# EVALUATION OF ANTIFUNGAL ACTIVITIES OF SOME MEDICINAL PLANT EXTRACTS AND FRACTIONS AGAINST CLINICAL ISOLATES OF *CANDIDA ALBICANS*

**BY**


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## ABSTRACT

The development of drug resistance by pathogenic yeast especially *Candida albicans,* has resulted in waste of financial resources globally. This study was aimed at evaluating the antifungal activities of some medicinal plant extracts and fractions against clinical isolates of *Candida albicans.* Four medicinal plants namely: *Vernonia amygdalina, Azardirachta indica*, *Moringa oleifera,* and *Vitellaria paradoxa* were used in this study. The leaves, stembark and root of each medicinal plants were successively extracted using reflux method to obtain crude extracts, the fractions were obtained by column and thin layer chromatography. Clinical samples used were obtained from high vaginal swab (25) and urine(5). All samples were cultured in Sabouraud Dextrose broth (SDB) and then on Sabouraud Dextrose Agar (SDA). Isolates were identified using standard microbiological and molecular techniques. Antifungal activities of the crude extracts and fractions were done by Agar Well Diffusion Method. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the extracts and fractions were also determined. Gas chromatography and mass spectrophotometer analysis (GCMS) were used to determine bioactive compounds in fractions. Acute and sub-acute toxicity studies were done using the method of Lorkes and Organization for Economic Corporation and Development (OECD) respectively. Antifungal activities of the crude extracts were assayed at varying concentrations of 40, 60, 80 and 100 mg/mL against eight strains of *Candida albicans*. The highest mean zone of inhibition (MZI) 21.00± 0.30 mm was observed for methanol leaf extract of *Vernonia amygdalina* against strain SC5314 (isolate B7). The lowest results for the MIC and MFC of the crude extracts were 6.25 and 25.0 mg/mL, respectively. The n-hexane, ethyl acetate and methanol crude extract of *Vernonia amygdalina* yielded 4, 6 and 6 fractions respectively while the n-hexane, ethylacetate and methanol crude extracts of *Azardirachta indica* gave 7 fractions each. Antifungal activity of the fractions against the strains were determined at a concentration of 5 and 10 mg/mL. The highest mean zone of inhibition (MZI) for the fractions was 16.00±0.80 mm which was recorded for fraction (F4) of *Vernonia amygdalina* leaf of n-hexane extract against strain P37005. The lowest value for MIC and MFC results for the active fractions were 1.25 and 3.125 mg/mL, respectively. The standard drug (fluconazole 1mg/mL) used had a higher inhibitory activity that ranged from 33.66±1.00 - 42.00±0.49 mm compared to the activity of the crude extracts and the fractions. Results of the GCMS revealed probable compounds that may have enhanced the activity of the extracts and fractions. Acute toxicity profile of the crude extracts of *Vernonia amygdalina* and *Azardirachta indica* on experimental rats showed no mortality for all groups exposed to the ,extract and as well no group had signs of toxicity. The sub-acute toxicity results on haematological and kidney parameters showed for both extracts were within the control value. Although, liver parameters and lipid profile showed significant increase ($P<0.05$) especially at doses of 600 mg/kgbw. Haematological and biochemical parameters for the active fractions were all within the control. The crude extracts of *Vernonia amygdalina* and *Azardirachta indica* leaf were the two active plants parts that showed significant activity.

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# CHAPTER ONE

# 1.0 INTRODUCTION

## 1.1 Background to the Study

Plants are used as valuable sources of food and medicine for the prevention of illness and maintenance of human health. In Nigeria, many indigenous plants are widely consumed as food or home remedies especially in the treatment or management of common diseases. The importance of plants in medicine remains even of greater relevance with the current global shift to obtain drugs from plant sources, as a result of which attention has been given to the medicinal value of herbal remedies for safety, efficacy and economy (Glombitza *et al*., 1993). Medicinal plants contains bioactive constituents with high therapeutic potentials; which also enable them to function as antioxidant, anti-inflammatory, antimicrobial and antipyretic agent . They have been used as a source for centuries as an alternative remedy for treating human diseases (Abayomi *et al*., 2013). Medicinal plants such as *Anchomanes difformis, Anisopus mannii Vernonia blumeoides and Pavetta crassipes* etc are continually being utilized as therapeutic agents in formulations for treating diseases in the traditional ethnomedicinal system (Abayomi *et al*., 2013).

