hidden in some chemical substances that produce a definite physiological action on the human body, and these chemical substances are known as secondary metabolites which can also be called phytochemicals (Hill, 1974).

The plant secondary metabolites vary from plant to plant, thus the plant kingdom provides a tremendous reservoir of various chemical substances with potential therapeutic purposes. These secondary metabolites constitute important sources of pharmaceutical drugs (Adebanjo and Adewumi, 1983).

The following are some of the active constituents occurring naturally in medicinal plants:-

2.3.1 Alkaloids

These are the most important among the secondary metabolites, they comprises the largest single class of secondary substances of which over 2000 are known (Godwin and Mercer, 1983). They are basic compounds that are derived from plants. In nature, alkaloids exist in large proportions in the seeds and roots of plants and often in combination with vegetable acids (Madziga *et al.,* 2010). It contains one or more nitrogen atoms (usually in a heterocyclic rings). The compounds have basic properties and are alkaline in reaction, turning red litmus paper blue. In fact, one or more nitrogen atoms that are present in an alkaloid, typically as 1°, 2° or 3° amines, contribute to the basicity of the alkaloid. The degree of basicity varies considerably, depending on the structure of the molecule, and presence and location of the functional groups (Sarker and Nahar, 2007). They react with acids to form crystalline salts without the production of water (Firn, 2010). Majority of alkaloids exist in solid form such as atropine, some as liquids containing carbon, hydrogen, and nitrogen. Most alkaloids are readily soluble in alcohol. They have great physiological

effect on man and other animals. Alkaloids are the most potent therapeutic compounds that have been manufactured as various allopathic drugs, including the pain-killer morphine and anti-malaria quinine. They are usually colourless and often optically active substances; alkaloid can be detected in fresh leaves by their bitter taste. In their native environment, they usually occur in the form of salts of simple organic acid, tartaric acid and citric acid and they are known to contain a lot of pharmacological properties (Trease and Evans, 1992).

The investigation of bioactivity of alkaloids reveals that they are used medically as antispasmodic, mydriatic, local anesthetic drug (Harborne, 1973). They are mostly used as anti-depressant (morphine), stimulants (caffeine), anesthetics (cocaine), anti-tumor (vinblastine), anti-malaria (quinine), anti-bacteria (berberine) and amoebicide (emetine) (Heinrichn *et al.*, 2004).

These are widely used in medicinal purposes which have positive and negative effects even to human beings. Most of the plants have alkaloids in different organs with different chemical configurations (Harbourne, 1984). Alkaloids are reported to have analgesic, anti-inflammatory and adaptogenic activities which help to alleviate pains, developed resistance against diseases and endurance against stress. They also have a protective role in animals (Edeoga *et al.* 2006).

Alkaloids are heterogeneous group of compounds which contain one or more nitrogen atom in acyclic system. The following are some of the examples of alkaloids:

i. Cordeine: this is widely used in the production of cough expectorant. It is active in depressing the cough centre in the medulla oblongata.

ii. Morphine: this can be used in the treatment of terminal cancer, and it can also be used to help to relieve severe pains at post operation discomfort, it can also be used as a pre anesthetic.

iii. Hernine: this can be used as a more potent analgesic than morphine which is used as an anti-tussive agent.

iv. Reserpine: this can be used as anti-hypertensive and psychotherapeutic analgesic (Edeoga *et al.* 2006).

2.3.2 Saponins

The term Saponin is derived from *Saponaria vaccaria (Quillaja saponaria),* a plant, which abounds in saponins and was once used as soap. Saponins therefore possess "soaplike" behavior in water, i.e they produce foam. On hydrolysis, an aglycone is produced, which is called sapogenin. There are two types of sapogenin: steroidal and triterpenoidal. There are two major groups of saponins and these include: steroid saponins and triterpene saponins. Saponins are regarded as high molecular weight compounds in which, a sugar molecule is combined with triterpene or steroid aglycone. Saponins are soluble in water and insoluble in ether, and like glycosides on hydrolysis they give aglycones. Saponins are extremely poisonous, as they cause heamolysis of blood and are known to cause cattle poisoning (Kar, 2007). They possess a bitter and acrid taste; besides causing irritation to mucous membranes they are mostly amorphous in nature, soluble in alcohol and water, but insoluble in non-polar organic solvents like benzene and n-hexane (Kar, 2007).

These are glycosides of both triterpenes and sterols. They are the surface active agents and they possess soap-like properties. They are usually detected by their ability to foam persistently and to haemolyse blood cells (Trease and Evans, 1989). It has been discovered that saponins and other flavonoid compounds at low concentrations inhibited the growth of

micro-organisms and they can also act as bactericidal agents at higher concentrations by coagulating protoplasm of the organisms. Saponins are sources of sapogenins which can be converted in the laboratory to animal sterols of therapeutic importance (Sofowora, 1993).

Saponins are terpene glycosides. Saponin is useful in medicine and pharmaceutical industry due to its foaming ability that produces frothy effect. Saponin is also used in the manufacture of shampoos, insecticides and various drug preparations and in synthesis of steroid hormones (Okwu, 2004). Generally, saponins are toxic, but researches have recently shown that consumption of saponins by human beings may be beneficial in reducing heart disease (by binding of saponins with plasma membrane and cholesterol). The presence of steroidal saponins could develop resistance to viral diseases. Finar (1989) reported that, saponins had expectorant action which is very useful in the management of upper respiratory tract inflammation. So these plants may be used to treat various ailments.

2.3.3 Terpenoids

Terpenoids form the largest group of plant products and are the most common ingredients in volatile oils.They are among the most widespread and chemically diverse groups of natural products. They are flammable unsaturated hydrocarbons, existing in liquid form commonly found in essential oils, resin or oleoresins (Firn, 2010). Terpenoids includes hydrocarbons of plant origin of general formula $(C_5H_8)n$ and are classified as mono-, di-, tri- and sesquiterpenoids depending on the number of carbon atoms. Examples of commonly important Monoterpenes include terpinen-4-ol, thujone, camphol, eugenol and menthol. Diterpenes are classically considered to be resins. The triterpenes include steroids, sterols and cardiac glycosides with anti- inflammatory, sedative, insecticidal or cytotoxic activity. Sesquiterpenes like monoterpenes, are major components of many essential olis (Martinez *et al.,* 2008). They include camphor, beta-carotene, and digitalin, which are

refered to as iso-prenoids due to the fact thet all terpenoids are derived from a 5-carbon precursor isoprene. Terpenoids are categorized as monoterpenoids and monoterpenoid lactones, sesquiterpenoids and sesquiterpenoids lactones, diterpenoids and triterpenoids. Out of all these, terpenoids form the largest compounds. These plant products possess interesting biological activities. They contain anti-insect, anti-fungal and anti-bacterial properties (Sofowora, 1993).

2.3.4 Essential oils

These are also called volatile oils. They have odoriferous substances that are selected in the secondary duct or cavity or in glandular hair. They have the tendency to evaporate on exposure to air even at ambient conditions and are therefore also referred to as volatile oils or ethereal oils (Martinez *et al*., 2008). They are widely distributed in various organs and tissues of plants and differ in chemical and physical compositions. They can serve as a mode of transportation to distribute medicine equally throughout the body, they act as antiseptics, stimulate tissues that they come in contact with, counter – irritants, increase saliva and stimulate the heart muscle (Evans, 2002).

Chemically, they are made of hydrocarbons, which are responsible for their odour and colour (Trease and Evans, 1989). Examples include oil of *Eucalyptus*; used for therapeutic actions, oil of lemon; used for flavouring and oil of rose; used for perfume purposes. Some examples of compounds from essential oils are murcene as from oil of bay; methanol from mint and carvone.

Essential oils have been associated with different plant parts including leaves, stems, flowers, roots or rhizomes. Chemically, a single volatile oil comprises of more than 200 different chemical components, and mostly the trace constituents are solely responsible for attributing its characteristic flavor and odour (Firn, 2010).

2.3.5 Glycosides

Glycoside is a generic term usually used for natural product that is chemically bonded to sugar(s) e.g cardiac glycosides. These are complex compounds in plants and they are confined to angiosperms (Godwin and Mercer, 1983).

Glycosides in general, are defined as the condensation products of sugars (including polysaccharides) with a host of different varieties of organic hydroxyl (occasional thiol) compounds (invariably monohydrate in character).

Glycosides are colourless crystalline of carbon, hydrogen and oxygen containing water-soluble phytoconstituents, found in cell sap (some contain nitrogen and sulphur). Glycosides are neutral in reaction and can be readily hydrolysed into its components with mineral acids. Glycosides are classified on the basis of type of sugar component, chemical nature of aglycone or pharmacological action. Chemically, glycosides contain a carbohydrate (glucose) and a non-carbohydrate part (aglycone or genins). They are complex groups which can be broken down to yield one or more sugar (glycones) (Kar, 2007; Firn, 2010).

2.3.6 Steroidal Compounds

Plant steroids (or steroid glycosides) also referred to as “cardiac glycosides” are one of the most naturally occurring plant phytoconstituents that have found therapeutic applications as arrow poisons or cardiac drugs (Firn, 2010).

Steroids are natural products in a class of widely distributed compounds, which develop and control the reproductive tracts in humans, and induce sexual reproduction in aquatic fungi. Therapeutically, steroids contribute cardiotonics, vitamin D precursors, anti inflammatory agents (corticosteroid) and anabolic agents (androgen). In plants steroid content is divided into steroid saponins which are very similar to triterpenoid saponins in

the terpenoid group. Steroid compound serve many functions for humans. Combined steroids derived from plants are used for medical purposes that range from topical antibiotics to relieving dysmenorrhea (Trease and Evans, 1992).

2.3.7 Tannins

This is a term used to describe a group of compounds in some plants which can 'tan' (convert) animal skin to produce leather. They are colourless and non crystalline compounds (Trease and Evans, 1989). They are widely distributed in plant flora. They are phenolic compounds of high molecular weight. Tannins are soluble in water and alcohol and are found in the root, stem, bark and outer layers of plant tissue. They are acidic in reaction, and the acidic reaction is attributed to the presence of phenolics or carboxylic group (Kar, 2007). They form complexes with proteins, carbohydrates, gelatin and alkaloids

Tannins are used as anti-septic and this activity is due to the presence of the phenolic group. They also serve as anti-microbial agents in plants. They form colloidal solutions in water and precipitate proteins from solutions. Two basic groups are usually recognized: hydrolysable tannins and condensed tannins. Hydrolysable tannins, upon hydrolysis, produce gallic acid and ellagic acid and these are called gallotannins or egallitannins. On heating, they form pyrogallic acid. Common examples of hydrolysable tannins include theaflavins (from tea), diadezein, genistein and glycitein (Kar, 2007).Tannins rich medicinal plants are used as healing agents in a number of diseases. Tannins were also reported to have demonstrated activity against bacteria.

2.3.8 Phenols

These are also called poly phenols or phenolic compounds and they are widely found throughout the plant kingdom. They are chemical compounds that occur ubiquitously as

natural colour pigments responsible for the colour of fruits of plants. Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL). They are very important to plants and have multiple functions. The most important role may be in plant defence against pathogens and herbivour predators, and thus are applied in the control of human pathogenic infections (Pwupponeu-Pima *et al.*, 2008). They are classified into (i) phenolic acids and (ii) flavonoid polyphenolics (flavones, flavonones, xanthones and catechins) and (iii) non- flavoned polyphenolics. Caffein is regarded as the most common of phenolics compounds distributed in the plant flora followed by chlorogenic acid known to cause allergic dermatitis among humans (Kar, 2007). They have an aromatic ring bearing one or more hydroxyl groups. The most common phenol is the flavonoids that constitute about 8,000 of the recognized phenols. Phenols can be broken down into phenyl propanoids, anthones, stilbenoid and quinines. Flavonoids are molecules responsible for the colour of fruits and flowers. The most important role may be in plant defence against pathogens and herbivore predators, and thus are applied in the control of human pathogenc infections (Pwupponen-Pinna *et al.*, 2008). They are beneficial to man as powerful anti-oxidants, stress modifiers, anti-allergic agents, anti-viral compounds, stimulant of protein synthesis, anti-inflammatory agents, vaso-propertive activity, diuretic, anti-spasmodic, anti-bacterial and anti-fungal (Evans, 2002).

Research has shown that polyphenols contribute to the prevention of cardiovascular diseases, cancers, osteoporosis and anti-oxidant character with potential health benefits.

Phenols are reported anti-tumour agents and are known to exhibit anti-viral and anti-microbial activities, hypotensive effects and anti-oxidant properties (Arts and Hollman, 2005).

2.3.9 Flavonoids

Flavonoids are important group of polyphenols widely distributed among the plant flora. Structurally they are made of more than one benzene ring (a range of C15 aromatic compounds) and numerous reports support their use as anti-oxidants or free radical scavengers (Kar, 2007). The compounds are derived from parent compounds known as flavans. Over four thousand flavonoids are known to exist as pigments in higher plants.

