ASSESSMENT OF THE PROXIMATE, PHYTOCHEMICAL, NUTRITIONAL AND ANTINUTRITIONAL COMPOSITION IN *Borreria stachydea (*DC)Hutch and Dalziel, *Cassia absus* Linn and *Aspillia kotschyi* (Sch.Bipex,Hochst)oliv

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DECLARATION

I declare that this thesis entitled **"Assessment of the Proximate, Phytochemical, Nutritional an Anti nutritional Composition in *Borreria stachydea (*DC)Hutch & Dalziel ,*Cassia absus* Linn and *Aspillia kotschyi* (Sch.Bipex,Hochst)oliv"** has been carried out by me in the Department of Chemistry Ahmadu Bello University Zaria. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for degree or diploma at this or any other Institution.

ABDU ADAMU UMAR	Signature	Date

CERTIFICATION

This thesis entitled **"ASSESSMENT OF THE PROXIMATE, PHYTOCHEMICAL, NUTRITIONAL AND ANTINUTRITIONAL COMPOSITION IN *Borreria stachydea* (DC) Hutch & Dalziel , *Cassia absus* Linn AND *Aspillia kotschyi* (Sch.Bipex,Hochst)Oliv"** by Abdu, Adamu UMAR meets the regulation governing the award of the degree of Doctor of Philosophy in Analytical Chemistry of Ahmadu Bello University, Zaria, Nigeria, and is approved for its contributions to knowledge and literary presentation

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DEDICATION

This work is dedicated to my beloved parents and my children for the sake of scientific research.

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LIST OF ABBREVIATION

AAS: Atomic Absorption Spectroscopy

AES: Atomic Emission spectroscopy

AOAC: Association of official Analytical Chemist

FAO: Food and Agricultural Organist ion

ICP: Inductively coupled Plasma

I ARC: International Agency for research on Cancer

MS : Mass Spectroscopy

RDA: Recommended Dietary Allowance

TLC: Thin layer Chromatography

XRF: X-Ray fluorescence Analysis

WHO: World Heath Organization

ABSTRACT

In this present work, the phytochemical constitution of *Borreria stachydea* , *Cassia absus* and *Aspillia Kotschyi* plants belonging to *rubiacea* , *leguminosae* and *Compositae* families respectively were investigated. The three plants methanolic and petroleum ether extracts were analyzed for alkaloids, tannins, saponins, steroids, terpenoids, flavonoids, glycosides and phenolic compounds. The study results showed that the petroleum ether extract of the three plants contained glycosides, steroids carbohydrates and alkaloid while the methanolic extracts of the three plants revealed the presence of carbohydrates, glycosides, cardiac glycosides, saponins, and flavonoids, triterpene, and alkaloids. Tannins were also observed to be present in the methanolic extract of *Borreria stachydea* only. The proximate analysis of the two plant materials *Borreria stachydea* and *Cassia absus* (whole) showed moisture content of 8.67 % and 6.67 % and ash content of 8.5 % and 8.0 % in *Borreria stachydea* and *Cassia absus* respectively. The crude protein was 8.08 % and 9.81 %, crude fibre 7.11 % and 3.17 %, crude fat 2.15 % and 2.11 %, and Nitrogen Free Extract (NFE) 65.59 % and 70.36 % in *Borreria stachydea* and *Cassia absus* methanolic extracts respectively. The petroleum spirit extracts of *Borreria stachydea* and *Cassia absus* showed crude protein of 5.63 % and 4.88 %, crude fibre 0.11 % and 2.65 %, crude fat 3.13 % and 1.94 % and NFE 89.27 % and 88.39 % in the two plants respectively On the other hand *Aspilla kotschyi* Proximate composition analysis showed moisture content of 5.7%, total ash of 4.03% ,crude protein 10.94%, fibre 9.06%,Fat value 0.83% and Nitrogen free extract of 70.19%. This study also assesses the presence of Calcium, Magnesium, Zinc, Lead, Chromium, Cadmium, in the three plants using AAS in the plants extracts average elemental concentrations for *Borreria stachydea* were 0.4272, 0.42625, 0.0193, 0.0003, 0.1425 and 0.0008 mg/Kg for Ca, Mg, Zn, Pb, Cr, and Cd respectively, while those for *Cassia absus* were 0.5093, 0.6423, 0.0320, 0.0010, 0.1616 and 0.0028 mg/Kg for Ca, Mg, Zn, Pb, Cr, and Cd respectively. *Aspilla kotschyi* extract showed the following elemental concentrations Calcium 0.3099, Magnesium 0.1380, Zinc 0.2449,Lead 0.0020,Chromium 0.0018, and Cadmium 0.0026. Other metals ; Se, Ag, Fe, Cu, Ni, As, Co, Mn and Al were determined from both the methanolic and petroleum spirit extracts of the three plants using micro wave plasma atomic emission spectroscopy technique. From the result, Silver, Copper, Nickel and Cobalt are of very negligible

concentrations in all the three plants, however Seleniun is found to be 0.780 (mg/kg) in *Casssia absus* methanolic extract , 0.556 (mg/kg) in *Borreria stachydea* methanolic extract, and 0.530 (mg/kg) in *Aspillia kotschyi* methanolic extract. Iron on the other hand was found to be 3.712 (mg/kg), in *Aspillia Kotschyi* metahanolic extract, while in *Borreria stachydea* petroleum spirit extract was 3.674 (mg/kg) . Arsenic was found to be 0.506 and 1.301 (mg/kg) in *Aspillia Kotschyi* extracts, 2.373(mg/kg) and 0.789(mg/kg) in *Borreria Stachydea* extracts, and in *Cassia absus* extract was found to be 3.315 (mg/kg). The concentration of Aluminium was found to be of 3.050(mg/kg) in *Aspillia* extract and 2.99(mg/kg) in *Borreria stachydea* extract. Sodium and potassium were determined in the three plants extracts using flame photometry. Sodium was found to be 50, 78 and 99 mg/l in *Borreria stachydea*, *Cassia absus* and *Aspillia kotschyi* respectively. The potassium level in the three plants were 870,860 and 895 mg/l respectively. Functional group analysis of the three plants extracts were also carried out using Fourier Transform Infrared Spectroscopy (FTIR) after the extract has been purified by column chromatography. The functional groups determined in the three extracts showed that the plant samples contained the following functional groups; O-H Alcohols, phenols , C-H for Alkanes , C=O Unsaturated aldehydes and ketones and C=O Aliphatic amines. The results of this study suggest some merit in the popular use of these plants in herbal medicine as these functional groups are known to have therapeutic effectiveness against wide array of microorganisms.

CHAPTER ONE

1.0 Introduction

Plants in general, contribute to the energy/calorie, mineral, vitamin and fibre contents of diets. Among plants, vegetables are excellent sources of minerals and contribute to the recommended dietary allowance (RDA) of the essential nutrients. Minerals are very important ingredients for normal metabolic activities of body tissues. They are constituents of bones, teeth, blood, muscles, hair and nerve cells. Vitamins cannot be properly assimilated without the correct balance of minerals (Sonni, 2002). Plants have occupied very important position in human life for a very long time. They provide food, medicine, fibre and fodder for man and his animals. A variety of plants have promising nutritive value which could nourish the ever increasing human population. They however remain underutilized due to lack of awareness and technologies for their utilization.

Currently, there is a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect, especially when compared with synthetic drugs. Thus, the search for new drugs with better and cheaper substitutes from plant origin is a natural choice. The medicinal value of these plants lies in some chemical substances that induce a definite physiological action in the human body (Edeoga *et al.*, 2005).

In Nigeria, numerous edible wild plants are exploited as sources of food, which provide adequate levels of nutrients for inhabitants. Some edible plants are also used in traditional medicine, some of these herbs are indigenous to Africa, America and India. The use of herbs is common element in ayurvedic, homoeopathic, naturopathic traditional oriented medicine

(Ogundipe, 2008). The pharmacological actions of crude drugs are determined by the nature of their constituents (Mukherjee, 2002).

Plants based drugs have been used against various diseases since time immemorial. The primitive man use herbs as therapeutic agents and medicaments, which they were able to produce easily. Nature has provided abundant plant wealth for all living creatures, which possess medicinal virtues. The most important values of some plants have been published in many books and journals but a large number of them remain unexplored. So there is need to explore their uses and conduct pharmacognostic and pharmacologicacal studies to ascertain their therapeutic properties (Mushtaq,*et al.*, 2009).

Traditional medicine as a major African socio cultural heritage, obviously in existence several hundred years, was once believed to be primitive and wrongly challenged with animosity, especially by the conventional or orthodox medical practioners. However today traditional medicine have been brought into focus for meeting the goals of a wider coverage of primary health care delivery, not only in Africa but also to various extents, in all countries of the world. Traditional medicine is the first choice health –care treatment for at least 80% of Africans who suffer from high fever and other common ailments(Eliyoba *et al.*, 2005).

Each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals. These nutrients are essential for the physiological function of human body. Such nutrients and biochemical's like carbohydrates, fats, and proteins play important roles in satisfying human needs for energy and life processes (Novac and Hasiber, 2000).

Plants generally contain compounds (such as saponins, tannins, oxalates, phylates, trypsin inhibitors and cynogenic glycosides) known as secondary metabolites which are biologically active (Soetan and Oyewale, 2009). Secondary metabolites may be applied in nutrition and as pharmacologically active agents (Soetan and Oyewale, 2009).Plants are also known to have high amounts of essential nutrients, vitamins, minerals, fatty acids and fibre(Gafar and Itodo, 2011). Phytochemical screening of plants materials makes detailed pharmacological and other academic studies possible as it offers information of a particular chemical compound responsible for some said activities in a given plant (Sofowora, 1982).

The phytochemical investigation of a plant may thus involve the following: extraction of the plant material, separation and isolation of the constituents of interest, characterization of the biosynthetic pathways to particular compounds and quantitative evaluation (Trease and Evans, 2000).

Not all the chemical compounds found in plants are of equal interest to the pharmacognosist. The so called “active principles” are frequently alkaloids or glycosides and these therefore deserve special attention. Other groups such as carbohydrates, fats and proteins are of dietic importance, and many others such as starches and gums are used in pharmacy but lack any marked pharmacological action. Other substances such as calcium oxalate, silica, lignin and coloring matters may be of assistance in the identification of drugs and the detection of adulteration (Trease and Evans, 2000).

Results from phytochemical analysis have shown that the general classes of compounds found in plants which are of medicinal interest include alkaloid, cardiac glycosides, saponins, tannins and anthraquinones (Sofowora, 1982).

Plant materials contain several chemicals which act against diseases and infections of human and animals when properly used. Plants contain different types of compounds such as resins, rubbers, gums, waxes, dyes, flavours, fragrances, proteins, amino acid, bioactive peptides, sugars, flavonoids and biopesticides (Muhammed *et al.*, 2006).

Today according to the World Health Organization (WHO) as many as 80% of the world people depend on traditional medicine for their primary health care needs (Leila *et al.*, 2011). High plants are sources of drugs, which have made important contribution to the welfare and quality of life in the urban as well as rural communities, especially in tropics and subtropics (Sofowora,1993).

In the ancient days of human existence, by instinct, intuition, or trial and error, man was able to identify various plants used to combat various ailments. In fact, it is the knowledge derived from the active components of these plant extracts that guided man to synthesize and use modern drugs in health care delivery. Presently, there is a renewed interest in the study of medicinal plants such that much percentage of pharmaceutical preparations are based on natural products from plants. Over the years, people have passed down knowledge of the types and applications of medicinal plants from generation to generation, often orally. The compilation of useful drugs derived from medicinal plant is impressive; these include; heart drugs, analgesics, anesthetics, antibiotics, anti-cancer and anti-parasitic compounds, anti-inflammatory drugs, oral contraceptic hormones, as well as laxative diuretics (Morris, 2004).

Available medical literature revealed that plants have been used for curative purposes (Ming,1999). There are many plants whose green leaves, roots and stems are used in herbal preparations for the treatment of various ailments(Ekop, 2007).Most of the times the potency of those plants is accounted for in terms of their organic constituents but, it is an established fact that there is a high relationship between the chelation of metals and some chemotherapeutic agents. The role of inorganic elements in animal and plant metabolism has long been established but the effect and an influence of these elements on administration of medicinal plants has received relatively little attention (Gwarzo *et al*., 2006).

It is a common knowledge that administration of plants claimed to have medicinal properties has largely been indiscrimate without due regard to possible side effects. It is thus important to determine the chemical content of these plants for pharmacological assays and other probable roles in the curative process. In addition to that, plants are generally the major sources of food and minerals to both man and animals. The different species of plants play important roles in the diet of inhabitants of arid and semi –arid savanna countries of Africa. They contain minerals in quantities compared favorably with World Health Organization (W.H.O.) standard (Baminas *et al.*, 1998).

From the environmental, pollution and toxicological point of view, many minerals including those that are essential to life can be toxic at high doses or in certain compound formulation. So the amount of minerals in living organism normally correlate significantly with amounts in the environment. Also, heavy metals do not degrade but accumulate in food and are a serious threat to human health if consumed. In fact, there are certain plants known to have special ability which enable them to take elements with the tendencies of accumulating same to dangerous levels, thus making the plants toxic (WHO, 1980, IRI 1989, Szefer and Szefer 1994 and Last, 1995). Likewise it is a known fact that different parts of plants contain trace elements that are harmful to the body of man at a certain concentrations (Sani, 2014).

Sewage from homes, industrial effluents into aquatic environment, smoke from industries and vehicles,parents materials from which the soil is formed; types of agrochemicals used in farming and waste disposal systems all contribute to the elemental composition of plants.

It is estimated that today, plants materials are present in or have provided the models for 50% of industrially manufactured drugs (Iwu,1994). Therefore there is greater tendency for the accumulation of a particular element as suggested by Kovac (1979) and Whitton (1989) and the extent of accumulation is a reflection of the concentration of such materials in the environment as well as species of the plant in use. It is an established fact that metals in particular are present in both plants and animals as components of simple salt and complexes performing various functions (Yalwa, 2002).They are required by plants in amounts that result in their classification as essential, beneficial and non-essential for proper growth and development (Daniel, 1990).

It is a common knowledge that administration of plants claimed to have medicinal properties has largely been indiscriminate without due regard to possible side effects, or long term accumulation of toxic compounds in the plants tissue which may accumulate and became harmful to human tissues. It is therefore important to determine the chemical content of these plants for pharmacological assays and other probable roles in the curative process.

In recent times focus on plant research has increased all over the world and a large body of evidence has accumulated to show immense potential of medicinal plants used in various pharmaceutical, cosmetic, agrochemical applications. Plants have been the subject of human curiosity and use for thousand of years (Ram *et al.,* 2004). These plants have played important roles in many centuries by providing food, shelter, clothing, agrochemicals, flavours and fragrances and more importantly medicines (Gurib- Fakim, 2006).

1.1 Mineral elements

Minerals are inorganic substances, present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life. Minerals are chemical constituents used by the body in many ways. Although they yield no energy, they have important roles to play in many activities in the body (Malhotra, 1998; Eruvbetine, 2003).

In addition, plants are the major sources of food and minerals to both man and animals. The different species of plant play important roles in the diet of the man and his animals. They contain minerals in quantities compared favorably with foods of World Health Organization (W.H.O.) standard (Barminas *et al*., 1998). Of particular importance among the parts of the plant are the leaves and the seeds containing trace elements at varying levels depending on many factors such as species, variety, stage of maturity and environment (Daun and Mc Gregor, 1991).

Moreover metals and in particular trace metals are major class contaminants in our present world arising principally from natural and anthropogenic sources. The anthropogenic sources are primarily from industrialization and mining activities which are paramount importance to ecosystem sustainability (Okorie and Egila, 2012).

1.2.0 Proximate Analysis

A method was developed for quantitative analysis of the different macronutrient in plant samples and feed known as proximate analysis and the method was developed in the year 1860 by Henneberg and Stohmann in Germany (Greenfield and Southgate, 2003).

Proximate Analysis is a partitioning of compounds in a feed into six categories based on the chemical properties of the compound. The six categories are;

i) Moisture

ii) Ash

iii) Crude protein (or Kjeldahl protein)

iv) Crude lipid

v) Crude fibre

vi) Nitrogen free extract (digestible carbohydrates)

In the proximate analysis of feed stuff's kjedahl nitrogen, ether extract, crude fibre and ash are determined chemically.

The determination of Nitrogen allows the calculation of the protein content of the sample. It is important to remember that proximate analysis is not a nutrient analysis rather it is a partitioning of both nutrients and non nutrients into categories based on common chemical properties.

Proximate and nutrient analyses of edible plant and vegetables play a crucial role in assessing their nutritional significance (Pandey *et al*., 2006). As various medicinal plant species are also used as food along with their medicinal benefits, evaluating their nutritional significance can help to understand the worth of these plant species (Pandey *et al*., 2006) For herbal drug's standardization, WHO also emphasize on the need and importance of determining proximate and micronutrients composition of the herbal plants. Such herbal formulations must pass through standardization processes (Niranjan and Kanaki, 2008).

1.3 Borreria stachydea [(Dc)Hutch &Dalziel]

This plant belongs to the phylum *magnoliophyta* and its class is *manoliopsida* and a member of the *Rubiaceae* family. It is found in Nigeria, Ghana, Sudan, Malaysia, India and several other nations of the world. A poultice of the whole plant is used to heal leg ulcer, wounds, urinary tract infections (Neoh, 2010). In Northern Nigeria the Hausa popularly know and call *Borreria stachydea* as "alkamar tururuwa" while Fulanis called it "fairare". The plant is used in Northern

Nigeria medicinally for urinary troubles, gonorrhoea ,etc and for women in childbirth, or to regulate the menses (www.wikipedia) June 2014.

1.4 Cassia absus Linn

This plant belongs to the family *leguminosae* and sub family *caesalpinonodeae*. It is found in tropical Asia, Australia, and Africa .It is a useful medicinal plant. It is regarded as a blood tonic, bitter astringent for the bowels and applied locally to heal ulcers (Hussain, *et al*, 2008). It is useful in the disease of eyes such as purulent conjunctivitis and opthalmia (Ghani, *et al*, 1997). *Cassia abus* Linn is popularly known as "fidili" in Hausa and in English is called "jasmeejaz"

1.5 Aspilla kotschyi

Aspilla kotschyi belongs to the family *compositae* which is also known as *Asteracea*. The common names of this plant in Nigeria are "Ja majina" (draw up mucus in hausa) or "Jinin barewa". Historically *Aspillia africana* was used in Mbaise and most Igbo speaking parts of Nigeria to prevent conception suggesting potential contraceptive and anti-fertility properties (Oluyemi, *et al.*, 2007).

1.7 Statement of Problem

The continuous increase in the use of plants in traditional medicine has become a cultural heritage for several hundred years in many developing nations including Nigeria. A knowledge of the available metabolites, nutrients in these plants sources and the establishment of the presence of the level of toxic anti nutritional substances in the plants will help to determine the suitability of using these plants as remedies for various diseases.

This study underlook the task of determining the nutrient composition, the secondary metabolites and anti nutritional substances in three locally used medicinal plants in Nigeria namely;

Borreria stachydea, Cassia absus and Aspillia kotschyi plants. The study highlighted the presence and suitability of using these plants locally as remedies for various diseases and sources of food.

1.8 Aims of the Research

The study aimed at achieving the following:

i. Phytochemical screening of the various extracts of the three different plants, *Borreria stachydea* {(D C Hutch & Daziel}, *Cassia absus* Linn and *Aspillia kotschyi* plants to determine the bioactive agents responsible for their physiological activity or otherwise.

ii. Evaluation of the nutritive components (Proteins, Lipids, carbohydrates, fibre and vitamins) and anti nutritive components (Cyanides, tannins, oxalates and phytates) in *Borreria stachydea* {(D C Hutch & Daziel},*Cassia absus* Linn and *Aspilia kotschyi* plants.

ii. Determine qualitatively and quantitatively the major, minor, trace and ultra trace elements in the above listed plants and

iii. Make comparative analysis, and come out with appropriate recommendations on their toxicity or otherwise.

The above aims are individually of special problem-solving relevance. They are also complimentary in helping to provide the much needed information on the scientific data of the plants extracts of *Borreria stachydea* {(DC Hutch & Daziel},*Cassia absus* Linn and *Aspilia kotschyi* which may find use as patentable and industrially exploitable compounds for drugs development, food and cosmetics industries among others..

1.9 Objectives of the Research

The objectives of the research are as follows:

i. Collection of the three plant samples
ii. Proper botanical identification of the plant at the herbarium
iii. Air drying, segregating and pulverizing the plants.
iv. Extracting using appropriate solvent of the pulverized plant parts
v. Phytochemical screening of the extracts of the three different plants
vi. Conducting proximate analysis of the three plants
vii. Determination of anti nutritive factors in the three plants
viii. Determination of major, minor, trace & ultra trace elements from the three different plants
ix. Purifying the three plants extract
x. Determination of Functional groups present in the three plants by Fourier Transform Infrared (FTIR) spectroscopy.
xi. Conducting statistical analysis on the results obtained so as to draw conclusions and recommendations on the use of the three plants.

1.10 Justification of the Research

Right from time immemorial, wild plants have occupied very important position in human life. They provide food, medicine, fibre and fodder for domestic animals. So many plants of promising nutritive values, which could nourish the ever increasing human population, remain underutilized due to lack of awareness and practicalization of technologies for their utilization. Many of them are even more resilient, adaptive and tolerant to adverse climatic conditions than the conventional foods plants. The need to explore these underutilized (lesser) wild foods in order to enhance availability of foods and create a balance between population growth and agricultural productivity, particularly in developing countries like Nigeria is imperative. Exploration of the nutro-chemical potentiality of the wild lesser known plants through analysis of proximate composition will alleviate the problem of nutrient deficiency and reduce degenerative diseases in Nigerian populace. In addition, the in-depth understanding of the nutro-chemical characteristics of these plants under investigation will ensure their judicious use as food in addition to their role in traditional medicine.

CHAPTER TWO

2.0 Literature Review

2.1 Description of *Borreria stachydea*

Borreria stachydea [(Dc) Hutch &Dalziel] is an erect, hairy and weedy herb, about 0.3048 metres in height with mauve flowers. Taxonomically it belongs to the phylum *magnoliophyta* and class *manoliopsida* and a member of the *Rubiaceae* family. It is found in Nigeria, Ghana, Sudan,Malaysia,India and several other nations of the world. A poultice of the whole plant is used to heal leg ulcer, wounds, urinary tract infections and was found to be highly antioxidative in nature .*Borreria* is relatively large genus of herbs or semi-shrubby plants. This genus consists of about 100 species distributed troughout the tropics (Neoh,2010).

2.1.1 Brief Taxonomy of *Borreria stachydea*

Kingdom: *Plantae*

Phylum: *Tracheophyta*

Class: *Magnoliopsida*

Family: *Rubiacea*

Genus: *Spermacoce Dill exl*

Species: *Spermacoce Stachydea Dc Borreria Stachydea Dc (hutch& dalziel)*

2.1.2.Local Names of *Borreria stachydea*

Hausa: Alkamar tururuwa
Fulani : Fairare
Hindi: Madanghanti
Tamil: nathaichoori
Telugu: Madona

Rubiaceae is a family of flowering plants commonly known as the coffee, madder or bed straw family. It consists of terrestrial trees, shrubs, or herbs that are recognizable by simple,opposite leaves, with interpepetiolar stipules. The family consists of about 13,000 species in 611 genera which makes it the fourth- largest angiosperm family. *Rubiaceae* have a cosmopolitan distribution, however, the largest species diversity is concentrated in the subtropics. (www.wikidpedia) 2014.

The distribution pattern of the family is very similar to the overall global distribution of plant diversity. However, the largest diversity is distinctly concentrated in the humid tropics and subtropics. An exception is the Rubieae tribe, which is cosmopolitan but centered in temperate regions. Only a few genera are pantropical (e.g. *Ixora*, *Psychotria*), many are paleotropical, while Afro-American distributions are rare (e.g. *Sabicea*). Endemic rubiaceous genera are found in most tropical and subtropical floristic regions of the world. The highest number of species is found in Colombia, Venezuela and New Guinea.

Rubiaceae consists of terrestrial and predominantly woody plants. Woody rubiaceous shrubs constitute an important part of the under storey of low- and mid-altitude rainforest. *Rubiaceae* are tolerant of a broad array of environmental conditions (soil types, altitudes, community structures, etc.) and do not specialize in one specific habitat type (although genera within the family often specialize).

In Northern Nigeria, the Hausa popularly know and call *Borreria stachydea* as “alkamar tururuwa” while Fulanis called it “fairare”.The plant is used in northern Nigeria medicinally for urinary tract infections, gonorrhoea ,etc and for women it is used to regulate menses.

Rubiacea is morphologically easily recognizable as a coherent group by a combination of characters; opposite leaves that are simple and entire, interpetiolar stipules, tabular sympetalous actinomorphic corollas and an inferior ovary.

A wide variety of growth forms are present: shrubs are most common, but members of the family can also be trees, Ilana’s or herbs. Some epiphytes are also present (e.g. Myrmecodia). The leaves are simple, undivided and entire blades are usually elliptic, with a cuneate base and an acute tip. In three genera (Pavetta, Psychotria, Sericanthe), bacterial leaf nodules can be observed as dark spots or lines on the leaves. The phyllotaxis is usually decussate: rarely whaled (e. g. fodogia): or rarely alternate resulting from the suppression of one leaf at each nod (e.g. sabicea sthenula) characteristic of rubiacea is the presence of stipules that are mostly fused to an interpetiolar structure on either side of the stem between the opposite leaves. Their inside surface often bears glands called “Collectors” which produce mucilagininious compounds protecting the young shoot. The whaled leaves of the herbaceous *Rubiacea* tribe have classically been interpreted as a true leaves plus interpetiolar leaf – like stipules. The inflorescence is a cyme rarely of solitary flowers e.g. Ruthmannia and is either terminal or axillary and paired at the nodes.

The flowers are usually bisexual and usually epigynous. The perianth is usually bisexual although the calyx is absent in some taxa (e g *Theligonum*). The calyx is 4 – 5 merous with basally fused lobes. The corolla is sympetalous 4 – 5 lobes, mostly actinomorphic, usually tabular, often white but also yellow, blue and rarely red. The stamens are 4 – 5 , alternipetalous and epipetalous. Anthers are longitudinal in dehiscence, but some genera are poricidal (e g *Rustia*). The gynoecium is syncarpous with an inferior ovary (rarely secondarily superior, eg. *Gaertnera Pegamea*) and 2 (3 – 5+) Carpels placentation is axial rarely parietal (e.g. *Gardenia*) ovules are anatropous to hemitropous, unitegmic with funicular obturator, 1 – many per carpel. Necteries are often present as a nectariferous disk atop the ovary. The fruits are a berry, capsule drupe, or schizocarp. Red fruits are fairly dominant yellow, orange or blackish fruits are equally common. Blue fruits are rather exceptional save in psychothecea and associated tribes. Most fruits are about one cm in diameter. Very small fruits are relatively rare and occur in herbaceous tribes: very large fruits are rare and confined to *Gardenieae.* The seeds are endospermous.

Proximate analysis of the leaves of *Borreria hispida* has shown that water soluble value was more which indicates the less contamination with metal ions (Patel, 2011). Similarly it was also reported that *Borreria verticillata* also from *Rubiacea* family the secondary metabolites (phytochemicals) were extracted using hexane,chloroform, acetone,ethylacetate and methanol in order of polarity. The phytochemical screening of the stem bark extract of the plant revelead the existing of terpene,cardiac glycosides,saponins, flavonoids and steroids.This showed that the *Borreria verticillata* stem bark are of medicinal value and due to their phytochemical content could be expoloited for use in pharmaceutical and cosmetic industries (Ushie, 2013).

The stem bark of *B. verticillata* are known to carry out important medicinal roles in human body as a result of the detection of flavonoids in the hexane, chloroform and ethyl acetate extracts from the stem bark. Flavonoids have inherent ability to modify the body's reaction to allergens, viruses and carcinogens. They show anti-allergic, anti-inflammatory, anti-microbial (Cushnie and Lamb, 2005).

It can be said that *B.verticillata* stem bark has economic value as well because saponins is used in the manufacture of shampoos, insecticides, preparation of various drug and steroidal synthesis (Sodipo and Akiniyi, 2000).

Plate 1.1 *Borreria stachydea* plant

2.2 Description of *Cassia absus* Linn

This plant belongs to the family *leguminosae* and sub family *caesalpininioideae*. It is found in tropical Asia, Australia, Africa and throughout India. The plant is a useful medicinal plant . It is regarded as a blood a tonic, bitter astringent for the bowels and applied locally to heal ulcers (Hussain, *et al*, 2008). It is useful in the disease of eyes such as purulent conjunctivitis and opthalmia (Ghani, *et al*, 1997). *C. abus* Linn is popularly known as “fidili” in Hausa and in English is called “jasmeejaz”

2.2.1 Brief Taxonomy of *Cassia absus:*

Kingdom: *Plantae*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Order*: Fabales*

Family: *leguminosae*

Sub family*: Caesalpininioideae*

Genus: *Cassia*

Species: *Absus*

2.2.2. Local Names of *Cassia absus*

Hausa: Fidili
Hindi: Chaksu
Bangali: Bankulthi,Banku
Gujarati: Chimed

It is an erect annual plant 30 -60cm high, distributed throughout India. All plants parts of the species are used in folk medicine. The leaves are bitter, acidic and astringent. The seeds are used in the treatment of opthalmia and skin infections. The leaves are used in treatment of constipation. The reported medicinal uses of roots are consistent with the presence of chrysophanol. (Hemen, *et al*., 2012).

Yadava, R. N. *et al.,* 2013 reported the uses of *Cassia absus* seed such as its diuretic and stimulant properties.This enable the treatment of ring worm; opthalmia skin affections. They also reported the isolation and structural elucidation of a new allelochemical 5,7, 4'-trihydroxy -8.3'-dimethoxyflavone-5-oα-L-rhamnopyranosy1-7-o-β-D-xylopyranosy1-(1-4)–Ogalactopyranoside. 1 long with two known compounds 3,5,7,4'-tetrahydroxy- 2', -5' – dimethoxy flavones of the 2 and luteolin 3 from methanolic extract of the seeds of this plant.