Medicinal plants are a source of great economic value in Africa continent and the world (Iwu, 1993; Sofowora, 2013). The ethnomedicinal resources of Africa remain largely unexploited. Medicinal plants have been used as natural chemotherapeutic agents that can be described as nature pharmacy for nearly 80% of people living in Africa (Tan *et al*., 2010). Nature has bestowed on us a very rich botanical wealth and a large number of diverse plants growing in different parts of the continent. In Nigeria, thousands of species are known to have medicinal value (Sofowora, 2013) and the use of different parts to cure specific ailments has been in vogue since ancient times (Sofowora, 2013). Herbal

medicine still remain the mainstay of 75-80 % of the whole population in the developing countries. Plant based medicines have been used for decades especially in the rural areas to prevent or even eliminate diseases worldwide and have proven to be promising in their actions (Robinson, 2006). Herbal medicines otherwise called herbal drugs are generally of natural plant parts such as the leaves, stem, flowers, stem bark, seeds and bulb (Robinson, 2006).

*Candida albicans* is a dimorphic fungus that grows both as yeast and filamentous cells and one of the few species of *Candida* that cause the infection candidiasis in humans (Zadik *et al*., 2010). *Candida albicans* have emerged as important cause of morbidity and mortality in immunocompomised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation). In addition, hospital-acquired infections by *Candida albicans* have become a major cause of public health concern (Tartorano and Gerald, 2010). *Candida albicans* is a common member of the human gut flora and is detectable in the gastrointestinal tract in 40% of healthy adults (Martins *et al*., 2014).

It is usually a commensal organism, but can become pathogenic in immunocompromise individuals under a variety of conditions. (Martins *et al*., 2014). Candidiasis is often observed in immunocompromised individuals, including HIV-infected patients. It commonly occurs on mucous membranes in the mouth or vagina but may affect a number of other regions of the body (Zadik *et al*., 2010).

In immunocompromised individuals, *Candida* infections in the esophagus occur more frequently than in healthy individuals and have a higher potential of becoming systemic, causing a much more serious condition, a fungemia called candidemia (Fidel, 2006; Pappas, 2006). Symptoms of esophageal candidiasis include difficulty swallowing, painful swallowing, abdominal pain, nausea, and vomiting (Yamada and Alpers, 2009).

## 1.2 Statement of the Research Problem

The development of drug resistance by pathogenic yeast especially *Candida albicans* has resulted in wastage of financial resources globally (Moen *et al*., 2009). *Candida albicans* represent the most common fungal pathogens that affect humans and produce a wide spectrum of disease ranging from superficial mucocutaneous to invasive illnesses such as hepatosplenic candidiasis. More so, it is a serious public health challenge with increasing medical and economic importance due to the high mortality rates and increased cost of care (Lim *et al*., 2012). Several drugs commonly used for the treatment of these infections tend to have toxic effect on the body (Pfaller *et al* ., 2012). The abuse of these antimycotic drugs that include: Nystatin, Itraconazole and Amphotericin B during treatment (Pfaller *et al*., 2012) has resulted to the development of drug resistance hence a significant drop in their efficacies.

## 1.3 Justification for the Study

Medicinal plant have played a major role in the production of biological compounds some of which can be used in drug development (Martins *et al*., 2014). Inspite of the influence of modern technology and orthodox medicine, plant-based medicine have been used for decades especially in rural areas to prevent or even eliminates disease worldwide and have prove to be promising in their actions (Bonjor and Farrokhi, 2004). Efforts in this regard have focused on plant because of their history and the fact that they are cheap and a good portion of the world population particularly in developing countries rely on plants for the treatment of infectious and non-infectious disease (Martins *et al*., 2014). Some of this medicinal plant include *Vernonia aqmygdalina* , *Azadirachta indica, Moringa oleifera* and *Vitellaria paradoxa.* They have also being reported to possess antimicrobial properties which have aid their uses in the treatment of some ailment such as stomach

disorder, fever symptoms, skin infection , cough and other respiratory disease (Martins *et al*., 2014).

The impact of these medicinal plants based and their relevance in treating disease could serve as a good source of antimycotic agent. The economic predicament of most developing countries like Nigeria necessitate the search for new herbal drugs that are safe, less toxic and can serve as an alternative source of medicine. Therefore the above reasonings are partly what prompted this investigation.