Flavonoids are 15 carbon compounds generally distributed throughout the plant kingdom (Harbourne, 1991). Flavonoids have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's raction to allergies, virus and carcinogens. More recent research has enabled scientists to group them into classes on the basis of similar protective functions as well as individual physical and chemical characteristics of the molecules. Flavonoids have been reported to be synthesized by plants in response to microbial infection and have been shown to have anti-bacterial activities. They show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity (Aiyelagbe and Osamudiamen, 2009).

2.3.10 Anthraquinones

These are derivatives of phenolics and glycoside compounds. They are solely derived from anthracene giving variable oxidized derivatives such as anthrones and anthranols (Firn, 2010). Other derivatives such as chrysophanol, aloe-emodin, rhein, salinos poramide, luteolin and emodin have in common a double hydroxylation at positions C-1 and C-8.

2.4 Botanical Description of *Phyllanthus*

The name *Phyllanthus* is a Greek word for "leaf flowers" (because the flowers of most of the species are borne on the leaves). *Phyllanthus* is one of the largest genera in the family

Euphorbiaceae (Spurge family), of the flowering plants. An estimate of the number of the species in this genus varies widely from 750 to 1200 (Cabieses, 1993).

The members of the genus *Phyllanthus,* are mostly upright or prostrate herbs or shrubs, often with milky acrid juice. The *Phyllanthus* species are commonly found around all tropical regions of Africa, Asia, America, Australia and Europe. The plant has a remarkable diversity of growth forms including annual and perennial herbs, shrubs, climbers, floating aquatics and pachycaulous succulents. Almost all the species have simple leaves. Some of the species have flattened leaf like stems called cladoles. It has a wide variety of floral morphologies and chromosome numbers and has one of the widest ranges of pollen types of many seed plants (Cabieses, 1993).

Most of the common species in West Africa are monoecious, with axillary cluster of spike-like inflorescences covered with minute green flowers. All flowers have 4 to 6 sepals, while male flowers have additional 3 to 6 stamens surrounded by a disc of glands, and female flowers has a pistil. The ovary cells may become divided by extra walls, and so may appear to have up to 6 separate 1- seeded cells, when the fruits burst open the seeds are hurled away. Seeds are triangular (like an orange segment), light brown, 1 mm long, with 5 to 6 ribs on the back (Morton, 1981). Fruits are red or black, 3- lobed and berry-like, drying and splitting open later.

Despite their variety, almost all *Phyllanthus* species express a specific type of growth called "phyllantoid branching " in which the vertical stems bear deciduous floriferous (flower bearing), plagiotropic (horizontal or oblique) stems. The leaves on the main vertical axes are reduced to scales called "cataphylls", while leaves on the other axes develop normally (Morton, 1981). *Phyllanthus* is widely distributed in all tropical and sub-tropical regions of the earth.

2.4.1 ***Phyllanthus discoideus*** (Baill.)

Phyllanthus discoideus is a synonym of *Magaritaria discoidea* (Baill.) G.L Webster.

This is a many-stemmed, densely branched spreading to somewhat deciduous shrub or tree up to 25m high (rarely more) often with a flattened crown. The stem bark is usually straight, rough, grey or brownish on top and reddish beneath (Pooley, 1993), flaking in irregular strips, the branches of young trees grow horizontally from the stem. Twigs are lenticellate. Petioles are 1-9mm long, grooved, glabrous to densely crisped-puberulous. Stipules are 2-13mm long, linear lanceolate or oblong, acute or obtuse, entire, membranous, soon falling. The leaves are alternate and produced on one plane (Pooley, 1993). Leaves blades are obovate to elliptic-lanceolate, obtuse or rounded to acutely acuminate, rounded to cuneate at the base, chartaceous, glabrous above and beneath. The inflorescence bracts 2-3mm long, ovate, chaffy, brownish or blackish. Male and female flowers are produced on separate trees with both types of flowers being small, greenish-yellow in colour and fragrant (Pooley, 1993). The male flowers are long, glabrous or sparingly pubescent; the female flowers are long. The fruit is a three-lobed capsule about 10mm in diameter and golden brown when ripe. The seeds are exo-testa fleshy and bright glossy metallic-blue or purplish-blue when fresh, drying papery and grayish – white; and endo-testa plano-convex and smooth (Pooley, 1993).

This species has a complicated taxonomic history with many synonyms, partially because of its morphological variability. These were formerly placed in the genus *Phyllanthus* and Euphorbiaceae family, four varieties of this plant are now recognized (Morton, 1981), these having in the past been treated variously as distinct species, subspecies. It is synonyms with typical *Margaritaria discoidea.*

This species is distributed from the coastal areas of the Eastern Cape, South Africa to Tropical Africa as far as Senegal in West Africa.

2.4.2 *Phyllanthus amarus*

Phyllanthus amarus (family: Euphorbiaceae) is widely distributed in all tropical and subtropical regions of the planet (Edeoga *et al*., 2006) and Paleobotanical studies have not found the exact geographic origin of this plant. This plant may be indigenous to the tropical Americas (Cabieses, 1993; Morton, 1981) and the Philippines or India (Cabieses, 1993). It is a common pantropical weed that grows well in moist, shady and sunny places (Cabieses, 1993).This is a glabrous herb with slender sub-woody stem. It is a widely distributed small, erect tropical annual herb or shrub that grows to a height of about 10 to 50 cm.

This is a common weed which can be found along the roads, in valley, on the riverbanks and near lakes. It can grow well in moist, shady and sunny places (Cabieses, 1993). The stem has green capsule and the plant blooms with flowers having 5 white sepals and apical acute anther. The fruit has green capsules and smooth and fruiting pedicels. The seeds are longitudinally rugose. It is locally called Iyin-Olobe (Yoruba, South-West Nigeria). (Adeneye *et al.,* 2006).

Pyllanthus niruri and *Phyllanthus amarus* are very closely related in appearance and in phytochemical structure. The major difference between these two is that *Phyllanthus niruri* has larger leaves and the plants as a whole is bigger when compared to *Phyllanthus amarus*. Reorganization of the *Phyllanthus* genus has been however classified as *Phyllanthus amarus* as type of *Phyllanthus niruri. Phyllanthus amarus* is usually misidentified with the closely related *Phyllanthus niruri* in appearance, phytochemical structure and history of use (Morton, 1981).

2.4.3 *Phyllanthus muellarianus* (Kuntze) Exell

Phyllanthus muellerianus (Kuntze) Exell, is a glabrous shrub or woody climber, occasionally arborescent, it is about 12 metre tall found in savannah and drier secondary forests common in coastal thickets and scrub and widespread in parts of tropical Africa (Pooley, 1993). The main branches are stout, angular, reddish tinged, branchlets 15-20 cm long, with several short axillary shoots; branch basis transformed into a pair of spines 4 cm long, purplish brown. Leaves alternate, distichous along lateral twigs, simple, glabrous, stipules lanceolate, 2 mm long, acuminate; 3-5 mm long; blade ovate, elliptical-ovate to lanceolate ovate, 3-9 cm x 2-4.5 cm, base cuneate to rounded, apex acute to obtuse, with 10-14 pairs of lateral veins. Inflorescence a false raceme on short axillary shoots, 2-6cm long, solitary or several together, with flowers in clusters having 2-3 male flowers and one female flower in each cluster. Flowers unisexual; perianth lobes 5, free, minutely warted, fleshy stamens 5, free unequal, anthers very knobbly, fleshy , ovary superior, ellipsoid, warty, 4-5 celled, styles 4-5, free, green becoming red, later black, 6- seeded. Seeds angular, 1mm long, with faint ridges, bright reddish or yellowish brown. It has been used traditionally in the treatment of various ailments (Pooley, 1993).

Phyllanthus muellerianus is commonly known in Nigeria as, 'Igbehen' = horns of fish (Edo), nkana (Efik), 'Kuma' = a thicket, from the habit (Hausa), 'Anya nnumi' = birds eye (Igbo), 'egu eza' (igbo), 'Arunjeran' or 'Arunyeran' (Yoruba) (Omodara *et al*., 2013). It is a shrub or climber, occasionally arborescent, of deciduous and secondary forest from Guinea-Bissau and Mali to West Africa. In Edo and Delta states, it is reported as weed of ricefield, by lack of timely cultivation. The stem seldom becomes large, clear potable water may be obtained from the cut stem and this sap is used in Sierra Leone to relieve ophthalmia and in

Nigeria for pain in the eyes or to remove foreign body. In Nigeria, twigs of the plant are used as chewing-stick after removal of the surface spines (Isawumi, 1978).

2.5 Ethno-Medicinal Utilities of *Phyllanthus*

The use of the genus *Phyllanthus* is largely attributed to the wide distribution of the plant in many tropical and subtropical countries, large number of species availability, diversity of secondary metabolites and their broad therapeutic use in folk medicine. Studies on the use of *Phyllanthus* plant in traditional medicine which in turn has revealed the potential and beneficial therapeutic actions of the plant in the management of certain illnesses and diseases such as inflammatory reactions, intestinal problems, hepatitis B, kidney and urinary problems (Calixto *et al.*, 1998).

Phyllanthus species has recently become a focal point of several studies due to their broad therapeutic use in folk medicine and their wide distribution as well as their diverse secondary metabolite entities (Kalidas and Mohan, 2009).

Phyllanthus is a genus that consists of species with medicinal value in which bioactive compound have been isolated and used in treating some bacterial and viral infections. *Phyllanthus* are regarded as a source of new anti-viral compounds and showed the versatility of this genus (Unander *et al.,* 1990).The genus *Phyllanthus* has been intensively studied clinically for its anti-viral effects. A systematic review of 22 randomized clinical trial showed that *Phyllanthus* species have positive effects on anti-viral activity and on liver biochemistry in chronic hepatitis B virus infection (Calixto *et al.,* 1998; Liu *et al*., 2001).

Phyllanthus appears to be promising in the treatments of patients with chronic Hepatitis B Virus (HBV) infection. Bioactive principles like alkaloids, tannins, flavonoids, lignans, phenols and terpenes have been isolated from various species of *Phyllanthus* and their compounds showed antinoniceptive activity (Cechinel *et al*., 2001). It was reported by Liu

et al. (2001) that the effectiveness and biosafety of genus *Phyllanthus* for chronic HBV infections.

In India, there has been an upsurge of interest in the *Phyllanthus* plants regarding their therapeutic potentials for the management of a number of diseases (Kumaran and Karunakaran, 2007). *Phyllanthus* species have been tested for their anti- HIV activity *in vitro* and *in vivo*. They inhibited the HIV-key enzymes like integrase, reverse transcriptase and protease (Calixto *et al*., 1998; Notka *et al*., 2004). The infusion of leaves, stem and roots of *Phyllanthus* species are commonly used in folk medicine for treating intestinal infections, diabetes, hepatitis B virus and alignment of the kidney and urinary bladder. Antiviral effects of the plant extracts against hepatitis B virus and possibly against the reverse transcriptase of retroviruses have been reported (Thygarajan *et al.,* 1988)

According to Liu *et al*. (2001), the *Phyllanthus* plant are commonly used to expel kidney stones, support kidneys, increase urination, relieve pain, protect and detoxify the liver, reduce spasms and inflammation, kill viruses and bacteria, aid digestion, reduce fever and blood sugar, lower blood pressure and cholesterol, treat malaria, prevent mutation, as a mild laxative and as a worm expellant.

The bruised leaf and fruit of a *Phyllanthus* plant is applied by the Indians as a dressing to abscesses. The root of a *Phyllanthus* plant is a Zigula (South Africa) gonorrhea remedy (Mdlolo *et al*., 2007). A *Phyllanthus* plant is a component of a Teita (South Africa) remedy for bubonic plague (Mdlolo *et al*., 2007). Such medicinal plants can be used in the management of HIV/AIDS (Adegoke and Adebayo, 2009).

Additional studies on callus and root extracts of different species of *Phyllanthus* have shown the presence of phyllemblin - tannin which has anti-microbial activity, and the hydrolyzable tannins inhibited DNA polymerase and reverse transcriptase, of geraniin and

its derivatives which showed high activity in the inhibitions of HIV reverse transcriptase and angiotensin-converting enzyme involved in diabetic complications (Unander, 1990).

In Brazil, infusion of leaves, stems and roots of *Phyllanthus* species has been used in folk medicine for treating intestinal infections, diabetes and disturbances of the kidney (Calixto *et al.,* 1998). An alcoholic extract of *Phyllanthus niruri* was found to reduce significantly the blood sugar in normal rats and in alloxan diabetes rats, and indicates its potential anti-diabetic action (Raphael and Kuttan, 2003). *Phyllanthus niruri* extract also showed inhibitory activities against angiotensin converting enzyme (ACE) and aldose reductase (AR), which play a significant role in the reduction of aldose to alditol under abnormal conditions such as diabetes (Shimizu *et al*., 1989). *Phyllanthus niruri* was also used as a hypoglycemic agent in traditional medicine to control non-insulin dependent *Diabetis mellitus.* Most *Phyllanthus* plants have been reported to have pharmacological properties e.g extracts of *Phyllanthus emblica* showed good inhibitory effects against *Staphylococcus aureus, Staphylococcus typhosa,* and *Candida albicans* (Asha *et al.,* 2004).