Plate 1. 2 *Cassia absus* plant

2.3 Description of *Aspillia kotschyi* (Sch.Bipex,Hochst)Oliv

Aspilla kotschi belongs to the family *compositae* which is also known as *Asteracea*. The common names of this plant in Nigeria are "Ja majina" i.e. to draw up mucus in Hausa or "Jinin Barewa". Historically *Aspillia africana* is used in Mbaise and in southern eastern Nigeria to prevent conception suggesting potential contraceptive and anti-fertility properties. (Oluyemi, *et al* 2007).

The name Asteraceae comes from Aster, the most prominent genus in the family that derives from the Greek dotrip, meaning star and is composed with its inflorescence star form. As for the term "compositae" more ancient but still valid it obviously makes reference to the fact that the family is one of the few angiosperms. Most members of Astracea are herbaceous, but a significant number are also shrubs, vines, or trees. The family has a world wide distribution and is most common in the arid and semi arid regions of subtropical and lowers temperate latitudes (Barkely, 2006).

It was reported that the leaf of *Aspilia africa* a plant that belongs to the same family effectively arrested bleeding from fresh wounds, inhibited microbral growth of known wound contaminants and accelerated wound healing process. (Okoli, *et al.*,2007)

Essiet and Akpan in 2013 reported that the stem of *Aspillia africana* contained metabolites such as saponins, tannins, flavonoid and cardiac glycosides .

Plate 1.3 *Aspillia kotschyi* plant

2.3.1 Brief Taxonomy of *Aspillia kotschyi*

Kingdom: *Plantae*

Division: *Agiosperm*

Class: *Eudicots*

Order: *Asterales*

Family: *Compositae*

Genus: *Aspillia*

Species: *Kotschyi(*sch.Bipex,Hochs*t)*oliv

2.3.2. Local Names of *Aspillia kotschyi*

Hausa: Jinin Barewa or Ja majina
Fulani : nyaki
English: Haemorrhage plant

2.4.0 Proximate Analysis

The proximate system for routine analysis of animal feedstuffs was devised in the mid nineteenth century at the Weende Experiment Station in Germany (Henneberg and Stohmann,1860, 1864). It was developed to provide a top level, very broad, classification of food components. The system consists of the analytical determinations of water (moisture), ash, crude fat (ether extract), crude protein and crude fibre. Nitrogen-free extract (NFE), more or less representing sugars and starches, is calculated by difference rather than measured by analysis (Greenfield and Southgate, 2003).

Although some of the methods used historically in the proximate system of analysis are not recommended for the preparation of food composition databases (e.g. crude fibre), it is useful to consider the concepts involved as they have dominated views on the composition of foods and food analysis. This system was developed at a time when the chemistry of most food constituents was only partially understood, and the growth of nutritional sciences have shown that for nutritional studies a more detailed and biochemically oriented approach to food analysis is needed. Nevertheless, proximate analysis, including the original methods, still forms the basis for feed analysis, and the analysis of foods for legislative purposes in many countries (Greenfield and Southgate, 2003).

Many people find the concept and term "proximates" useful to represent the gross components that make up foods; the actual analytical methods then become independent. Others believe that the definition of proximates is based on the original methods prescribed by Henneberg and Stohmann, and that method substitution, e.g. dietary fibre instead of crude fibre, negates the use of the term.

The aim of food composition databases should be to include all nutrients or other bioactive food components that are known or believed to be important in human nutrition. This ideal can rarely be achieved, especially where resources are scarce, and therefore decisions must be made on priorities. Some measure of selectivity is both desirable and practicable, particularly in respect of analytical work, which constitutes the major demand on resources.

The following considerations, in addition to the availability of resources, will govern the selection of nutrients and other food components:

a) the basic need for information;

b) health problems in the country concerned;

c) the state of current thinking in the nutritional and toxicological sciences;

d) the availability of existing data;

e) the existence of adequate analytical methods;

f) the feasibility of analytical work;

g) national and international nutrition-labelling regulations.

2.4.1 Nitrogen and nitrogenous constituents

2.4.2 Total Nitrogen

Lakin's (1978) review still provides a comprehensive account of the analysis of nitrogen and nitrogenous constituents, and the methods are discussed briefly by Sullivan (1993). On total nitrogen values using a nitrogen conversion factor (FAO/WHO, 1973), with all factors being recorded at the food level in the database. Values can also be based on the total nitrogen minus the non-protein nitrogen multiplied by a specific factor related to the amino acid composition of the food, or as the sum of amino acids. New amino acid data used in conjunction with the ratio of total amino acid residues to aminoacid nitrogen seem to suggest that the nitrogen conversion factor should be lowered. Sosulski and Imafidon (1990) suggest a global conversion factor of 5.7 and Salo-Väänänen and Koivistoinen (1996) of 5.33, both with individual factors for different foods and food groups.

At this time no new international agreement on conversion factors had yet been reached.

The proximate system, where “protein” is measured as total nitrogen multiplied by a specific factor, continues to dominate food composition studies. Most cited values for “protein” in food composition databases are in fact derived from total nitrogen or total organic nitrogen values. In the majority of cases, total nitrogen is measured using some version of the Kjeldahl (1883) method (which measures total organic nitrogen). In this method the organic matter is digested with hot concentrated sulphuric acid. A “catalyst mixture” is added to the acid to raise its boiling point, usually containing a true catalytic agent (mercury, copper or selenium) together with potassium sulphate. All organic nitrogen is converted to ammonia, which is usually measured by titration or, more rarely, colorimetrically. In the original method, a relatively large analytical portion (1–2 g) was used, but this requires large amounts of acid.

Micro-Kjeldahl methods are much more commonly used as they produce a reduced amount of acid fumes and also require less acid and catalyst mixture. Environmental considerations exert considerable pressure to ensure the safe disposal of mercury and, especially, to minimize acid usage (Green field and southgate, 2003).

The micro methods can be automated at several levels (Egan, Kirk and Sawyer, 1987; Chang, 1998). Automation of the distillation and titration stages works well but automation of the digestion has proved quite difficult.

The Dumas method measures the total nitrogen as nitrogen gas after complete combustion of the food. Comparison of the results obtained with those obtained using the Kjeldahl method shows good agreement (King-Brink and Sebranek, 1993). The method has been successfully automated and, although the instrumentation is expensive, a high throughput of samples is possible, with good precision. The equipment uses very small analytical portions, and a finely divided analytical portion is essential.

2.4.3 Total fat

Values for total lipids vary considerably with analytical method (see Chapters 6 and 7) and

may be of limited nutritional significance; nevertheless, they are widely used and should beincluded at all levels of the database.

2.4.4 Fat (-acylglycerols). Inclusion of this item is desirable in the reference database, primarily for use in the calculation of food energy value, and also because of the interest in triacylglycerols from animal and vegetable sources. The widespread and increasing use of mono- and acylglycerols in manufactured foods is an additional reason for its inclusion.

The values obtained for total fat or total material soluble in lipid solvents are very method dependent. Carpenter, *et al*., (1993), in their review for the AOAC of methods for nutritional labelling, set out the nature of the problems encountered. Gurr (1992) and Gurr, *et al*., (2002), discuss in detail the methods available for separating the different classes of lipids.

The classical method is based on continuous extraction performed on dried samples of food in a Soxhlet extractor, sometimes preceded by acid hydrolysis. This technique is time consuming and subjects the extracted lipids to long periods at high temperatures. Its main drawback, however, is that it yields incomplete lipid extractions from many foods, especially baked products or those containing a considerable amount of structural fat. The extractant used is often petroleum spirit (which is less flammable that diethyl ether and less likely to form peroxides), which requires completely dry analytical portions and the removal of mono and disaccharides. Values obtained using this method require close scrutiny before their inclusion in a database and their continued use is not recommended.

Other solvents, for example, trichloroethylene, are used in a number of automated systems of the 'Foss-Let' type; these appear to give more complete extractions (Pettinati and Swift,1977). The use of mixed polar and non-polar solvents has been shown to extract virtually all the lipids from most foods. In the case of baked (cereal) products, however, incomplete Extraction of fat may occur. Chloroform–methanol extraction is well known (Folch, Lees and Stanley, 1957; Bligh and Dyer, 1959); this combines the tissue-penetrating capacity of alcohol with the fat-dissolving power of chloroform. The resultant extracts are complete but may also contain non-lipid materials and require re-extraction to eliminate these. This extraction method is preferred when the extract is to be subsequently measured for fatty acids and sterols (Shepherd, Hubbard and Prosser, 1974). The method is effective for composite foods and is included in the AOAC Official Methods. It has been shown to be useful for foods such as brain and egg that are rich in phospholipids (Hubbard *et al.*, 1977). The measurement of lipids after acid (Weibull and Schmid methods) or alkaline (Röse-Gottlieb method) treatment also provides good extraction from many foods. These techniques are recognized as regulated methods by the AOAC and the European Union. Alkaline methods are almost exclusively used for dairy foods and are the approved method for such foods. The extracts from acid and alkaline treatments are not suitable for fatty acid analysis because some oxidation and losses due to (acid) hydrolysis of fats may occur. The AOAC has adopted methods for determining total fat (also saturated, unsaturated and monounsaturated fats) in foods using acid hydrolysis and capillary gas chromatography (Ngeh-Ngwainbi, Lin and Chandler, 1997; House, 1997) to comply with the Nutrition Labeling and Education Act (NLEA) definition of fat as the sum of fatty acids expressed as triacylglycerols.

Lipid classes show strong carbonyl absorption bands in the infrared region. NIR has been used for legumes (Hunt *et al.*, 1977a) and various other foodstuffs (Cronin and McKenzie, 1990). The effective use of the method depends on extensive calibration against comparable matrices using another approved method; for this reason the technique is most commonly applied in routine analyses of large numbers of very similar samples, for foods such as cereals and dairy products.

2.4.5 Protein

Since the development of the proximate system of analysis, "crude protein" values have been calculated by multiplying the total nitrogen (N) by a certain factor. This factor was originally 6.25, based on the assumption that proteins contained 16 percent of N. It has been known for a considerable time that proteins of plant origin (and gelatin) contain more N and therefore require a lower factor. Jones, Munsey and Walker (1942) measured the nitrogen content of a wide range of isolated proteins and proposed a series of specific factors for different categories of food. These factors have been widely adopted and were used in the FAO/WHO (1973) review of protein requirements. Several authors have criticized the use of these traditional factors for individual foods (e.g. Tkachuk, 1969). Heidelbaugh *et al.*, (1975) evaluated three different methods of calculation (use of the 6.25 factor, use of traditional factors and summation of amino acid data) and found variations of up to 40 percent. Sosulski and Imafidon (1990) produced a mean factor of 5.68 based on the study of the amino acid data and recommended the use of 5.70 as a factor for mixed foods.

In principle, it would be more appropriate to base estimates of protein on amino acid data (Southgate, 1974; Greenfield and Southgate, 1992; Salo-Väänänen and Koivistoinen, 1996) and these were incorporated in the consensus document from the Second International Food Data Base Conference held in Lahti, Finland, in 1995, on the definition of nutrients in food composition databases (Koivistoinen *et al.*, 1996).

If these recommendations are to be adopted, the amino acid data should include values for free amino acids in addition to those for protein amino acids because they are nutritionally equivalent. The calculations require very sound amino acid values (measured on the food) as discussed below, and involve certain assumptions concerning the proportions of aspartic and glutamic acids present as the amides and correction for the water gained during hydrolysis. Clearly, this approach would not be very cost-effective when compared with the current approach.

At the present time it is probably reasonable to retain the current calculation method, recognizing that this gives conventional values for protein and that the values are not for true protein in the biochemical sense. However, it is important to recognize also that this method is not suitable for some foods that are rich in non-amino non-protein nitrogen, for example cartilaginous fish, many shellfish and crustaceans and, most notably, human breastmilk, which contains a substantial concentration of urea.

A number of direct methods for protein analysis have been developed for specific foods based on reactions involving specific functional groups of the amino acids present; these are thus not applicable to the measurement of proteins in general. Such methods include formol titration (Taylor, 1957) and the biuret reaction (Noll, Simmonds and Bushuk, 1974). A widely used group of colorimetric methods is based on reaction with Folin's reagent, one of the most widely used biochemically in the dairy industry (Lowry *et al.*, 1951; Huang *et al.*, 1976). These methods are most commonly calibrated with bovine serum albumin, which is available at high purity.

2.4.6 Carbohydrates

The range of carbohydrates found in the human diet illustrates the nature of the task facing the analyst who wishes to follow the recommendations published by FAO/WHO (1998) for measuring the carbohydrates in foods separately. Not all types of carbohydrates are, of course, present in all types of foods.

The distinctive metabolic and physiological properties of the different carbohydrates emphasize the fact that for nutritional purposes it is inadequate to consider the carbohydrates as a single component of foods.

The calculation of "carbohydrate by difference" using the Weende proximate system of analysis described at the beginning of the chapter was a reflection of the state of knowledge of carbohydrate chemistry at the time. Moreover, the system was designed for animal feedstuffs, especially for ruminants, and most of the carbohydrates (except lignin-cellulose of which crude fibre was an approximate measure) would therefore be digested in the rumen. For nutritional purposes carbohydrates can be considered as falling into three groups based on the degree of polymerization:

• sugars (mono- and disaccharides);

• oligosaccharides (polymers of three to nine monosaccharide or uronic acid units);

• polysaccharides (polymers containing more that nine units), which fall into two broad categories: α-glucans (starches, starch hydrolysis products and glycogen) and a much more diverse group of non-α-glucans (non-starch polysaccharides [NSPs], which are the major constituents of dietary fibre).

These broad chemical groupings do not correspond precisely with physiological properties or with analytical fractions. The analyst faced with the analysis of carbohydrates, particularly NSPs, is "bound to make a compromise between the ideal of separating the many components and measuring them or a scheme that is entirely empirically based" (Southgate, 1969). In many cases, a food contains a limited range of carbohydrates and simpler procedures can be used for its analysis (Southgate, 1991).

2.4.7 Total ash

Nutritionally, there is little value in recording ash values other than to provide an approximate estimate of the total inorganic material and to check for replication in the destruction of the matrix. A value for total ash is, of course, essential when it is necessary to calculate carbohydrate "by difference". In dry ashing, the food is incinerated in a crucible, usually made of silica, although porcelain (can be used but less suitable) or platinum (very expensive but the least reactive) can be used. The food matrix must be destroyed by heating gently at first to char the sample and then at 500 °C in a muffle furnace (Wills, Balmer and Greenfield, 1980) to prevent foaming of lipids (and sugars) until a white (or light grey) residue is produced. Heating above 500 °C can result in the loss of alkali metals. The general procedure is described by Osborne and Voogt (1978) and in the AOAC Official Methods (Sullivan and Carpenter, 1993).

In the case of "wet ashing" acid digestion, the food sample is heated with acid – usually a mixture of nitric and sulphuric acids. Perchloric acid is often included in the digesting acid mixture although this introduces the risk of explosion and the procedure must be carried out in a fume hood designed for the use of perchloric acid. Wet ashing offers the advantage that no reactions with the crucible can occur that can lead to the formation of insoluble silicates. Digestion can be carried out in a Kjeldahl flask but this requires a larger quantity of acid. Particularly for trace element analysis, digestion is best carried out in a sealed container. Tubes designed for this purpose are available from most laboratory equipmments suppliers. They are made from resistant glass and have a cap with a plastic insert to provide an inert gas-tight seal. The analytical portion and the acid are placed in the tube, which is then capped and may be heated in a conventional or microwave oven. The tube is then allowed to cool completely before the gases are released with care.

For trace element analyses, the acids used must be of the highest analytical quality; blanks should be run as a matter of course and digestion of the reference materials should be included.

The most widely used instruments are atomic absorption spectrophotometers, which are suitable for the analysis of most cations of nutritional interest. The more simple flame photometers can be used for the analysis of Sodium and Potassium.

Plasma emission instruments such as inductively coupled plasma spectrometers are available that permit the analysis of a wide range of elements and have the capacity to handle a large number of samples and analytes (McKinstry, Indyl and Kim, 1999). They do, however, require high initial capital expenditure and routine maintenance. Ihnat (1982;1984) provides a detailed review of the application of these methods to foods. Sullivan (1993) discusses the use of these techniques in the AOAC's*Methods of analysis for nutrition labeling* (Sullivan and Carpenter, 1993).

2.4.8 Dietary fibre

Dietary fibre are considered as part of the carbohydrates in foods. The major problem in the choice of method lies in the definition of dietary fibre and its interpretation in an analytical context. The term was first used in 1953, by Hipsley, to describe the sum of the hemicelluloses, cellulose and lignin in food, in other words the components of plant cell walls in foods. Trowell, in 1972, took up the term for "the indigestible components of the plant cell wall in foods". Both these terms were too vague to use as a basis for an analytical method and in 1976 Trowell *et al.*, (1976) proposed that it be defined as "the sum of the plant polysaccharides and lignin that are not digested by the enzymes of the gastrointestinal tract".

This was closely analogous to the "unavailable carbohydrates" as defined by Mc Cance and Lawrence (1929) and measurable by the procedures proposed by Southgate (1969). In this method the aim was to measure the carbohydrates specifically using colorimetric techniques. Englyst developed this approach using the more specific GLC methods, which gave values for the non-starch polysaccharides and incorporated a stage to convert resistant starch to non-enzymatically resistant starch. The procedure was developed in a series of collaborative studies and the most recent protocols are described by Englyst, Quigley and Hudson (1994) and Southgate (1995). This method measures only the NSPs and does not include lignin. In other parts of Europe, especially Sweden and Switzerland, and in the United States, the focus was directed at the "indigestibility of the polysaccharides and lignin". A gravimetric method was developed where the residue after starch removal is weighed to give a measure of total dietary fibre (TDF); this has evolved into the Official AOAC Method No. 982.29 (Prosky *et al.*, 1992). The method requires correction of the residue for undigested protein and for mineral contamination; total nitrogen and ash in the residue are measured and deducted to give the TDF values. These include lignin, resistant starch and all other indigestible carbohydrates (Guillon *et al.*, 1998). A modification has been introduced to include the measurement of indigestible oligosaccharides.

The Englyst NSP and the AOAC TDF procedures are not very robust, especially where levels low are present (Southgate, 1995). The NSP method uses analytical portions of 100–200 mg and the preparation and homogeneity of these portions is absolutely critical. The mixing procedures also require close attention during the execution of the method.

The AOAC gravimetric procedure requires great skill when measuring low levels but gives good precision with high-fibre foods such as bran and whole meal products. The residue also includes heat-induced artifacts.

In many countries, the choice of method for nutrition labelling will be defined by legislation. Nutritionally specific measurement of the different carbohydrate fractions is the preferred approach. The measurement of soluble and insoluble fractions is highly method dependent and the FAO/WHO (1998) review concluded that there was no physiological justification for recording separate values based on solubility.

It is important to recognize that the hypothesis concerning the protective effects of dietary fibre was based on differences between diets (Burkitt and Trowell, 1975), i.e. it was a statement about the protective effects of diets that were rich in foods containing plant cell walls in a relatively unprocessed state. These diets are rich in many other components in addition to dietary fibre.

2.5.0 Mineral Elements:

Plants usually contain in varying proportions the full range of mineral element which are present in the soil in which they are grown (Luh and woodroof, 1975). Among the factors influencing the mineral composition of leafy green vegetables, soil fertility (or type of quality of fertilizer used) is perhaps the most important. Most of the earlier studies have shown that Nigerian vegetables contain appreciable amounts of minerals.Specifically, these vegetables are in low sodium, but relatively high potassium.(Oguntona,1998).

Minerals are inorganic nutrients, usually required in small amounts from less than 1 to 2500 mg per day, depending on the mineral. As with vitamins and other essential food nutrients, mineral requirements vary with animal species (Soetan *et .al* ,2010). Minerals are inorganic substances, present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life.

Minerals are chemical constituents used by the body in many ways. Although they yield no energy, they have important roles to play in many activities in the body (Malhotra, 1998;

Eruvbetine, 2003). Every form of living matter requires these inorganic elements or minerals for their normal life processes (Hays and Swenson, 1985; Ozcan, 2003).

2.5.1 Classification of Minerals:

Minerals maybe broadly classified as macro (major) or micro (trace) elements. The third category is the ultra trace elements. The macro-minerals include calcium, phosphorus, sodium and chloride, while the micro-elements include iron, copper, cobalt, potassium, magnesium, iodine zinc, manganese, molybdenum, fluoride, chromium, selenium and sulfur (Eruvbetine, 2003).

The macro-minerals are required in amounts greater than 100 mg/dl and the micro-minerals are required in amounts less than 100 mg/dl (Murray *et al.,* 2000). The ultra trace elements include boron, silicon, arsenic and nickel which have been found in animals and are believed to be essential for these animals. Evidence for requirements and essentialness of others like cadmium, lead, tin, lithium and vanadium is weak (Albion Research Notes, 1996).

The mineral elements are separate entities from the other essential nutrients like proteins, fats, carbohydrates, and vitamins. Animal husbandry had demonstrated the need for minerals in the diet (Hegsted *et al.*,1976).

Soetan *et al*.,2011 reported that, Micronutrient deficiencies are a major public health problem in many developing countries, with infants and pregnant women especially at risk. Infants deserve extra concern because they need adequate micronutrients to maintain normal growth and development .

The micronutrient deficiencies which are of greatest public health significance are iron deficiency, causing varying degrees of impairment in cognitive performance, lowered work capacity, lowered immunity to infections, pregnancy complications e.g. babies with low birth weight, poor learning capacity and reduced psychomotor skills (Batra and Seth, 2002).

2.5.2 The importance of the mineral elements

Underwood, 1971 and Darby, 1976 reported that the importance of mineral elements in human, animal and plant nutrition has been well recognized. Deficiencies or disturbances in the nutrition of an animal cause a variety of diseases and can arise in several ways (Gordon, 1977). When a trace element is deficient, a characteristic syndrome is produced which reflects the specific functions of the nutrient in the metabolism of the animal. The trace elements are essential components of enzyme systems. Simple or conditioned deficiencies of mineral elements therefore have profound effects on metabolism and tissue structure. (Rao and Rao,1981; Simsek and Aykut, 2007).

The significance of the mineral elements in humans, animals and plants nutrition cannot be over emphasized. The presence of mineral elements in animal feed is vital for the animal's metabolic processes. Grazing livestock from tropical countries often do not receive mineral supplementation except for common salt and must depend almost exclusively upon forage for their mineral requirements (McDowell *et al*., 1984).

Mineral deficiencies or imbalances in soils and forages account partly for low animal production and reproductive problems. Soil acidity and season are factors affecting mineral uptake by plants. Plants use these minerals as structural components in carbohydrates and proteins; organic molecules in metabolism, such as magnesium in chlorophyll and phosphorus in ATP; enzyme activators like potassium, and for maintaining osmotic balance. Calcium is highly implicated in the maintenance of firmness of fruits (Olaiya, 2006) and its requirements in fruits are related to cell wall stability and membrane integrity (Belakbir *et al*., 1998).

Partwardhan, 1961; Deosthale and Belavady, 1978 reported that Mineral elements play important roles in health and disease states of humans and domestic animals. For example, iron deficiency anaemia and goitre due to iodine deficiency are reported to be problems of public health importance in some communities.

Wood, 2000 and O' Connor, 1995 also reported that Trace elements of significance to people with HIV are zinc and selenium. Selenium is an antioxidant that increases immune function. Zinc, usually taken to stimulate the immune system, has been reported to weaken immune system function and lower calcium levels in HIV – positive men.

2.5.3 Biochemical functions of mineral elements in humans and animals

The basic functions performed by the minerals are: they are structural components of body tissues, are involved in the maintenance of acid-base balance and in the regulation of body fluids, in transport of gases and in muscle contractions (Malhotra, 1998; Murray *et al.,*2000).

Knowledge of the biochemistry and functions of the mineral elements in humans, animals and plants will assist plant physiologists and breeders/geneticists, to give priority to mineral elements of importance in health and disease of humans and animals when selecting desirable traits in diets and this will also assist food industries, dieticians, human and animal nutritionists, and veterinarians to be aware of the effects of different processing methods/techniques on these important mineral elements .

2.5.4 Calcium

Calcium functions as a constituent of bones and teeth, regulation of nerve and muscle function. In blood coagulation, calcium activates the conversion of prothrombin to thrombin and also takes part in milk clotting. It plays a vital role in enzyme activation. Calcium activates large number of enzymes such as adenosine triphosphatase (ATPase), succinic dehydrogenase, lipase etc. It is also required for membrane permeability, involved in muscle contraction, normal transmission of nerve impulses and in neuromuscular excitability. A reduced extracellular blood calcium increases the irritability of nerve tissue, and very low levels may cause spontaneous discharges of nerve impulses leading to tetany and convulsions (Hays and Swenson, 1985; Malhotra, 1998; Murray *et al*., 2000).

In plants, calcium is taken up in the ionized form (as Ca^{2+}) , the leafy parts are relatively high in calcium and low in phosphorus, whereas, the reverse is true of the seeds. Legumes, in general, have higher calcium content than grasses (Merck, 1986). In children, calcium deficiency causes rickets due to insufficient calcification by calcium phosphate of the bones in growing children. The bones therefore remain soft and deformed by the pressure of body weight. In adults, it causes osteomalacia, a generalized demineralization of bones. It may also contribute to osteoporosis, a metabolic disorder resulting in decalcification of bone with a high incidence of fracture, that is, a condition where calcium is withdrawn from the bones and the bones become weak and porous and then breaks (Hays and Swenson, 1985; Malhotra, 1998; Murray *et al*., 2000).

2.5.5 Zinc

Zinc is widely distributed in nearly all plants and its concentration in soil varies widely. The mean concentration in the earth's crust is 500ppm .Electrolytic methods, titrimetry, polarography, colorimetry, turbidmetry, spectrochemical (Ekwumengbo 2003) are some of the reported method of zinc analysis. Scott (1960) observed that flame atomic absorption photometry has greater sensitivity than flame photometry for zinc analysis. Other reported methods for zinc analysis in soil and plant samples are X-Ray fluorescence XRF (Pazsit and Nagy, 1979), neutron activation analysis (Funtua *et al.*, 2003 and Dim et al 2004): atomic fluorescence and flame photometry (Simobuco and Filho, 1994).

It is estimated that over 7% of all proteins in plants and animals require zinc for their proper function (Prasad, 2004).The human body can tolerate quite high dietary intakes of zinc without any deleterious health consequences. Without fortification or supplementation, it is difficult to consume more than 20 mg of zinc per day from commonly available foods and the toxic threshold is well in excess of 100 mg per day (Plum *et al*., 2010). At high dietary zinc intakes (>50 mg Zn/day), copper absorption by the gut may be reduced and this may have consequences for copper status and resulting effects on health. Calcium has long been known to reduce the toxicity of transition metals like zinc and in high concentration, it may reduce zinc absorption (Prasad, 2004).

2.5.6 Lead

Lead is a naturally occurring metal found deep in the ground. It occurs in small amounts in ore, along with other elements such as silver, zinc or copper. It is bluish gray in color and mostly used to make batteries Even though it is found in small amounts, there is an abundant supply of lead throughout the earth because it is widespread and easy to extract and work with (ASTDR, 1999).

Lead is usually found in soil and plant samples and the mean concentration in the earth's crust is about 16ppm (Turekian and Scott, 1967) .In the presence of other elements, Lead has been determined gravimetrically, titrimetrically, electrolytically, calorimetrically, spectrographically, polarographyically Linear sweeps oscillo-polarographyically and spot-test respectively (Ekwumemgbo. 2003). Others are laser induced breakdown spectrometry (Wisbrun *et al.*, 1993).

Lead toxicity indicates that, depending on the dose, lead exposure in children and adult can cause a wide spectrum of health problems ranging from convulsion, coma, renal failure and death at the high end to subtle effects on metabolism and intelligence at the low end of exposure (WHO, 1991).

2.5.7. Manganese

Manganese was found to be a constant component of plants, animals and soil and has been shown to be an essential requirement by plants and microorganisms. It has a mean concentration in the earth's Crust of about 900-1000ppm. Attempts to demonstrate its essentiality to laboratory animals proved difficult but the metal was determined calorimetrically in soil and plants samples by Dobritsykaya (1969) .

The use of methods like gravimetric, electrolysis, polarography, spectrography, absorptiometry, AAS and X-ray fluorescence (Ekwumengbo, 2003) for the analysis of manganese in soil and plants led to the identification of specific functions of the metal in plants and animals. It was observed that the spectrographic methods are more suitable than the chemical methods for the analysis of manganese in soil samples (Burrell *et al*., 1954). Also (Gouhui *et al.*, 1994 and Simabuco *et al.*, 1994) used XRFs to analyze the element in plant and soil samples.

2.5.8 Copper

Copper, a reddish coloured metal, is a relatively rare element, accounting for only 0.0068% of the earth's crust by mass. Copper is found in nature in the elemental state. Its most important ores are sulphide such as chalcopyrite $CuFeS_2$(Karle, 1977). Copper occurs in rocks, soil, dust, water, air, plants and animals.

The presence of copper in plant and animal tissues was recognized early in the nineteenth century but this was thought to be the result of accidental contamination and the concentration was about 100ppm in both mentioned tissues and earth's crust (Kiple and Coneeorles, 2000). The element was also analyzed calorimetrically (Chiba and Watanabe,``1972) in plant and soil samples. The universal distribution of the element in plants prompted the hypothesis that copper was a catalyst participating in life processes (Kiple and Coneeoles, 2000) and this led to some spectrochemical studies of the metal (Kashun 1969). Some other methods that have been used in the determination of copper for possible sources of the metal due to its dietary importance includes neutron activation analysis (Funtua *et al.*, 2003 : Dim *et al.*, 2004); the dropping mercury electrode technique (Reed and Cummings,1941) and Scot (1960) who used flame atomic absorption photometry.

Copper is an essential element in human nutrition and the intake from food is normally 1mg/day. It forms part of the various proteins and enzymes. The liver, nervous system, pancreas and bones are rich in copper. Its highest concentration is in the liver and brain (Wong, 1996). Copper is essential element in plant needed in micro level however at higher levels, it may be toxic to plants.