### 1.4 Aim and Objectives of the Study

The aim of this study was to evaluate antifungal activities of *Vernonia amygdalina*, *Azadirachta indica*, *Moringa oleifera*, and *Vitellaria paradoxa* extracts and fractions against clinical isolates of *Candida albicans*. This was with a view to elucidating some of their pharmacologic roles as medicinal plants.

The objectives of the study were to:

i. isolate and identify *Candida albicans* from clinical samples

ii. screen quantitatively for phytochemical components of the leaf, stembark and roots of the selected medicinal plants

iii. determine antifungal activities of the crude extracts and fractions of the plants

iv. determine the minimum inhibitory/minimum fungicidal concentrations of the active crude extracts and fractions of the above mentioned plant components.

v. identify and elucidate the most active fractions using GCMS.

vi. evaluate the toxicological characteristics of the active plant extracts and fractions of the plants

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

Fungi are widespread in the environment, some are associated with animals and humans as commensals, but they may become pathogenic after alteration of host immune system (Koehler *et al*., 1999). Therapeutic applications of immunosuppressive drugs, the use of broad spectrum antibiotics in various clinical conditions and other predisposing factors are responsible for an increasing number of immune compromised patients and consequent opportunistic infections globally. A weakened or impaired immune system provides favorable conditions for pathogenic and non-pathogenic microorganisms to thrive. Acquired Immunodeficiency Syndrome due to human immunodeficiency virus (HIV-I and HIV-II) is one of the major contributing factors for the increasing number of patients with fungal infections (Nyirjesy and Sobel, 2013).

Candida species has a wide distribution and ranges from pure saprobes to being pathogens in many humans (Okungbowa *et al*., 2000). *Candida* species causes a wide variety of disorders that include thrush, vulvo vaginitis, urinary tract candidiasis, mucocutaneous candidiasis and invasive candidiasis (Okungbowa *et al*., 2000; Isibor *et al*., 2005). The genus *Candida* contains more than 150 species but only a few are regarded as being medically important to humans (Isibor *et al*., 2005). However, more than 90 % of invasive infections are caused by *Candida albicans, Candida glabrata, Candida parapsilosis*, *Candida tropicalis* and *Candida krusei* (Pfaller *et al*., 1996). The most pathogenic of these species is *Candida albicans* (Isibor *et al*., 2005). *Candida albicans* is responsible for 50–90% of all cases of candidiasis in humans (Martins *et al*., 2014).

*Candida albicans* is a dimorphic fungus that grows both as yeast and filamentous cells and one of the few species of *Candida* that cause the infection candidiasis in humans (Martins *et al*., 2014). *Candida albicans* have emerged as important causes of morbidity

and mortality in immunocompromised patients (e.g., AIDS, cancer chemotherapy, organ or bone marrow transplantation (Tartorano and Gerald, 2010).

*Candida albicans* is a common member of the human gut flora and is detectable in the gastrointestinal tract in 40% of healthy adults (Martins *et al*., 2014). It is usually a commensal organism, but can become pathogenic in immunocompromise individuals under a variety of conditions. Overgrowth of the fungus results in candidiasis (Martins *et al*., 2014). Candidiasis is often observed in immunocompromised individuals, including HIV-infected patients. It commonly occurs on mucous membranes in the mouth or vagina but may affect a number of other regions of the body (Zadik *et al*., 2010). In immunocompromised individuals, *Candida* infections in the esophagus occur more frequently than in healthy individuals and have a higher potential of becoming systemic, causing a much more serious condition, a fungemia called candidemia (Fidel, 2006; Pappas, 2006). Symptoms of esophageal candidiasis include difficulty swallowing, painful swallowing, abdominal pain, nausea, and vomiting (Yamada and Alpers, 2009).

## 2.1 An Overview of Candidiasis

Candidiasis is a fungal infection that is caused by a yeast( a type of fungus) called Candida

(Goncalves *et al*., 2016). Candida normally lives inside the body, in places such as the mouth, throat, gut and Vagina and on skin. When it affects the mouth, it is commonly called thrush (Pappas *et al*., 2016). Signs and symptoms include white patches on the tongue or other areas of the mouth and throat. Other symptoms may include soreness and problems swallowing. When it affects the vagina, it is commonly called a yeast infection. Signs and symptoms include genital itching, burning, and sometimes a white "cottage cheese-like" discharge from the vagina. Less commonly the penis may be affected, resulting in itchiness. Although very rarely, the infection may become invasive spreading

throughout the body, resulting in fevers along with other symptoms depending on the parts of the body affected (Pappas *et al*., 2016).