Extracts of *Phyllanthus emblica* L. have been reported to possess several pharmacological actions e.g analgesic, anti-inflamatory, anti-oxidant, chemoprotective, and hypolipidaemic and anti HIV-1 (Human immunodeficiency virus -1), (Asha *et al.,* 2004).

Hexane extracts of *Phyllanthus madaraspatensis* revealed significant hepato-protective effect on carbon tetrachloride and thio acetamide induced liver damage in rats (Santos *et al.,* 1994).

Glycosides isolated from *Phyllanthus acuminatus* have been reported to exert anti-tumor activity on murine P-388, lymphocytic leukemia and B-16 melanoma cell lines (Lee *et al.,* 2003).

2.5. 1 Ethno-Medicinal Utilities of *Phyllanthus discoideus*

The *Phyllanthus discoideus* plants are used in traditional medicine across Africa. A leaf decoction is taken in Ivory Coast for blennorhea and for poisoning, while in Ubangi a decoction of roots and leafy twigs is also used for blennorhea. A wash of the decotion is a stimulant in case of general fatigue.The Fula people use the bark for toothache In Central Africa, a decotion for post-partum pains, and in /the Republic of Congo for stomach and kidney complaints and to facilitate parturition (Adedapo *et al*., 2009). In Malawi the powdered bark extract is applied to swellings and inflammation for quick relief (Adedapo *et al*., 2009)

Oral administration of aqueous extracts at various concentrations showed no acute toxicity in rats and no adverse change in behavior, suggesting that it may be safe for pharmacological uses (Adedapo *et al*., 2009).

The Lyophilized Aqueous Extract (LWE) from the leaves of *Phyllanthus discoideus* was found to show anti-bacterial activity. The alkaloid fraction obtained from LWE also inhibited the growth of *Escherichia coli, Enterococcus faecium, Pseudomonas aureginosa, Staphylococcus aureus* and *Mycobacterium smegmatis* (Mensah *et al.*, 2008).

2.5.2 Ethno-Medicinal Utilities of *Phyllanthus amarus*

In traditional medicine, it is used for its hepatoprotective, anti-diabetic, anti-hypertensive, analgesic, anti-inflammatory and anti-microbial properties (Adeneye *et al.*, 2006). *Phyllanthus amarus* was studied for it's phytochemical analysis and it was shown that it contains lignans, niranthin, nirtetralin, phyltertralin and other compounds like alkamide, alkaloid, terpenoid, flavonoid (Akinjogunia *et al*., 2010).

Phyllanthus amarus is also used in the treatment of stomach disorders, skin diseases and cold (Iwu, 1993). It has anti-diarrhea effect and it also has anti-viral activity against

hepatitis B virus (Thyagarajan *et al.,* 1988; Meixa *et al.,* 1995), anti-carcinogenic (Joy and Kutten, 2000) and anti-mutagenic activities (Joy and Kutten, 1998) has been established. It also has anti-nociceptive and anti-inflammatory activities (Kassuya *et al.,* 2003), anti-diabetic and anti-lipidemic potentials (Adeneye *et al.,* 2006).

Phyllanthus amarus are now being used conventionally to treat jaundice, diabetes, gonorrhea, irregular menstruation, tachycardia, dysentery, spasmodic cough, itchiness, arthritis, otitis, swelling, skin ulcer and weakness of male organ (Calixto *et al.,* 1998; Rao and Alice, 2001).

Phyllanthus amarus blocks DNA polymerase in the case of hepatitis B virus during replication. It also blocks reverse transcriptase in HIV infection and in diabetes complication. It blocks angiotensin-converting enzymes involved (Bensky and Gamble, 1993). Its leaves and whole plant are usually used for the treatment of gonorrhea, jaundice, rickets and asthma (Schlage *et al.* 2002). It is mostly used by traditional healers (Leaman *et al.,* 1995).

Interest in this plant has been heightened by reports of anti-viral activities and its potential as a remedy for hepatitis B-virus infection. Different plant parts are also ethno-botanically reported to have various therapeutic activities, e.g., leaves as expectorant, diaphoretic and the seeds as carminative, laxative, tonic to the liver, diuretic, diaphoretic, useful in bronchitis, ear-ache, griping, opthalmia and ascites. The fresh roots and leaves have been reported to be potent remedy for jaundice (Kirtikar and Basu, 2001). A variety of natural products have been found to inhibit activities of unique enzymes and proteins crucial to the life cycle of HIV including efficient intervention with the reverse transcription process, but also virus binding, the integrase or protease (Jung *et al*., 2000; Cos *et al*., 2004**).** *Phyllanthus amarus* extract also has good anti-oxidant properties which help in removal of

free redicals from the human body (Raphael and Kuttan, 2003; Rai *et al*., 2007). The phenolic constituents of *Phyllanthus amarus* mitigated the effect of anti-mycin A-induced mitochondrial apoptotic cascade (Guha *et al*., 2010).

Also, there is a claim on the use of aerial part of *Phyllanthus amarus* to improve libido or fertility in men, by traditional medicine practitioners. Based on this claim a study came up to establish for the first time, a scientific information on the effectiveness of *Phyllanthus amarus* as fertility agent by its effects on the sperm parameters of male guinea pigs (Obianime and Uche, 2009).

It is commonly known as "Bhuiamlki" in India and has long been used for the treatment of liver, kidney and bladder problems. In a number of countries, the aerial part of *Phyllanthus amarus* is highly valued in traditional medicine for its healing properties (Foo and Wong, 1992). Phyllanthin and hypophyllanthin present in this plant are reported as therapeutically active constituents and served as a hepato-protective agent. This species is also used in the most popular Ayurvedic formulations, Chyawanprash, which is consumed at large scale, not only in India but also throughout the world**.** Aerial parts of *P. amarus* exhibited marked anti-inflammatory properties and suggest that these lignans are the main active principles responsible for the traditional application of this plant for the inflammatory complaints (Kassuya *et al*., 2005).

In South Indian, an infusion of the leaves of *Phyllanthus niruri* is given for headache (Kirtikar and Basu, 1987). An extract of the callus culture of *Phyllanthus niruri* showed analgesic activity (Santos *et al*., 1994). Methanol and ethanol extracts of dried callus tissue of *Phyllanthus niruri* administered intraperitonially (10 mg/kg) to mice showed anti-nociceptive effects on 5 different models of nociception. Main compounds identified in the extracts of *Phyllanthus niruri* such as flavonoids, tannins, terpenes, sterols, alkaloids and

phenols were found to be responsible for the anti-nociceptive activity (Santos *et al.*, 1994; Catapan *et al*., 2000). Phytosterols, quercetin, gallic acid, ethyl ester and geraniin were identified in *Phyllanthus caroliniensis* and among them quercetin, gallic acid, ethyl ester and some flavonoids were found to have antinociceptive action in mice (Filho *et al*., 1996). The fresh roots and leaves have been reported to be potent remedy for jaundice (Kirtikar and Basu, 2001). An aqueous extract of *Phyllanthus niruri* was found to inhibit the hepatitis B virus (Thyagarajan *et al*., 1988) and also inhibits endogenous DNA polymerase of hepatitis B virus and binds to the surface antigen of hepatitis B virus *in vitro* (Venkateswaran *et al*., 1987). Aqueous extracts containing tannin, lignan and other isolated compounds from *Phyllanthus* species have been tested for their anti - HIV activity *in vitro* and *in vivo*. They inhibited the HIV-key enzymes like integrase, reverse transcriptase and protease (Thyagarajan *et al.,* 1988; Calixto *et al*., 1998; Notka *et al*., 2004). *Phyllanthus amarus* was also proved to be potential plant for the treatment of hepatitis B by suppressing the growth and replication of the virus (Mehrota *et al*., 1991, Lee *et al*., 1996). The most recent research on *Phyllanthus niruri* reveals that, its isolated molecule niruriside's anti-viral activity extends to human immunodeficiency virus by inhibiting the reverse transcriptase enzyme. Its anti-viral activity extends to HIV-1 RT inhibition (Notka *et al*., 2004).

2.5.3 Ethno-Medicinal Utilities of *Phyllanthus muellerianus*

In Tangayinka the decoction of *Phyllanthus muellerianus* is taken by draught for hard abscesses, and the powdered dried root and bark is sprinkled on wounds as a dressing (Burkill, 1984). After removal of surface spines, twigs are used as chewing sticks in Nigeria and in Ivory Coast to prevent tooth-ache (Burkill, 1984). In Congo, the dried bark powder is taken for colds and sinusitis (Burkill, 1984). Together with powdered roots, a bark decoction in draught and enema is used for throat troubles and glandular fevers

(Burkill, 1984). *Phyllanthus muellerianus* is widely used to treat intestinal troubles. An infusion of the shoot is taken to treat severe dysentery. In Sierra Leone, a leaf decoction is taken to treat constipation. In Ghana and Nigeria cooked roots, sometimes with maize meal or other plants, are taken to treat severe dysentery (Burkill, 1994). In Tanzania, roots are pounded in water and the liquid is drunk to treat diarrhea. Boiled roots are also applied as enema to treat stomach ache (Burkill, 1994). In Congo powdered roasted roots with palm oil are taken to treat stomach problems. In Cote d'Ivoire and Burkina Faso, twigs are sucked to prevent toothache. Powdered roots are used as a snuff and a bark decoction is taken to treat a sore throat, cough, pneumonia and enlarged glands. Pulped leafy twigs are rubbed on the body to treat paralysis (Burkill, 1994).

In West Africa the sap from the thick hollow stem is applied as eye drops to treat pain in the eyes, eye infections or to remove foreign body. In Nigeria a root bark decoction is taken as an alternative to treat fever. A twig and root decoction is taken to treat jaundice and urethral discharges (Burkill, 1994).Throughout West Africa pounded leaves are applied as wound dressing. In Cote d'Ivoire the leaves are eaten together with the young leaves of *Funtumia elastic* (Preuss) Stapf, to improve male fertility. In Ghana and Nigeria leaves boiled with palm fruit are given to women after delivery as a general tonic. In Cameroon a maceration of the leaves and roots is used to wash the body to treat rash with fever in children. In D.R. Congo, leaf decoction is taken to treat anaemia and also used as a mouthwash to treat toothache. A leaf extract is used as a bath and a vapour bath to treat veneral diseases (Burkill, 1994). Cooked leaves are applied to the gums to treat toothache. The leaves are used as fodder. In Sierra Leone and Nigeria leaves are sometimes cooked with food or in food as seasoning. In Zambia the wood is used for rafters and other

construction work. It is also used to make fish traps and basketry. Fruit pulp is used as a hair fixative. In Gabon it is used in magic to lift taboos (Burkill, 1994).

The roots are widely used for intestinal troubles. The root is cooked with maize meal for severe dysentery in Ghana. Powdered root charcoal with palm-oil is taken in Congo for stomach-upsets and as antemetic. In Nigeria a root - decoction is used as a febrifuge and an infusion of roots and leaves is given to children in Togo suffering from eruptive fevers. In Nigeria, the young root with young leafy twigs is given for jaundice and as a mild purgative and to treat dysentery and urethra discharge. The leaves are an occasional supplement, cooked with food or in soup in Sierra Leone and South Nigeria. Freshly pounded leaves are used for wound-dressing and leaf-sap is used as treatment for fever and skin-eruptions.Leaf sap is widely used in instillation for eye- troubles. Also in Ivory Coast the leaves made into an eye-pad on the lids, leafy twigs prepared with a pulp are rubbed topically on the body in Ivory Coast to cure paralysis. A leaf-decoction serves in Ubangi as a mouth-wash for toothache after which the cooked leaves are applied to the gums (Burkill, 1994).

In Ghana, the root is cut into small pieces with those of Psycholtriacalva (Rubiaceae) and *Harrisonia abyssinica* (Simaroubaceae) and decocted and the liquid drunk to treat cough.

This prescription is also given for whooping cough. An infusion of the flowers is cooling and gently aperient. The fruits are edible and are eaten by some people. The pulp provides a hair fixative used in Ubangi. *In vitro* anti-microbial properties of the chloroform and methanol extract of *Phyllanthus amarus* and *Phyllanthus muellerianus* using human pathogenic micro-organisms were evaluated respectively. The leaf methanol extract of *Phyllanthus amarus* and the leaf chloroform extract of *Phyllanthus muellerianus* exhibited antimicrobial properties. The chloroform extract of *Phyllanthus muellerianus* displayed sensitivity higher than the *Phyllanthus amarus* against *Candida albicans* but inhibited the

growth of only *Staphylococcus aureus* (gram positive) and *Escheria coli* (gram negative) (Burkill, 1994) .