Chronic copper poisoning does not pose major health hazard, although there were reports of its occurrence in infants resulting from contaminated food or water pipe (Karle, 1977). Diets deficient in copper had resulted in anaemia (Philip, 1990). Copper is associated with intestinal enzyme activity and is carried to the body tissues in the blood plasma. Highest concentration of copper is found in the liver and certain areas of the central nervous system particularly the brain (Philip, 1990). Inhalation of dusts, fumes or mists of copper salts can cause congestion and irritation of the upper respiratory path, nausea, melaena, coma and even death (Czavderna, 1985;

IOSHIC, 1999). Copper intoxication results in haemolytic crisis and the release of haemoglobin in urine (Karle, 1977). Failure to excrete copper in the bile at a rate necessary to maintain its zero balance in the body results in a disease called Wilson's disease. Chronic exposure to copper may lead to skeletal lesion and severe psychiatric disturbance (Waish, 1987).

2.5.9 Chromium

Chromium in the environment arises from both natural and anthropogenic sources. It is present in drinking water, food, soil, air, dust, paint, ceramics and textile products. In soil, chromium (III) predominates. Chromium (VI) can easily be reduced to chromium (III) by organic matter. For example, its occurrence in soil is often resulted from human activities (Anderson, 2000). Chromium exists in micro and macro quantities in soils but in micro quantity in plants. Cruf (1964), Johnson and Simons (1977) described procedures that permit rapid multi-element analysis of plant materials by direct reading emission spectroscopy, the method does not require any sample treatment and allows analysis of twenty-one elements. Its discovery and nutritional importance was first recognized in 1954 by Curran when he found that, the metal enhanced the synthesis of cholesterol and fatty acids and was later identified as the active component of the glucose tolerance factor, which alleviate the impaired glucose tolerance in rats fed certain diets apparently deficient in chromium (Schwatz and Mertz, 1959).

Even though essentiality of the element chromium has not been biochemically defined, there are clinical evidences that showed the element might be responsible for some cases of impaired glucose tolerance, high blood glucose, low blood glucose and refractoriness to insulin ultimately diabetes (Kiple and Coneeorles, 2000).

Several spectroscopic techniques like colorimetric and AAS (Lerner *et al*.,1978) direct reading spectrometry and ICP excitation (Moselly *et al*., 1978) neutron activation analysis (Rustamov *et* *al*., 1974), XRFs (Schneider, 1970) for environmental analysis of chromium have been reported for chromium analysis. Plants and animals have also been recognized to contain the element as a cellular component. Its toxicity in plant leaves starts from 1-4 ppm (Anderson *et al*., 1997) while it is twenty times more in the roots.

Chromium accumulates in aquatic life, adding to the danger of eating fish that may have been exposed to high levels of chromium (ASTDR, 2004). Studies have shown that the ingestion of 1-5 g of "chromate" resulted in severe acute effects such as gastrointestinal disorders, haemorrhagic diathesis, and convulsions. Death may occur following cardiovascular shock (Janus and Krajnc, 1990).

2.5.10 Selenium

Plant and soil samples usually contain selenium in trace amounts and the mean content in soil samples is about 0.3ppm (Aubert and Pinta, 1977). The more important forms of selenium in soil are basic selenite (SeO_3^{-2}), calcium selenate ($CaSeO_4$) and organic selenium compounds derived from decayed vegetation (Williams and Byers, 1936).

Ravokovitch and Margolin (1957) reported the highest concentration of selenium top soil, while lane (1966) showed that fluorescence analysis of selenium gave results, which were excellent agreement with those obtained by neutron activation analysis, AAS (Stanton, 1974); ICP- AES (Mcquaker *et al*, 1979); XRFs and ICP-MS (Simabuco and Filho, 1994 and Fujikawa *et al*., 1995) are some of the techniques reported on the analysis selenium in environmental samples.

2.5.11 Cadmium

Cadmium derives its toxicological properties from its chemical similarity to zinc, an essential micronutrient for plants and animals. Cadmium exposure is encountered in industries dealing with pigment, metal plating, some plastics and batteries. Cadmium pollution can result in significant human exposure to cadmium through the ingestion of contaminated foodstuffs especially grains, cereals and leafy vegetables (Prasad, 2004). It is a dangerous element because it can be absorbed through the alimentary tract; penetrate through placenta during pregnancy and damage membranes and DNA (WHO, 2004). Once in the human body, it may remain in the metabolism between 16 to 33 years and is connected to several health problems such as renal damages and abnormal urinary excretion of protein (Wong, 1996). Cadmium's effect on the kidney can have metabolic effects with pathologic consequences (Hu, 2002). In particular, the loss of calcium caused by cadmium's effect on the kidney can be severe enough to lead to weakening of the bones. Increased cadmium burden in the population was found to be predictive of an increased risk of cadmium with an oxidation state of +2 (Mc Cluggage, 1991). It is chemically similar to zinc and occurs naturally with zinc and lead in sulphide ores.

Cadmium metal is used mainly as anticorrosive electroplated on steel. Cadmium sulphide is commonly used as pigments in plastics. Cadmium compounds are used in electric batteries, electronic components, nuclear reactors, component in manufacture of solder, bearing alloys, amalgam in dentistry, and worm treatments for swine and poultry (Wong, 1996). Cadmium is also present as an impurity in several products including phosphate fertilizers, detergents and refined petroleum products (Friberg *et al*., 1986). Cadmium is present in drinking water, food, soil, air, dust and some fertilizers. Its presence in the environment arises from both natural and anthropogenic sources (WHO, 1989). Cadmium concentrations in humans increase with age. Both kidney and liver act as cadmium stores. The biological half-life in humans is in the range 10-35 years. The estimated lethal oral dose for humans is 350-3500 mg of cadmium (Krajnc, 1987). Cadmium causes organ damage, reproductive and blood disorders, interfering with mineral metabolism.

Cadmium is a potentially carcinogen. Organic forms of cadmium are potentially bio-accumulative with resultant genetic damage. In humans, cadmium interferes with the metabolism of calcium and phosphorus causing a painful bone disease (Osteomalacia, Osteoporosis) (Mench *et al.*, 1994). Cadmium is bio persistent and once absorbed by an organism, remains resident for many years (over decades in humans) although it is eventually excreted. The average daily intake of cadmium for humans is estimated as 0.15µg from air and 1µg from water (ASTDR, 1999). Smoking a packet of 20 cigarettes can lead to the inhalation of around 2- 4 µg of cadmium but level may vary widely (WHO, 1989). In the general non-smoking population, the major exposure pathway is through food by the addition of cadmium to agricultural soil from various sources (atmospheric deposition and agrochemicals fertilizer application) and uptake by food and foldder crops. Additional exposure to humans arises through cadmium in ambient air and drinking water (IARC, 1987; WHO, 1992).

2.5.11.1 Cadminium Toxicity

Human disease has occurred in some special situations, such as itai-itai disease in Japan, and renal dysfunction and increased occurrence of bone effects in Belgium and in China as a result of long-term effects related to cadmium pollution of the general environment. Long-term exposure to cadmium via air, food, or water increases the cadmium concentration in the kidneys and gives rise to kidney disease. Other adverse effects that may be caused by cadmium exposure are lung damage, bone effects, liver dysfunction, and reproductive toxicity.

Chronic cadmium poisoning occurs as a result of long-term intake of food with increased cadmium concentrations or long-term inhalation of airborne cadmium in industry. Inhalation of cadmium affects the lungs. The most prominent adverse health effects upon long-term exposure to cadmium, regardless of exposure route, are renal tubular dysfunction and glomerular disturbance.One of the most extreme forms of disease related to cadmium exposure by the oral route is itai-itai disease, described in Japan in the 1960s. Itai-itai disease is characterized by osteomalacia,osteoporosis, renal tubular dysfunction, mal absorption, and anemia(Nordberg, 2003).

2.5.12 Cobalt

Cobalt is a chemical element with symbol Co and atomic number 27. It is found naturally in chemically combined form. It is an essential trace dietary mineral for all animals. Cobalt is naturally occurring element found in rocks, soil, water, plants and animals. Elemental cobalt is a hard, silvery grey metal (ATSDR, 2004). However, it is usually found in the environment combined with other elements such as oxygen, sulphur and arsenic. It is used in production of alloys that are used in the manufacture of aircraft engines, magnets, grinding and cutting tools, artificial hip and knee joints (ATSDR, 2004). Cobalt compounds are also used to colour glass, ceramics and paints and used as a drier for porcelain enamel and paints. It enters the environment from natural sources and burning of coal or oil or the production of cobalt alloys (Wong, 1996). ^{60}Co is used as a source of gamma rays for sterilizing medical equipment and consumer products, radiation therapy for treating cancer patients and for manufacturing plastics.

According to Prasad (2004), cobalt cannot be destroyed, it can change form or attach to or separate from particles. Food and drinking water are the largest sources of exposure to cobalt, also working in industries that use cutting or grinding tools: mine, smelt, refine or process cobalt metals or ores are other ways of exposure to cobalt. It can benefit or harm human health (WHO, 1989). Cobalt is beneficial to humans because it is part of vitamin B_{12}. Exposure to high levels of cobalt results in lung and heart diseases and dermatitis. It is a key constituent of cobalamin which is known as vitamin B_{12}, the primary biological reservoir of cobalt as an ultratrace element (Harada, 1994). Bacteria in the guts of ruminant animals convert cobalt salts into vitamin B_{12}, a compound which can only be produced by bacteria or arches. The minimum presence of cobalt in soils therefore markedly improves the health of grazing animals and an uptake of 0.20mg/kg a day is recommended for them, as they can obtain vitamin B_{12} in no other way (Schwarz *et al.*, 2000).

In the early 20th century, during the development for farming of the North Island Volcanic Plateau of New Zealand, cattle suffered from what was termed “bush sickness”. It was discovered that the volcanic soils lacked cobalt salts which was necessary for cattle (Schwarz *et al.*, 2000). The ailment was cured by adding small amounts of cobalt to fertilizers. Non-ruminant herbivores produce vitamin B_{12} from bacteria in their colons which again make the vitamin from simple cobalt salts. Animals that do not follow these methods of getting vitamin B_{12} from their own gastrointestinal bacteria or that of other animals must obtain the vitamin pre-made in other animal products in their diet and they cannot benefit from ingesting simple cobalt salts. In human, B_{12} exists with two types of alkyl ligand: methyl and adenosyl. These are essential in the metabolism of proteins and fats in humans (Smith *et al.*, 1999).

Although far less common to others metallo-proteins (e.g. those of zinc and iron), cobaltoprotiens are known aside from B_{12}. These proteins include *methionine, aminopeptidase*; an enzyme that occurs in humans and other mammal which does not use the corrin ring of B_{12} but binds to cobalt directly. Another non-corrin cobalt enzyme is *nitrit hydratase* an enzyme in bacteria that are able to metabolize nitriles (Kobayashi *et al*., 1999). After nickel and chromium, cobalt is a major cause of contact dermatitis (Basketter *et al.,* 2003).

2.5.13 Iron

Iron is the fourth most abundant metal in the earth's crust of which it accounts for about 5% by mass. Iron is most commonly found in nature in the form of its oxides (Knepper, 1981). Iron is used as constructional material, inter alia for drinking–water pipes. Iron oxides are used as pigments in paints and plastics. Other compounds of iron are used as food colours and for the treatment of iron deficiency in humans. Various iron salts are used as coagulants in water treatment (APHA, 1985; NRC, 1989). Iron is present in drinking water, food, soil, air and dust. Iron in the environment arises from both natural and anthropogenic sources. Iron occurs as a natural constituent in plants and animals. Liver, Kidney, fish and green vegetables contain 20-150 mg/kg whereas red meats and egg yolks contain 10-20 mg/kg (Philip, 1990). Rice, fruits and vegetables have low iron contents (WHO, 1999).

Iron is an essential element in human nutrition, and in the formation of red blood cells. It forms an important constituent of haemoglobin and intercellular enzyme system. Estimates of the minimum daily requirement for iron depends on age, sex, physiological status and iron bioavailability which ranges from about 5 to 10 mg/day (FAO/WHO, 1988). The effects of toxic doses of iron include; depression, rapid and shallow respiration, coma, convulsions, respiratory failure and cardiac arrest. Chronic iron overload results primarily from a genetic disorder (haemochromatosis) characterized by increased iron absorption and from diseases that require frequent transfusions (Bothwell, 1979).

2.6.0 Fourier Transform Infrared (FTIR) Spectroscopy for Material Identification

Fourier Transform Infrared Spectroscopy, also known as FTIR Analysis or FTIR Spectroscopy, is an analytical technique used to identify organic, polymeric, and in some cases, inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties. The test is performed in absorbance, but can be converted to transmittance. Both qualitative and quantitative information about the test sample can be provided.

2.6.1 Valuable Analysis with FTIR Spectroscopy

i. Assessing purity

ii . Identifying:

a) Base polymer composition
b) Additives
c) Organic contaminants
d) General type of material being analyzed when there are unknowns

2.6.2 Analyzing Samples in a Variety of Forms

i)Solids placed on a crystal

ii) Liquids placed between two sodium chloride plates

iii) Thin film placed in a cassette

iv) Powdered sample mixed with pottasium bromide and placed in a pellet

FTIR spectroscopy compliments the wide-range of instrumental and wet chemistry services performed at LTI for materials characterization, qualitative and quantitative element analysis and failure analysis on a variety of materials including metals, powdered metals, ores, ferroalloys, composites and ceramics. Laboratory Testing Inc. is fully capable of preparing test specimens for all analysis.

2.6.3 Advantages of FTIR

i. Better speed and sensitivity than dispersive I R-spectrometer
ii. Provide an automatic calibration in accuracy of better than $0.01cm^{-1}$ and therefore need no external calibration
iii. Un-modulated stray light and sample emission are not detected
iv. Equipped with a power computerized data system that can perform library searching , baseline correction, smoothing, integration, transformation etc
v. Require no sample preparation and can analyze samples in solids, liquid or gaseous form using different accessories.

CHAPTER THREE

3.0.0 Materials and Methods

3.1.0: Sample Collection and Identification.

The plants in this work {*Borreria stachydea* D.C. Hutch & Dalziel, *Cassia absus* Linn, and *Aspillia kotschyi* (Sch.Bipex,Hochst) oliv} were collected from Ajide forest of Okpokwo Local Government area of Benue State Nigeria in March 2015.

Plate iv: Region Map of Benue State showing Okpokwu Local Government Area

Plate V: Ajide forest image

Okpokwo is a Local Government Area of Benue state, Nigeria.Its headquarters is in the town of Okpoga.It has an area of 731km^2. Ajide forest is under the okopokwu Local Government Area.

The Plants were identified at the Herberium Unit of the Department of Biological Sciences Ahmadu Bello University (ABU) Zaria. Voucher specimen numbers were assigned to the plants as follows: *Borreria stachydea* (2756), *Cassia absus* (3106),and *Aspillia kotschyi (*2956) respectively .

3.2.0 : Sample Treatment

The fresh plants leaves were washed thoroughly and carefully with distilled water and air dried for five days in an oven at $60^{O}C$ for 12 hours. After drying, the samples were pulverized mechanically using wooden mortar and pestle.

3.3.0 : Extraction of Plant Materials

Of the three powdered plants materials, 200g each were carefully weighed and loaded into soxhlet extractor. Each powdered plant material was separately extracted with methanol and petroleum ether (60-80) for 72 hours. The extracts were concentrated in vacuo at $40^{O}C$ using rotary evaporator followed by air drying.

3.3.1 Materials for Phytochemical Analysis

The reagents used in phytochemical analysis are as follows; Methanol, 10% Ammonium solution, Phenyl hydrazine, Chloroform solution, Resorcinol crystals, 5% KOH and 20% KOH, 95% Ethanol, concentrate Lead acetate solution, 1% 3, 5-dinitrobenzoic acid, 20% Sodium hydroxide solution, 1% Ferric chloride solution, Dilute sulphuric acid, concentrated sulphuric acid, 10% Sodium Nitrate, Pyridine, Acetic anhydride, Concentrated Nitric acid, Glacial acetic acid, 1% Picric acid solution and 10% Tannic acid solution.

Other reagents prepared for the anlaysis are;

(i) **Molisch's Reagent**: which was prepared by dissolving 10% solution of alpha- napthol in alcohol.

(ii) **Wagner's Reagent**: This was prepared by dissolving iodine (2.0g) and potassium iodide (3g)in distilled water and sufficient amount of water was added and made up to 100ml mark.

(iii) **Dragendorff's Reagent:** Bismuth nitrate (0.85g) was dissolved in $10cm^3$of acetic and 40 cm^3 distilled water was added to give a stock solution (A). 8.0g of potassium iodide was equally dissolved in 20 cm^3 of distilled water to give a stock solution (B). 5 cm^3 of each of the stock solution (A) and (B) were mixed with 20 cm^3 of 33% acetic acid followed by 100ml distilled water to give the Dragendorff's reagent.

(iv) **Ammonical Silver Nitrate Solution;** Silver nitrate (3.0g) was dissolved in 50 cm^3 of distilled water, to that solution ammonia solution was added drop wise until the initial precipitate of silver oxide dissolved. This resulting solution was made up to 100 cm^3 in a volumetric flask.

(v) **Meyers Reagent:** This was prepared by dissolving potassium iodide (4.0g) in distilled water to give solution A. Another solution B was also formed by dissolving Mercuric chloride ($Hgcl_2$) (1.36g) in $60cm^3$ of distilled water and the two were mixed together and made up to $100cm^3$ volumetric flask.

(vi) **Iodine solution** : Iodine crystals (2.5g) was added to 0.5g of pottasium iodide (KI) and 25 cm^3 of distilled water was added to the mixture to give iodine solution.

(vii) **Fehling Solution:** Solution A was prepared by dissolving 7.0g copper sulphate and 0.1 cm^3 of sulphuric acid with sufficient distilled water and made up to 100 cm^3 mark. Solution B was prepared by dissolving 35.2g of sodium hydroxide in sufficient distilled water and made up to 100 cm^3 mark. Equal volumes of solutions (A) and (B) were mixed immediately before use.

(viii) **Barfoeds Reagent:** Crystallized neutral copper acetate (13.3g) was dissolved in 200 cm^3 of 1% acetic acid solution.

3.3.2 Preliminary Phytochemical Screening:

Preliminary photochemical screening was carried out on each of the petroleum ether and methanol extracts of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* plants using standard procedures (Sofowora,1993,Trease and Evans 1989,Ayoola *et al.*, 2005).

The results for the photochemical screening on the extracts are summarized in Table 4.1.

3.3. 2.1 GENERAL TEST FOR ALKALOIDS:

3.3.2.2 Extraction of the Alkaloid from the Plant Material:

1gm each of the powdered plant materials were respectively put in small beakers, $5cm^3$ of concentrated ammonium solution was added and mixed thoroughly. This was allowed to stand for 10 minutes so that the liquid can penetrate the plant material. Sufficient quantity of a mixture of chloroform and ethanol (ratio 1:1) was added just to soak and suspend the powder. This was allowed to stand for another 20 minutes with occasional stirring with glass rod. The mixtures were filtered through a plug of cotton wool. The filtrate was evaporated to dryness using a water bath. They were then transferred into small separatory funnels and shaken respectively with $3cm^3$ dilute sulphuric acid. They were allowed to separate into two layers. The chloroform (lower) layer was discarded.

The layers in the funnels were completely made alkaline by adding concentrated ammonia solution and tested with litmus paper. This was shaken with $3cm^3$ chloroform and the layer was allowed to separate. The chloroform (lower) layers were drained off by passing them through a plug of absorbent cotton wool in small beakers. This was repeated and the two respective chloroform portions were combined and evaporated to dryness. The following tests were then carried out on the residues of the extracts.

3.3.2.3 ***Test for Alkaloids in the Extracts***:

The residue from the above processes were dissolved in $3cm^3$ ethanol and divided into un equal portions (i.e 1ml and 2ml) respectively.

3.3.2.4 ***Test for alkaloids in the Extract of Borreria stachydea, Cassia absus Linn, and Aspillia kotschyi plants:***

The residues obtain from above were dissolved in $3cm^3$ ethanol and divided into two unequal portion $1cm^3$ and $2cm^3$respectively. The $2cm^3$-portion was neutralized with dilute sulphuric acid and few drops of this solution was treated with the following alkaloid reagents for instance Meyers reagent, Dragendoff's reagent, 1% picric acid solution, Wagner's reagent and 10% tannnic acid solution.

All the alkaloid test in the methanolic extract of the three plants material using the various reagents proved positive as shown in Table 4.1 .Various coloured precipitate were obtained viz, Mayer's reagent gave a dirty cream colour, Dragendooff's reagent gave blue-black precipitate, tannic acid gave orange precipitate ,as well as picric acid solution which showed the presence of alkaloid in the plants.

3.3.3 TEST FOR TANNINS:

a). Ferric chloride Test

To a portion of each of the three plant extracts, 3-5 drops of ferric chloride solution was added. A greenish black precipitate indicate the presence of condensed tannins, while hydrolysable tannins give a blue or brownish- blue precipitate (Evans,1996). A greenish black precipitate was observed only in *Borreria stachydea* methanolic extract.

3.3.4 TEST FOR GLYCOSIDES

To a portion of each of the three extracts, $5cm^3$ of dilute sulphuric acid was added separately and boiled on water bath for 10 -15 minutes. This was then cooled and neutralized with 20%

KOH. Each of the three extract was divided into two portions for further analysis as indicated below.

i. To the first portion of each of the plants extract, 5cm^3 of a mixture of Fehling's solution A and B was added and boiled, a brick red precipitate shows the release of reducing sugar as a result of hydrolysis of Glycoside (Evans,1996).

ii. To the second portion of each of the three plant extract about 3cm^3 of Ferric chloride Solution was added, a green to blue color was produced because of the release of phenolic aglycones due to the hydrolysis(Evans, 1996).

3.3.5 TEST FOR SAPONINS:

Frothing test

1gm each of the powdered plant materials of the three plant material were shaken with 10cm^3 of distilled water, warmed and allowed to stand for 30 minutes. Frothing was observed which persisted after 30 minutes.

3.3.6 TEST FOR CARDIAC GLYCOSIDES

Keller-Kiliani Test

A portion of each of the three plant extracts was dissolved in 1cm^3 of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1cm^3 of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. A pale green color was observed in the acetic acid layer which indicates the presence of cardiac glycosides (Evans, 1996).

3.3.7 TEST FOR STEROIDS/TRITERPENES.

Lieberman – Bur chard Test

To a portion of each extract equal volume of acetic anhydride ($2cm^3$) was added and mixed gently. $1cm^3$ of concentrated sulphuric acid ($1cm^3$) was carefully added down the test tube. Color change were observed immediately and over a period of an hour blue to blue green color in the upper layer and reddish pink color which indicate the presence of triterpene (Evans,1996).

3.3.7.1 Salkowski Test:

To a small portion of the extracts 2-3 drops of concentrated sulphuric acid was added at the side of the test tube. A reddish brown colour was observed at the interphase which indicates the presence of unsaturated sterols (Evans,1996).

3.3.7.2 Keller-Kiliani Test (cardiac glycosides)

A small quantity of each of the extract was dissolved in a $2cm^3$ of glacial acetic acid containing one drop of ferric chloride solution. These were then treated with concentrated sulphuric acid. A brown ring was obtained at the interface between glacial acetic acid layers and that of sulphuric acid and a violetring appeared below the brown ring.

3.3.8 TEST FOR CARBOHYDRATES;

3.3.8.1 Fehlings Test (Test for free Reducing sugars)

Each extract (0.1g) was mixed with $5cm^3$ of equal mixture of fehling's solutions A and B and boiled in a water bath. Brick red precipitate was formed in all the methanolic extracts of the plants indicating the presence of reducing sugar (Evans, 1996).

3.3.8.2 Molisch Test (standard test for carbohydrates)

Each extract (0.5g) was dissolved in 3cm^3 of distilled water and a few drops of Molisch's reagent was added. Concentrated sulphuric acid (2ml) was carefully added from the side of the test tube to form a lower layer. A reddish coloured ring was observed at the interphase (Evans,1996).

3.3.9 TEST FOR FLAVONOIDS:

3.3.9.1 ShinodaTest

A portion of each of the three extract was dissolved in 1-2cm^3 of 50% methanol in the heat Metallic magnesium chips and few drops of concentrated hydrochloric acid were added. Appearance of red color indicates the presence of flavonoids (Evans,1996).

3.3.9.2 Sodium hydroxide Test

Few drops of 10% sodium hydroxide were added to each of the three different plant extracts. Yellow coloration indicates presences of flavonoid (Evans,1996)

3.3.10 TEST FOR ANTHRAQUINONES

Borntragers Test

This is used to test the presence of free and combined anthraquinones. Each extract (0.5g) was taken in a dry test-tube, and shaken with 5cm^3 of chloroform for five (5) minutes and filtered. Ammonia solution10% was added and shaken again. No change in colouration of the solution nor precipitate was observed to show the presence of free anthraquinones.

3.3.11 TEST FOR TANNINS

Two methods were used in the testing for tannins.

a) To 10cm^3 of freshly prepared 10% Potassium hydroxide in a beaker 0.5g of extract was added and shaken . A dirty precipitate observed indicated the presence of tannins.

b) About 0.5g each of the three extract was separately boiled in $10cm^3$ of water in a test tube and then filtered. A few drops of 0.1 % ferric chloride was added and the solution observed for brownish green or a blue black colouration.

3.3.12 Chemical Test for fixed oils and fats

Fixed oils and fats were confirmed by the chemical test for glycerin which was produced by their hydrolysis as follows;

Using sodium hydroxide: each of the three samples of the three different plants were separately mixed with 1% copper solution. Five drops of100% sodium hydroxide was added. A clear blue solution was obtained which indicated the presence of fixed oils.

3.4.0 Determination of anti nutritional Components

3.4.1 Determination of total phenols by spectrophotometric methods;

Each of the fat free sample of the three plants was boiled separately with 50 cm^3 of ether for the extraction of the phenolic components for 15 minutes. About $5cm^3$ of each of the three extract was pipette into a 50 cm^3 flask then 10 cm^3 of distilled water was added. About $2cm^3$ of ammonium hydroxide solution and 5 cm^3 of concentrated amyl alcohol was also added. The three samples was made up to mark and left to react for about 30 minutes for colour development. This was measured at 505nm.

3.4.2 Determination of oxalate

Total oxalate was determined as described by Day and Underwood (1986). 1.0g of each of the three plants sample was weighed separately into 100ml conical flasks. $75cm^3$ sulphuric acid (3mol/l) was added and stirred. This was filtered using a what man No. 1 filter paper. About $25cm^3$ of the filtrate was taken and titrated while hot against 0.05mol/l of potassium permanganate solution until a faint pink color persisted for at least 30 seconds.

The oxalate content was then calculated by taking 1cm^3 of 0.05mol/l of potassium permanganate as equivalent to 2.2mg oxalate (Ihekoronye and Ngoddy,1985; Chinma and Igyov, 2007; and Dahovenon-Ahoussi *et. al*, 2012).

3.4.3 Determination of crude hydrogen cyanide

This was done by the alkaline titration method (A.O.A.C., 1995) . 10g each of the ground three samples separately was soaked in a mixture of 200cm^3 of distilled water and10cm^3 of orthophosphoric acid. The mixture was left overnight to release all bounded hydrocyanic acid. The mixture was distilled until 150cm^3 of the distillate was collected. 20cm^3 of the distillate was taken into a conical flask containing 40 cm^3 of distilled water. 6 cm^3 of 6mol/dm^{-3} aqueous ammonia and 2cm^3 of 5% potassium iodide solution were added. The mixture was titrated against 0.02 mol/dm^{-3} silver nitrate to faint but permanent turbidity.

Result was calculated as 1m 0.02 mol/dm^{-3} $AgNO_3$ = 1.08g HCN.

3.4.4 Determination of phytates

This was done by soaking separately 4.0g each of the three different plants ground samples in100cm^3 of 20% hydrochloric acid for 5 hours and filtered. 25 cm^3 of the filtrate was placed in a conical flask and 5cm^3 of 0.3%ammoniumthiocynate solution was added. The mixture was titrated with standard Iron(111) chloride solution until a brownish yellow color persisted for five minutes(Reddy *et al*.,1982).

Results were calculated as 4 moles of Fe: 6 moles of phytic acid.

3.5.0 Determination of Nutritional Content

The three plants samples both petroleum sprit ether 60-80 and methanolic extract were analyzed for nutritional composition (moisture, ash, crude proteins, crude fat, crude fibre and nitrogen free extract). Result are shown on Table 3a and b respectively.

3.5.1 Determination of Moisture Content:

The moisture content in all the three samples was determined by drying the fresh samples at 110^0C at constant weight in an oven:

$$\text{Percentage moisture content (\%)} = \frac{\text{original weight-final weight x 100}}{\text{Original weight}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.1$$

3.5.2 Determination of Ash Content

The ash content was determined in both the three different plant samples by incineration of the samples in a muffle furnace at 550^{o}C for 8 hours. The Association of Official Analytical Chemists (AOAC, 1980) method was used. Porcelain crucibles were washed and dried in an oven to a constant weight at 110^{o}C for 10 minutes. They were allowed to cool in a desiccators and weighed. 2.0 g of each of the three plants samples were weighed into the porcelain crucibles and reweighed. The crucibles containing the samples were transferred into a muffle furnace, which was set at 550^{o}C for 8 hours to ensure proper ashing. They were then removed and allowed to cool in the desiccators then weighed. The percentage ash content was calculated.

$$\text{Percentage ash content (\%)} = \frac{\text{Weight of ash residue X 100}}{\text{Dry weight of samples}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.2$$

3.5.3 Determination of Crude Protein

This was done by the use of Microkjedahl nitrogen method as described by AOAC (1980) which involves the digestion of a given weight of the three different plant samples with concentrated

H_2SO_4 and a catalyst to convert any organic Nitrogen to ammonium sulphate in solution, followed by the decomposition of ammonium sulphate with NaOH. The ammonium liberated was distilled into 5 % boric acid. The nitrogen from ammonia liberated was deduced from titration of a trapped ammonia with 0.005N HCl using methylene red and methylene blue (double indicator solution) indicators.

$$\textit{Percentage Nitrogen (\%)} = \frac{\textit{Vol.acid(ml) – Nomality of acid x1.4}}{\textit{Weight of sample}} \ldots\ldots\ldots\ldots 3.3$$

Therefore *Percentage crude protein (%) = % Nitrogen x 5.3*

3.5.4 Determination of Crude Fibre:

This was done by obtaining the loss in weight on ignition of the residue of dried plant samples after digestion of fat free samples with 1.25% each of sulphuric acid and sodium hydroxide solution under specified condition.