Candida is a genus of yeast and is the most common cause of fungal infections worldwide (Manolakaki *et al*., 2010). Many species are harmless commensals or endosymbionts of host including humans; however, when mucosal barriers are disrupted or the immune system is compromised they invade and cause disease (Kourkoumpetis and Thermistoklis, 2011). They are said to be an opportunistic pathogenic yeast that is a common member of the human gut flora. It can also survive outside the human body. It is detected in the gastrointestinal tract and mouth in 40–60 % of healthy adults. It is usually a commensal organism, but it can become pathogenic in immunocompromised individuals under a variety of conditions. *Candida albicans* is one of the few species of the genus Candida that causes the human infection candidiasis, which results from an overgrowth of the fungus. Candidiasis is for example often observed in HIV-infected patients. *Candida albicans* is the most common fungal species isolated from biofilms either formed on (permanent) implanted medical devices or on human tissue. *Candida albicans*, *Candida tropicalis, Candida parapsilosis*, *and Candida glabrata* are together responsible for 50–90 % of all cases of candidiasis in humans. A mortality rate of 40 % has been reported for patients with systemic candidiasis due to *Candida albicans* (Martins *et al*., 2014).

### 2.1.1 Signs and symptoms /clinical presentation of candidiasis

Signs and symptoms of candidiasis vary depending on the areas affected (Dolin *et al*., 2010). Most candidal infections result in minimal complications such as redness, itching, and discomfort, though complications may be severe or even fatal if left untreated in certain populations. In healthy (immunocompetent) persons, candidiasis is usually a localized infection of the skin, fingernails or toenails (onychomycosis), or mucosal membranes, including the oral cavity and pharynx (thrush), esophagus, and the genitalia (vagina, penis, etc.) (Martins *et al*., 2014). Less commonly in healthy individuals, the

gastrointestinal tract, urinary tract and respiratory tract are sites of candida infection (Pappas *et al*., 2016).

Infection of the vagina or vulva may cause severe itching, burning, soreness, irritation, and a whitish or whitish-greay cottage cheese-like discharge. Symptoms of infection of the male genitalia (balanitis thrush) include red skin around the head of the penis, swelling, irritation, itchiness and soreness of the head of the penis, thick, lumpy discharge under the foreskin, unpleasant odour, difficulty retracting the foreskin (phimosis), and pain when passing urine or during sex (Pappas, 2006).

Common symptoms of gastrointestinal candidiasis in healthy individuals are anal itching, belching, bloating, indigestion, nausea, diarrhea, gas, intestinal cramps, vomiting, and gastric ulcers (Martins *et al*., 2014).

### 2.1.2 Pathogenicity of *Candida albicans*

An infection caused by Candida is termed candidiasis or candidosis. Mycoses caused by these fungi show a wide spectrum of clinical presentations and can be classified as superficial, as with cutaneous and mucosal infections, to deep, widespread and of high severity, as is the case with invasive candidiasis. According to Colombo & Guimara˜es (2003), the main transmission mechanism is through endogenous candidaemia, in which Candida species that constitute the microbiota of various anatomical sites under conditions of host weakness behave as opportunistic pathogens.

Another mechanism for transmission is exogenous, and this occurs mainly through the hands of health professionals who care for patients. Also indicated in the spread of infection are health-care materials, such as contaminated catheters and intravenous solutions (Ingham *et al*., 2012). Candida species are considered important pathogens due to their versatility and ability to survive in various anatomical sites (Calderone and Fonzi, 2001). It was believed decades ago that yeasts passively participated in the process of

pathogenesis in the establishment of fungal infection. Thus, weakness or an immunocompromised host was considered the only mechanism responsible for the establishment of opportunistic infection (Tamura *et al*., 2007). Candida species are eukaryotic opportunistic pathogens that reside on the mucosa of the gastrointestinal tract as well as the mouth, oesophagus and vagina (Kim and Sudbery, 2011; Lim *et al*., 2012).