2.6 Phytochemicals

"Phyto" is the Greek word for plants. There are many classes of phytochemicals and they have been found helpful to the human body in a variety of ways. Phytochemicals may protect humans from various diseases. Phytochemicals are non-nutritive and naturally occuring plant chemicals that have protective or disease preventive properties (Hill, 1974). Primary metabolites comprise common sugars, amino acids, proteins and chlorophyll; while secondary metabolites consist of alkaloids, flavonoids, tannins, and so on. Plants have ability to synthesize aromatic substances which are mainly secondary metabolites, of which at least 12,000 has been isolated, a number assumed to be less than 10% of the total. In many cases, these substances serve as the molecules of plant defense against predation by micro-organisms, insects and herbivores. Furthermore, some of them may be involved in plant odour (terpenoids), pigmentation (tannins and quinines) e.t.c. It is now clear that, the medicinal values of these plants lie in the bioactive phytochemical constituents that produce definite physiological effects on the human body (Hill, 1974). The plants are considered as biosynthetic laboratory for a multitude of compounds that exert physiological effects. Secondary metabolites are the compounds which are responsible for imparting therapeutic effects.

The plants of genus *Phyllanthus* have been reported to contain potential phytoconstituents like flavonoids, tannins, alkaloids and triterpenoids in earlier studies (Calixto *et al*., 1998). Phytochemical analysis is now acted as the essential part towards discovery of useful drugs.

Table 2.1: Structural Features of some Phytochemicals

Phytochemical	Structural features	Example (s)	Activities
Phenols and polyphenols	C3 side chain, -OH groups, phenol ring	Catechol, Epcatechin, Cinnamic acid	Antimicrobial, Anthelmintic, Antidiarrhoeal
Quinones	Aromatic rings, two ketone substitutions	Hypericin	Antimicrobial
Flavones, Flavonoids and Flavonols	Phenolic structure, one carbonyl group Hydroxylated phenol, C6-C3 unt linked to an aromatic ring Flavones + 3 hydroxyl group	Abysinone, Chrysin, Quercetin, Rutin, Totarol	Antimicrobial, Antidiarrhoeal
Tannins	Polymeric phenols (Mol. Wt 500-3000)	Ellagitamin	Antimicrobial, Anthelmintic, Antidiarrhoeal
Coumarins	Phenols made of fused benzene and α-pyrone rings	Warfarin	Anitimicrobial
Terpenoids and essential oils	Acetate units + fatty acids, extensive branching and cyclized	Capsaicins	Antimicrobial, Antidiarrhoeal
Alkaloids	Heterocyclic nitrogen compounds	Berberine, piperine, palmatine, Tetrahydropalmatine	Antimicrobial, Anthelmintic, Antidiarrhoeal
Lectins and Polypeptides	Proteins	Mannose- specific agglutinin, fabatin	Antimicrobial
Glycosides	Sugar + non carbohydrate moiety	Amygdalin	Antidiarrhoeal
Saponins	Amphipathic glycosides	Vina- ginsenosides-R5 and -R6	Antidiarrhoeal

Source: Cowan,1999; Kumar and Bhardwaj, 2012.

2.7 Phytochemistry of *Phyllanthus*

Among the widely studied species of *Phyllanthus* are *Phyllanthus ninuri, Phyllanthus urinaria, Phyllanthus emblica, Phyllanthus amarus* and *Phyllanthus sellowianus.* Several compounds such as alkaloids, tannins, flavonoids, lignans, phenols and terpenes have been isolated and identified in various species of *Phyllanthus* and have shown anti-nociceptive action and other therapeutic activities in mice (Rojas *et al.*,2006). Some of the compounds reported include: Ellargic acid, geraniin, phyllemblin and phyllanthoside.

The phytochemical analysis of ethanolic leaf extract of *Phyllanthus amarus* showed the presence of alkaloids, cyanogenic glycosides, saponins, tannins and oxalate.

Alkaloids are known to have a lot of pharmacological properties. They are mostly used as anti-depressant (morphine), stimulants (caffeine), anaesthetic (cocaine), anti-tumor (viriblastine), anti-malaria (quinine), anti-bacterial (berberine) and amoebicide (emetine) (Bruneton, 1995; Cowan, 1999; Heinrich *et al.*, 2004).

Flavonoids are known to have anti-inflammatory, anti-allergic, anti-viral anti-spasmodic and diuretic effect (Bruneton, 1995; Van Wyk *et al.*, 1997; Cowan, 1999). Saponins are known to be immune boosters. Extracts of plants rich in saponins are said to demonstrate anti-inflammatory, hemolytic, allelopathic, cholesterol lowering and anti-cancer properties Tannins are good anti-microbial agent with precipitate protein thereby providing water proof layer on the skin when used externally or protect the underlying layers of the skin and limit the loss of fluid. They are also known to be good anti-viral agents (Bruneton, 1995; Cowan, 1999).

2.8 Choice of Solvents

Successful determination of biologically active compounds from plant materials is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes :- low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate (Ellof, 1998).

The choice of solvent is influenced by what is intended with the extract, since the end product will contain traces of residual solvent, the solvent should be non toxic. The choice will also depend on the targeted compounds to be extracted (Ncube *et al.*, 2008; Das *et al.*, 2010). The following are some of the solvents that can be used in research work for extractions of Phytocemicals -:

a. Water **(**Aqueous**)**

b. Acetone

c. Chloroform

d. Hexane

e. Petroleum Ether

f. Methanol

g. Ethanol

2.9 Methods of Extraction

The variation in different extraction methods that will affect quantity and quality of secondary metabolites composition of an extract depends upon the following:-

(a) type of extraction (a) time of extraction (c) temperature (d) nature of solvent (e) solvent concentration, and (f) polarity (Cowan, 1999).

Extraction (as the term is pharmaceutically used) is the separation of medicinally active portion of plant (and animal) tissues using selective solvents through standard procedures. The products so obtained from plants are relatively complex mixtures of metabolites, in a liquid or semi solid state or (after removing the solvent) in dry powder form, and are intended for oral or external use. These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semi solid) extracts or powdered extracts.

Extraction methods use pharmaceutically involves the separation of medicinally active/inert components by using selective solvents. During extractions, solvents diffuse into the solid plant material and solubulize compounds with similar polarity (Ncube, 2008). The purpose of standardized extraction procedures for medicinal plant parts is to attain the therapeutically desired portion and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization may be used as medicinal agent as such in the form of tinctures or fluid extracts or further processed to be incorporated in any dosage form such as tablets and capsules. These products contain complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans (Handa *et al.,* 2008).

2.9.1 Extraction by Maceration

The extraction of phytochemicals of plants can be carried out by the maceration method. The basic principle here is to grind (pulverize) the plant material (dry or wet) into fine powder, this helps to increase the surface area for extraction, thereby increasing the rate of extraction. Earlier studies reported that solvent to sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal (Das *et al.,*2010). In maceration (for fluid extracts), whole or coarsely powdered plant parts is kept in contact with the solvent in a stoppered

container for a defined period with frequent agitation, until soluble matter is dissolved. This method is best suitable for use in case of the thermonabile drugs (Ncube *et al.,* 2008)

2.9.2 Extraction by Percolation

This is a procedure that is used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator is generally used. The solid ingredients are moistened with an appropriate amount of the specific menstrum and allowed to stand for approximately 4 hours in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstrum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24hours. The outlet of the percolator then is opened and the liquid contained inside is allowed to drip slowly. Additional menstrum is added as required until the percolate measures about three – quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting (Handa *et al.,* 2008).

CHAPTER THREE

3.0 Materials and Methods

3.1 Scope of Study

The research was carried out in the Pharmacognosy laboratory of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. The research work focused on the detection and determination of the Phytochemicals in the methanol and aqueous crude extracts of the roots, stem and leaves of the selected three species of the genus *Phyllanthus i.e. Phyllanthus discoideus, Phyllanthus amarus* and *Phyllanthus muellerianus*

3.2 Plant Collection and Identification

Matured whole plants of *Phyllanthus amarus* were collected from the Botanical gardens, Ahmadu Bello University, Zaria, while the matured plant parts of *Phyllanthus muellarianus* and *Phyllanthus discoideus* were collected in the bush in the outskirts of Zaria towards Kaduna. The three *Phyllanthus* species were identified and authenticated in the Herbarium section, Department of Biological Sciences, Ahmadu Bello University, Zaria.

3.3 Preparation of the Plant Parts

After the collection and identification of the *Phyllanthus* species, the plant parts (the roots, stem and leaves) were washed thoroughly with tap water in order to remove the dust and soil particles. Then the plants parts were air dried under the shade to prevent ultra-violet rays from inactivating the chemical constituents (Das *et al.*, 2010; Ncube *et al*., 2008). The individual species parts were later pulverized (ground into powder form) separately, using pestle and mortar.

3.4 Procedure for Preparation of the Crude Extracts

The procedure that was used for the extraction of the active ingredients from the plant parts in this research work was extraction by percolation. Percolators were generally used. Each of the powdered samples of the root, stem and leaf of *Phyllanthus discoideus, Phyllanthus amarus* and *Phyllanthus muellarianus* was divided into two parts, "A"s and "B"s. The "A"s were moistened with 70% methanol in a closed container and allowed to stand for about 4 hours, after which the mass was packed into a percolator and 70% methanol was added until the material was saturated. Additional solvent was added from the 70% methanol to form a shallow layer above the mass and then the top of the percolator was closed, and the mixture was allowed to macerate in the closed percolator for 24hours. The outlet or tap of the percolator was then opened and the liquid contained inside was allowed to drip slowly into a container. Additional 70% methanol was added as required until the percolate measures about three – quarters of the required volume of the finished product. The marc was then pressed and the expressed liquid was added to produce the required volume (Sofowora, 2006).The filtered liquids were placed in the water bath to evaporate it, in order to get the methanol crude extracts.

The "B"s were moistened with aqueous solvent in a closed container and allowed to stand for about 4 hours after which the mass was packed into a percolator and the aqueous solvent was added until the material was saturated. Additional solvent was added from the aqueous solvent to form a shallow layer above the mass and then the top of the percolator was closed, and the mixture was allowed to macerate in the closed percolator for 24 hours. The outlet or tap of the percolator was opened and the liquid contained inside was allowed to drip slowly into a container. Additional aqueous solvent was added as required until the percolate measures about three – quarters of the required volume of the finished product.

The marc was then pressed and the expressed liquid was added to produce the required volume (Sofowora, 2006).The filtered liquids were placed in the water bath to evaporate it, in order to get the aqueous crude extracts.

3.5 Qualitative Phytochemical Screening of the Plant Extracts

The phytochemical examination was carried out for the roots, stem and leaves extracts for *Phyllanthus discoideus*, *Phyllanthus amarus* and *Phyllanthus muellarienus* using the standard methods. Each of the concentrated extracts was subjected to qualitative tests for the identification of its various phytochemical constituents as per standard procedures (Harbourne, 1973; Trease and Evans, 1989) and also by characteristics colour changes as described by Sofowora (1993).

(i) Detection of Alkaloids: - Each of the methanol and aqueous extracts of the roots, stem and leaves of the three species of *Phyllanthus* were dissolved individually in dilute hydrochloric acid and filtered. The following tests were carried out on them to detect the presence of alkaloids

(a) Mayer's Test: filtrates were treated with Mayer's reagent (Potassium Mercuric Chloride). The formation of a yellow colour precipitate indicates the presence of alkaloids.

(b) Dragendroff's Test: - This test was used as the confirmatory test for detection of alkaloids. Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate confirms the presence of alkaloids.

(ii) Detection of Flavonoids: - The following tests were carried out on extracts of each of the three *Phyllanthus* species.

(a)Alkaline Reagent Test: - Each of the extracts was treated with few drops of Sodium Hydroxide solution. Formation of intense yellow colour which becomes colourless on the addition of dilute acid indicates the presence of flavonoids.

(b)Lead Acetate Test: - This test served as the confirmatory test for flavonoids. Each of the extracts was treated with few drops of Lead Acetate solution Formation of yellow colour precipitate confirms the presence of flavonoids.

(iii) Detection of Tannins: - 1% gelatin solution containing Sodium Chloride was added to each of the extracts of the three *Phyllanthus* species. The formation of white precipitate indicates the presence of tannins.

(iv) Detection of Saponins: - The following tests were carried out on the extracts of each of the three *Phyllanthus* species to detect the presence of Saponins.

(a) Froth Test: - Each of the extracts of the three *Phyllanthus* species were diluted with distilled water to 20 ml and they were shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence saponins.

(b) Foam Test: - This served as confirmatory test for saponins. 0.5 gm of each of the extract was shaken with 2 ml of water. The production of foam which persisted for ten minutes, confirms the presence of saponins.

(v) Detection of Phenolic compounds: To a small portion of the extract of each of the three *Phyllanthus* species was added 1ml of 10% $FeCl_2$ and mixed together. The presence of blue precipitate confirms the presence of Phenolic compounds.

(vii) Detection of Anthraquinones: To the extract of each of the three *Phyllanthus* species, Magnesium Acetate solution was added the development of pink colour indicates the presence of Anthraquinone.

(viii) Detection of Triterpenes: - Each of the three extracts of the *Phyllanthus* were dissolved in water and treated with 3-4 drops Copper Acetate solution. The formation of emarald green colour indicates the presence of Triterpenes.