Percentage crude fibre (%) = weight of dried fat free sample-weight of ash x100 …………..3.4

3.5.5 Determination of Crude Fat :

This was done by exhaustive soxhlet extraction of a known amount (2.0g) of each plant sample with Petroleum sprit ether (40-60^{o}C) and methanol mixed in the ratio 1:1 for 2 hours (AOAC,1980).The detailed procedure is as follows: 2g of each sample were placed into separate extraction thimbles and then covered with cotton wool. The extraction thimbles containing the samples were placed in the extraction jackets. Clean dried 500ml round bottom flask containing few anti bumping granules were weighed and 150ml of petroleum ether and 150 ml of methanol was poured into each flask fitted with soxhlet extraction units. The round bottom flask and the condenser were connected to the soxhlet extractor and cold water circulation was put on. The heating mantle was switched on while the heating rate was adjusted until the solvents were refluxing at a steady rate. Extraction was carried out for two hours. The solvents were recovered and the oil was dried in the oven at 70^{o}C for one hour .The round bottom flask and oil were cooled and then weighed. The lipid content in all the three plants were separately calculated using the formula below:

$$\textit{Percentage of fat (\%)} = \frac{\textit{weight of the fat X 100}}{\textit{Weight of sample}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.5$$

3.5.6 Determination of Free nitrogen extract (NFE) (Dutcher *et al*, 1951) :

Carbohydrate as nitrogen free extract (NFE) was calculated by difference as:

NFE= 100- (crude protein +crude lipid +ash +moisture +crude fibre).

3.6.0 Determination of Mineral Content:

The minerals, Calcium(Ca),Magnesium (Mg), Zinc(Zn), Lead(Pb), Chromium(Cr) and Cadmium (Cd) were determined by following the method of Madison 1971 with modification, using the Varian AA240 FS Model Atomic Absorption spectrophotometer.(Results are presented on Table 4a, b, and c)

Digestion; The fine crushed extract (1g) of each of the three plant extract were digested separately with 20 cm^3 of a mixture of concentrated nitric acid and hydrochloric acid (3:1 by vol). The contents were heated on a hot plate at 100^{o}C for 45 minutes. This was then filtered while still warm through a whatman 45 paper into a 100cm3 conical flask and made up to the mark with distilled water.

Minerals were measured from this solution at the appropriate wavelength using a Varian AA240 FS Model Atomic Absorption spectrophotometer and quantified by reference to Standard curves (figure 3.1- 3.6) below made from standard mineral solutions. The amount of each of themetal was calculated from the equation:

$$\text{Amount of mineral (ppm)} = \frac{\text{graph reading x vol dilution}}{\text{Weight of sample}} \quad \ldots\ldots\ldots\ldots\ldots\ldots..3.6$$

3.6.1 Determination of other Minerals Using MP- EAS

The following elements Selenium (Se), Silver (Ag),Iron (Fe), Copper (Cu), Nickel (Ni), Arsenic (As), Cobalt (Co), Manganese (Mn) and Aluminum (Al) were determined from both the methanolic and petroleum sprit extracts of the three plants using micro wave plasma atomic emission spectroscopy technique. The instrument used in this determination is MY15150003 MP-EAS spectrometer. The Agilent 4200 MP-AES is a new atomic emission spectrometer that features a microwave plasma as the emission source. This source is sustained with nitrogen gas, avoiding the need for more expensive flammable and oxidizing gases used with traditional flame atomic absorption (AA) spectrometers. In addition, the Agilent 4200 MP-AES features a scanning spectrometer with a CCD detector for multi element measurements, providing sub-ppb detection limits for many elements. (Results are shown on table 4d).

a) Operating Conditions for MP-EAS

1. Standard Nebulizer

MP-AES: Agilent 4200

Gas Supply Nitrogen . Nebulizer Gas Pressure: 100 to 240 kPa

Solution Uptake: 1.4 mL/min (pumped) . Pump Tubing: 1.02 mm i.d. PVC

Nebulizer: Glass Concentric. Spray Chamber: Glass Cyclonic

Read Times: 10 s; 30 s for As and Se. Replicates: 7

Background correction: Auto

2. Ultrasonic Nebulizer

Nebulizer System: CETAC U5000AT+

Gas Supply: Nitrogen

Nebulizer Gas Pressure: 125 to 240 kPa

Heater Temp: 140°C

Cooler Temp: 3°C

Solution Uptake: 0.85 mL/min (pumped)

Pump Tubing: 0.76 mm i.d. PVC

Read time: 10 seconds (all elements)

Replicates: 7

Background correction: Auto

3.6.1.1 Experimental Parameters for MP-EAS

Reagents:

Nitric Acid, Baker Instra-Analyzed Reagent, J.T.

Baker, Phillipsburg N.J. USA

Hydrochloric Acid, BDH Aristar Plus, VWR,

Radnor, PA USA

Agilent Technologies QCSTD-27 multi element standard

Various single-element standards, Inorganic

3.6.1.2 Blank and Standards Preparation:

Reagent blanks and calibration standards were prepared by weight in low density polyethylene (LPDE) bottles in 1.0 % (v/v) nitric acid or 1% (v/v) hydrochloric acid . Standard concentrations were 0.250 and 0.500ppm.

3.6.1.3 Ultrasonic Nebulizer (USN) Setup, Calibration, and Instrument Detection Limits:

The standard nebulizer and spray chamber were first removed from the Agilent 4100 MP-AES. Using an interface kit, the ultrasonic nebulizer was connected to the MP-AES through a nebulizer

gas line and a sample out line. The nebulizer gas line contained a flow restriction fitting and the sample out line a 12/5 glass adapter for direct connection to the torch. Sample solution flow to the ultrasonic nebulizer transducer was from the host MP-AES peristaltic pump. Setup of the ultrasonic nebulizer took only 5 minutes.The reagent blank and the two calibration standards were then introduced to the MP-AES with the ultrasonic nebulizer.

Instrument detection limits (IDLs) are calculated as 3 times the standard deviation of the blank concentration. Note that data was obtained under non-clean room conditions.

Calibration curves for Se, Ag, Fe, Cu, Ni, As, Co, Mn and Al are given below;

3.7.0 Determination of Sodium and Potassium using Flame Photometry

Sodium and potassium were determined from the methanolic extracts of the three different plants samples using flame photometery. The standard solutions were prepared as described for AAS. Gas pressure ,slit width and other settings were selected as recommended for the instrument.

A calibration curve was prepared from the standard range after setting the top standard to a suitable scale deflection and the zero ppm starndard to zero .

The samples solutions was aspirated in to the flame under the same conditions as the standards. The top zero and and an intermediate standards were checked frequently.The instrument atomizer and the burner was flushed frequently with water particularly at the end of the run. The calibration curve was used to determined the concentrations of the minerals. Blank determination and subtractions made were necessary.(Results were shown on Table 4).

3.8.0 Thin layer chromatography

The one way ascending technique was used. Samples were analyzed by TLC were dissolved in suitable organic solvent(s) and applied on pre-coated silica gel TLC aluminium plates (silica gel F_{254}) as spots with the aid of capillary tubes at one end of the plate in a straight line, about 2cm above the bottom edge and 1.5cm away from the sides. The spots were dried and the plates placed in a chromatank containing the mobile phase that has been prepared in the tank at least 30 minutes earlier. The mobile phase ran along the TLC plate in an ascending manner due to capillary action, carrying with it the components of the extract or the mixture. When the mobile phase reached the desired distance, the plate was removed, the solvent front was marked and the plate was allowed to dry. The separated compounds were identified by observing the chromatogram under ultra-violet light for fluorescence. This was followed by spraying with anisaldehyde in conc. H_2SO_4 and heating at 120°C for 5mins for visibility of fluorescent bands. The colour raction was recorded and the relative retention factor (Rf) value was calculated using the formulae below. This method was used for all TLC analysis (Ghani, 1990) .

$$Rf = \frac{\text{Distance travelled by the samples (streak) from the starting point}}{\text{Distance travelled by the solvent from the starting point}} \quad \ldots\ldots\ldots\ldots\ldots..3.7$$

a) Choosing Solvents

Solvent system for use as mobile phases for the three different plants extracts as mobile phases in the column chromatography was determined as described above in TLC by varying the different ratio of the solvents. The separation began by using low polarity solvent which allowed the material to absorb the stationary phase then slowly switching the polarity of the solvent to desorb the compounds and allow them to travel with the mobile phase. The polarity of the solvents was changed gradually. Some solvent system combinations used are hexane/ethyl acetate. Ethyl acetate methanol, Methanol/hexane at different rations .Often conducting the TLC with the solvent combinations for the column chromatography was established . (figures of TLC plates are attached as appendix) .

3.8.1 Chromatographic Separation:

Column chromatography was performed in order to purify the plant materials for functional group analysis. Column chromatography of the extract of *Borreria stachydea* (B), *Cassia absus* Linn,(C) and *Aspillia kotschyi* (C) plants was carried out using Silica gel [(60-120mesh) (500g)]. The silica gel was loaded in a column (58.5inch length, 1.2inch id) in slurry of n-hexane. The silica gel was washed several times with n-hexane and later with chloroform to remove oily materials. At the end of the parking the tap was locked and the column was allowed to stand for 24hours. The three different plant sethyl acetate , methanol, and n hexane extracts seperately extract of was mixed thoroughly with 50g of silica in a beaker using spatula until the mixture becomes homogenous. The mixture was then carefully loaded on to the column that was already packed. Additional silica gel (10g) was added on top to serve as a protective layer.

The column was eluted using solvents and solvent mixtures of increasing polarity, in the following order with n-hexane (100% 150ml), n-hexane:ethylacetate (9:1 3:2; 1:1; 6 100% ethyl acetate 150ml each and washed finally with acetone). The eluents were collected in 10 drops per minutes that is aliquots of 15ml each in beakers, a total of 140 fractions were obtained, evaporated to dryness and analyzed on TLC (Sharma and Achaya, 1988). The pulled ethyl acetate, hexane and methanol extracts of the three plants were subjected for functional group analysis.

3.9.0 Functional Group Analysis using Fourier Transform Infrared Spectroscopy(FTIR)

The obtained samples from column chromatography of the three plants i.e the methanol ethyl acetate, and hexane were subjected to Infrared spectroscopy using Fourier transform IR-spectrometer FTIR. Thus infrared spectroscopic analysis is an important tool for compound identification and structural elucidation or confirmation.

The test was performed in absorbance, and was converted to transmittance. FTIR analysis was accomplished by Attenuated Total Reflectance (ATR), Nujol and thin film. Enough sample was used in order to obtain an absorption spectrum of the FTIR analysis. The samples were prepared by shaving some material off the samples that were thin enough to obtain a good spectrum.

The first step was the collection of background spectra to subtract from the test spectra to ensure the actual sample is all that is analyzed. Next, the samples was analyzed by LTI's fully-computerized Fourier Transform Infrared Spectroscopy system which generates the absorbance spectra showing the unique chemical bonds and the molecular structure of the sample material. This profile is in the form of an absorption spectrum which shows peaks representing components in higher concentration. Absorbance peaks on the spectrum indicate functional groups (e.g. alkanes, ketones, acid chlorises). Different types of bonds, and thus different functional groups, absorb infrared radiation of different wavelengths. Although the analysis was performed in absorbance, it can be converted to transmittance, since they are simply the inversions of each other. (Refer to Appendix 1-9 for the spectrum while the interpretation is at Table 4.16 to 4.24).

CHAPTER FOUR

4.0 RESULTS

4.1 **Yields of crude extracts**

Table 4.1: Percentage extraction of the plants materials:

Plant	Extract	% yield	Colour/texture
Borreria stachydea	Methanol	18.6	Dark brown sticky
	Pet-ether	14.6	Light brown jelly
Cassia absus	Methanol	8.7	Greenish brown
	Pet-ether	7.1	Light green paste
Aspillia kotschyi	Methanol	6.4	Dark greenish paste
	Pet-ether	4.8	Greenish paste

4.2 **Preliminary phytochemical screening of methanolic and petroleum spirit extracts of *Borreria stachydea, Cassia absus,* and *Aspillia kotschyi* Plants.**

This research studied the proximate, phytochemical, nutritional and antinutritional compositions of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants. Table 4.2 shows the results of Preliminary phytochemical screening of Methanolic and Petroleum spirit extracts of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.

Table 4.2 : Results of Preliminary phytochemical screening of Methanolic and Petroleum spirit extracts of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.

Table 4.2 below shows the results of Preliminary phytochemical screening of Methanolic extracts of *Borreria stachydea*, *Cassia absus* , and *Aspillia kotschyi* Plants.

Constituent	Plants extracts					
	B Meth	B Pet	Cass Meth	Cass Pet	Asp Meth	Asp Pet
Carbohydrate						
• Molisch test	+	+	+	+	+	+
• Fehlings test	+	-	+	-	+	-
Glycosides						
• Saponins	+	-	+	-	-	-
• Cardiac glycosides	+	-	+	-	+	-
• Steroids	-	+	-	+	-	-
• Triterpenes	-	-	-	-	+	-
• Anthraquinones	+	-	-	-		-

Key: (+) = positive
(-) = negative
B Meth = *Borreria stachydea* methanolic extract
B Pet = *Borreria stachydea* petroleum spirit extract
Cass Meth= *Cassia absus* methanolic extract
Cass Pet = *Cassia absus* petroleum spirit extract
Asp Meth = *Aspillia kotschyi* methanolic extract
Asp Pet = *Aspillia kotschyi* petroleum spirit extract

Table 4.3 : Preliminary phytochemical screening of Methanolic and Petroleum spirit extracts of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.

Constituent	Plants extracts

	B Meth	B Pet	Cass Meth	Cass Pet	Asp Meth	Asp Pet
Flavanoids						
• Shinoda test	+	-	+	-	+	-
• Sodium hydroxide	+	-	+	-	+	-
Tannins						
• Ferric chloride	+	-	-	-	-	-
• Lead acetate	-	-	-	-	-	-

Key: (+) = positive
(-) = negative

B Meth = *Borreria stachydea* methanolic extract
B Pet = *Borreria stachydea* petroleum spirit extract
Cass Meth= *Cassia absus* methanolic extract
Cass Pet = *Cassia absus* petroleum spirit extract
Asp Meth = *Aspillia kotschyi* methanolic extract
Asp Pet = *Aspillia kotschyi* petroleum spirit extract

Table 4.4 : Preliminary phytochemical screening of Methanolic and Petroleum spirit extracts of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.

Constituent	Plants extracts					
	B Meth	B Pet	Cass Meth	Cass Pet	Asp Meth	Asp Pet
Alkaloid						
• Dragendoff's	+	-	+	-	+	+
• Mayers	+	-	+	-	+	+

Steroids • Liberman buchard • Salkowoski	- -	+ +	- -	+ +	- -	- -

Key: (+) = positive B Meth = *Borreria stachydea* methanolic extract
(-) = negative B Pet = *Borreria stachydea* petroleum spirit extract
Cass Meth= *Cassia absus* methanolic extract
Cass Pet = *Cassia absus* petroleum siprit extract
Asp Meth = *Aspillia kotschyi* methanolic extract
Asp Pet = *Aspillia kotschyi* petroleum spirit extract

4.3 Quantitive phytochemical screening of Methanolic extracts of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.

The three plants methanolic extracts were subjected to quantitative phytochemical analysis which shows the following in table 4.3

Table 4.5: Results of Quantitive phytochemical screening of Methanolic extracts of *Borreria stachydea, Cassia absus* , and*Aspillia kotschyi* Plants.

Phytochemical Constituent(%)	*Borreria stachydea*	*Cassia absus*	*Aspillia kostschyi*
Alkaloid	7.316	7.972	4.412
Flavonoid	8.745	9.921	2.895
Cardiac glycoside	5.244	4.550	4.494
Tannins	17.7		

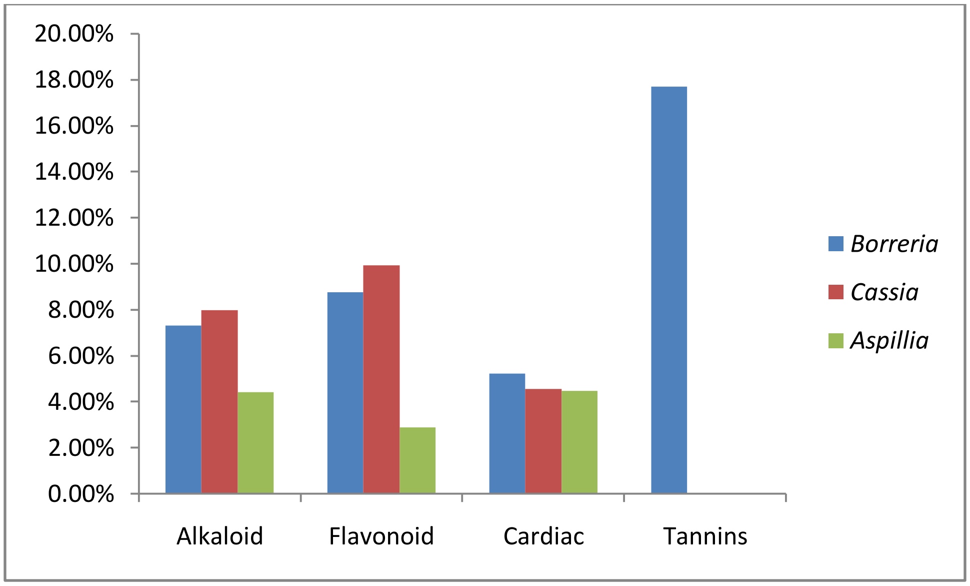


Figure 4.1: Bar chart representing quantitive phytochemical screening of methanolic extracts of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants

4.4 Proximate composition of methanolic extracts of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.

The proximate composition results of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants Methanolic and Pet ether extracts were shown in Table 4.4 a and 4.4 b respectively below;

Table 4.6: Results of proximate composition of methanolic extracts of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.

Components (%)	*Borreria stachydea*	*Cassia absus*	*Aspillia kotschyi*
Crude protein value	8.08	9.81	10.94
Crude Fibre value	7.11	3.17	9.06
Crude fat value	2.15	2.11	0.83
Nitrogen free extract	65.59	70.36	70.19
Ash	8.50	8.0	4.03
Moisture	8.67	6.67	5.7

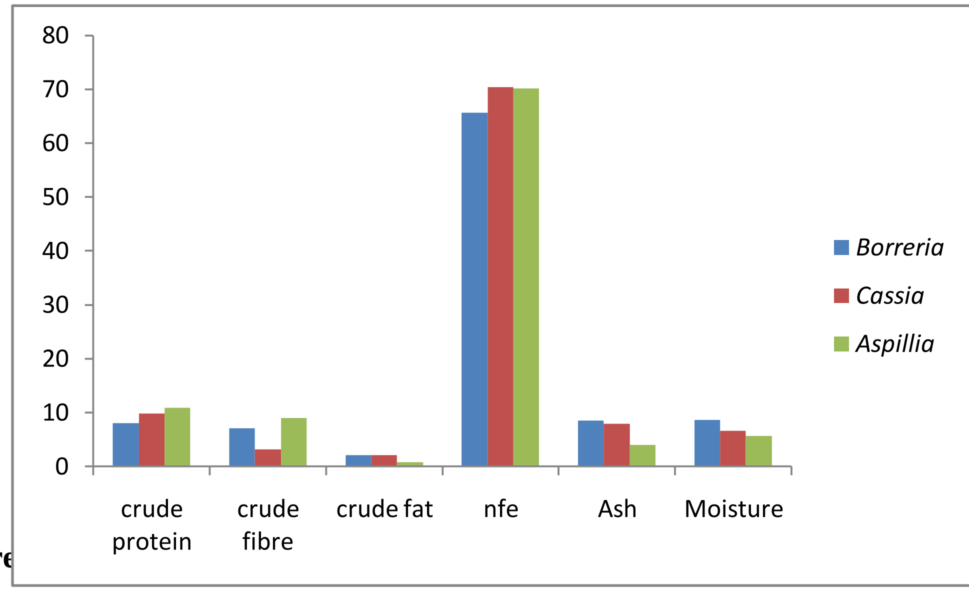


Figure **anolic extracts of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.**

Table 4.7: Results of proximate composition of petroleum spirit extracts of *Borreria stachydea, Cassia absus,* and *Aspillia kotschyi* Plants.

Components value(%)	*Borreria stachydea*	*Cassia absus*	*Aspillia kotschyi*
Crude protein value	5.63	4.88	11.94
Crude Fibre value	0.11	2.65	12.06
Crude fat value	3.13	1.94	1.78
Nitrogen free extract	89.27	88.39	70.19

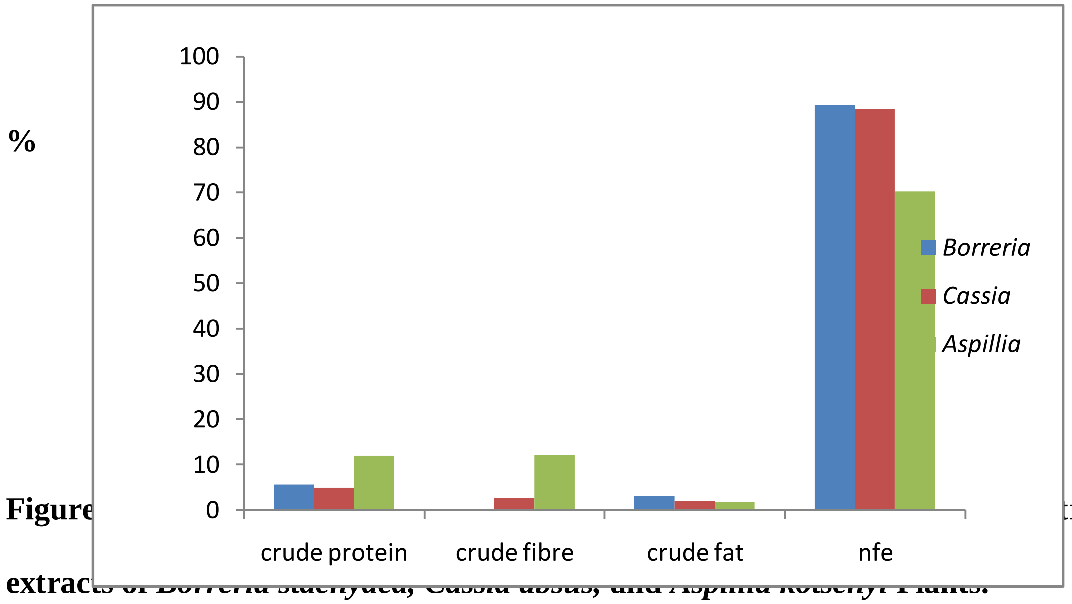


Figure … troleum spirit extracts of *Borreria stachydea*, *Cassia absus*, and *Aspillia kotschyi* Plants.

4.5 Elemental Analysis of *Borreria stachydea, Cassia absus and Aspillia kotschyi* methanolic and petroleum spirit extracts using Atomic Absorption spectrometry

Tables 4 .8, 4.9 and 4.10, shows the elemental concentrations in *Borreria stachydea, Cassia absus* and *Aspillia kotschyi* methanolic and petroleum sprit extracts respectively. Six elements were determined using Atomic Absorption spectrometry. The elements are as follows; Calcium, Magnesium, Zinc, Lead, Chromium, Cadmium.

Table 4 .8 : Elemental concentrations in both of methanolic and petroleum spirit extracts (mg/kg) of *Borreria stachydea* Plant

Element	Methanolic Extract	Petroleum Sprit Extract
Calcium	0.4458±0,0014	0.4086±0.0595

Magnesium	0.3677±0.0015	0.4848±0.0009
Zinc	0.0199±0.0012	0.0186±0.0007
Lead	0.0000±0003	0.0005±0.0006
Chromium	0.1613±0.0011	0.1236±0.0018
Cadmium	0.0004±0.0003	0.0012±0.0004

Table 4 .9: Elemental concentrations in both of Methanolic and petroleum spirit extracts (mg/kg) of *Cassia absus* Plant

Element	Methanolic Extract	Petroleum Sprit Extract
Calcium	0.6102±0.0023	0.4083±0.0003
Magnesium	0.8769±0.001	0.4076±0.0003
Zinc	0.0389±0.0015	0.0251±0.0004
Lead	0.0019±0.0005	0.0001±0.0006
Chromium	0.0023±0.0008	0.3209±0.0019
Cadmium	0.0042±0.0017	0.0013±0.0005

Table 4 .10: Elemental concentrations in both of methanolic and petroleum spirit extracts (mg/kg) of *Aspillia kotschyi* Plant

Element	Methanolic Extract	Petroleum Sprit Extract
Calcium	0.3099±0.0056	0.0796±0.0022
Magnesium	0.1380±0.0009	0.8401±0.0100
Zinc	0.2449±0.0005	0.0435±0.0020
Lead	0.0020±0.0005	0.0009±0.0005
Chromium	0.0018±0.0012	0.0051±0.0004
Cadmium	0.0026±0.0004	0.0023±0.0004

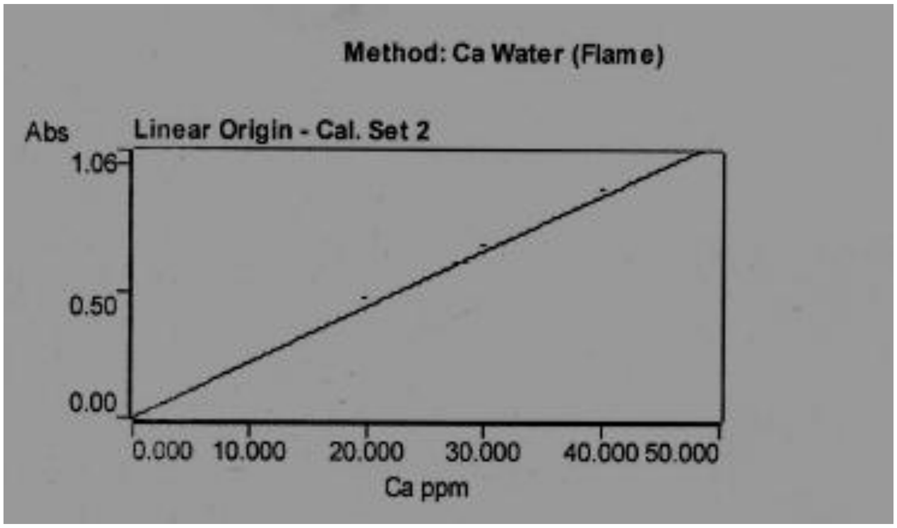


Figure 4.4: Calibration curve for the determination of Calcium

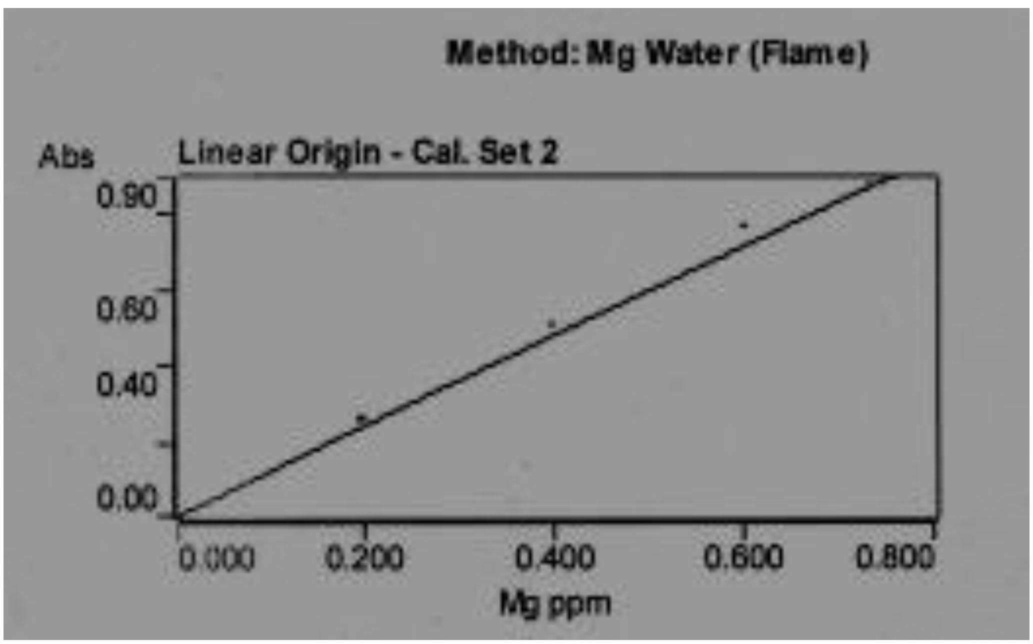


Figure 4.5: Calibration curve for the determination of Magnesium

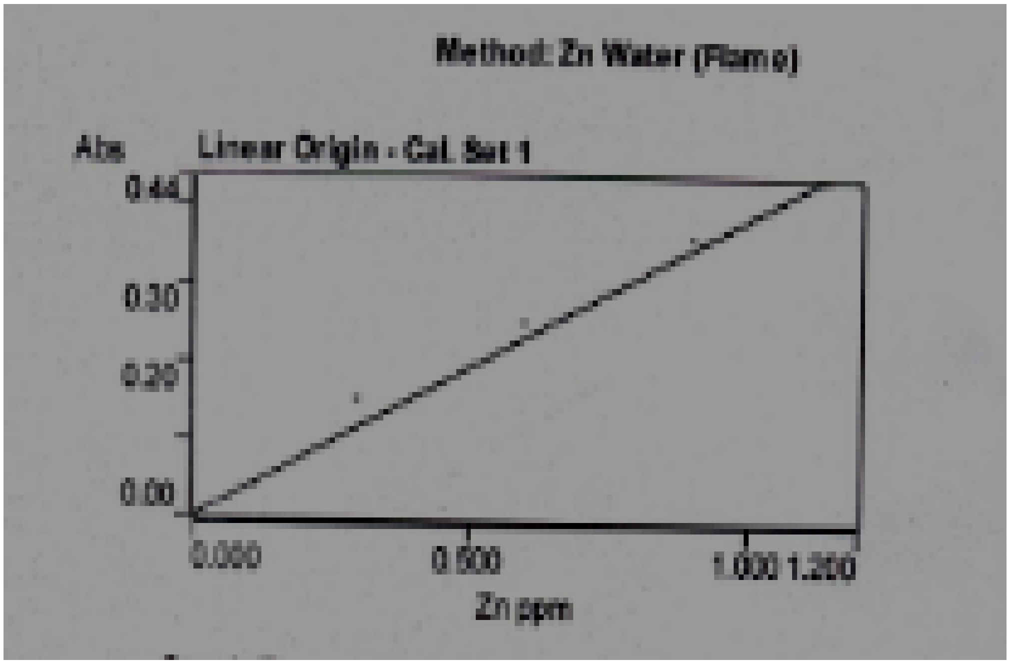


Figure 4.6: Calibration curve for the determination of Zinc

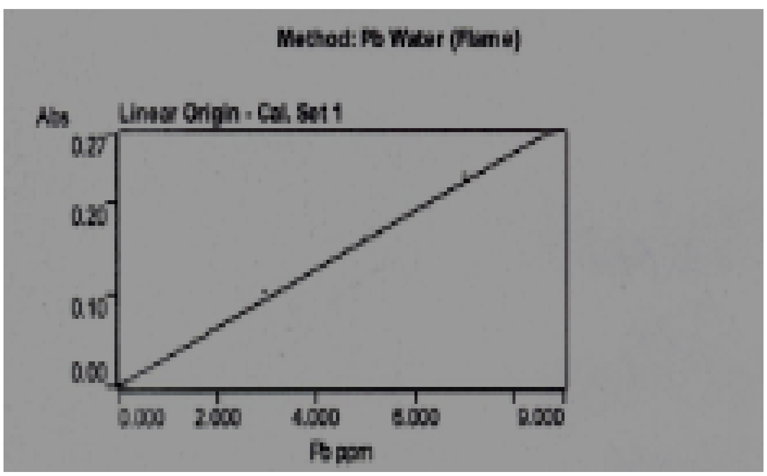


Figure 4.7: Calibration curve for the determination of Lead

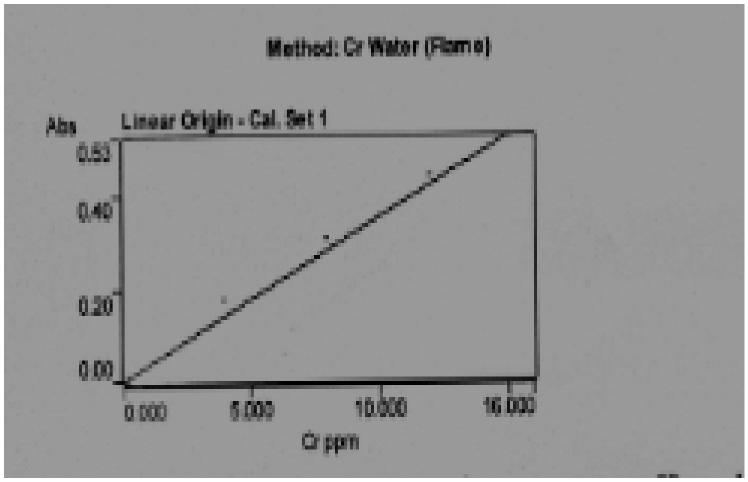


Figure 4.8: Calibration curve for the determination of Chromium

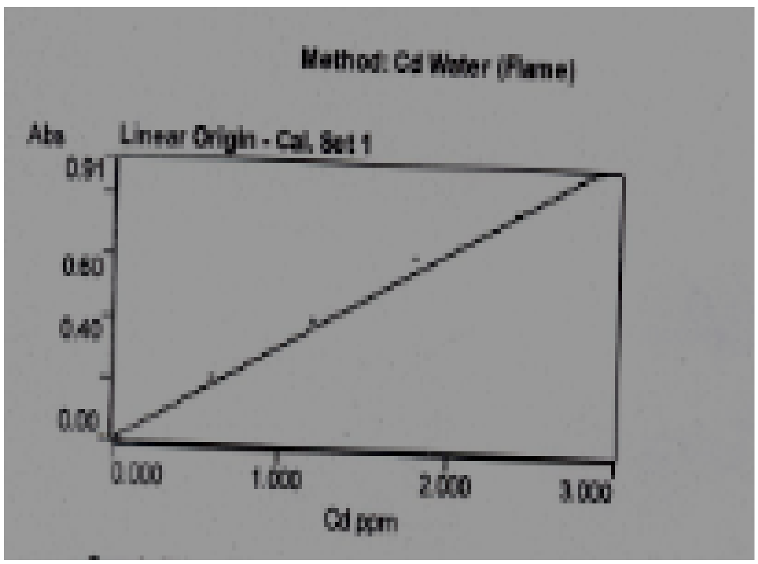


Figure 4.9: Calibration curve for the determination of Cadmium

4.6 Determination of other minerals in *Borreria stachydea, Cassia absus* and *Aspillia kotschyi* methanolic extract using Micro Wave Plasma Atomic Emission Spectroscopy technique (MP- EAS)