Although this commensal organism normally colonizes mucosal surfaces in an asymptomatic manner, it can become one of the most significant causes of a disabling and lethal infection (Wisplinghoff *et al*., 2006; Vincent *et al*., 2009). In the early 1980s, fungi emerged as major causes of nosocomial infections, mainly affecting immunocompromised patients or those who were hospitalized for long periods due to serious underlying diseases (Klotz *et al*., 2007). Candida pathogenicity is facilitated by a number of virulence factors, the most important of which are those for adherence to host tissues, biofilm formation and secretion of hydrolytic enzymes (e.g. proteases, phospholipases and haemolysins).

Furthermore, although there has been extensive research to identify pathogenic factors in fungi, particularly in *Candida albicans* (Silva *et al*., 2007). Virulence in *Candida albicans* and other pathogens includes host recognition, which enables the pathogen to bind to host cells and proteins. Additionally, degradative enzymes play a special role in virulence. Fungal invasion is facilitated more by the transition between yeast cells and filamentous growth than by yeast growth (Cullen and Sprague, 2012). The primary factor in the fungal colonization of human tissues is adherence to host surfaces; this process is controlled and induced by several cellsignalling cascades in both the fungus and the environment. In addition, Candida species can adhere to the surfaces of medical devices and form biofilms. The initial attachment of Candida cells is mediated by non-specific factors (hydrophobicity and electrostatic forces) and promoted by specific adhesins present on the

surface of fungal cells that recognize ligand such as proteins, fibrinogen and fibronectin (Li *et al*., 2003).

The phenomenon of adhesion is exhibited by specialized surface proteins, called adhesins, that specifically bind to amino acids and sugars on the surface of other cells or promote adherence to abiotic surfaces (Kojic and Darouiche, 2004).

### 2.1.3 Causes of candidiasis

Candida requires moisture for growth, notably on the skin (Bassetti *et al*., 2010). For example, wearing wet swimwear for long periods of time is believed to be a risk factor (CDC, 2014). In extreme cases, superficial infections of the skin or mucous membranes may enter into the bloodstream and cause systemic Candida infections. Factors that increase the risk of candidiasis include HIV/AIDS, mononucleosis, cancer treatments, steroids, stress, antibiotic usage, diabetes, and nutrient deficiency. Hormone replacement therapy and infertility treatments may also be predisposing factors. Treatment with antibiotics can lead to eliminating the yeast's natural competitors for resources in the oral and intestinal flora; thereby increasing the severity of the condition (Bassetti *et al*., 2010). A weakened or undeveloped immune system or metabolic illnesses are significant predisposing factors of candidiasis. Almost 15 % of people with weakened immune systems develop a systemic illness caused by *Candida* species (Choo *et al*., 2010).

### 2.1.4 Diagnosis of candidiasis

Diagnosis of a yeast infection is done either via microscopic examination or culturing. For identification by light microscopy, a scraping or swab of the affected area is placed on a microscope slide. A single drop of 10 % (KOH) solution is then added to the specimen. The KOH dissolves the skin cells, but leaves the *Candida* cells intact, permitting visualization of pseudohyphae and budding yeast cells typical of many *Candida* species. Symptoms of vaginal candidiasis are also present in the more common bacterial vaginosis

(Lim *et al*., 2012). Aerobic vaginitis is distinct and should be excluded in the differential diagnosis (Donders *et al*., 2002). In a 2002 study, only 33 % of women who were self-treating for a yeast infection actually had such an infection, while most had either bacterial vaginosis or a mixed-type infection (Ferris *et al*., 2002**).**

For the culturing method, a sterile swab is rubbed on the infected surface. The swab is then streaked on a culture medium. The culture is incubated at room temperature for 72hours for several days, to allow development of yeast. The characteristics (such as morphology and colour) of the colonies may allow initial diagnosis of the organism causing disease symptoms (Sri Kumar and Nagaraja, 2010).

Candida species can also be identified by molecular techniques (Lim *et al*., 2012). These techniques include polymerase chain reaction a process that amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. It is an easy and cheap tool to amplify a focused segment of DNA, useful for such purposes as the diagnosis and monitoring of genetic diseases, identification of criminals (in the field of forensics), and studying the function of a targeted segment of DNA (Moustafa *et al*., 2010). PCR is a useful tool for studying etiologic agents of infectious diseases and can be regarded as a method of choice due to its rapidity, sensitivity and reproducibility.