(ix) Detection of Sterols: - The following tests were carried out on the extracts of each of the three *Phyllanthus* species to detect the presence of polysterols.

(a) Salkowski Test: - Each of the extract were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

(b) Libermann Burchard's Test: - Each of the extracts were treated with few drops of Acetic Anhydride, and cooled. Concentrated Sulphuric acid was added. Formation of brown rings at the junction indicates the presence of phytosterols.

3.6 Quantitative Dertermination of the Phytochemicals in the Plant Extracts

The quantitative analysis for the determination of the total phytochemicals of the roots, stem and leaves of *Phyllanthus discoideus, Phyllanthus amarus* and *Phyllanthus muellerianus* were carried out using standard procedures (with some modifications). A spectrophotometer was used to determine the Tannins and the Phenol contents on the basis of UV spetra through absorption maxima at individual wavelength of every biocomponent **(**The total Phenol contents were gotten using standard calibration curve).

(a) Determination of the quantity of Alkaloids using Harborne (1973) method: 2g each of the extracts of the roots, stem and leaves of the three species of *Phyllanthus* were put in a 250ml beaker; 80ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. It was then filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium was then added in drops to the extract until the precipitation was complete. The whole solution was collected and allowed to settle and then the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue which is the alkaloid was dried and weighed.

(b) Determination of the quantity of Saponnins the by the Obadoni and Ochuko (2001) method**:** 2gm each of the extracts of the roots, stem and leaves of the three species of the *Phyllanthus* were put into a conical flask and 20cm of 20% aqueous ethanol was added. It was then heated over the water bath for 4 hours with continuous stirring at 55°C. This mixture was then filtered and the residue was re-extracted with another 200ml of 20% ethanol. This combined extracts were reduced to 40ml over water bath at about 90°C. These concentrates were transferred into a 250ml separator 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 the water bath. After evaporation the samples were dried in the oven to a constant weight to give the saponins which was then calculated.

(c) Determination of the quantity of Tannins by the Van-Burden and Robinson (1981) method: 500mg each of the extracts of the roots, stem and leaves of the three species of the *Phyllanthus* were weighed into a 50ml plastic bottle. 50ml of distilled water was added and then shaken vigorously or in a mechanical shaker for one hour. This was then filtered into a 50ml volumetric flask and made up to the mark. 5ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120nm within 10 minutes to get the total Tannins.

(d) Determination of the quantity of Flavonoids by the method of Bohm and Kocipai-Abyazan (1984): 2gm each of the extracts of the roots, stem and leaves of the three species of the *Phyllanthus* were extracted repeatedly with 20ml of 80% aqueous methanol at room temperature. The whole solution was filtered using filter paper No 42 (125mm). The filtrate

was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight, and the weight was calculated.

(e) Determination of the quantity of Phenolic compounds by spectrophotometric method: 100mg each of the extracts of the roots stem and leaves of the three species of the *Phyllanthus* were weighed and dissolved in 100ml of triple distilled water (TDW). Then 1ml of this solution was transferred to a test tube and then 0.5ml 2N of the Folin-Ciocalteu reagent and 1.5ml 20% of Na_2CO_3 solution was added and the volume was made up to 8ml with TDW, followed by vigorous shaking and was made to stand for 2 hours. Then the absorbance was taken at 765 nm. The data was used to estimate the total phenolic content using a calibration curve obtained from various diluted concentrations of gallic acid.

3.7 Statistical Analysis

- Results were subjected to statistical analysis using One Way Analysis of Variance (ANOVA) to compare the quantity of the phytochemicals of the different parts of the three species of *Phyllanthus*.
- The DMRT (Duncan Multiple Range Test) used to separate the means where there was significant difference. Results with $P < 0.05$ were accepted as significant, while results with $P \geq 0.05$ were accepted as not significant.
- The t-test was used also for the comparison of the aqueous and methanol extracts of the different parts of the three species of *Phyllanthus.*
- Data were expressed as mean ± standard error of mean (S.E.M).

CHAPTER FOUR

4.0 RESULTS

4.1 Qualitative Phytochemical Screening of the Methanol Extracts

The Qualitative Phytochemical screening of the methanol extracts of the plants parts (roots, stem and leaves) of the three *Phyllanthus* species showed the presence of medicinally active constituents such as alkaloids, flavonoids, tannins, saponins, phenols, glycosides, anthraquinones, triterpenes and sterols at varying levels/intensities (Table 4.1).

The Phytochemical screening showed very deep presence of flavonoids in the roots of *Phyllanthus discoideus,* and alkaloids in the stem of *Phyllanthus muellerianus.*The Phytochemical screening also showed the presence and deep presence of most of the Phytochemicals. It also showed the absence of phenols in the roots, stem and leaves of *Phyllanthus discoideus* and *Phyllanthus amarus* and in the leaves of *Phyllanthus muellerianus*. It also showed the absence of anthraquinones in the roots, stem and leaves of *Phyllanthus amarus*, and also the absence of triterpenes in the roots and stem of *Phyllanthus amarus*. It also showed the absence of sterols in the leaves of *Phyllanthus muellerianus* and *Phyllanthus discoideus* (Table 4.1).

4.2 Qualitative Phytochemical Screening of the Aqueous Extracts

The qualitative phytochemical screening of the aqueous extracts of the plant parts (roots, stem, and leaves) of the three *Phyllanthus* species showed the presence of medicinally active constituents such as alkaloids, flavonoids, tannins, triterpenes and sterols at varying levels/intensities. The Phytochemical screening did not reveal very deep presence of any of the Phytochemicals, but it revealed deep presence and presence of most of the Phytochemicals (Table 4.2).

Table 4.1: The Phytochemical Screening (Qualitative) of the Methanol Extracts of the Three Species of *Phyllanthus*.

Plant parts	Phytochemicals	*P discoideus*	*P. amarus*	*P. muellerianus*
Roots	Alkaloids	+	+	++
	Flavonoids	+++	++	++
	Tannins	++	+	++
	Saponins	++	++	+
	Phenols	-	-	-
	Glycosides	++	+	+
	Anthraquinones	+	-	++
	Triterpenes	++	-	++
	Sterols	++	+	+
Stem	Alkaloids	+	+	+++
	Flavonoids	+	+	++
	Tannins	++	++	+
	Saponins	++	++	++
	Phenols	+	+	+
	Glycosides	++	++	++
	Anthraquinones	+	-	++
	Triterpenes	++	-	++
	Sterols	++	+	++
Leaves	Alkaloids	+	+	++
	Flavonoids	++	+	++
	Tannins	++	+	+
	Saponins	+	+	+
	Phenols	-	-	-
	Glycosides	+	+	++
	Anthraquinones	+	-	++
	Triterpenes	++	++	++
	Sterols	-	+	-

NB: + : present, ++ : Deeply Present, +++ : Very deeply present, □ : Absent

Table 4.2: The Phytochemical Screening (Qualitative) of the Aqueous Extracts of the Three Species of *Phyllanthus*

Plant parts	Phytochemicals	*P. discoideus*	*P. amarus*	*P. muellerianus*
Roots	Alkaloids	+	+	+
	Flavonoids	++	++	+
	Tannins	+	+	++
	Saponins	++	+	+
	Phenols	-	-	-
	Glycosides	++	+	++
	Anthraquinones	+	-	++
	Triterpenes	++	-	++
	Sterols	++	+	++
Stem	Alkaloids	+	+	+
	Flavonoids	+	+	+
	Tannins	+	++	+
	Saponins	++	+	+
	Phenols	-	-	-
	Glycosides	++	++	++
	Anthraquinones	+	-	++
	Triterpenes	++	-	++
	Sterols	++	+	++
Leaves	Alkaloids	+	+	+
	Flavonoids	++	+	+
	Tannins	++	+	+
	Saponins	+	+	+
	Phenols	+	-	-
	Glycosides	+	+	++
	Anthraquinones	+	-	++
	Triterpenes	++	+	+
	Sterols	+	+	-

NB: +: present, ++: Deeply Present, +++: Very deeply present, □ : Absent

The Phytochemical screening revealed the absence of alkaloids in the leaves of *Phyllanthus muellerianus* and in the roots of *Phyllanthus discoideus*, flavonoids in the roots and stem of *Phyllanthus amarus* and the stem only of *Phyllanthus muellerianus*, phenols in the roots

4.3 Quantitative Analysis of The Phytochemicals in Methanol Extracts of Some *Phyllanthus* Species.

The Analysis of Variance (ANOVA) for the quantitative analysis of phytochemicals in methanol extracts of three *Phyllanthus* species (Table 4.3) showed some significant differences and some not significant differences of the Phytochemicals in their roots, stem and leaves.

Phyllanthus discoideus plants had the highest contents of alkaloids, tannins and phenols in the leaves, and flavonoids and saponins in the roots. The roots had the lowest content of most of the phytochemicals.

The highest alkaloids content observed in the leaves and stem was significantly higher than the lowest in the root.The highest tannins content observed in the leaves was only significantly higher than the lowest in the roots. The highest saponins content observed in the roots was comparable to the lowest in the stem. The lowest phenols content observed in the roots was only significantly lower than the highest in the leaves.

Phyllanthus amarus plants had the highest content of alkaloids, tannins and phenols in the stem, flavonoids in the roots and saponins in the leaves. The roots had the lowest content of most of the phytochemicals (Table 4.3).

The highest alkaloids content observed in the stem was only significantly higher than the lowest in the leaves. The highest flavonoids observed in the roots is comparable to that in the stem, but significantly higher than the lowest in the leaves. The highest tannins observed in the stem was only significantly higher than the lowest in the roots. The lowest

Table 4.3: Quantitative Analysis of the Phytochemicals in the Methanolic Extracts of the Three Species of *Phyllanthus*

Species	Phytochemicals[mg/g]	Roots	Stem	Leaves	S.E ±
Phyllanthus	Alkaloids	0.18b	0.50a	0.50a	±0.057
discoideus	Flavonoids	0.74a	0.60b	0.72a	±0.023
	Tannins	0.11b	0.17a	0.18a	±0.012
	Saponins	0.22a	0.17a	0.18a	±0.009
	Phenols	0.05b	0.08b	0.12a	±0.011
Phyllanthus	Alkaloids	0.43b	0.58a	0.53ab	±0.025
amarus	Flavonoids	0.58a	0.54a	0.22b	±0.058
	Tannins	0.20b	0.26a	0.25a	±0.011
	Saponins	0.05b	0.06b	0.20a	±0.025
	Phenols	0.04b	0.08a	0.05ab	±0.007
Phyllanthus	Alkaloids	0.48c	0.91a	0.76b	±0.066
muellarianus	Flavonoids	0.78a	0.71b	0.80a	±0.016
	Tannins	0.34a	0.28b	0.29b	±0.011
	Saponins	0.17a	0.09b	0.16a	±0.014
	Phenols	0.09a	0.09a	0.10a	±0.006

NB: Means with the same letter(s) in each row, under each species are not significantly different (P=0.05), using DMRT.

saponins observed in the roots was only significantly lower than the highest in the leaves. The highest phenols observed in the stem was only significantly higher than the lowest in the roots.

Phythanthus muellerianus plants had the highest content of alkaloids in the stem, flavonoids and phenols in the leaves and tannins and saponins in the roots. The stem had the lowest content of most of the Phytochemicals.

The highest content of alkaloids observed in the stem was significantly higher than that of the leaves and the roots and that of the leaves was significantly higher than the lowest in the roots. The highest content of flavonoids observed in the leaves was only significantly higher than the lowest in the stem. The lowest content of tannins observed in the stem was only significantly lower than the highest in the roots. The highest saponins content observed in the roots was only significantly higher than the lowest in the stem. The highest phenols content observed in the leaves was comparable to the lowest in the stem and roots (Table 4.3).

4.4 Quantitative Analysis of the Phytochemicals in Aqueous Extracts of Some *Phyllanthus* Species

The Analysis of Variance (ANOVA) of the quantitative determination of the phytochemicals in aqueous extracts in the three *Phyllanthus species* (Table 4.4) shows some significant differences and some not significant differences of the Phytochemicals in their roots, stem and leaves.

Phyllanthus discoideus plants had the highest content of alkaloids in the roots and flavonoids, tannins, saponins and phenols in the leaves. The stem had the lowest content of most of the phytochemicals. The highest alkaloids content observed in the roots was significantly higher than that in the leaves and stem and that of the leaves was significantly

Table 4.4: Quantitative Analysis of the Phytochemicals in the Aqueous Extracts of the Three Species of *Phyllanthus*.