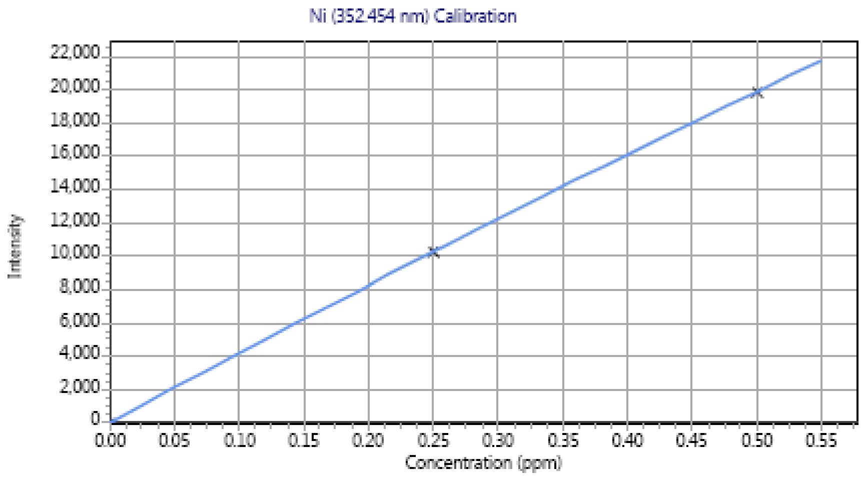
Table 4 .6 shows the elemental concentrations of both methanolic and petroleuem sprits extracts of *Borreria stachydea, Cassia absus* and *Aspillia kotschyi* . Nine elements were determined using micro wave plasma atomic emission spectroscopy technique. The elements are as follows Selenium, Silver, Iron, Copper, Nickeli, Arsenic, Cobalt, Manganese and Aluminium.

Table 4.11 Results of Elemental concentrations of both Methanolic and petroleum spirits extracts of *Borreria stachydea, Cassia absus* and *Aspillia kotschyi* Plant.

Element(mg/kg)	A1	A2	B1	B2	C1	C2
Se	0.394±1.29	0.530±4.50	0.408±6.16	0.556±3.34	0.436±3.33	0.780±3.44
Ag	0.007±5.52	0.06±3.53	0.008±1.12	0.001±2.96	0.001±9.41	0.000±52.83
Fe	2.093±0.79	3.712±0.27	3.674±0.21	1.194±0.65	0.343±0.93	2.198±0.83
Cu	0.129±0.39	0.034±0.78	0.036±0.89	0.013±0.66	0.023±0.49	0.012±0.48
Ni	0.028±1.47	0.016±1.36	0.017±0.76	0.068±0.41	0.045±0.94	0.334±0.34
As	0.506±1.87	1.301±2.90	0.789±1.23	2.373±2.94	0.741±6.31	3.315±0.89
Co	--------	---------	-----------	---------------	--------------	----------
Mn	0.169±0.34	0.111±0.28	0.109±0.92	0.104±0.09	0.039±0.29	0.079±0.13
A l	0.814±0.08	3.050±0.63	2.992±0.50	0.858±0.23	0.095±0.88	0.135±0.96

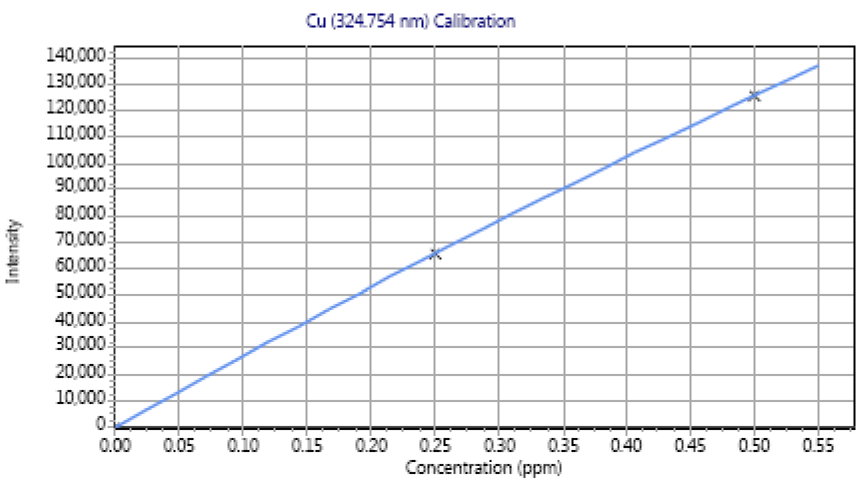
KEY:

A1 = *Aspillia kotschyi* methanolic extract
A2 = *Aspillia kotschyi* petroleum spirit extract
B1 = *Borreria stachydea* methanolic extract
B2 = *Borreria stachydea* spirit extract
C1 = *Cassia absus* methanolic extract
C2 = *Cassia absus* petroleum spirit extract



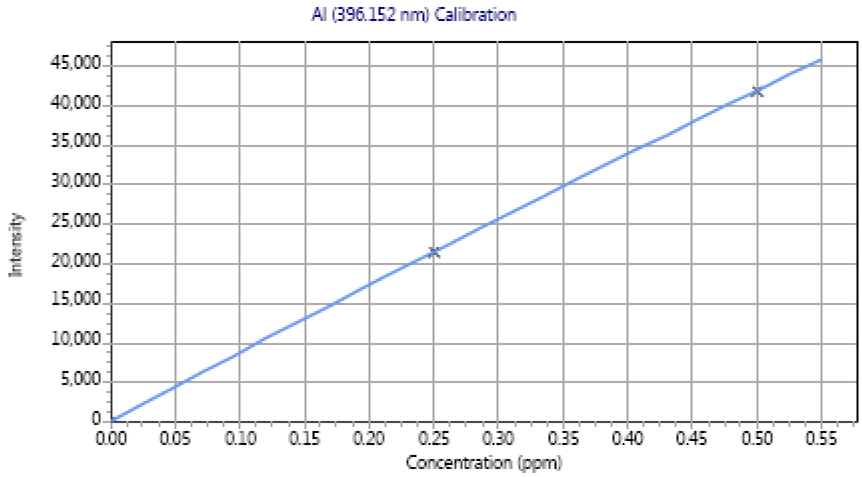
Ni (352.454 nm)
Intensity = (42350.755 * Concentration + 0.000) / (1 + 0.128 * Concentration)
Correlation coefficient: 1.00000

Figure 4.10: Calibration curve for determination of nickel.



Cu (324.754 nm)
Intensity = (276051.691 * Concentration + 0.000) / (1 + 0.194 * Concentration)
Correlation coefficient: 1.00000

Figure 4.11: Calibration curve for determination of copper



Al (396.152 nm)
Intensity = (88132.718 * Concentration + 0.000) / (1 + 0.104 * Concentration)
Correlation coefficient: 1.00000

Figure 4.12: Calibration curve for determination of aluminium

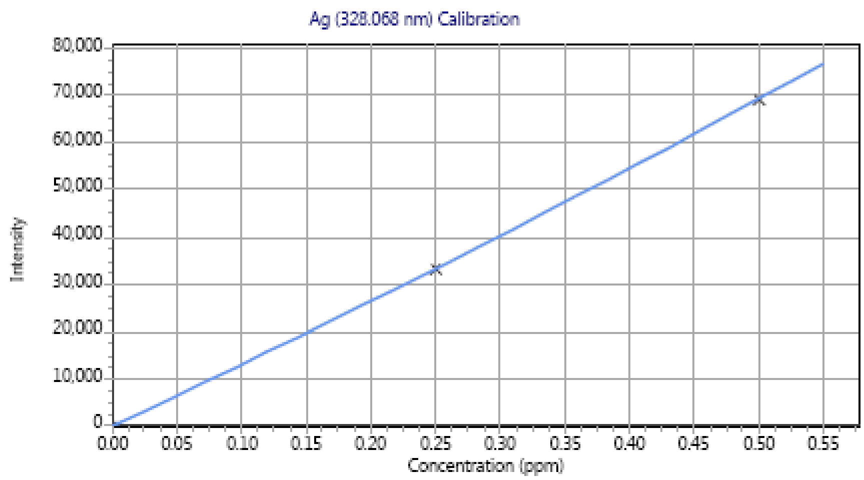
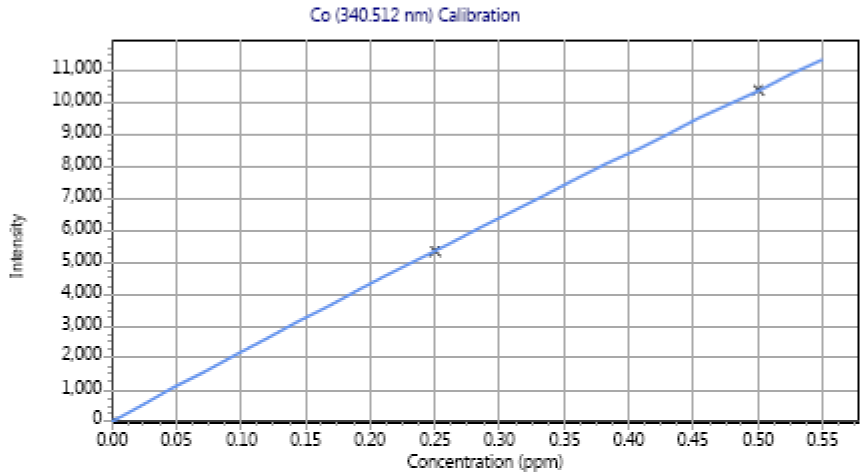
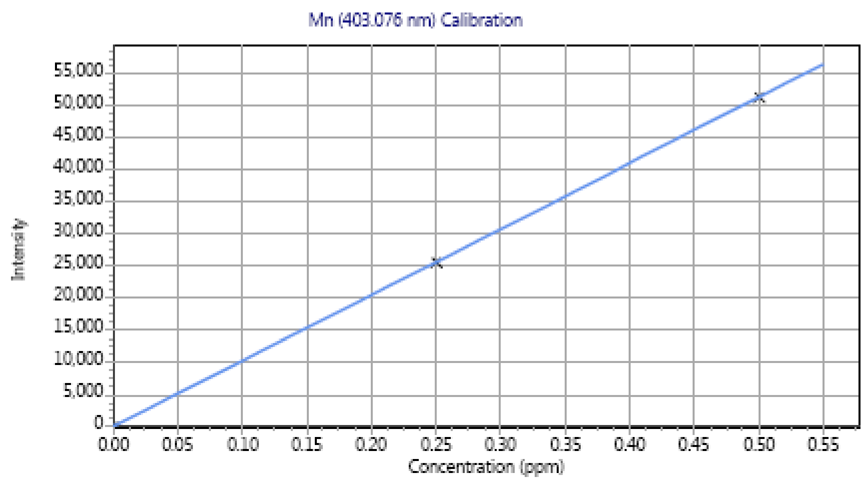


Figure 4.13: Calibration curve for determination of silver



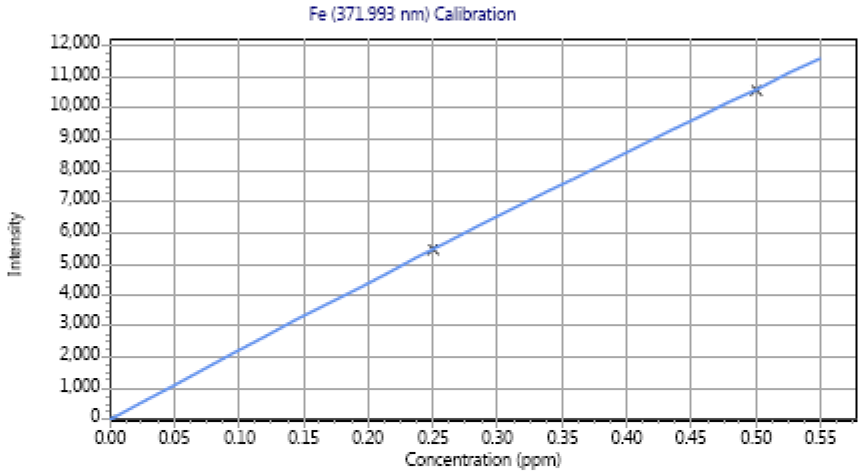
Co (340.512 nm)
Intensity = (22236.722 * Concentration + 0.000) / (1 + 0.138 * Concentration)
Correlation coefficient: 1.00000

Figure 4.14: Calibration curve for determination of cobalt



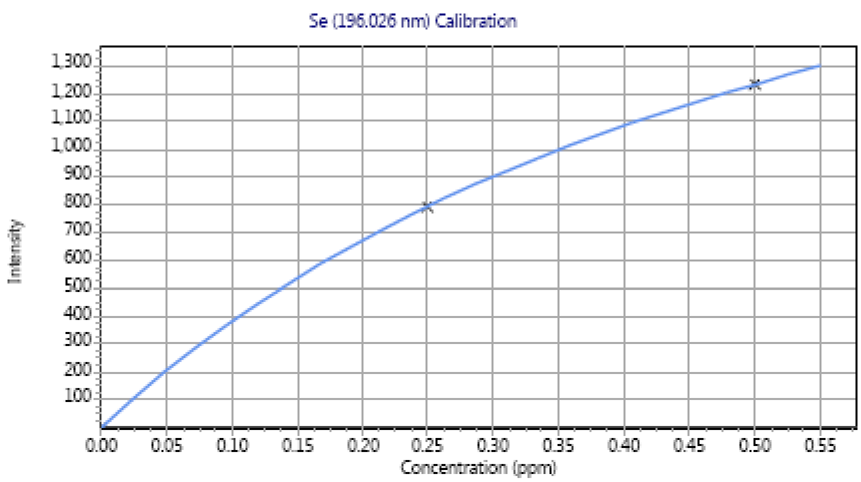
Mn (403.076 nm)
Intensity = (102062.490 * Concentration + 0.000) / (1 - 0.014 * Concentration)
Correlation coefficient: 1.00000

Figure 4.15: Calibration curve for determination of manganese



Fe (371.993 nm)
Intensity = (22541.897 * Concentration + 0.000) / (1 + 0.123 * Concentration)
Correlation coefficient: 1.00000

Figure 4,16: Calibration curve for determination of iron



Se (196.026 nm)
Intensity = (4443.516 * Concentration + 0.000) / (1 + 1.600 * Concentration)
Correlation coefficient: 1.00000

Figure 4.17: Calibration curve for determination of selenium

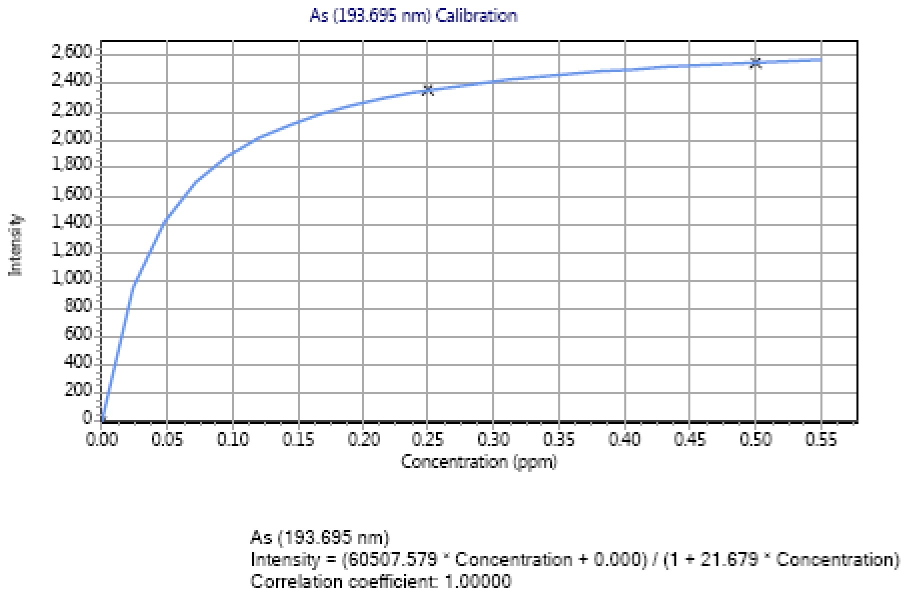


Figure 4.18: Calibration curve for determination of arsenic

4.7 Determination of Sodium and Potassium using Flame Photometry

Sodium and potassium were determined from the methanolic extracts of the three different plants samples using flame photometery. The result is shown on Table 4.12:

Table 4.12: Amount of Analyzed Sodium and Potassium (mg/l) in plant samples using Flame Photometry

Element (mg/l)	*Borreria stachydea*	*Cassia absus*	*Aspillia kotschyi*
Na	50	78	99

K	870	860	895

4.8 Determination of anti nutritional factors in *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plant

The following anti nutritional factors oxalate, phytate and cynide were determined from the three different plants and the results were shown on Table 4.13.

Table 4.13 : Results of Anti nutritional factors (mg/g) in *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.

Constituent(mg/g)	*Borreria stachydea*	*Cassia absus*	*Aspillia kotschyi*
Oxalate	7.8	18.4	6.8
Phytate	28.4	21.4	14.1
Hyrogencynide	6.8	8.9	4.4

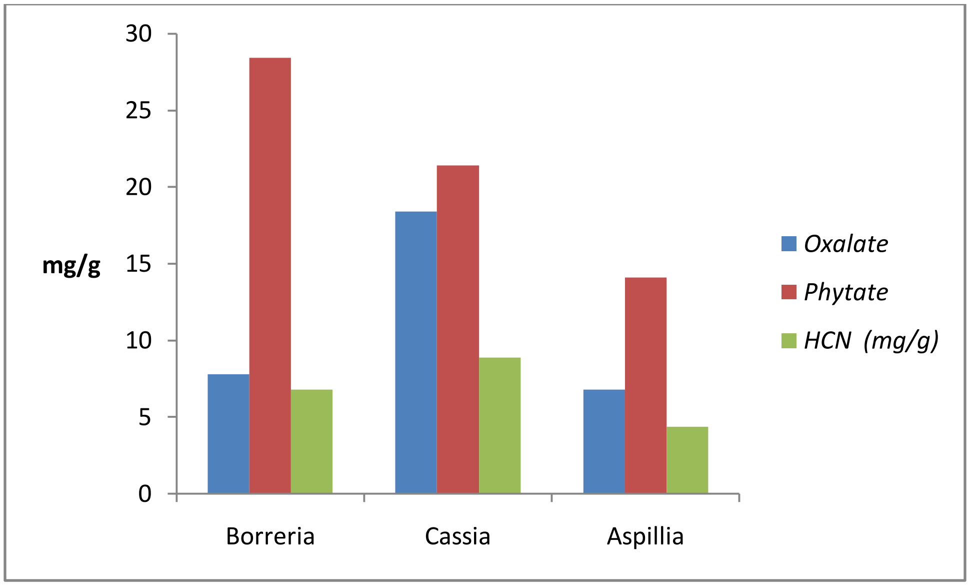


Figure 4.19: Bar Chart showing results of antinutritional factors (mg/g) in *Borreria stachydea, Cassia absus* and *Aspillia kotschyi* plants

4.9 Determination of nutritional factors in *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.

Vitamin A and C contents were respectively determined in the three plants materials and the results are in Table 4.14

Table 4.14 : Results of nutritional factors (mg/l) in *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.

Constituent (mg/l)	*Borreria stachydea*	*Cassia absus*	*Aspillia kotschyi*
Vitamin A	224	164	232

Vitamin C	8.0	6.66	9.3

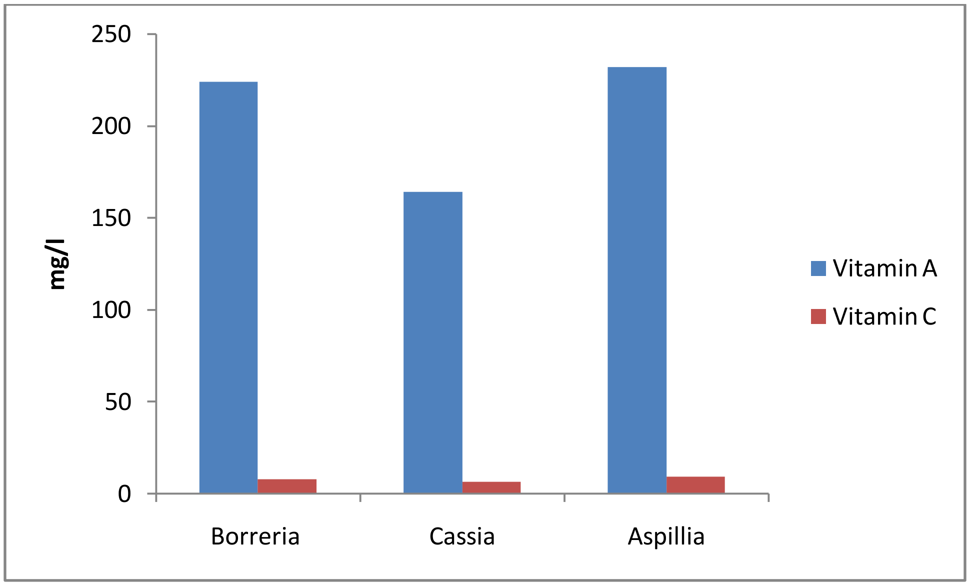


Figure 4.20: Bar chart showing results of nutritional factors in *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.

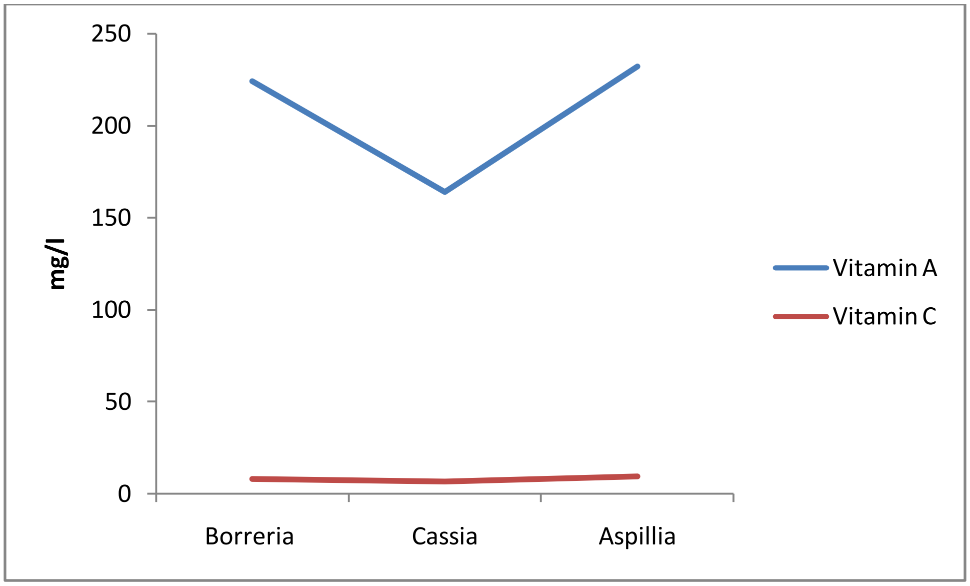


Figure 4.21: Line chart showing results of nutritional factors in *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.

4.10 Column Chromatography

Column chromatography was performed in order to purify the plant materials for functional group analysis. Table4.10 showed the extract yield after pulling of the different fractions collected from the three plants namely Borreria *stachydea* , *Cassia absus* Linn, and *Aspillia kotschyi* plants.

Table 4.15 Summary of fractions yield collected from column chromatography

Plant	Extract	Fraction Yield (g)
Borreria stachydea	Ethyl acetate	29.62
	N hexane	30.01
	methanol	32.29
Cassia absus	Ethyl acetate	31.98

	N hexane	30.03
	methanol	35.91
Aspillia kotschyi	Ethyl acetate	32.00
	N hexane	29.22
	methanol	31.91

4.11 Functional Group Analysis using Fourier Transform Infrared Spectroscopy(FTIR)

The obtained samples from column chromatography of the three plants i.e the methanol ethyl acetate, and hexane were subjected for Infrared spectroscopy using Fourier transform IR-spectrometer FTIR . Figures 4.22- 4.30 show the spectrum of run samples in the three plants.