### 2.1.5 Prevention of candidiasis

A diet that supports the immune system and is not high in simple carbohydrates contributes to a healthy balance of the oral and intestinal flora (Mulley and Goroll, 2006). While yeast infections are associated with diabetes, the level of blood sugar control may not affect the risk (Mobley *et al*., 2011). Wearing cotton underwear may help to reduce the risk of developing skin and vaginal yeast infections, along with not wearing wet clothes for long periods of time (CDC, 2014).

Oral hygiene can help prevent oral candidiasis when people have a weakened immune system (Jurden *et al*., 2012). For people undergoing cancer treatment, chlorhexidine mouthwash can prevent or reduce thrush (Jurden *et al*., 2012). People who use inhaled corticosteroids can reduce the risk of developing oral candidiasis by rinsing the mouth with water or mouthwash after using the inhaler (Jurden *et al*., 2012). For women who experience recurrent yeast infections, there is limited evidence that oral or intravaginal probiotics help to prevent future infections (Jurden *et al*., 2012).

### 2.1.6 Treatment of candidiasis

Candidiasis is treated with antifungal medications; these include Sertaconazole, Tioconazole, Clotrimazole, Nystatin, Fluconazole, Voriconazole, Amphotericin B, and Echinocandins. Intravenous Fluconazole or an intravenous Echinocandin such as Caspofungin are commonly used to treat immunocompromised or critically ill individuals (Pappas *et al*., 2016).

The 2016 revision of the clinical practice guideline for the management of candidiasis lists a large number of specific treatment regimens for *Candida* infections that involve different *Candida* species, forms of antifungal drug resistance, immune status, and infection localization and severity (Pappas *et al*., 2016). Gastrointestinal candidiasis in immunocompromised individuals is treated with 100– 200 mg fluconazole per day for 2–3 weeks (Pappas *et al*., 2016). Mouth and throat candidiasis are treated with antifungal medication. Oral candidiasis usually responds to topical treatments; otherwise, systemic antifungal medication may be needed for oral infections. For infections of the mouth, treatment with topical clotrimazole or nystatin is usually effective. Oral or intravenous fluconazole, itraconazole, or amphotericin B can be used if these do not work (CDC, 2014).

A number of topical antifungal medications may be used for vaginal infections including clotrimazole (CDC, 2014). In those with widespread disease, an echinocandin such caspofungin or micafungin is used (Pappas *et al*., 2016). A number of weeks of intravenous amphotericin B may be used as an alternative. In certain groups at very high risk, antifungal medications may be used preventatively (Pappas *et al*., 2016).

### 2.1.7 Epidemiology of candidiasis

Several Candida species are commensal and colonize the skin and mucosal surfaces of humans. Critically ill or otherwise immunocompromised patients are more prone to develop both superficial and life-threatening *Candida* infections. Candida infections also constitute the most common fungal infections in AIDS patients (Fidel, 2006). These patients predominantly develop oropharyngeal candidiasis, which can lead to malnutrition and interfere with the absorption of medication. *Candida albicans* is the predominant cause of invasive fungal infections and represents a serious public health challenge with increasing medical and economic importance due to the high mortality rates and increased costs of care and duration of hospitalization (Lim *et al*., 2012). Although *Candida albicans* is the most prevalent species involved in invasive fungal infections, the incidence of infections due to non-albicans species is increasing. In a study with 2019 patients at major North American medical centres, a predominance of non-albicans species was observed; although *Candida albicans* was the most frequently isolated species, it was followed by *Candida glabrata* and other non *Candida albicans* species. This change in epidemiology could be associated with severe immunosuppression or illness, prematurity, exposure to broad-spectrum antibiotics and older patients. In European countries, an analysis showed that more than half of the cases of candidaemia were caused by *Candida albicans*, and the incidence rates for non-albicans candidaemia infections were 14 % each for *Candida glabrata* and *Candida parapsilosis,* 7 % for *Candida. tropicalis* and 2 % for *C. krusei*

(Tortorano and Gerald, 2006). Changes in the epidemiology have also been observed in Latin American countries. In Chile, the prevalence of *Candida. albicans* has changed, and a progressive increase of non-albicans infection has been observed; *Candida parapsilosis* was the most frequent species, followed by *Candida tropicalis* and *Candida glabrata*. All isolates were susceptible to amphotericin B; however, 50 % of the *Candida. glabrata* isolates were resistant to fluconazole (Lim *et al*., 2012).