Species	Phytochemicals[mg/g]	Roots	Stem	Leaves	S.E ±
Phyllanthus	Alkaloids	0.44a	0.510c	0.20b	±0.051
discoideus	Flavonoids	0.34b	0.27b	0.44a	±0.026
	Tannins	0.23b	0.17c	0.36a	±0.029
	Saponins	0.21a	0.18a	0.22a	±0.010
	Phenols	0.03a	0.01b	0.05a	±0.007
Phyllanthus	Alkaloids	0.26b	0.31a	0.28ab	±0.011
amarus	Flavonoids	0.44a	0.35b	0.17c	±0.040
	Tannins	0.16c	0.30a	0.21b	± 0.021
	Saponins	0.05b	0.04b	0.14a	±0.016
	Phenols	0.02a	0.05a	0.02a	±0.007
Phyllanthus	Alkaloids	0.09a	0.12a	0.12a	±0.008
muellarianus	Flavonoids	0.25b	0.09c	0.36a	±0.040
	Tannins	0.20a	0.17a	0.15a	±0.021
	Saponins	0.21a	0.18a	0.22a	±0.010
	Phenols	0.01b	0.06a	0.04a	±0.009

NB: Means with the same letter(s) in each row, under each species are not significantly different (P=0.05), using DMRT.

higher than the lowest in the stem.

The lowest flavonoids content observed in the stem was only significantly lower than the highest in the leaves. The highest tannins content observed in the leaves was significantly higher than in the roots and stem. The highest saponins content observed in the leaves was comparable to that in the stem and roots. The highest phenols content observed in the leaves was only significantly higher than the lowest in the stem.

Phyllanthus amarus plants had the highest content of alkaloids, tannins and phenol in the stem, flavonoids in the roots and saponins in the leaves. The roots had the lowest contents of most of the phytochemicals.

The highest alkaloids content observed in the stem was only significant higher than the lowest in the roots. The highest flavonoids content observed in the roots was significantly higher than that of the stem and leaves, and that of the stem was significantly higher than the lowest in the leaves. The highest tannins content observed in the stem was significantly higher than that of the leaves and roots and that of the leaves was significantly higher than the lowest in the roots. The lowest saponins content observed in the stem was only significantly lower than the highest in the leaves. The highest phenols content observed in the stem was comparable to that of the roots and leaves.

Phyllanthus muellerianus plants had the highest content of alkaloids in the stem and leaves, flavonoids and saponins in the leaves, tannins in the roots, and phenols in the stem. The leaves had the highest content of most of the phytochemicals. Most of the Phytochemicals in *Phyllanthus muellarianus* has comparable content of most of the phytochemicals in the roots, stem and leaves. (Table 4.4).

The highest alkaloids content observed in the stem and leaves was comparable to the lowest in the roots.

The highest flavonoids content observed in the leaves was significantly higher than that of the stem and roots, and that of the roots was significantly higher than the lowest in the stem. The highest tannins content observed in the roots was comparable to the lowest in the leaves. The highest saponins content observed in the leaves was comparable to the lowest in the stem. The highest phenols content observed in the stem was only significantly higher than the lowest in the roots (Table 4.4).

4.5: Quantitative Analysis of Phytochemicals in *Phyllanthus* Species in Methanol and Aqueous Extracts.

The combined Analysis of Variance (ANOVA) of the Phytochemicals in both methanol and aqueous extracts of the three *Phyllanthus* species (Table 4.5) showed some significant differences and some not significant differences of the Phytochemicals in their roots, stem and leaves in the combination of the two solvents.

Phyllanthus discoideus plants had the highest content of alkaloids, saponins in the roots. The stem had the lowest content of most of the phytochemicals while the highest content of most of the Phytochemicals was found in the leaves. The highest alkaloids content determined in the leaves was significantly higher than the lowest in the stem and roots.The highest flavonoids and tannins content observed in the leaves were significantly higher than that in the roots and stem, and that of the roots was significantly higher than the lowest in the stem. The lowest saponins content observed in the stem was only significantly lower than the highest in the roots. The highest phenols content observed in the leaves was only significantly lower than the lowest in the stem (Table 4.5).

Phyllanthus amarus plants had the highest content of flavovoids, tannins and phenols in the stem, alkaloids and saponins in the leaves. The roots had the lowest contents of most of the phytochemicals.

Table 4.5: Quantitative Analysis of the Phytochemicals in the Methanol and Aqueous Extracts (Combined ANOVA).

Species	Phytochemicals [mg/g]	Roots	Stem	Leaves	S.E ±
Phyllanthus	Alkaloids	0.30b	0.30b	0.35a	±0.054
discoideus	Flavonoids	0.54b	0.44c	0.58a	±0.025
	Tannins	0.17b	0.17c	0.27a	±0.021
	Saponins	0.22a	0.18b	0.20b	±0.010
	Phenols	0.03a	0.01b	0.05a	±0.009
Phyllanthus	Alkaloids	0.04b	0.04b	0.08a	±0.018
amarus	Flavonoids	0.35b	0.45a	0.41a	±0.049
	Tannins	0.18c	0.28a	0.23b	±0.016
	Saponins	0.05b	0.05b	0.17a	±0.021
	Phenols	0.03a	0.07a	0.04a	±0.007
Phyllanthus	Alkaloids	0.29b	0.52a	0.44a	±0.037
muellarianus	Flavonoids	0.52b	0.40c	0.58a	±0.028
	Tannins	0.27a	0.23a	0.22a	±0.016
	Saponins	0.19a	0.14b	0.19a	±0.012
	Phenols	0.05b	0.08a	0.07ab	±0.008

NB: Means with the same letter(s) in each row, under each species are not significantly different (P=0.05), using DMRT.

The highest alkaloids content observed in the leaves was significantly higher than the lowest in the roots and stem. The highest flavonoids content observed in the stem was only significantly higher than that of roots. The highest tannins content observed in the stem was significantly higher than that of the roots and the leaves, and that of the leaves was significantly higher than the lowest in the roots. The highest saponins content observed in the leaves was significantly higher than the lowest in the stem and roots. The highest phenols content observed in the stem was not significantly higher than the lowest in the roots.

Phyllanthus muellerianus plants had the highest content of alkaloids and phenols in the stem, flavonoids in the leaves, tannins in the roots and saponins in the roots and leaves.

The highest alkaloids content observed in the stem was only significantly higher than the lowest in the roots. The highest flavonoids contents observed in the leaves was significantly higher than that of the stem and roots, and that of the roots were significantly higher than the lowest in the stem. The highest tannins content observed in the roots was comparable to the lowest in the leaves. The highest saponins content observed in the roots and leaves was significantly higher than the lowest in the stem. The highest phenols observed in stem was only significantly higher than that of the roots (Table 4.5).

4.6: Quantitative Analysis of the Phytochemicals in the Methanol and Aqueous Extracts of the *Phyllanthus* Species Irrespective of the Plant Parts.

The Analysis of Variance (Combined ANOVA) of the quantitative analysis of the phytochemicals in the methanol and aqueous extracts of the combination of the roots, stem and leaves (Table 4.6) showed some significant differences and some not significant differences of the Phytochemicals extracted by the two solvents used.

Table 4.6: Quantitative Analysis of the Phytochemicals in the Roots, Stem and Leaves (Combined ANOVA) for each Solvent.

Species	Phytochemicals [mg/g]	Methanol Solvent	Aqueous Solvent	S.E±
Phyllanthus discoideus	Alkaloids	0.39a	0.25b	±0.054
	Flavonoids	0.69a	0.35b	±0.025
	Tannins	0.15a	0.25a	±0.021
	Saponins	0.20a	0.19a	±0.010
	Phenols	0.08a	0.01a	±0.009
Phyllanthus Amarus	Alkaloids	0.51a	0.28b	±0.018
	Flavonoids	0.45a	0.32b	±0.049
	Tannins	0.24a	0.22a	±0.016
	Saponins	0.10a	0.08b	±0.021
	Phenols	0.06a	0.03b	±0.007
Phyllanthus muellarianus	Alkaloids	0.72a	0.11b	±0.037
	Flavonoids	0.76a	0.23b	±0.028
	Tannins	0.76a	0.23b	±0.016
	Saponins	0.20a	0.14b	±0.012
	Phenols	0.09a	0.04b	±0.008

NB: Means with the same letter(s) in each row, under each species are not significantly different (P=0.05), using DMRT.

Phyllanthus discoideus plants**:** Methanol solvent extracted the higher contents of alkaloids, flavonoids, saponins and phenols than the aqueous solvent. The higher alkaloids content extracted by methanol solvent was significantly higher than that extracted by aqueous solvent. The higher flavonoids content extracted by methanol solvent was significantly higher than that extracted by aqueous solvent. The higher tannins content extracted by aqueous solvent was not significantly higher than that extracted by methanol solvent.

Phyllanthus amarus plants: Methanol solvent extracted higher content of alkaloids, flavonoids, tannins, saponins and phenols from *Phyllanthus amarus* than aqueous solvent. The higher tannins content extracted by methanol solvent was comparable to that extracted by aqueous solvent.

The aqueous solvent extracted lower content of all the Phytochemicals than the methanol solvent. Methanol solvent extracted significantly higher alkaloids, flavonoids, saponins and phenols contents than those extracted by aqueous solvent.

Phyllanthus muellerianus plants: Methanol solvent extracted significantly higher contents of alkaloids, flavonoids, tannins, saponins and phenols from *Phyllanthus muellarianus* than aqueous solvent. The aqueous solvent extracted the lower contents of all the phytochemicals than methanol solvent (Table 4.6).

4.7 Quantitative Analysis of the Phytochemicals Extracted from the Plant Parts by Methanol and Aqueous Solvents Irrespective of the *Phyllanthus* Species.

The Analysis of Variance (ANOVA) of the quantitative analysis of the phytochemicals extracted from the plant parts by methanol and aqueous solvents irrespective of the plant parts showed the some significance differences and the not significant differences of most of the phytochemicals extracted from their roots, stem and leaves (Table 4.7).

Table 4.7: Quantitative Analysis of the Phytochemicals Extracted by Methanol and Aqueous Solvents from Plant Parts of the *Phyllanthus* Species Combined

Solvent	Phytochemicals[mg/g]	Roots	Stem	Leaves	S.E ±
Methanol	Alkaloids	0.36c	0.66a	0.60b	±0.039
	Flavonoids	0.70a	0.62b	0.58c	± 0.034
	Tannins	0.22b	0.24a	0.24a	±0.040
	Saponins	0.15b	0.11c	0.18a	±0.012
	Phenols	0.06c	0.08b	0.09a	±0.006
Aqueous	Alkaloids	0.26a	0.18b	0.20b	±0.022
	Flavonoids	0.34a	0.24b	0.32a	±0.022
	Tannins	0.20a	0.21a	0.24a	±0.015
	Saponins	0.17b	0.13b	0.19a	±0.014
	Phenols	0.18 b	0.38 a	0.36 a	±0.004

NB: Means with the same letter(s) in each row, under each solvent are not significantly different (P=0.05), using DMRT.

Methanol Solvent: The combined ANOVA of the data showed that, methanol solvent extracted more of tannins, saponins and phenols from the leaves than from the stem and roots, but more of alkaloids stem and flavonoids from the roots. The roots had the lowest content of most of the Phytochemicals. The highest alkaloids content extracted from the stem by methanol was significantly higher than that from the leaves and roots, and that from the leaves was significantly higher than the lowest from the roots.

The highest flavonoids content extracted from the roots by methanol was significantly higher than that from the stem and leaves, and that from the stem was significantly higher than the lowest from the leaves.

The highest tannins content extracted from the stem and the leaves by methanol was significantly higher than the lowest from the roots.

The highest saponins content extracted from the leaves by methanol was significantly higher than that from the roots and stem, and that from the roots was significantly higher from the lowest from the stem

The highest phenol content extracted from the leaves by methanol was significantly higher than that of the stem and roots, and that of the stem were significantly higher than the lowest from the roots (Table 4.7).

Aqueous solvent: Aqueous solvent extracted the highest contents of alkaloids and flavonoids in the roots, tannins and saponins in the leaves, phenols in the stem. The stem had the lowest content of most of the Phytochemicals.

The highest alkaloids content extracted from the roots aqueous solvent was significantly higher than that of the leaves and stem, and that of the leaves was comparable to the lowest in the stem.

The highest flavonoids content extracted from roots by aqueous solvent was only significantly higher than the lowest in the stem. The highest tannins content extracted from the leaves by the aqueous solvent was comparable to the lowest in the roots. The highest saponins content extracted from the leaves by aqueous solvent was significantly higher than that of the stem and the roots, and that of the roots was comparable to the lowest in the stem. The highest phenols content extracted from the stem was comparable to that of the leaves and that of the leaves was significantly higher than the lowest in the roots (Table 4.7).

4.8 Quantitative Analysis of the Phytochemicals Extracted by Methanol and Aqueuos Solvents from the *Phyllanthus* Species Irrespective of the Parts.

The Analysis of variance (ANOVA) of the quantitative analysis of Phytochemicals extracted by methanol and aqueous solvents from the *Phyllanthus* species irrespective of their parts is as shown in (Table 4.8) revealed some significant differences and not significant differences of most of the phytochemicals from the three species.

Methanol solvent: Methanol solvent extracted the highest contents of alkaloids, flavonoids, tannins and phenols from *Phyllanthus muellarianus* and saponins from *Phyllanthus discoideus.* The *Phyllanthus amarus* had the lowest extraction of most of the Phytochemicals.