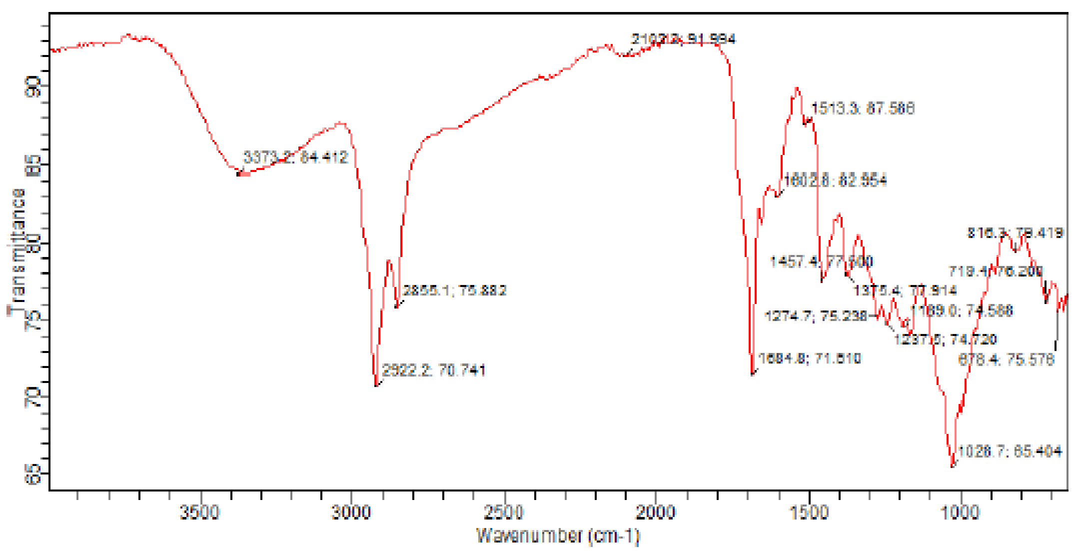


Figure 4.22 : Ethyl acetate extract spectrum of *Borreria stachydea*

Table 4.16 Interpretation of Ethyl acetate extract spectrum of *Borreria stachydea*

Wave number cm^{-1}	Bond	Functional group	Compared frequency range cm^{-1}
3328	O-H stretch, H-bonded	Alcohols,phenols	3500-3200
2926	C-H	Alkanes	3000-2850
1703	C=O stretch	Unsaturated aldehydes and ketones	1710-1665
1036	C-N	Aliphatic amines	1250-1020

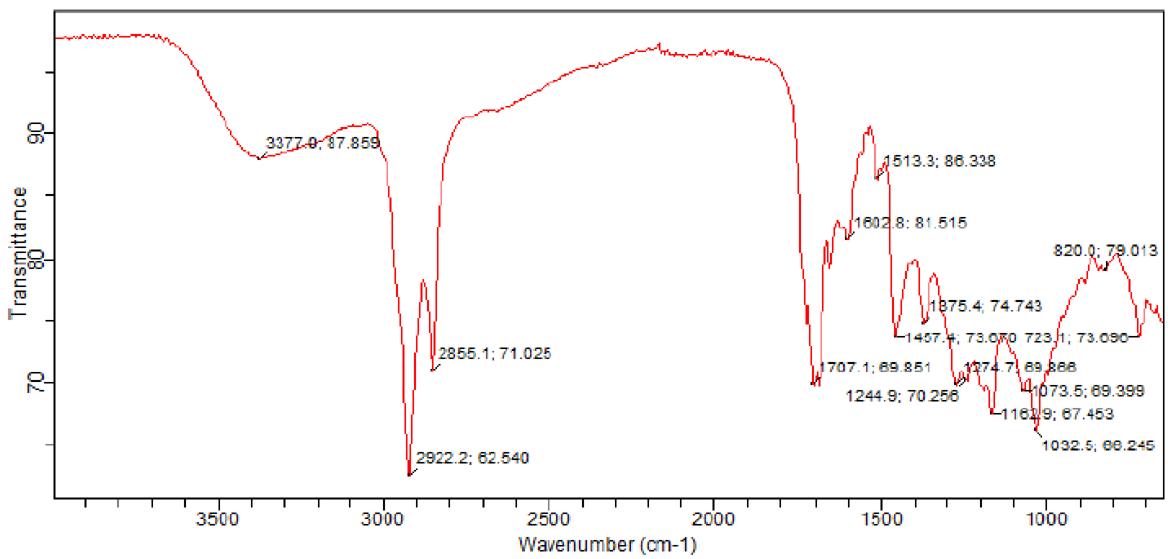


Figure 4.23 : N hexane extract spectrum of *Borreria stachydea*

Table 4.17 Interpretation of N hexane extract spectrum of *Borreria stachydea*

Wave number cm^{-1}	Bond	Functional group	Compared frequency range cm^{-1}
3377	O-H stretch, H-bonded	Alcohols,phenols	3500-3200
2922	CH_3	Methyl	2800-2960
1707	C=O stretch	Unsaturated aldehydes and	1710-1665

		ketones	
1457	C-H bend	Alkanes	1470-1450
1274	C-N Strech	Aliphatic amines	1250-1020
1032	C-N	Aliphatic amines	1250-1020

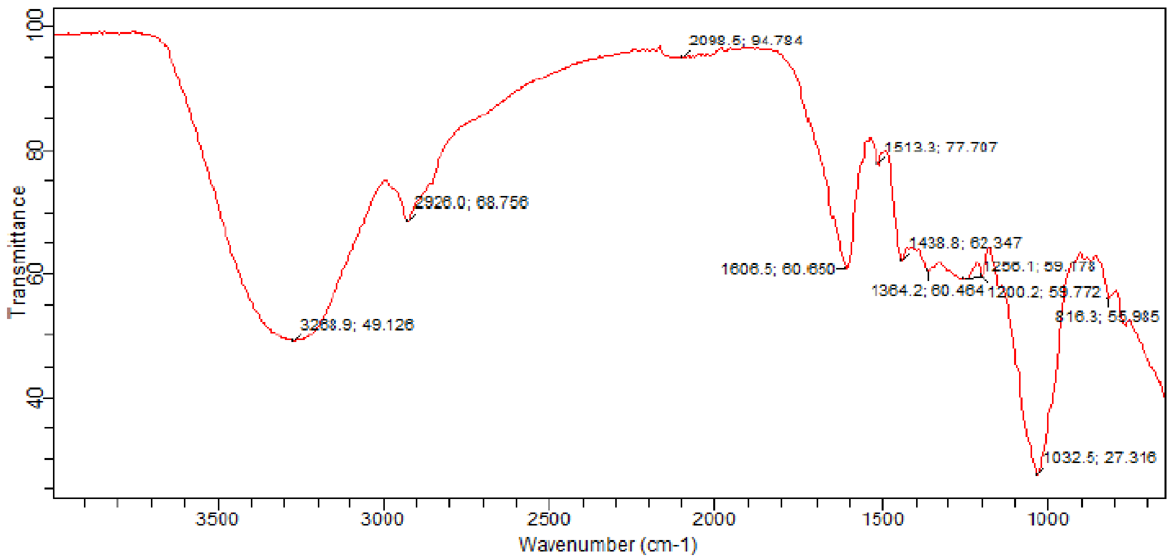


Figure 4.24 : Methanol extract spectrum of *Borreria stachyde*

Table 4.18 Interpretation of methanol extract spectrum of *Borreria stachydea*

Wave number cm^{-1}	Bond	Functional group	Compared frequency range cm^{-1}
3200	O-H stretch, H-bonded	Alcohols,phenols	3500-3200
2920	C-H stretch	alkanes	3000--2850
1606	C=O stretch	Unsaturated aldehydes and ketones	1710-1665
1304	C-H bend	Alkanes	1470-1450
1513	N-O Strech	Nito compounds	1550-1475

1032	C-N	Aliphatic amines	1250-1020

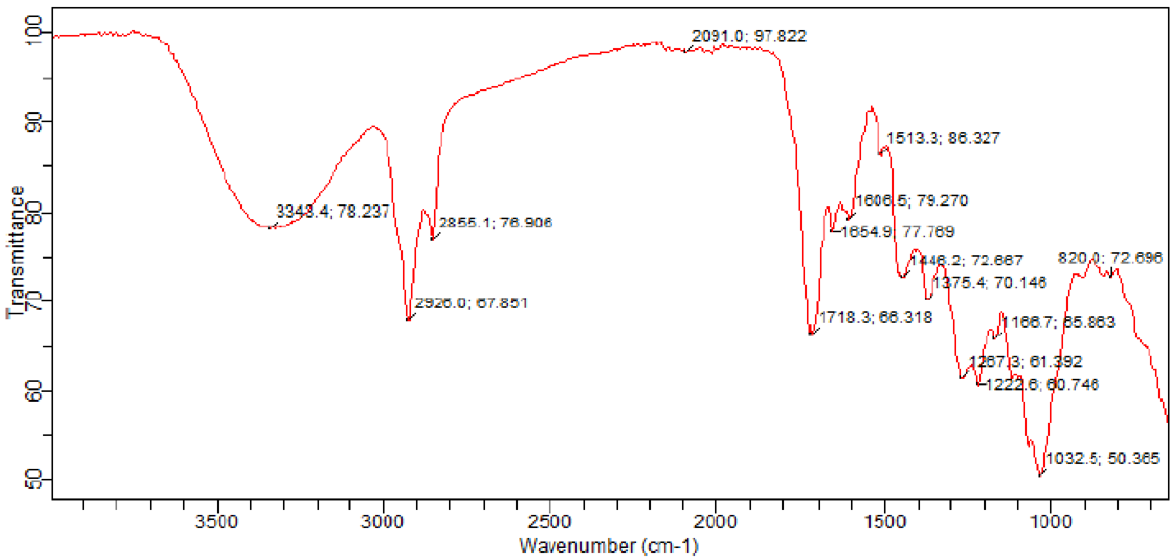


Figure 4.25 : Ethyl acetate extract spectrum of *Cassia absus*

Table 4.19 Interpretation of Ethyl acetate extract spectrum of *Cassia absus*

Wave number cm^{-1}	Bond	Functional group	Compared frequency range cm^{-1}
3348	O-H stretch, H-bonded	Alcohols,phenols	3500-3200
2926	C-H	Alkanes	3000-2850
1718	C=O stretch	Unsaturated esters	1730-1715
1036	C-N		1250-1020

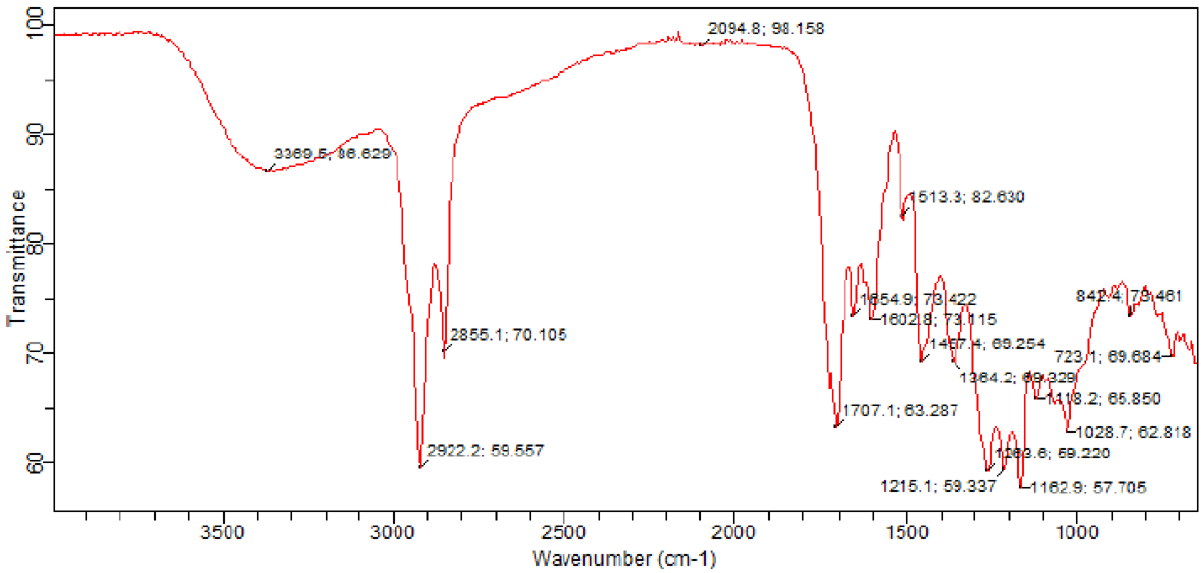


Figure 4.26 : N hexane extract spectrum of *Cassia absus*

Table 4.20 Interpretation of N hexane extract spectrum of *Cassia absus*

Wave number cm^{-1}	Bond	Functional group	Compared frequency range cm^{-1}
3380	O-H stretch, H-bonded	Alcohols,phenols	3500-3200
2922	CH_3	Methyl	2800-2960
1707	C=O stretch	Unsaturated aldehydes and ketones	1710-1665
1457	C-H bend	Alkanes	1470-1450
1203	C-N Strech	Aliphatic amines	1250-1020
1162	C-N	Aliphatic amines	1250-1020

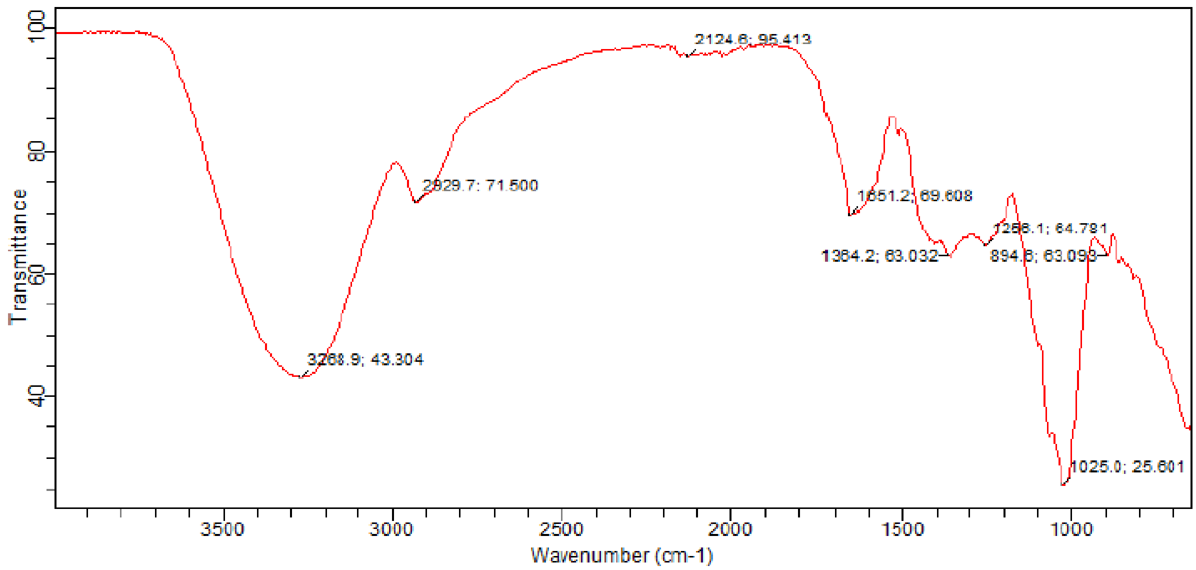


Figure 4.27 : Methanol extract spectrum of *Cassia absus*

Table 4.21 :Interpretation of methanol extract spectrum of *Cassia absus*

Wave number cm^{-1}	Bond	Functional group	Compared frequency range cm^{-1}
3208	O-H stretch	Carboxylic acids	3300-2500
2829	C-H stretch	alkanes	3000--2850
1661	C=O stretch	Unsaturated aldehydes and ketones	1710-1665
1304	C-H bend	Alkanes	1470-1450
1025	C-N	Aliphatic amines	1250-1020

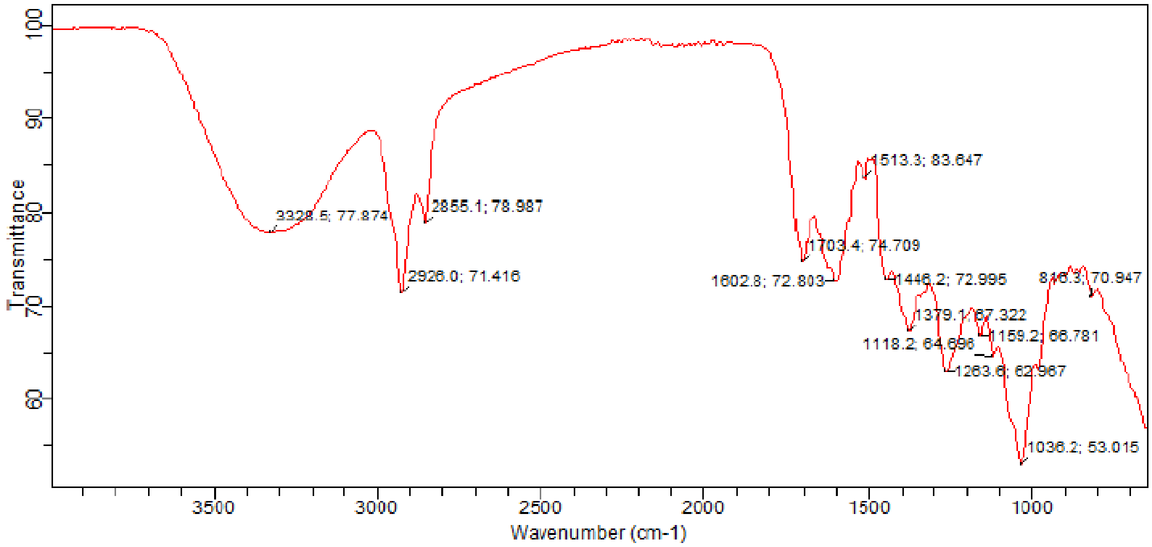


Figure 4.28: Ethyl acetate extract spectrum of *Aspillia kotschyi*

Table 4.22 Interpretation of Ethyl acetate extract spectrum of *Aspillia kotschyi*

Wave number cm^{-1}	Bond	Functional group	Compared frequency range cm^{-1}
3328	O-H stretch, H-bonded	Alcohols,phenols	3500-3200
2926	C-H	Alkanes	3000-2850
1703	C=O stretch	Unsaturated aldehydes and ketones	1710-1665
1036	C-N	Aliphatic amines	1250-1020

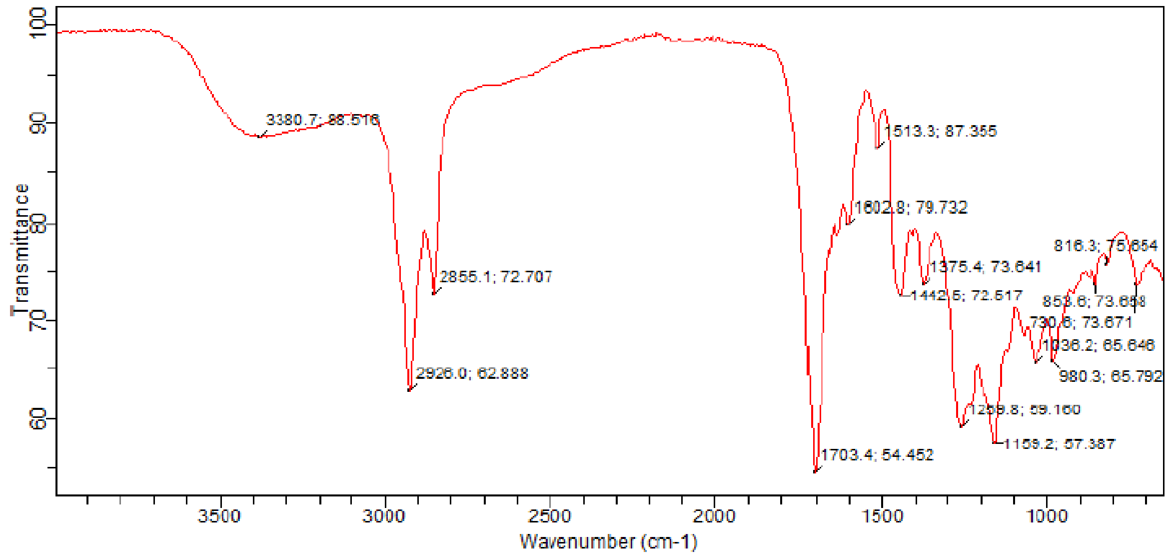


Figure 4.29 : N hexane extract spectrum of *Aspillia kotschyi*

Table 4.23 Interpretation of N hexane extract spectrum of *Aspillia kotschyi*

Wave number cm^{-1}	Bond	Functional group	Compared frequency range cm^{-1}
3380	O-H stretch, H-bonded	Alcohols,phenols	3500-3200
2922	CH_3	Methyl	2800-2960
1703	C=O stretch	Unsaturated aldehydes and ketones	1710-1665
1442	C-H bend	Alkanes	1470-1450
1259	C-N Strech	Aliphatic amines	1250-1020
980	C-H bend	Alkenes	1000-650

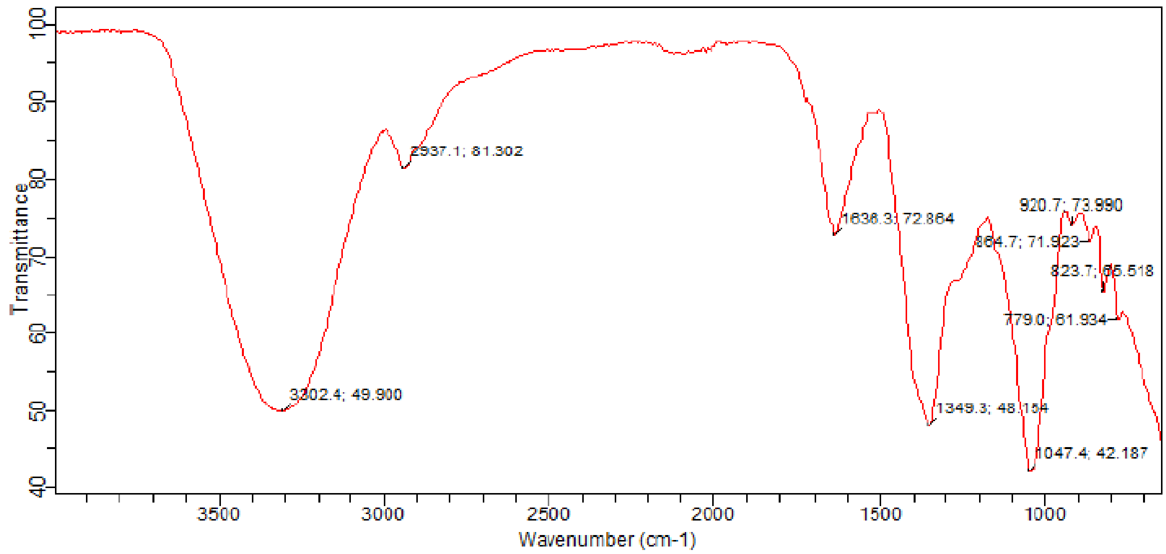


Figure 4.30 : Methanol extract spectrum of ***Aspillia kotschyi***

Table 4.24 :Interpretation of methanol extract spectrum of ***Aspillia kotschyi***

Wave number cm^{-1}	Bond	Functional group	Compared frequency range cm^{-1}
3302	O-H stretch	Alcohols, phenols	3500-3200
2837	C-H strech	alkanes	3000-2850
1636	C=O stretch	Unsaturated aldehydes and ketones	1710-1665
1360	C-H bend	Alkanes	1470-1450
1047	C-N	Aliphatic amines	1250-1020

CHAPTER FIVE

DISCUSSION

5.0 Yields of crude extracts

The various yields of crude extracts for the three different plants namely *Borreia stachydea*, *Cassia absus*, and *Aspillia kotschyi* are shown on Table 4.1. From the table the solvent which give the highest value of the extract was methanol. Therefore methanol was the best extracting solvent compared to Petroleum spirit 60-80^{o}C solvent used. Generally methanol extracted the highest quantity of the three different plants materials followed by Petroleum spirit 60-80^{o}C . On this note, it was established that different types of solvents extract different types of plants constituents and this is due to their different polarities. This feature enables the extraction of plant constituents to be more selective with optimal results.

5.1 Preliminary phytochemical screening of Methanolic and Petroleum spirit extracts of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.

The extracts of the three different plants materials namely *Borreria stachydea*, *Cassia absus* , and *Aspillia kotschyi* were screened for the presence of secondary metabolites alkaloids, flavonoids, carbohydrates tannins, sterols, and terpenoids among others.

Table 4.2 summarizes the results obtained from phytochemical screening of Methanolic and Petroleum spirit extracts of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.

5.1.1 Phytochemical screening of methanolic and petroleum spirit extracts of *Borreria stachydea, Cassia absus* :

This showed that the petroleum ether extract of the two plants contained glycosides, and steroids while the methanolic extracts of the two plants revealed the presence of carbohydrates, glycosides, cardiac glycosides, saponins, and flavonoids. Tannins were also observed to be present in the methanolic extract of *Borreria stachydea* only. The presence of these secondary metabolites has contributed to its medicinal value as well as physiological activity (Sofowara,1993).Various studies show that saponins although non toxic can generate adverse physiological responses in animals that consume them.

Presence of those phytochemicals justifies the use of the plant in curing certain diseases. Saponins act as an immune booster, lowers blood cholesterol and anti- intestinal cancer agent (Sodipo *et al*, 2009). The presence of flavonoids which are hydroxylated phenolics in the two plants might be responsible for their therapeutic effectiveness against wide array of microorganisms, probably due to their ability to complex with extracellular and soluble proteins and to complex with the bacterial cell wall. Flavanoids compound are potent water soluble antioxidants and free radical scavengers, that prevent oxidative cell damage and have strong anti cancer activity (Del- Rio *et al* 1997:Cowan, 1999, Okwu, 2004).

5.1.2 Phytochemical screening of Methanolic and Petroleum spirit extracts of *Aspillia kotschyi* plant ;

The results of phytochemical analysis of the two extracts of *Aspillia kotschyi* plant is shown in Table 4.2 to 4.4 in chapter four. This showed that the petroleum ether extract of the plants contained carbohydrates, and alkaloids while the methanolic extracts of the plant revealed the presence of carbohydrates, cardiac glycosides, flavonoids,triterpene, and alkaloid. The presence of these secondary metabolites contributed to its medicinal value as well as physiological activity (Sofowara,1993). Flavonoid which was detected has a wide range of pharmacological effects including antioxidant, anti-inflammation, antiplatelet, anti-allergic, cytoxicity and reduce risk for heart disease (Mohammad *et al.*,2013). The identified phytoactive compound in the plant are well known for their pharmacological activities ranging from antibacterial and antifungal (Trease and Evans ,1993).

5.1.3 Quantitaive **phytochemical screening of methanolic extracts of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.**

Table 4.5 in chapter four summarizes the quantitative phytochemical screening of the methanolic extract of the three plants. The results showed that *Borreria stachydea* is the only one that contained tannins with a value of 17.7%. Tannins is found to be absent in the remaining two plants(i.e *Cassia absus and Aspillia kotschyi*) . The nutritional effects of tannins are mainly related to their interaction with protein due to the formation of complexes (Laurena *et al.*, 1984).Tannin-protein complexes are insoluble and protein digestibility is decreased (Carnovale *et al.*, 1991).

5.2 Proximate composition of methanolic extracts of *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants.

The result from proximate analysis showed that *Borreria stachydea* methanolic extract has the highest crude fibre content of 7.11% compared with *Cassia absus* plant (Table 4.6). Nutritionally, this is of beneficial effect since it had been reported that food fibre aids absorption of trace elements in the gut (Kelsy, 1981) and reduce absorption of cholesterol (Le Veille and sanbelich ,1966). Crude fibre is very essential for the digestion of food materials in the food canal of animals, and thus reduces the risk of some cancers (Manalisha *et al*.,2013). *Cassia absus* methanolic extract showed higher crude protein content. Nutritionally *Cassia absus* is beneficial as protein as it contains amino acids utilized by the cells of the body to synthesize all the numerous proteins required for the function of the cell and also to provide energy (Robison,1978).

Borreria stachydea petroleum spirit extract also showed moderate percentage composition of fat, total ash, and moisture contents. The total fat content in *Borreria stachydea* if further analyzed may contain fatty acids as well as vitamins. The plant can be considered as a poor source of lipids as it contain 3.13 % and 2.15 % of crude fat as compared to reported values of (8.3-27.0%) in some vegetables consumed in West Africa (Ifon and Bassir,1980). The percentage of moisture content varies from 6.67 % in *Cassia absus* to 8.67 % in *Borreria stachydea*. The moderate moisture content provides for an activity of water soluble enzyme and co enzyme needed for metabolic activities of these leafy vegetables (Iheanacho and Udebani,2009). *Borreria stachydea* and *Cassia absus* have relatively similar values of ash contents of 8.5 % and 8.0 % respectively. This confirms that there are minerals in the two plants.

The proximate analysis of the crude sample of *Aspillia kotschyi* plant showed moisture of (5.7%) and ash content of (4.03%). The *Aspillia kotscyi* methanolic extract proximate analysis result is shown in Table 4.6. The result showed that it contains crude protein (10.94%), crude fibre (9.06%) , crude fat(0.83%),free nitrogen(70.19%).Crude protein has the highest value while crude fat has the least. The crude protein and crude fibre contents are higher in the methanolic extract of *Aspillia kotschyi* . The plant methanolic extract showed higher crude protein content. Nutritionally *Aspillia kotschyi* plant is beneficial as protein contain amino acids utilized by the cells of the body to synthesize all the numerous proteins required for the function of the cell and also to furnish energy (Robinson,1978). From the results also one can see that the fibre content in the plant material showed that nutritionally this is of beneficial effect since it had been reported that food fibre aids absorption of trace elements in the gut (Kelsay,1981) and reduce absorption of cholesterol(Le Veille and Sanberlich, 1966). Crude fibre is also very essential for the digestion of food materials in the food canal of animals (Manalisha *et. al*, 2013). The total fat content in *Aspillia kotschyi* if further analyzed may contain fatty acids as well as vitamins. Ash content of 4.03% dry matter (DM) was obtained as a result of *Aspillia kotschyi*.

Ash in food contributes for residue remaining after all the moisture has been removed as well as organic material(fat, protein, carbohydrates, vitamins, organic acids etc)have been incinerated at a temperature of about 500^{o}C .Ash content generally taken to be a measure of mineral content of original food (Onwuka, 2005).).The percentage of moisture content obtained in the plant is 5.7%. The moderate moisture content provides for an activity of water soluble enzyme and co enzyme needed for metabolic activities of the plant (Iheanacho and Udebuahi, 2009).

5.3 Elemental Analysis of *Borreria stachydea*, *Cassia absus* and *Aspillia kotschyi* methanolic and petroleum spirit extracts using Atomis Absorption spectrometry

Mineral elements such as Calcium, Zinc, and Magnesium were determined in the two plants. The mean concentration of calcium is in the order of 0.6102 and 0.4458 mg/kg in *Cassia absus* and *Borreria stachydea* methanolic extracts respectively (Tables 4.8 and 4.9). Calcium is good for growth and maintenance of bones, teeth and muscles (Dosunmu,1997). Therefore these plants could provide veritable sources of calcium. Other mineral elements detected at reasonable concentrations in Tables 4.8 and 4.9 are magnesium and zinc. The concentration of these metals found in the plants is advantageous since certain inorganic mineral elements (potassium, zinc, calcium etc) play important roles in the maintenance of normal glucose tolerance and in the release of insulin from beta cells of islets of Langerhans (Choudhary,1999).

Chromium was observed to be higher than the WHO recommended limit of 0.05 mg/L. However, lead and cadmium were relatively of low concentrations. Therefore consuming these plants may be harmful since it has been reported that some of these minerals like lead, chromium and cadmium are highly toxic even at low concentration (Asaolu, *et al*., 1997 and Rao,1998).