The highest alkaloids content extracted from *Phyllanthus muellarianus* was significantly higher than that of *Phyllanthus amarus* and *Phyllanthus discoideus*, and that of *Phyllanthus amarus* was significantly higher than the lowest from *Phyllanthus discoideus*.

The highest flavonoids content extracted from *Phyllanthus muellerianus* was significantly higher than that of *Phyllanthus discoideus* and *Phyllanthus amarus*, and that of *Phyllanthus discoideus* was significantly higher than the lowest from *Phyllanthus amarus*.

Table 4.8: Quantitative Analysis of the Phytochemicals in Roots, Stem and Leaves (Combined ANOVA) for the Three Species

Solvents	Phytochemicls [mg/g]	*Phyllanthus discoideus*	*Phyllanthus amarus*	*Phyllanthus muellerianus*	S.E±
Methanol	Alkaloids	0.39c	0.51b	0.72a	±0.039
	Flavonoids	0.69b	0.45c	0.76a	±0.034
	Tannins	0.15c	0.24b	0.30a	±0.014
	Saponins	0.19a	0.10c	0.14b	±0.012
	Phenols	0.08a	0.06b	0.09a	±0.011
Aqueous	Alkaloids	0.25a	0.28a	0.11c	±0.022
	Flavonoids	0.35a	0.32b	0.23c	±0.022
	Tannins	0.25a	0.22a	0.17b	±0.015
	Saponins	0.20a	0.08b	0.20a	±0.014
	Phenols	0.03a	0.03a	0.04a	±0.004

NB: Means with the same letter(s) in each row, under each solvent are not significantly different (P=0.05), using DMRT.

The highest tannins content extracted from *Phyllanthus muellerianus* was significantly higher than that of *Phyllanthus amarus* and *Phyllanthus discoideus*, and that of *Phyllanthus amarus* was significantly than the lowest from *Phyllanthus discoideus*.

The highest *saponins* content extracted from *Phyllanthus discoideus* was significantly higher than that of *Phyllanthus muellerianus* and *Phyllanthus amarus*, and that of *Phyllanthus muellerianus* was significantly higher than the lowest from *Phyllanthus amarus*.

The highest phenols content extracted from *Phyllanthus muellerianus* was comparable to that of *Phyllanthus discoideus* but significantly higher than the lowest from *Phyllanthus amarus.*

Aqueous solvent: Aqueous solvent extracted the highest contents of flavonoids, tannins and saponins from *Phyllanthus amarus*, flavonoids, tannins, from *Phyllanthus discoideus, saponins* from *Phyllanthus discoideus. Phyllanthus amarus* had the lowest extraction of most of the phytochemicals.

The highest alkaloids content extracted by aqueous solvent from *Phyllanthus amarus* was significantly higher than that extracted by *Phyllanthus discoideus*, and that of *Phyllanthus discoideus* was significantly higher than the lowest from *Phyllanthus muellerianus*.

The highest flavonoids content extracted by aqueous solvent from *Phyllanthus discoideus* was significantly higher than that extracted from *Phyllanthus amarus* and *Phyllanthus muellarianus,* and that of *Phyllanthus amarus* was significantly higher than the lowest from *Phyllanthus amarus*.

The highest tannins content extracted by aqueous solvent from *Phyllanthus discoideus* was only significantly higher than the lowest from *Phyllanthus meullerianus*.

The highest saponins content extracted by aqueous solvent from *Phyllanthus discoideus* and *Phyllanthus muellerianus* was significantly higher than the lowest from *Phyllanthus amarus*.

The highest phenols content extracted by aqueous solvent from *Phyllanthus muellerianus* was comparable to the lowest in *Phyllanthus discoideus* and *Phyllanthus amarus* (Table 4.8)

4.9: Quantitative Analysis of the Phytochemicals Extracted from the Roots, Stem and Leaves Irrespective of the Species and the Solvent.

The Analysis of Variance (combined ANOVA) of the data irrespective of the solvent used and the *Phyllanthus* species (Table 4.9) showed the significance differences and not significant differences of most of the phytochemicals extracted from three species by the combination of solvents.

The data revealed the higher extraction of alkaloids from the stem, flavonoids from the roots, tannins and saponins from the leaves and phenols from the stem and leaves. The roots had the lowest extraction of most of the Phytochemicals.

The highest alkaloids content extracted from the stem was comparable to that of the leaves, but was significantly higher than that of the roots. The highest flavonoids content extracted from the roots was significantly higher than that of the leaves and stem, and that of the leaves was significantly higher than the lowest from the stem. The highest tannins content extracted from the leaves was comparable only to that of the stem and that of the stem was comparable to the lowest in the roots. The highest saponins content extracted from the leaves was significantly higher than that of the roots and stem, and that of the roots was significantly higher than the lowest from the stem. The highest phenols extracted from the stem and leaves were significantly higher than the lowest from the roots (Table 4.9).

Table 4.9: Quantitative Analysis of the Phytochemicals in the Combination of Solvent and Species.

Phytochemicals[mg/g]	Roots	Stem	Leaves	S.E ±
Alkaloids	0.31b	0.42a	0.40a	±0.034
Flavonoids	0.52a	0.43c	0.45b	±0.040
Tannins	0.21b	0.23ab	0.24a	±0.027
Saponins	0.15b	0.12c	0.19a	±0.042
Phonols	0.04b	0.06a	0.06a	±0.008

NB: Means with the same letter(s) in each row, under each species are not significantly different (P=0.05), using DMRT.

4.10 Quantitative Analysis of the Phytochemicals Extracted from the *Phyllanthus* Species Irrespective of their Parts and the Solvent.

The combined ANOVA of the data irrespective of the plant parts and the solvent (Table 4.10) showed some significant differences not significant differences of most of the phytochemicals by the combination of the solvents from the combined plant parts. The highest extraction of alkaloids and tannins was from *Phyllanthus muellerianus,* flavonoids and saponins from *Phyllanthus discoideus* and phenols from *Phyllanthus discoideus* and *Phyllanthus muellerianus*. *Phyllanthus amarus* had the lowest of most of Phytochemicals extracted.

The highest alkaloids content extracted from *Phyllanthus muellerianus* was comparable to that of *Phyllanthus amarus* but significantly higher than the lowest from *Phyllanthus discoideus*. The highest flavonoids extracted from *Phyllanthus discoideus* was significantly higher than that of *Phyllanthus muellerianus* and *Phyllanthus amarus* and that of *Phyllanthus muellerianus* was significantly higher than the lowest from *Phyllanthus amarus*. The highest tannins content extracted from *Phyllanthus muellerianus* was comparable to that of *Phyllanthus amarus* but significantly higher than the lowest from *Phyllanthus discoideus*. The highest saponins content extracted from *Phyllanthus discoideus* was significantly higher than that of *Phyllanthus muellerianus* and *Phyllanthus amarus* and that of *Phyllanthus muellerianus* was significantly higher than the lowest from *Phyllanthus amarus*. The highest phenols content extracted from *Phyllanthus discoideus* and *Phyllanthus muellerianus* was significantly higher than the lowest in *Phyllanthus amarus* (Table 4.10).

Table 4.10: Quantitative Analysis of the Phytochemicals in the Combination of Solvents and Parts [Roots, Stem and Leaves].

Phytochemicals [mg/g]	***Phyllanthus discoideus***	***Phyllanthus amarus***	***Phyllanthus muellerianus***	**S.E±**
Alkaloids	0.31b	0.40a	0.41a	±0.031
Flavonoids	0.52a	0.38c	0.50b	±0.028
Tannins	0.20b	0.23a	0.24a	±0.014
Saponins	0.20a	0.09c	0.17b	±0.013
Phenols	0.06a	0.04b	0.06a	±0.005

NB: Means with the same letter(s) in each row, under each species are not significantly different (P=0.05), using DMRT.

4.11 Quantitative Analysis of the Phytochemicals Extracted by Methanol and Aqueous Solvents Irrespective of the *Phyllanthus* Species and their Parts.

The Analysis of Variance (combined ANOVA) of the data on the phytochemicals extracted by methanol and aqueous solvents irrespective of their species and their parts (Table 4.11) showed some significant and not significant differences of the phytochemicals from the combination of parts and species. There was higher extraction of alkaloids, flavonoids, tannins and phenols by the methanol solvent than the aqueous solvent.. Saponins in the aqueous solvent was however higher than that of methanol solvent. Generally aqueous solvent had the lowest extraction of almost all the Phytochemicals.

The higher alkaloids, flavonoids and phenols contents extracted by the methanol solvent were significantly higher than that of the aqueous solvent. The higher tannins content extracted by methanol solvent was comparable to that extracted by aqueous solvent. The higher saponins content extracted by aqueous solvent was significantly higher than the one that was extracted by methanol solvent (Table 4.11).

Table 4.11-: Quantitative Analysis of the Phytochemicals in the Combination of Species and Parts (Roots, Stem and Leaves).

Phytochemicals[mg/g]	Methanol solvent	Aqueous solvent	S.E ±
Alkaloids	0.54a	0.21b	±0.021
Flavonoids	0.63a	0.30b	±0.017
Tannins	0.23a	0.22a	±0.018
Saponins	0.14b	0.16a	±0.029
Phenols	0.08a	0.03b	±0.009

NB: Means with the same letter (s) in each row, under each species are not significantly different (P=0.05), using DMRT.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Occurrence and Variance in Phytochemicals of the Three *Phyllanthus* Species

Nature has been the source of medicinal agents for thousands of years and since the beginning of man. In Nigeria medicinal plant-based industry is growing annually for pharmaceuticals, phytochemicals, neutraceuticals, cosmetics and other valuable products (Kumar and Bhardwaj, 2012).

The results of the qualitative phytochemical screening of this research work on the aqueous and methanol extracts of the plant parts (roots, stem and leaves) of the three selected *Phyllanthus* species (*Phyllanthus discoideus, Phyllanthus amarus and Phyllanthus muellerianus*) revealed the presence of medicinally active secondary metabolites such as alkaloids, flavonoids, tannins, saponins, phenols, glycosides, anthraquinones, triterpenes and sterols in most of the plant parts, although to varying intensities. This is similar to the work of Mehta *et al*. (2013) on the phytochemical analysis of *Phyllanthus fraternus* which revealed the presence of medicinally active constituents like tannins, alkaloids, terpenes, sterols and saponins. Mdlolo et *al* (2007), also undertook studies on phytochemical constituets and antimicrobial studies on two South Africa *Phyllanthus* species. The phytochemical screening of the two species of *Phyllanthus* revealed the presence of secondary metabolites which are of medicinal interest. This research work therefore, supports the claims on the folkloric use of the plant parts of this *Phyllanthus* species in herbal medicine.

Lakshmi and Venkata (2012), also researched on the phytoconstituents of *Phyllanthus* species, the petroleum ether, methanol and water extracts of different parts (leaf, shoot, stem bark, root and root bark) of fifteen species of *Phyllanthus* were screened for

phytochemical constituents, the presence of alkaloids, coumarins, flavonoids, phenols, steroids, glycosides, saponins and others, are common compounds in all the tests Other similar research works to this, were carried out by Ganesh and Vennila (2011), (Irshad *et al.,* 2010), Olawale-Abulude (2007), Obianime and Uche (2009), Burkill (1994), Victor and Chidi (2009), and Alagesaboopathi and Sirakumar (2011).

Preliminary qualitative test according to Mallikharjunah *et al*. (2007) is useful in the detection of bioactive principles and subsequently may lead to drug discovery and development.

5.2 Interpecific Variation of Phytochemicals in the Three *Phyllanthus* Species

The results obtained in this study suggest that the identified phytochemical compounds may be the bioactive constituents responsible for the efficacy of the plants parts of the species studied. The presence of some of these compounds has also been confirmed to have antimicrobial activity. Hence, it could be inferred that the plant extracts could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection. This is similar to the findings of Victor and Chidi (2009), and Alagesaboopathi and Sirakumar (2011).

The methanol extracts of the roots revealed the absence of phenols in the three *Phyllanthus* species, anthraquinones and triterpenes in *Phyllanthus amarus* and very deep presence of flavonoids only in *Phyllanthus discoideus.* The stem revealed the absence of anthraquinones and triterpenes in *Phyllanthus amarus*.The leaves revealed the absence of phenols and anthraquinones in *Phyllanthus amarus* only and sterols in *Phyllanthus discoideus* and *Phyllanthus muellarianus*. Intra- specific variation in phyto-constituents has been documented extensively among plants (Khan *et al*., 2010)

The aqueous extract of the roots revealed the absence of phenols, anthraquinones and triterpenes in *Phyllanthus amarus* and phenols only in *Phyllanthus discoideus* and *Phyllanthus muellarianus*.The stem revealed the absence of anthraquinones and triterpenes in *Phyllanthus amarus*, phenols in *Phyllanthus discoideus*.There were no phenols and anthraquinones in the leaves of *Phyllanthus amarus*, phenols and sterols were absent from the leaves of *Phyllanthus muellarianus*. Other similar works carried out that were related to the findings of this present research work includes, Qualitative phytochemical screening of two species of *Avicennia* (Ganesh and Vennila, 2011) and three curcubits (Irshad *et al*., 2010) revealed the presence of alkaloids, flavonoids, tannins, saponins and phenols at various intensities in the two species, and this occurrence was in a manner suggesting close relationship of the two species studied. Similar results were obtained in this research work which confirmed the relatedness of the investigated three *Phyllanthus* species as well as revealed their potentials in the drug industry. Burkill (1994), also reported the presence of tannins and saponins (at various intensities) in the leaves of *Jatropha gossypifolia* and *Jatropha multifida* respectively which is also similar to the results of this research work.