5.4 Determination of other minerals in Borreria *stachydea, Cassia absus* and *Aspillia kotschyi* methanolic extract using Micro Wave Plasma Atomic Emission Spectroscopy technique (MP- EAS)

Table 4.11 showed the results of the other elements determined using micro wave plasma atomic emission spectroscopy technique from the three plants. The elements determined are;Selenium, Silver, Iron, Copper, Nickeli, Arsenic, Cobalt, Manganese and Aluminium. From the result Silver, copper, nickel and Cobalt are of very negliglble concentrations in all the three plants . However Seleniun is found to be 0.780±3.44 (ppm) in *Casssia absus* methanolic extract , 0.556±3.34 (ppm) in *Borreria stachydea* methanolic extract, and 0.530±4.50 (ppm) in *Aspillia kotschyi* methanolic extract. It was reported by Aubert and Pinta, 1977 that Plant and soil samples usually contain selenium in trace amounts and the mean content in soil samples is about 0.3ppm. The concentration of selenium determined in these three plants is slightly higher than the reported value.

Iron on the other hand was found to be 3.712±0.27 (ppm), in *Aspillia kotschyi* metahanolic extract, 3.674±0.21 (ppm) in Borreria stachydea pet ether extract. Role of iron in human body includes heamoglobin formation, electron transport, oxygen transport, enzyme activator. Iron is present in drinking water, food, soil, air and dust. Iron in the environment arises from both natural and anthropogenic sources. It also occurs as a natural constituent in plants and animals.

As pointed above Iron is an essential element in human nutrition, and in the formation of red blood cells. It forms an important constituent of haemoglobin and intercellular enzyme system. Estimates of the minimum daily requirement for iron depends on age, sex, physiological status and iron bioavailability which ranges from about 5 to 10 mg/day (FAO/WHO, 1988). From the values determined all the three plants are good source of iron.

Arsenic was found to be 0.506±1.87(ppm) and 1.301±2.90 (ppm) in *Aspillia kotschyi* extracts, 2.373±2.94(ppm) and 0.789±1.23(ppm) in *Borreria stachydea* extracts, and 3.315±0.89 (ppm) in *Cassia absus* extract.

Arsenic exists both in inorganic and organic forms and also in different valence states. Inorganic arsenic is significantly more toxic than organic arsenic compounds such as dimethylarsinate, and in turn the trivalent forms of arsenic, e.g. arsenic trichloride, are more toxic than the pentavalent arsenates. The latter are considered to be toxic only after metabolic conversion to the trivalent form of arsenic. This pattern of toxicity is also seen for certain other metallic compounds in the body.

The concentration of aluminium was found to be of the range of 3.050±0.63ppm in *Aspillia* extract and 2.992±0.50 ppm in *Borreria stachydea* extract. Aluminum being the third abundant element in the Earth's crust in the form of silica and alumino silicate, only a small quantity of it exist in a soluble form which is capable of influencing biological systems (Silva,2012).Its bioavailability is mainly restricted to soils that are acidic in nature there by making the alumino silicate clays and aluminium hydroxide minerals to dissolve there by releasing aluminium cation available to plants grown on such soils (Silva,2012).

5.5 Sodium and Potassium contents in the analyzed plants using Flame Photometry

Table 4.12 revealed the quantities determined for sodium and potassium from the three plants extracts. Sodium is found to be 50,78, and 99 mg/l in *Borreria stachydea*, *Cassia absus* and *Aspillia kotschyi* respectively.The potassium level in the three plants is 870, 860 and 895 respectively. The normal concentration of sodium and potassium in plants is 203ppm and 55.0ppm respectively.

Potassium works alongside sodium to maintain a normal blood pressure. Potassium is known as an electrolyte, and this helps to maintain a healthy balance of fluids in the body. It also helps to transmit electrical pulses to allow for proper nerve and muscle function.

Potassium is an electrolyte found in our body that has many significant functions. According to the American Heart Association, it keeps a normal water balance between cells and body fluids. It also aids nerve conduction and muscle contraction. Potassium plays a key role in keeping the heart beating at a normal rhythm.

Potassium is an essential mineral micronutrient and is the main intracellular ion for all types of cells. It is important in maintaining fluid and electrolyte balance in the bodies of humans and animals (Pohl, *et al*; 2013). Potassium is necessary for the function of all living cells, and is thus present in all plant and animal tissues. It is found in especially high concentrations within plant cells, and in a mixed diet, it is most highly concentrated in fruits. The high concentration of potassium in plants, associated with comparatively very low amounts of sodium there, From the result of this work there is high concentration of potassium in the plants which means that heavy crop production rapidly depletes soils of potassium, and agricultural fertilizers consume 93% of the potassium chemical production of the modern world economy.

5.6 Anti nutritional factors of the analyzed plants *Borreria stachydea, Cassia absus* , and *Aspillia kotschyi* Plants;

Table 4.13 revealed the concentrations of the anti nutritional factors in the three plants. These factors include phytates, oxalates, and hydrogen cyanide. These factors can exert adverse physiological effects when ingested by man and animals .The factors can be destroyed by heat or some other suitable treatment (Liener,1980).In addition to above, Oxalate and phytates binds trace elements and macro elements such as zinc, calcium, magnesium and iron in the gastrointestinal tract and making these dietry minerals unavailable for absorption and utilization by the body while tannins make proteins unavailable to the body and cyanides are poisonous to man and animal (Kala and Mohan,2012).

Phytates

The amount of phytate in *Borreria stachydea* is 28.4 mg/g, 21.4mg/g in *Cassia absus* and 14.1 mg/g in *Aspilla kotchyi* plants. Presently there is no reported phytate value of these three plants from the available literature which can be compared. Looking at the values above the phytate values are moderate in the three plants which is of the range of 28 to 14 mg/g and justified the claim that the phytic acid content in plants is highly variable and most of the seed, grains and legumes contain it in variable small amounts as see in the Table 4.20 below.

Table 5.1 : Values of phytic acid in some seed, grains and legumes.

Food	Phytic Acid
Almonds	0.4 - 9.4%
Beans	0.6 - 2.4%
Brazil nuts	0.3 - 6.3%
Hazelnuts	0.2 - 0.9%
Lentils	0.3 - 1.5%
Maize, corn	0.7 - 2.2%
Peanuts	0.2 - 4.5%
Peas	0.2 - 1.2%
Rice	0.1 - 1.1%
Rice bran	2.6 - 8.7%
Sesame seeds	1.4 - 5.4%
Soybeans	1.0 - 2.2%
Tofu	0.1 - 2.9%
Walnuts	0.2 - 6.7%
Wheat	0.4 - 1.4%
Wheat bran	2.1 - 7.3%

Source:Authantunutrition.com

In other words phytic acid reduces mineral absorption during the meal, but doesn't have any effect on subsequent meal. However when you eat high phytate foods with most of your meals, minerals deficiencies may develop over time.

Oxalates

The amount of oxalates in *Borreria stachydea* is 7.8 mg/g, 18.4mg/g in *Cassia absus* and 6.8 mg/g in *Aspilla kotchyi* plants. Presently there is no reported determined oxalate value of these three plants from the available literature which can be used for comparison. The amount of oxalate ingested may be an important risk factor in the development of Idiopathic calcium oxalate nepholithiasis. Reliable food tables listing oxalate contents of food are currently not available.

Hydrogen cyanide

Amount of hydrogen cyanide determined in this work is 6.1mg/g, 8.9mg/g and 4.4mg/g for *Borreria stachydea* , *Cassia absus* and *Aspilla kotchyi* plants respectively.

If one is exposed to cyanide, many factors will determine whether he can be harmed. These factors include the dose (how much), the duration (how long), and how you come in contact with it. Here populace are using these three plants as medicine but the cyanide concentration is low but still there is need to consider any other chemicals they are exposed to and their age, sex, diet, family traits, lifestyle, and state of health for healthy life

It was also reported that many plant materials, such as cassava roots, lima beans, and almonds, naturally contain low-to-moderate levels of cyanide. The concentration of hydrogen cyanide in unpolluted air is less than 0.2 parts of hydrogen cyanide per million (ppm; 1 ppm is equivalent to 1 part by volume of hydrogen cyanide in a million parts by volume of air). 0.011 ppm (1 ppm is equivalent to 1 part by weight in 1 million parts by volume of water) .

5.7 Vitamin content in the analysed plants (*Borreria stachydea, Cassia absus* , and *Aspillia kotschyi*).

Vitamin A and C contents were respectively determined in the three plants materials and the results are in Table 4.14 Vitamin A is found to be 224 mg/l, 164mg/l and 232 mg/l in *Borreria stachydea* , *Cassia absus* and *Aspilla kotschyi* plants respectively. Vitamin A is an important parameter in determining food quality of plants most especially vegetables. With the level of the vitamin A in these plants they can be considered as a source of vitamin A.

Vitamin C which is otherwise known as ascorbic acid was found to be 8.0mg/l, 6.66mg/l nd 9.3 mg/l in *Borreria stachydea* , *Cassia absus* and *Aspilla kotchyi* plants respectively. These values are extremely lower than the daily dietary amount of vitamin C in a 100g sample of the food for an average man or for a woman during pregnancy and lactation (60mg) (Food and Nutrition Board, 1969).

5.8 Functional Group Analysis using Fourier Transform Infrared Spectroscopy(FTIR)

Tables 4.16, 4.17 and 4.18 shows the functional groups determined in the three extracts of *Borreria stachydea.* All the three extracts showed that the plant material contained the following functional groups; O-H Alcohols, phenols , C-H for Alkanes , C=O Unsaturated aldehydes and ketones and C=O Aliphatic amines.

On the other hand Tables 4.19 - 4.21 shows the functional groups determined in the three extracts *of Cassia absus* plant. All the extracts of this plant shows the presence of –OH alcohols, phenols,C-H,Alkanes, C=O unsaturated esters, C-N amines, and CH_3 methyl.

Table 4.22 to 4.24 shows the functional groups determined in the three extracts *of Aspillia kotschyi* plant. The plant extracts shoed the presence of –OH alcohols, phenols, C-H, Alkanes, C=O unsaturated esters, C-N amines, and CH_3 methyl.

Functional groups in biological molecules play an important role in the formation of molecules like DNA, proteins, carbohydrates, and lipids. The major determined functional groups in these three plants are hydroxyl, methyl, carbonyl, carboxyl, and amines.

Functional Group is a group of atoms in a molecule which gives the characteristic property and reaction. It is also the basis of naming a certain molecule or entity of chemicals. In pharmacy, functional group is not just only to use in naming a drug, but it is the important part of the drug that exhibits a biological action into the human body or against another molecule. . Important functional groups in pharmacy are hydroxyl group, carbonyl group which is divided into ketone or aldehyde, carboxyl group etc and all these groups were obtained in these plants.

In pharmacy, the functional group is the part of a molecule that imparts the activity and action of the drug. It is the group that considers to have affinity and ability to bind to a receptor. It is the main part of a drug that acts on the microorganisms to either destroy or prevent them such as Fluoro and Chloro in Flucloxacillin and Cloxacillin, respectively. It is also the reason why drug interaction happens.

Thus, the significance of functional groups in the pharmacy is more than just naming or reaction of a chemical to another chemical, but it is the main group that provides action and importance of a certain drug. It will be the basis to distinct and identify a drug and its mode of action, or the site of action, classification and drug category from another drug. This reason supports the use of these three plant material as curative for some diseases.

5.9 Conclusion

Plant based drugs have been used against various diseases since time immemorial. The primitive man used herbs as therapeutic agents and medicaments, which they were able to produce easily. Nature has provided abundant plant wealth for all living creatures, which possess medicinal virtues. The most important values of some plants have been published but a large number of them remain unexplored. So there is need to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties (Mushtaq *et al.*, 2009). Plants in general contribute to the mineral, vitamin and fibre contents of diets. Among the plants, vegetables are excellent sources of minerals and contribute to the recommended dietary allowance (RDA) of these essential nutrients. Minerals are very important ingredients for normal metabolic activities of body tissues. They are constituents of bones, teeth, blood, muscles, hair and nerve cells. Vitamins cannot be properly assimilated without the correct balance of minerals (Sonni, 2002). Food and Agricultural Organisation (1986) report indicated that at least one billion people are thought to use wild plant in their diet (Burlingame, 2000)

Traditional medicine as a major African socio cultural heritage, obviously in existence for several hundred years, was once believed to be primitive and wrongly challenged with animosity, especially by the conventional or orthodox medical practitioners. However today, traditional medicine have been brought into focus for meeting the goals of a wider coverage of primary health care delivery, not only in Africa but also to various extents, in all countries of the world. Traditional medicine is the first choice health-care treatment for at least 80% of Africans who suffer from high fever and other common ailments (Elujoba *et al*., 2005).

Each medicinal plant species has its own nutrient composition besides having pharmacologically important phytochemicals. These nutrients are essential for the physiological functions of human body. Such nutrients and biochemicals like carbohydrates, fats, and proteins play an important role in satisfying human needs for energy and life processes (Novack and Haslber, 2000). Fortunately chemical composition diversity in plants also includes many compounds that are beneficial to humans; vitamins, antioxidants, anticarcinogens and many other compounds with medicinal value (Novack and Haslberger, 2000).

Phytochemical analysis revealed that the two plants *Borreriia stachydea* and *Cassia absus* contain similar constituents which are useful for medicinal properties. However more analysis such as thin layer chromatography, GC-MS, NMR , need to be done to ascertain the actual components of the plants.

From this work it is shown that, the presence of those phytochemicals justifies the use of the *Aspillia kotschyi* in curing certain diseases. The presence of flavonoids which are hydroxylated phenolics in the plant might be responsible for the therapeutic effectiveness against a wide array of microorganisms, probably due to their ability to complex with extracellular and soluble proteins and to complex with the bacterial cell wall. The work also shows that plant examined is good source of fibre and can decrease the concentration of high cholesterol level in body.

5.10 Recomendations

This work has shown that the plants examined *Borreria stachydea* and *Cassia absus* are good sources of fibre, which implies that they can decrease high cholesterol levels in the body. But care has to be taken to avoid the excessive use of the plants as medicinal therapies because they contain low concentration of some heavy metals such as lead and chromium. Long and excessive usage may lead to their bio-accumulation of such metals, which may eventually become toxic to the human body.

Moreover Important functional groups were determined which are useful in pharmacy such as hydroxyl group, carbonyl group which is divided in to ketone or aldehyde, if further analysis is carried out on the plant extracts the structure of the components present can be deduced.

Pharmacological studies of the extracts need to be undertaken to give further knowledge into their ethno-medical efficacy.

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APPENDICES

APPENDIX I : FTIR spectrum of hexane extract of *Aspillia kotschyi*

Sample ID:EB 29.22g HEXANE A
Sample Scans:16
Background Scans:16
Resolution:8
System Status:Good
File Location:C:\Program Files\Agilent\MicroLab PC\Results\EB 29.22g HEXANE A_2016-10-18T14-04-25.a2r

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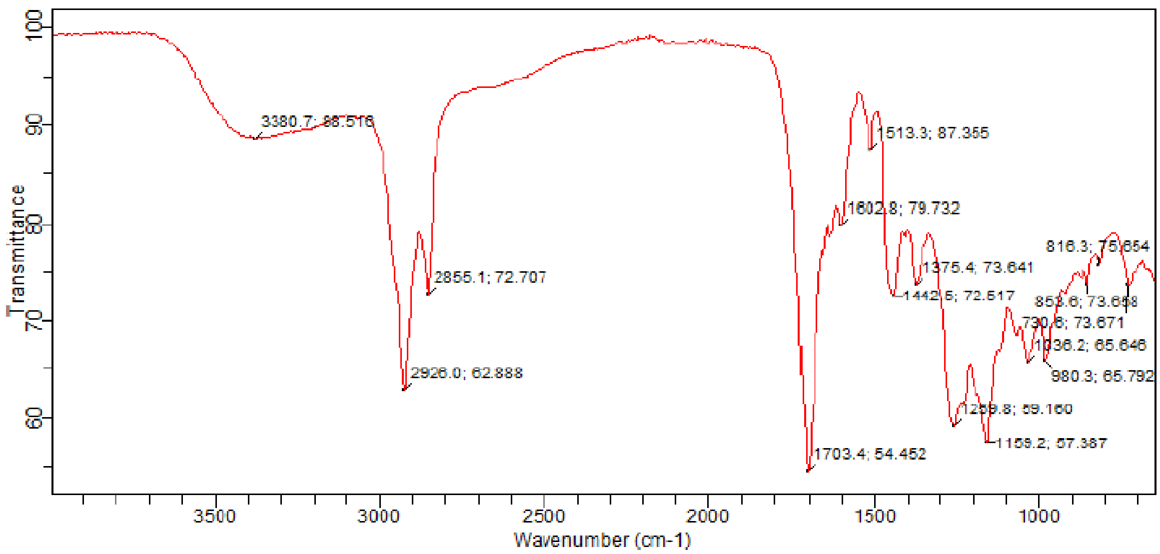
Apodization:Happ-Genzel



APPENDIX ii: FTIR spectrum of ehyl acetate extract of *Borreria stachydea*

Sample ID:EB 29.62g ETHYL ACETATE B
Sample Scans:16
Background Scans:16
Resolution:8
System Status:Good
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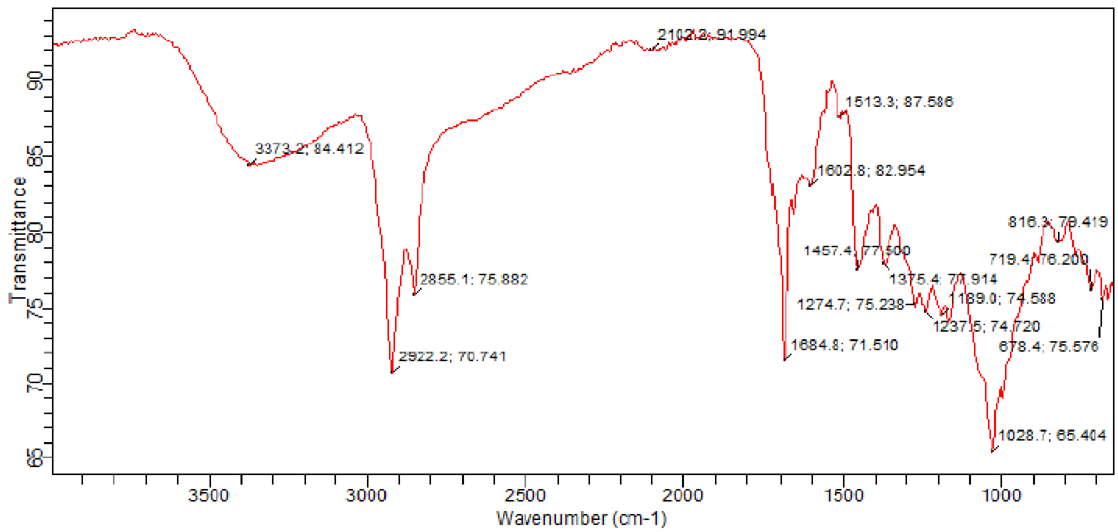

APPENDIX iii: FITR spectrum of hexane extract of *Casssia absus*

Sample ID:EB 30.03g HEXANE C
Sample Scans:16
Background Scans:16
Resolution:8
System Status:Good

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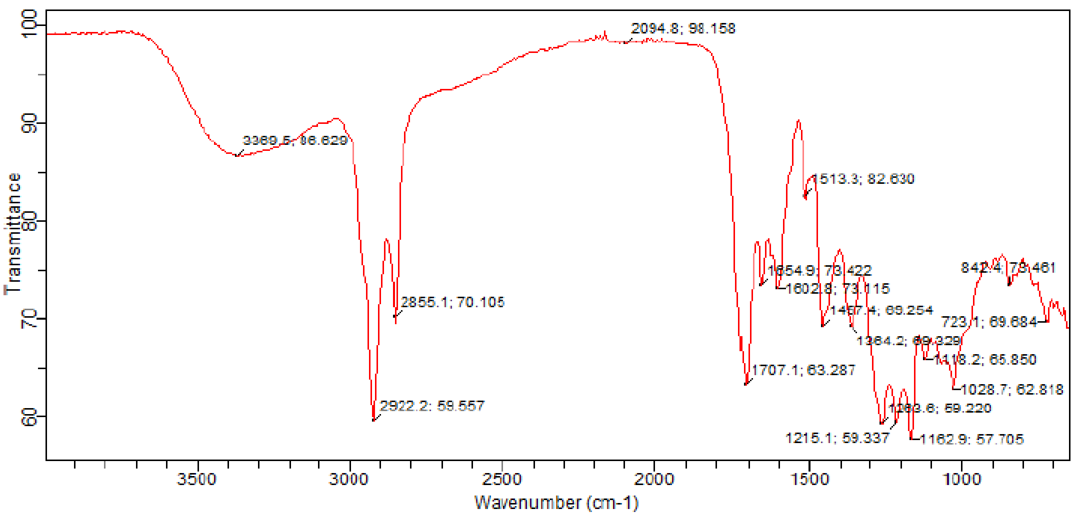

APPENDIX iv : FITR spectrum of hexane extract of *Borreria stachydea*

Sample ID:EB 30.09g HEXANE B
Sample Scans:16
Background Scans:16
Resolution:8
System Status:Good
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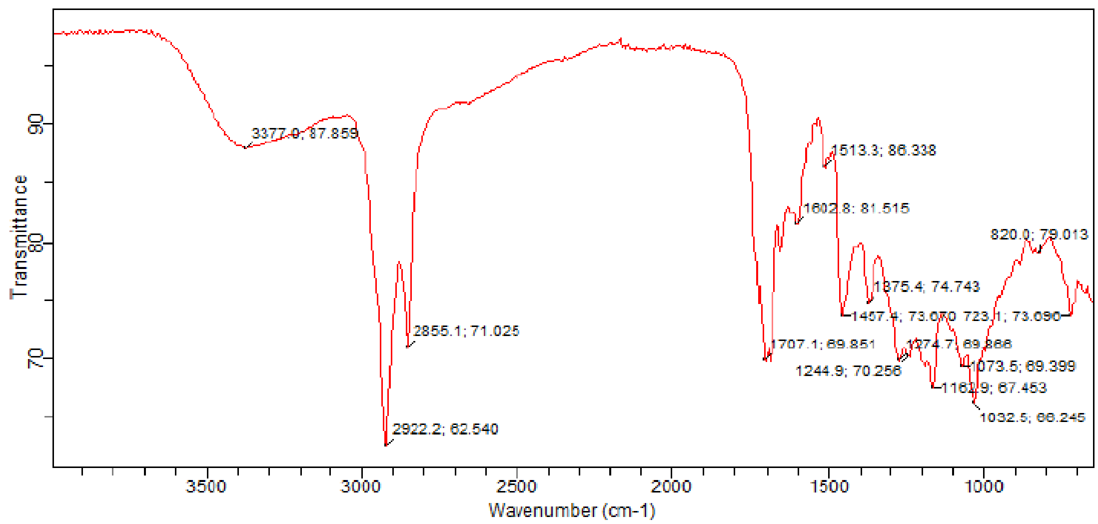

APPENDIX v: FTIR spectrum of methanol extract of *Aspillia kotschyi*

Sample ID:EB 31.92g ETHYL METHANOL A
Sample Scans:16
Background Scans:16
Resolution:8
System Status:Good

Method Name:Transmittance Method
User:Admin
Date/Time:2016-10-18T14:00:54.486+01:00
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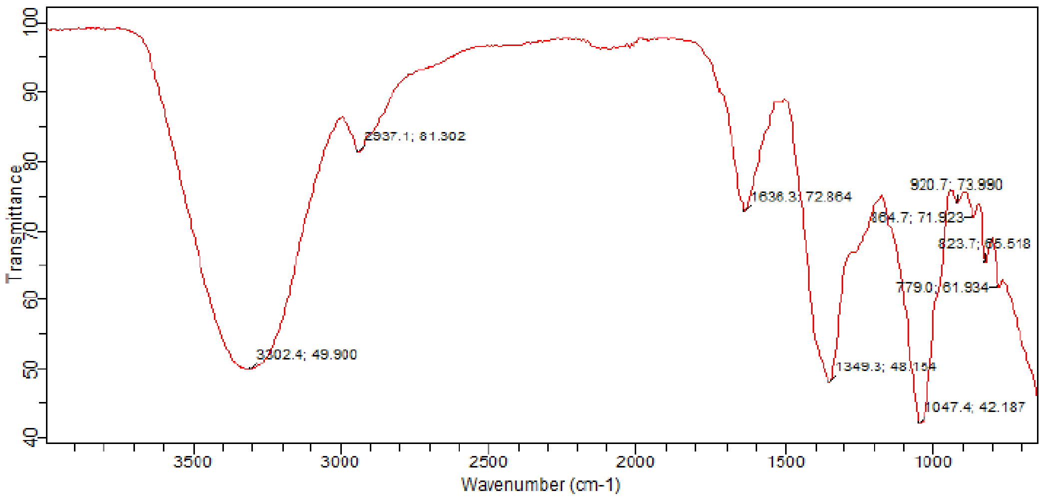

APPENDIX vi: FTIR spectrum of ethylacetate extract of *Cassia absus*

Sample ID:EB 31.98g ETHYL ACETATE C
Sample Scans:16
Background Scans:16
Resolution:8
System Status:Good

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User:Admin
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Range:4000 - 650
Apodization:Happ-Genzel

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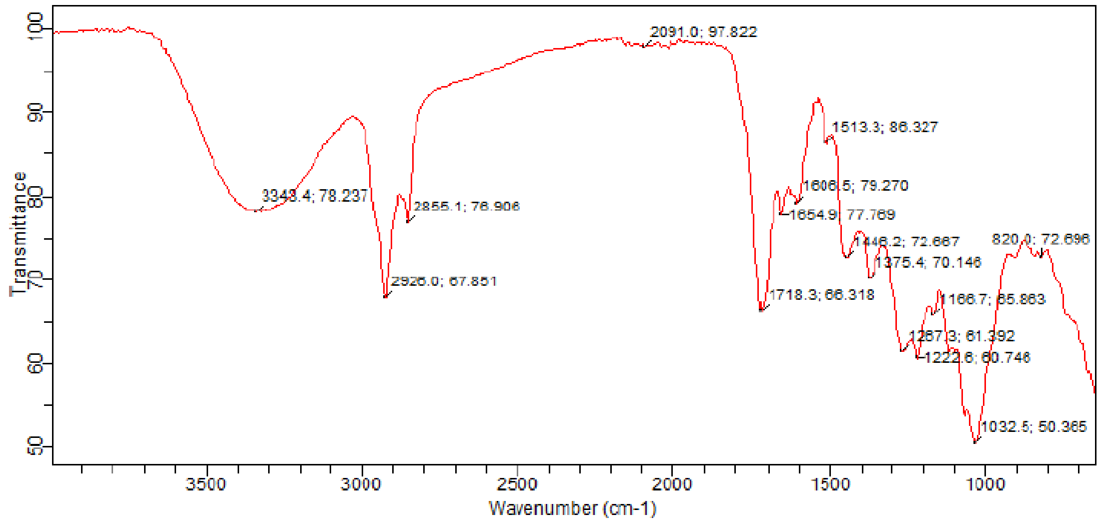

APPENDIX vii: FTIR spectrum of ethyl acetate extract of *Aspillia kotschyi*

Sample ID:EB 32.06g ETHYL ACETATE A
Sample Scans:16
Background Scans:16
Resolution:8
System Status:Good
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User:Admin
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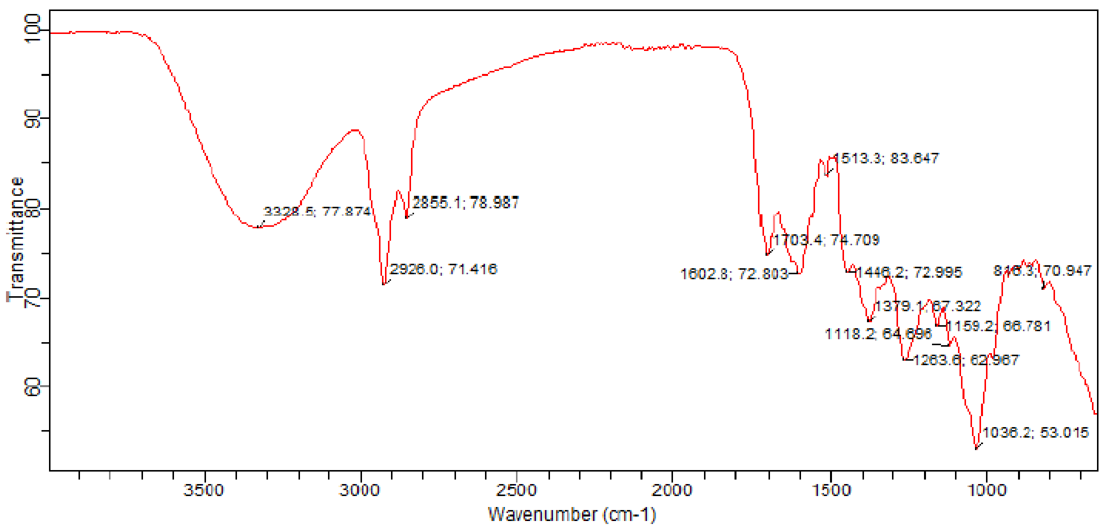

APPENDIX viii: FTIR spectrum of methanolic extract of *Borreria stachydea*

Sample ID:EB 32.29g METHANOL B
Sample Scans:16
Background Scans:16
Resolution:8
System Status:Good
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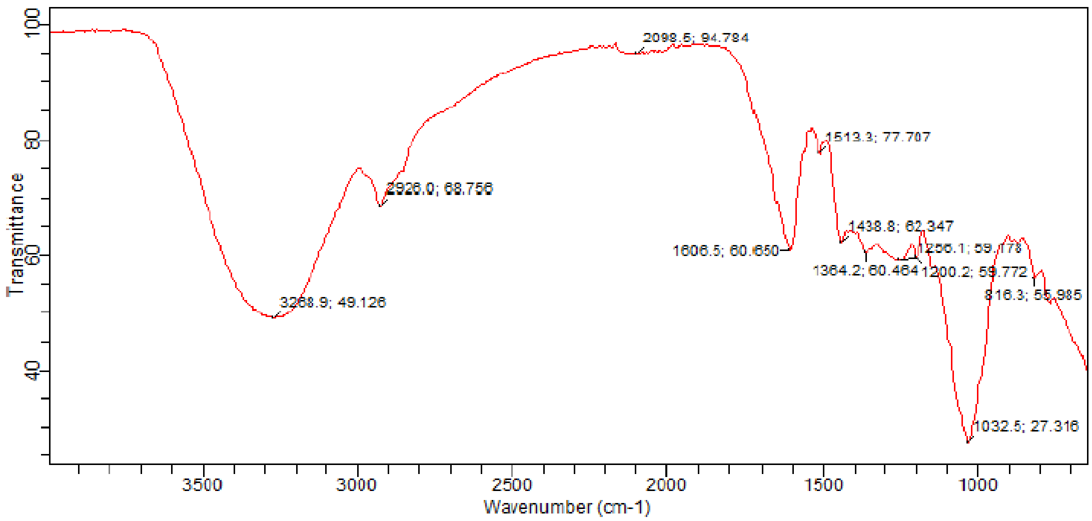