Olawale-Abulude (2007), undertook phytochemical screening of leaves of twenty-eight woody species from different plant families in Nigeria and discovered the presence of tannins, alkaloids and flavonoids in all the samples. Thus these secondary metabolites seem cosmopolitan in plants but to varying degrees and types.

5.3 Varying Intensities of Phytochemicals in the Different Parts of the Three *Phyllanthus* Species

The different degrees of the occurrence of the phytochemicals in plant parts confer taxanomic usefulness on them. This is similar to the findings of Nyananyo *et al*. (2010) on some Niger Delta plants.

Obianime and Uche (2009), in their study on The Phytochemical constituents and the effects of methanol extracts of *Phyllanthus amarus* leaves on the homornal parameters of male guinea pigs discovered the presence of phytochemicals in the leaves of *Phyllanthus amarus* to include: flavonoids, tannins, saponins, alkaloids, terpenoids, steroids and cardiac glycosides.These are similar to the findings of this research work.

The methanol extracts of the three species of *Phyllanthus* revealed that in *Phyllanthus discoideus,* the highest extracted phytochemical was the flavonoids followed by alkaloids in the stem and leaves and saponins in the roots, followed by tannins and the lowest was the phenols. In *Phyllanthus amarus,* alkaloids are highest in the stem and leaves, followed by flavonoids with the highest in the roots, followed by tannins, followed by saponins in the roots and leaves, the lowest is phenols. In *Phyllanthus muellerianus,* flavonoids is the highest in roots and leaves, followed by alkaloids in the stem, followed by tannins, then saponins and the lowest is the phenol. These findings are also similar to the research work carried out by Herin *et al.,* 2012, on the qualitative and quantitative analysis of phytochemicals in five *Pteris* species in which alkaloids and flavonoids had the highest content in the extractions. This is also similar to the findings of Kumar and Bhardwaj (2012) in Comparative, Qualitative and Quantitative Chemotypic Characterization among North India *Tribulus terrestris.*

The aqueous extracts of the three *Phyllanthus* species revealed that in *Phyllanthus discoideus,* alkaloids has the highest concentration in the roots and the stem, followed by flavonoids which has the highest concentration in the leaves, followed by tannins and then saponins, the lowest concentration was that of phenols. In *Phyllanthus amarus,* flavonoids has the highest content in the roots and the stem, followed by alkaloids which has the highest content in the leaves, followed by tannins and then saponins, the lowest contents is

phenols. In *Phyllanthus muellerianus*, flavonoids has the highest content in the roots and leaves, followed by saponins which has the highest content in the stem, followed by tannins and then by alkaloids, the lowest content is phenols. This is also similar to the findings of Mdlolo *et al*., 2007, on phytochemical constituents and antimicrobial studies of two South African *Phyllanthus* species. A higher percentage of alkaloid, saponin and flavonoid were observed in the quantitative analyses of the two *Phyllanthus* species studied.

The combined ANOVA of the data on the methanol and aqueous extracts of the three *Phyllanthus* species revealed that in *Phyllanthus discoideus*, flavonoids has the highest concentration, followed by alkaloids, followed by saponins and then tannins, the lowest concents is phenols. In *Phyllanthus amarus*, flavonoids has the highest concentration, followed by tannins, followed by saponins and then alkaloids, the lowest concentration is phenols. In *Phyllanthus muellerianus*, flavonoids has the highest concentration, followed by alkaloids, followed by tannins and then saponins, the lowest concentration is phenols. This is similar to the findings of Nwokocha *et al*. (2011), who researched on the comparative phytochemical screening of *Jatropha* species in the Niger delta and discovered that, among the five groups of phytochemicals investigated from the leaf, roots, seed and stem of the species phenols were found to be the lowest in concentration. The differences in the concentrations of the phytochemicals in the different *Phyllanthus* species are likely to be due to the fact that the variance in their compositions, establishes the fact that the plants under study are not the same (even though they belong to the same genus), and they are not likely to have the same medicinal potentials. These secondary metabolites are known to exhibit medicinal activity as well as physiological activity.

The quantitative analysis of the phytochemicals extracted by methanol revealed that, in *Phyllanthus discoideus*, flavonoids has the highest concentration, followed by alkaloids and

followed by saponins and then tannins, the lowest is phenols. In *Phyllanthus amarus*, alkaloids has the highest concentration, followed by flavonoids, followed by tannins and then saponins, the lowest is phenols. In *Phyllanthus muellerianus*, flavonoids and tannins has the highest contents, followed by alkaloids and then saponins, the lowest is phenols.

5.4 Varying Intensities of Phytochemicals Extracted by Different Solvents

The quantitative analysis of the phytochemicals in the three species of *Phyllanthus* under study showed that the methanol solvent extracted the highest contents of flavonoids in the roots and stem, followed by alkaloids in the leaves, followed by tannins, followed by saponins, the lowest is phenols. The aqueous solvent extracted the highest contents of flavonoids in the roots and phenols in the stem and leaves, followed by alkaloids in the roots and tannins in the stem and leaves, the lowest is the saponins. Among the two solvents used for the extraction, methanol extracted the highest content of the phytochemicals in general, this may be due to the fact that methanol solvent exhibited positive reaction for maximum number of phytoconstituents than the aqueous solvent. This result is similar to the findings of Lakshmi and Venkata (2012), on the phytochemical constituents of *Phyllanthus* species from Eastern Ghats of Pradesh, India. Three solvents, methanol, petroleum ether and aqueous were used for the extraction of 15 *Phyllanthus* species, and the methanol solvent extracted the highest content of the phytoconstituents followed, by petroleum ether and the lowest phytoconstituents were extracted by aqueous. Herin *et al.* (2012) also observed that the *Pteris* species studied showed varied degree of phytoconstituents with reference to the solvents used for the plant extracts.

In the quantitative analysis of phytochemicals in three species of *Phyllanthus* under study, the methanol solvent extracted the highest flavonoids from *Phyllanthus discoideus* and *Phyllanthus muellerianus*, followed by alkaloids from *Phyllanthus amarus*, followed by

tannins from *Phyllanthus amarus* and *Phyllanthus muellarianus* and saponins from *Phyllanthus discoideus*, the lowest is the phenols. The aqueous solvent extracted the highest flavonoids, followed by alkaloids and tannins, followed by saponins, the lowest is phenols from *Phyllanthus discoideus.*The highest flavonoids, followed by alkaloids, followed by tannins, followed by saponins, the lowest is phenols from *Phyllanthus amarus.* The highest flavonoids, followed by saponins, followed by tannins, followed by alkaloids, the lowest is phenols from *Phyllanthus muellarianus*.

Among the two solvents used in this study (methanol and aqueous), the highest content of the Phytochemicals were extracted by methanol solvent.

In the quantitative analysis of the total phytochemicals (combination of methanol and aqueous solvents), the highest content of phytochemicals extracted from the roots,stem and leaves is flavonoids, followed by alkaloids, followed by tannins, followed by saponins, the lowest is the phenols.

In the quantitative analysis of the phytochemicals (combination of methanol and aqueous solvents), the highest phytochemical extracted is flavonoids from *Phyllanthus discoideus* and *Phyllanthus muellarianus*, followed by alkaloids from *Phyllanthus amarus*, followed by tannins, followed by saponins, the lowest is phenols.

In the quantitative analysis of the phytochemicals from the three species and their parts, the highest content of flavonoids, alkaloids, tannins and phenols were extracted by methanol solvent and the highest saponins was extracted by aqueous solvent.

It is noteworthy that while all these phytochemicals are present in the different *Phyllanthus* species connoting taxanomic affinity, the differences in their concentrations uniquely confers individualism on each species and thus support their being treated as taxanomic species.This agrees with Irshad *et al*. (2010) report in which they carried out the

phytochemical screening of *Lagenaria siceraria*, *Luffa cylindrical* and *Cucumis maxima.*, their findings establishes a closer relationship between the *Lagenaria siceraria* and *Luffa cylindrical* than *Cucumis maxima.*

Flavonoids have been shown to possess many pharmacological properties such as: anti-oxidant activities, anti-inflammatory activities, anti- cancer activities and anti- microbial effects, hence, since the *Phyllanthus* species in this study possess flavonoids at very high concentration in their different plant parts, the flavonoids may be said to have a contributory effect to the Pharmacological effects the plant possesses (Joy and Kuttan, 1998; Kassuya *et al*., 2003; Adeneye, 2006). Numerous studies have shown that saponins possess the unique property of precipitating and coagulating the red blood cells, therefore the moderate intensity of saponins in the different parts of the three *Phyllanthus* species also indicate that the plants will not be deleterious to users if consumed as drug for the treatment of sicknesses and diseases. They were also revealed to contain high contents of alkaloids which suggest their anti-oxidant potentials, and it also represents a class which affects the Central Nervous System (CNS), reduces appetite and behaves as diuretic (Okwu, 2004). The moderate level of their tannins may also suggest the usage of these plants in herbal medicine, because it might not interfere with dietry iron absorption nor interfere with digestive enzymes (Eleazu *et al*., 2012). The tannins also help to hasten the healing of wounds and inflamed mucous membrane.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Phyllanthus plants belong to the family Euphorbiaceae. It is used as herbal medicine. The present study shows the qualitative and quantitative analysis of phytochemicals in the roots, stem and leaves of *Phyllanthus discoideus, Phyllanthus amarus* and *Phyllanthus muellerianus.* Their qualitative analysis revealed the appearance of phytochemicals such as alkaloids, flavanoids, tannins, saponins, phenols, glycosides, anthraquinones, triterpenes and sterols, where as their quantitative analysis gave approximate idea of the quantity these phytochemicals in the extracts.

Phytochemical survey is now acted as the first step towards the discovery of useful drugs. The Phytochemical constituents present in *Phyllanthus* plant act as a potential source of useful medicine for the treatment of various diseases.

Present study provides evidence that the methanol and aqueous extracts of the three selected species of the *Phyllanthus* plants contain medicinally important bioactive compounds and this justifies the use of these plant species in traditional medicine for treatment of diseases (Mehta *et al*., 2013).

The qualitative analysis carried out to detect the phytochemicals present in the methanol and aqueous extracts of the plants in this study showed that alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, glycosides, anthraquinones, triterpenes and steroids are present in all the plant parts of the three *Phyllanthus* species, although at various intensities. The methanol extracts of the plant parts showed more of the very deep presence, deep presence and presence of most of the phytochemicals than in the aqueous extracts.

The quantitative analysis carried out to determine the approximate quantity of the phytochemicals in the methanol and aqueous extracts of the plant parts in this study, revealed the variance in the composition of the phytochemicals in the three *Phyllanthus* species under study, and this establishes the fact that the three plants are not the same and are not likely to have the same medicinal potentials. The highest content of the phytochemicals was extracted from *Phyllanthus muellarianus*, followed by *Phyllanthus discoideus*, and the lowest was from *Phyllanthus amarus*. These phytochemicals are known to exhibit medicinal activity as well as physiological activity on organisms (Sofowora, 1993). The Percentage of richness of bioactive compounds in this present study were revealed to be more in the leaves, followed by the stem and then the lowest in the roots.

Among the two solvents used in this study (methanol and aqueous), methanol extracts exhibited the highest concentration of the Phytochemicals.

It can be concluded that the phytochemical analysis of the three *Phyllanthus* species researched in to, yielded a set of qualitative and quantitative pharmaco-botanical parameters or standards (such as the part of the plants that has the highest composition of the phytochemicals, the solvent that extracted the highest content of the phytochemicals), and this can serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies.

6.2 Recommendations

There is a need for further research on the selected *Phyllanthus* species, for identification, isolation, purification and characterization of their bioactive compounds using different detectors and chromatographic techniques such as liquid chromatography – mass spectrometry to provide a greater insight to the phytochemical composition of the plants.

The three selected *Phyllanthus* species in this study produced a wide range of phytochemicals, which may prove to be invaluable in development of drugs, flavours, fragrances, dyes, anti-oxidants and insecticides, it is therefore important to locate the role of these phytochemicals and unravel their biosynthesis through future research.

Since it is being anticipated that phytochemicals from medicinal plants like the three *Phyllanthus* species studied, need to be widely researched and processed for the treatment of infections and diseases, efforts should be geared up to exploit the biomedical applications of these screened plants due to the presence of the set of phytochemicals for their full utilization.

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