APPENDIX ix: FTIR spectrum of methanolic extract of *Cassia absus*

Sample ID:EB 35.91g METHANOL C
Sample Scans:16
Background Scans:16
Resolution:8
System Status:Good

Method Name:Transmittance Method
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Apodization:Happ-Genzel

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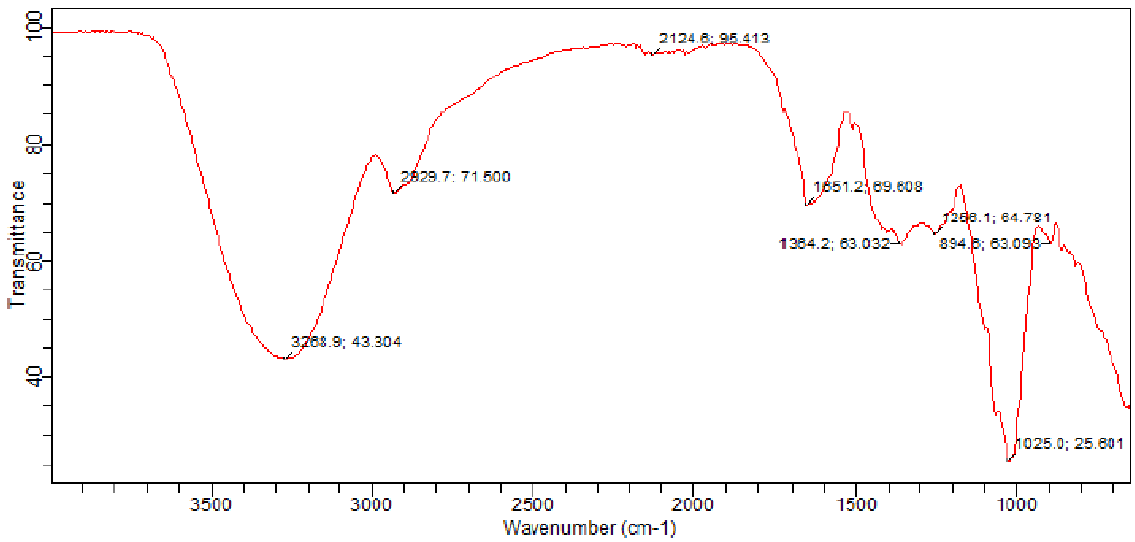
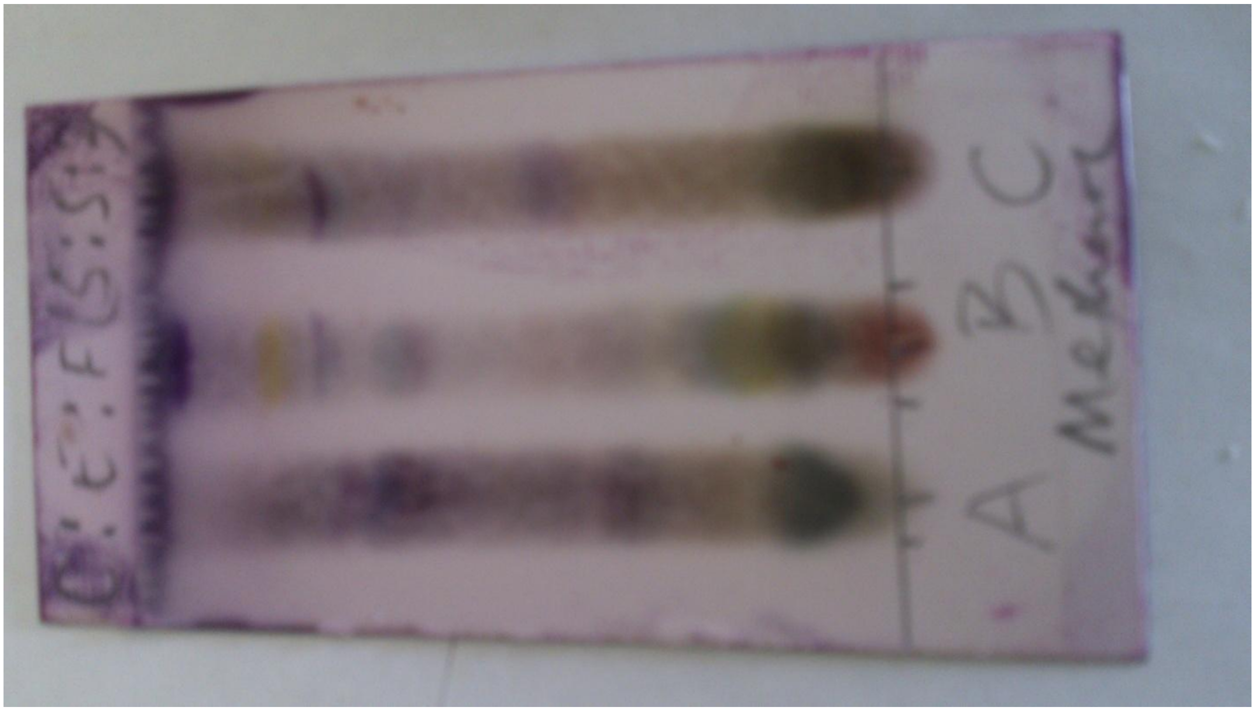
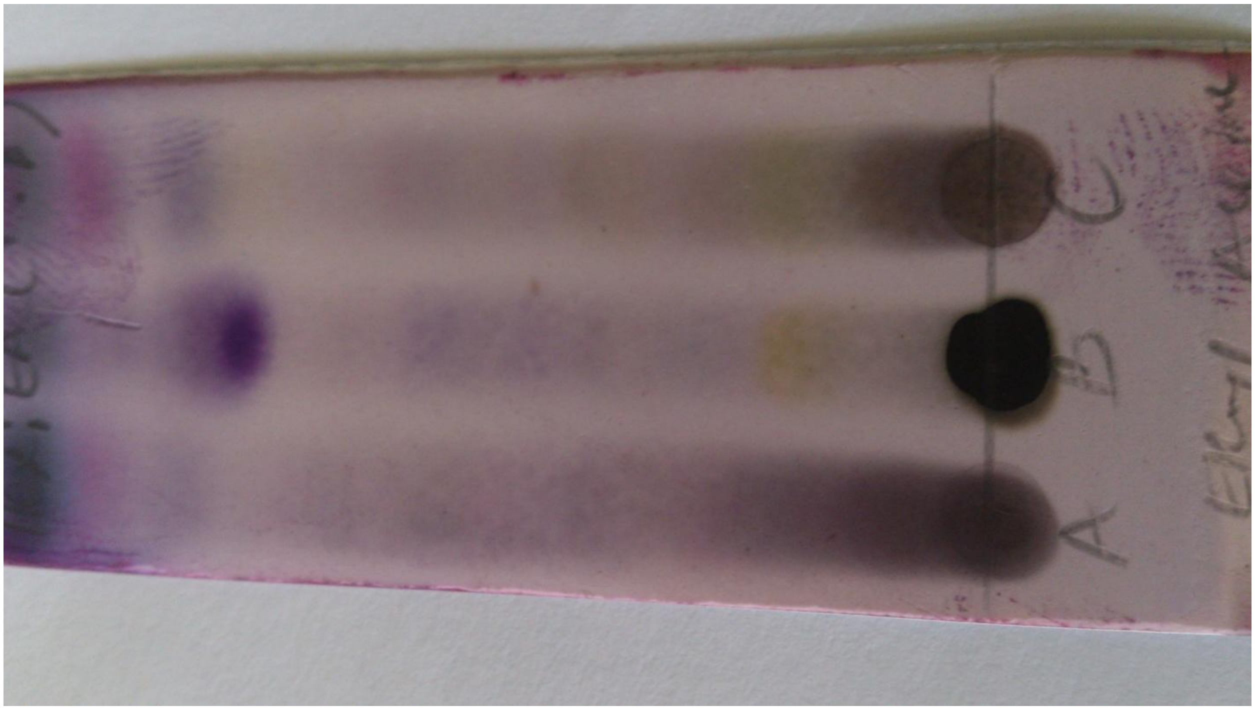


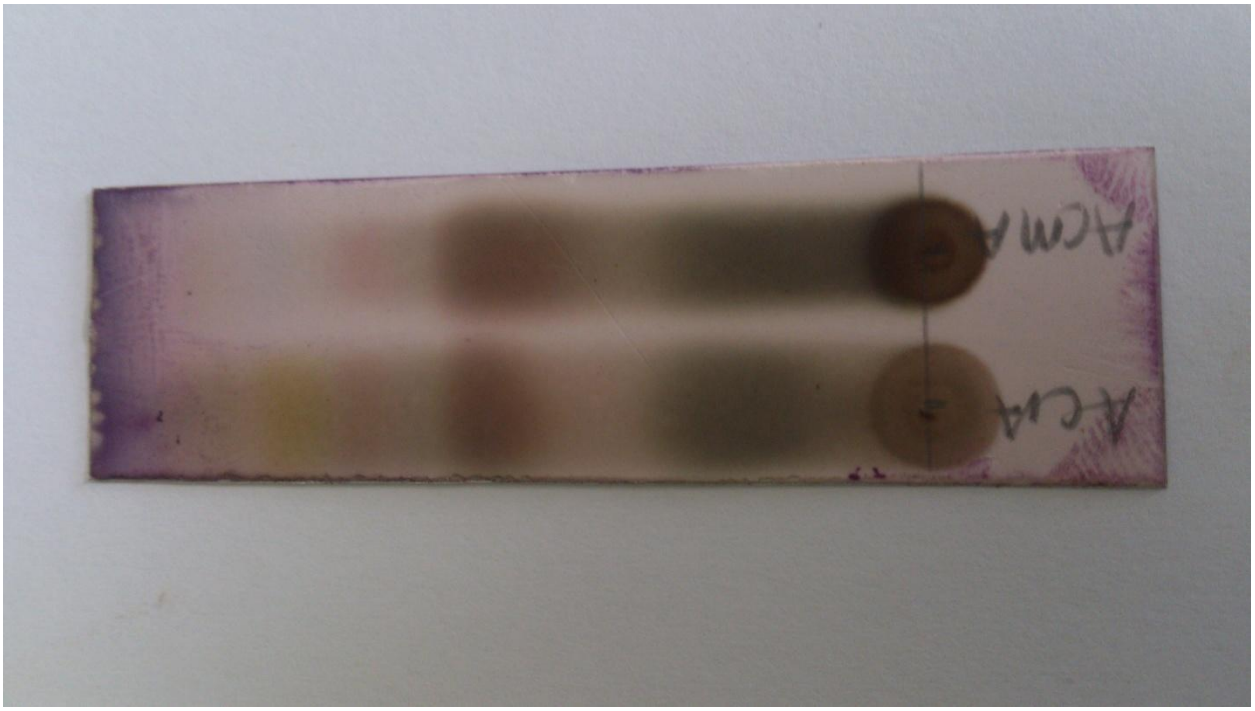
APPENDIX (Xa) : SAMPLES OF TLC PLATES



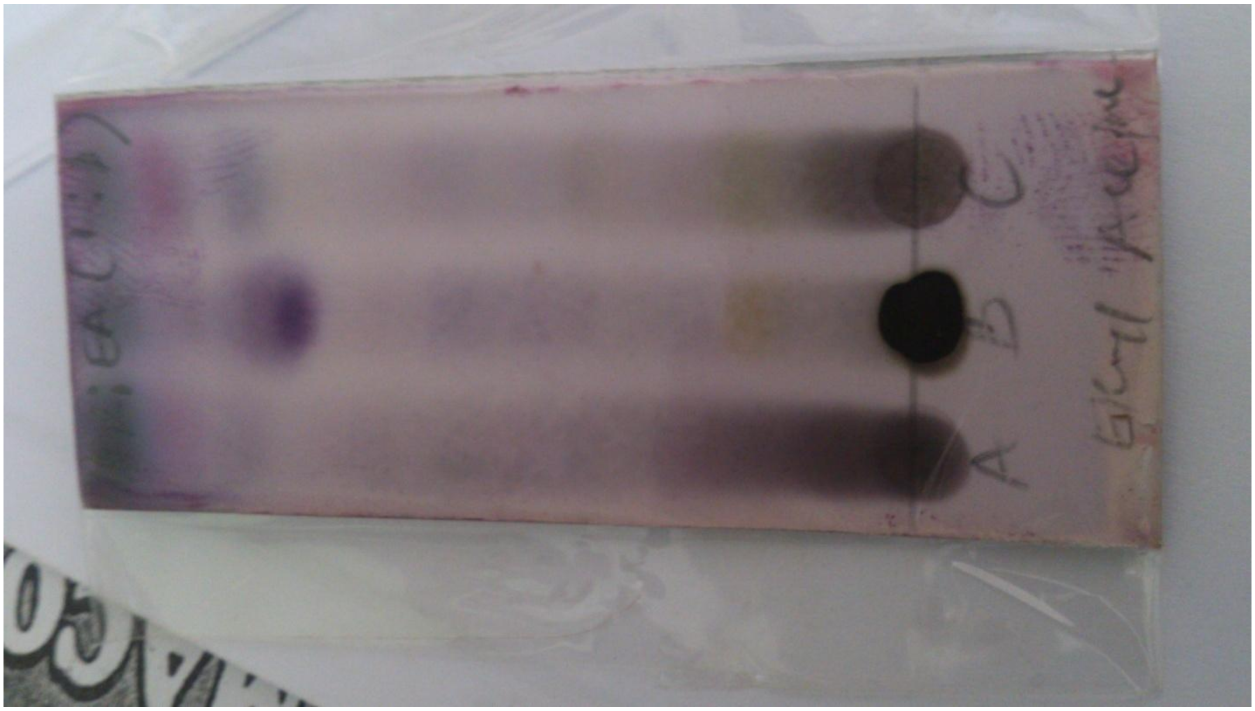
APPENDIX (Xb): SAMPLES OF TLC PLATES



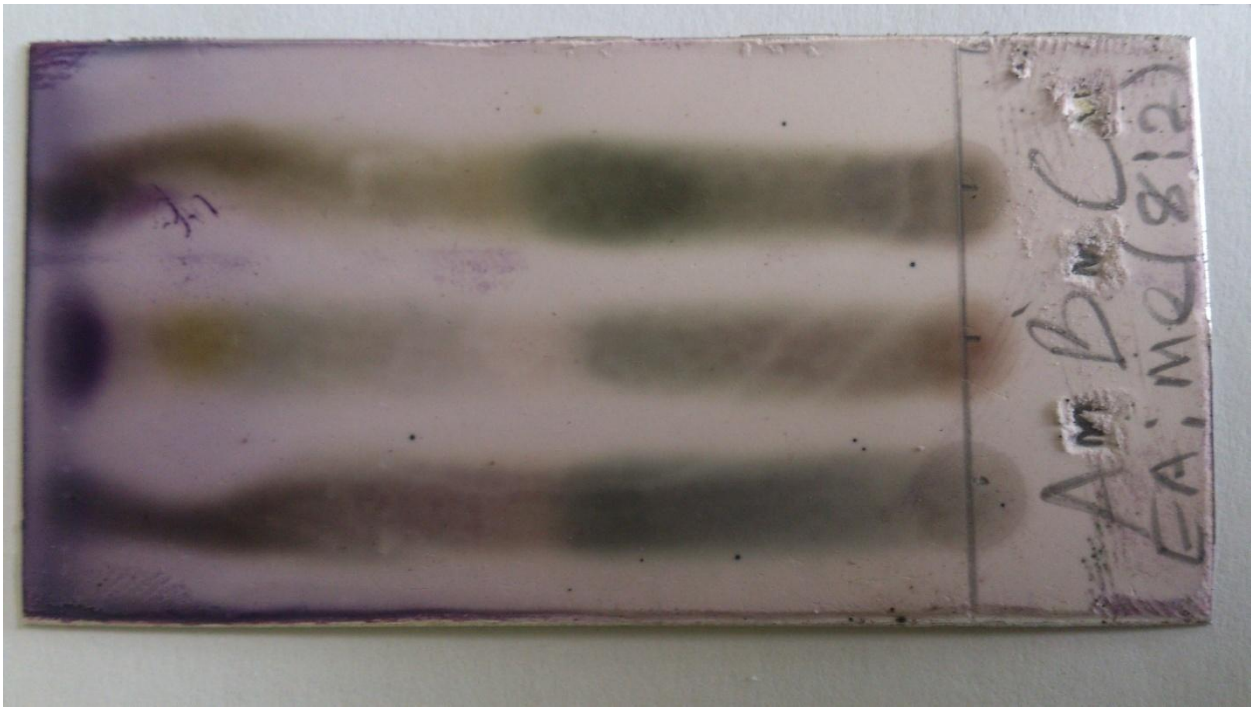
APPENDIX (Xc): SAMPLES OF TLC PLATES



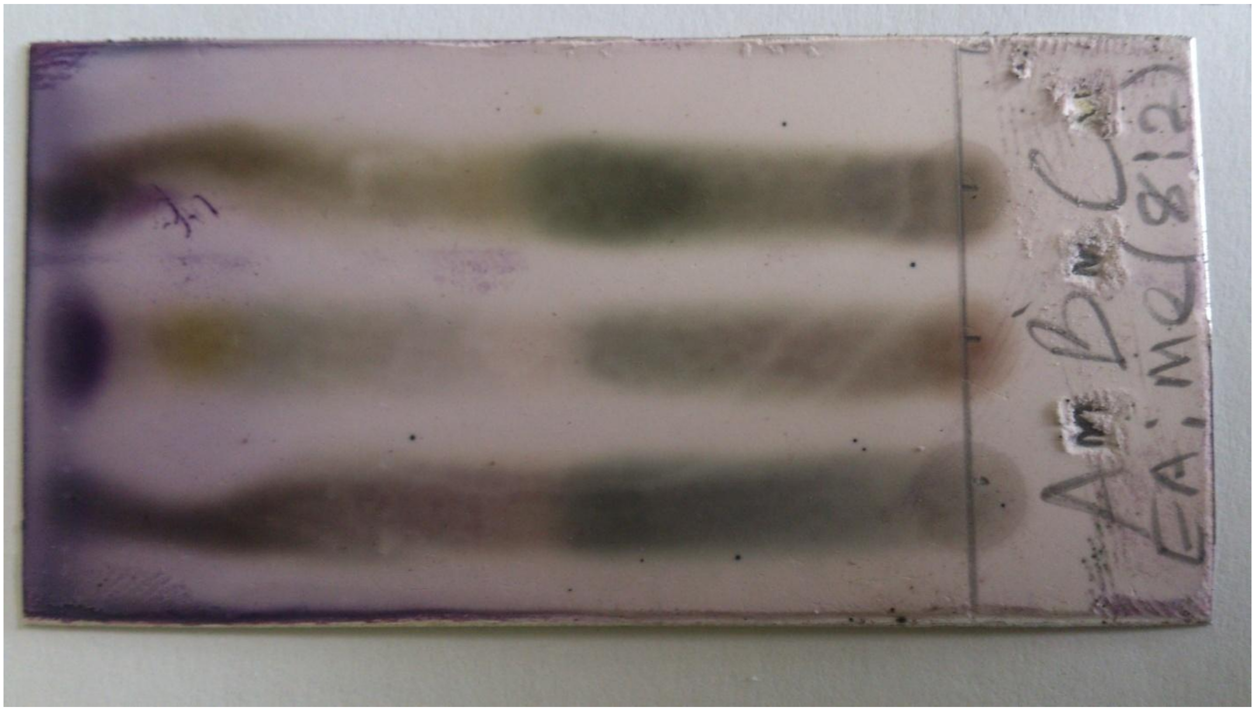
APPENDIX(Xd): SAMPLES OF TLC PLATES



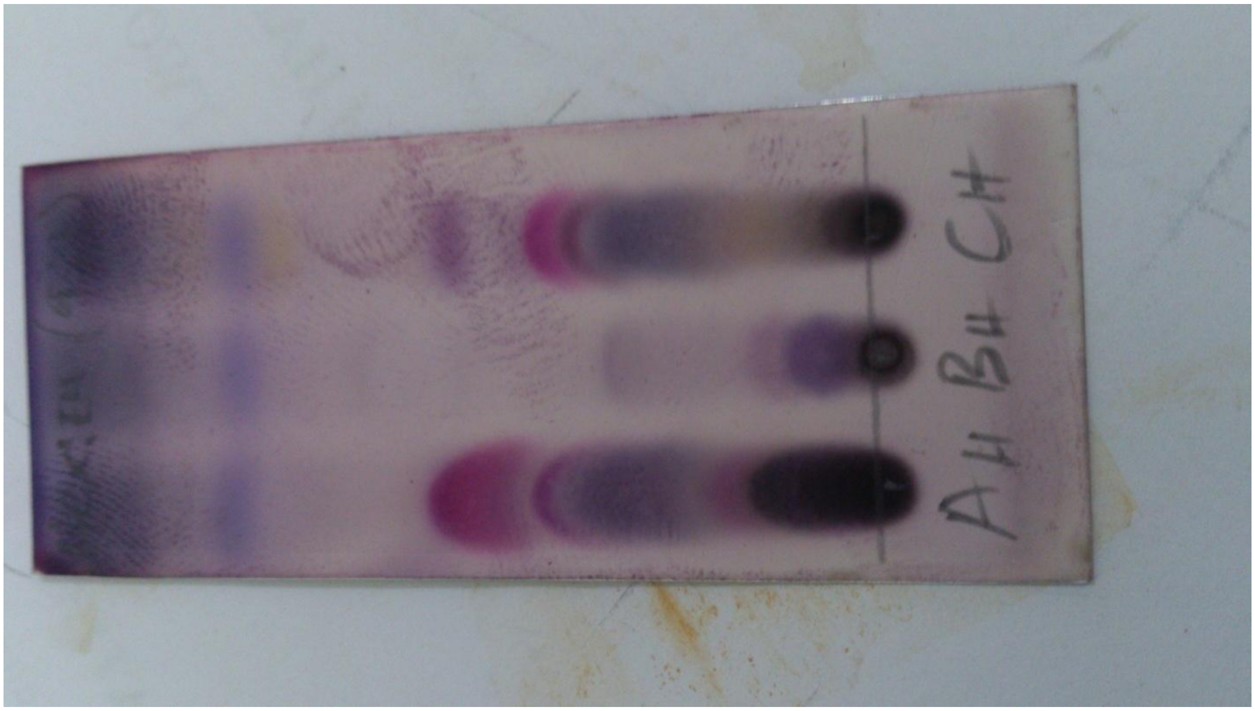
APPENDIX (Xe): SAMPLES OF TLC PLATES



APPENDIX (Xf): SAMPLES OF TLC PLATES



APPENDIX (Xg): SAMPLES OF TLC PLATES



APPENDIX (Xi): MPAES ANALYSIS RESULT

MULTI - USER SCIENCE LABORATORY AHMADU BELLO UNIVERSITY ZARIA

MP -AES AGILENT 4200 ANALYSIS RESULT

Instrument used: MY15150003

Date Time	Label	Element Label (nm)	Conc	%RSD	Unadjusted Conc	Intensity	%RSD
11/1/2016 13:43:16	Standard 1	Ag (328.068 nm)	0.250 (ppm)	N/A	0.250 (ppm)	33295.057	0.37
11/1/2016 13:43:16	Standard 1	Fe (371.993 nm)	0.250 (ppm)	N/A	0.250 (ppm)	5467.901	0.62
11/1/2016 13:43:16	Standard 1	Cu (324.754 nm)	0.250 (ppm)	N/A	0.250 (ppm)	65815.313	0.27
11/1/2016 13:43:16	Standard 1	Ni (352.454 nm)	0.250 (ppm)	N/A	0.250 (ppm)	10258.172	0.19
11/1/2016 13:43:16	Standard 1	As (193.695 nm)	0.250 (ppm)	N/A	0.250 (ppm)	2356.345	2.52
11/1/2016 13:43:16	Standard 1	Co (340.512 nm)	0.250 (ppm)	N/A	0.250 (ppm)	5373.421	0.13
11/1/2016 13:43:16	Standard 1	K (766.491 nm)	0.250 (ppm)	N/A	0.250 (ppm)	133850.827	0.48
11/1/2016 13:43:16	Standard 1	Mn (403.076 nm)	0.250 (ppm)	N/A	0.250 (ppm)	25606.278	0.65
11/1/2016 13:43:16	Standard 1	Al (396.152 nm)	0.250 (ppm)	N/A	0.250 (ppm)	21473.296	0.64
11/1/2016 13:43:16	Standard 1	Na (588.995 nm)	0.250 (ppm)	N/A	0.250 (ppm)	576099.220	0.47
11/1/2016 13:48:14	Standard 2	Se (196.026 nm)	0.500 (ppm)	N/A	0.500 (ppm)	1234.199	2.72
11/1/2016 13:48:14	Standard 2	Ag (328.068 nm)	0.500 (ppm)	N/A	0.500 (ppm)	69304.514	0.29
11/1/2016 13:48:14	Standard 2	Fe (371.993 nm)	0.500 (ppm)	N/A	0.500 (ppm)	10620.013	0.86
11/1/2016 13:48:14	Standard 2	Cu (324.754 nm)	0.500 (ppm)	N/A	0.500 (ppm)	125801.790	0.84
11/1/2016 13:48:14	Standard 2	Ni (352.454 nm)	0.500 (ppm)	N/A	0.500 (ppm)	19897.095	0.38
11/1/2016 13:48:14	Standard 2	As (193.695 nm)	0.500 (ppm)	N/A	0.500 (ppm)	2555.373	0.55
11/1/2016 13:48:14	Standard 2	Co (340.512 nm)	0.500 (ppm)	N/A	0.500 (ppm)	10399.349	0.54
11/1/2016 13:48:14	Standard 2	K (766.491 nm)	0.500 (ppm)	N/A	0.500 (ppm)	95652.289	0.13
11/1/2016 13:48:14	Standard 2	Mn (403.076 nm)	0.500 (ppm)	N/A	0.500 (ppm)	51395.161	0.31
11/1/2016 13:48:14	Standard 2	Al (396.152 nm)	0.500 (ppm)	N/A	0.500 (ppm)	41882.324	0.94
11/1/2016 13:48:14	Standard 2	Na (588.995 nm)	0.500 (ppm)	N/A	0.500 (ppm)	491679.937	0.32
11/1/2016 13:51:18	Sample A1	Se (196.026 nm)	0.394 (ppm)	2.10	0.394 (ppm)	1073.360	1.29
11/1/2016 13:51:18	Sample A1	Ag (328.068 nm)	0.007 (ppm)	5.52	0.007 (ppm)	947.209	5.52
11/1/2016 13:51:18	Sample A1	Fe (371.993 nm)	2.093 o (ppm)	0.81	2.093 (ppm)	42497.073	0.79
11/1/2016 13:51:18	Sample A1	Cu (324.754 nm)	0.129 (ppm)	0.40	0.129 (ppm)	34685.063	0.39
11/1/2016 13:51:18	Sample A1	Ni (352.454 nm)	0.028 (ppm)	1.48	0.028 (ppm)	1169.447	1.47
11/1/2016 13:51:18	Sample A1	As (193.695 nm)	0.506 o (ppm)	20.29	0.506 (ppm)	2555.529	1.87
11/1/2016 13:51:18	Sample A1	Co (340.512 nm)	-0.036 u (ppm)	1.73	-0.036 (ppm)	-806.745	1.73
11/1/2016 13:51:18	Sample A1	K (766.491 nm)	#### (ppm)	N/A	#### (ppm)	####	N/A

11/1/2016 13:51:18	Sample A1	Al (396.152 nm)	0.814 o (ppm)	0.08	0.814 (ppm)	66860.650	0.08
11/1/2016 13:51:18	Sample A1	Na (588.995 nm)	Uncal (ppm)	N/A	Uncal (ppm)	4405682.692	1.30
11/1/2016 13:54:12	Sample B1	Se (196.026 nm)	0.408 (ppm)	10.12	0.408 (ppm)	1094.449	6.16
11/1/2016 13:54:12	Sample B1	Ag (328.068 nm)	0.008 (ppm)	1.11	0.008 (ppm)	980.223	1.12
11/1/2016 13:54:12	Sample B1	Fe (371.993 nm)	3.674 o (ppm)	0.21	3.674 (ppm)	74145.428	0.21
11/1/2016 13:54:12	Sample B1	Cu (324.754 nm)	0.036 (ppm)	0.90	0.036 (ppm)	9842.701	0.89
11/1/2016 13:54:12	Sample B1	Ni (352.454 nm)	0.017 (ppm)	0.77	0.017 (ppm)	703.300	0.76
11/1/2016 13:54:12	Sample B1	As (193.695 nm)	0.789 o (ppm)	9.66	0.789 (ppm)	2679.950	1.23
11/1/2016 13:54:12	Sample B1	Co (340.512 nm)	-0.018 u (ppm)	1.19	-0.018 (ppm)	-394.435	1.19
11/1/2016 13:54:12	Sample B1	K (766.491 nm)	Uncal (ppm)	N/A	Uncal (ppm)	1749497.104	0.36
11/1/2016 13:54:12	Sample B1	Mn (403.076 nm)	0.109 (ppm)	0.92	0.109 (ppm)	11163.445	0.92
11/1/2016 13:54:12	Sample B1	Al (396.152 nm)	2.992 o (ppm)	0.51	2.992 (ppm)	240258.493	0.50
11/1/2016 13:54:12	Sample B1	Na (588.995 nm)	#### (ppm)	N/A	#### (ppm)	####	N/A
11/1/2016 13:57:42	Sample C1	Se (196.026 nm)	0.436 (ppm)	5.70	0.436 (ppm)	1140.511	3.33
11/1/2016 13:57:42	Sample C1	Ag (328.068 nm)	0.001 (ppm)	9.41	0.001 (ppm)	132.947	9.41