According to the Brazilian Network Candidaemia Study, *Candida. albicans* accounted for 40.9 % of cases in Brazil, followed by *Candida tropicalis* (20.9 %), *Candida. parapsilosis* (20.5 %) and *Candida. glabrata* (4.9 %). Other species have been isolated in healthy people and patients. *Candida dubliniensis* was usually found in combination with other yeast species, especially *Candida albicans*. A high prevalence of *Candida dubliniensis* in the oral cavities of HIV-infected and AIDS patients has also been reported (Lim *et al*., 2012) since the first description of *Candida dubliniensis* from the oral cavities of HIV-positive patients from Ireland subsequent epidemiological studies have revealed that this species is prevalent globally in association with human and nonhuman habitats with a possibility of inter-host transmission. The species has now been reported from other body sites/specimens, such as the vagina, urine, skin and gastrointestinal tract of both HIV-positive and HIV negative patients (Lim *et al*., 2012).

## 2.2 Selected Medicinal Plants

Medicinal plants contain complex active components which makes them useful. The presence of certain bioactive components like alkaloids, saponins, tannins and flavonoids makes them relevant pharmacologically especially in their use as antimicrobial, antimalarial, antitumourigenic , antihelminth and hypolipidaemic (Izevbigie *et al*., 2014).

### 2.2.1 *Vernonia amygdalina*

*Vernonia amygdalina* (Plate I ) is a member of the Asteraceae family, a small shrub that grows in tropical Africa. *Vernonia. amygdalina* typically grows to a height of 2–5 m (6.6–16.4 ft). The leaves are elliptical and up to 20 cm (7.9 in) long. Its bark is rough (Jeh and Ejike, 2011) *Vernonia. amygdalina* is commonly called bitter leaf in English because of its bitter taste. The cooked leaves are a staple vegetable in soups and stews of various cultures throughout equatorial Africa. African common names include grawa (Amharic), ewuro (Yoruba), etidot (Ibibio), onugbu (Igbo), ityuna (Tiv), oriwo (Edo), chusar-doppki (Hausa), mululuza (Luganda), labwori (Acholi), olusia (Luo), andndoleh (Cameroon) (Kokwaro and John, 2009; Egedigwe, 2010).

The stem and root divested of the bark are used as chewing sticks in Nigeria. More importantly, the leaves are a very popular soup vegetable and have even been reported to be consumed by goats in some parts of Nigeria (Aregheore *et al*., 1998). All parts of the plant are pharmacologically useful (Aregheore *et al*., 1998). The roots and the leaves are used in ethnomedicine to treat fever, hiccups, kidney problems and stomach discomfort among several other uses (Hamowia and Saffaf, 1994; Burkill, 1995). Both aqueous and alcoholic extracts of the stem, bark, roots and leaves are reported to be antimicrobial and extensively used as a anpurgative, antimalarial and in the treatment of eczema (Kupcham, 1997). The plant has acquired special relevance recently, having been proved in human medicine to possess potent antimalarial and anthelminthic properties (Abosi and Raseroka, 2013) as well as anti tumorigenic properties (Izevbigie *et al*., 2014). The plant has also an amazing anti parasitic efficacy in zoo pharmacognosy as it is easily recognized and used for self medication by parasitized chimpanzees (Huffman, 2003). Despite these varied uses of the plant, there has been insufficient information on its toxicological potentials on the animal system of diabetic model.

**Plate I: Whole plant of *Vernonia amygdalina***

**Plate II: Leaf sample of *Vernonia amygdalina***
**Source: Field photograph**

**Plate III :** ***Vernonia amygdalina*** **root R: Root sample S:Stem sample**
**Source: Field photograph**

### 2.2.2 *Moringa oleifera*

*Moringa oleifera* (Plate II) is the most widely cultivated species of the genus Moringa (Olson, 2010) which is the only genus in the family Moringaceae. English common names include: moringa, drumstick tree (from the appearance of the long, slender, triangular seed-pods), horseradish tree (from the taste of the roots, which resembles horseradish), ben oil tree, or benzoil tree (from the oil which is derived from the seeds). It is a fast-

growing, drought-resistant tree, native to the southern foothills of the Himalayas in northwestern India, and widely cultivated in tropical and subtropical areas where its young seed pods and leaves are used as vegetables. It can also be used for water purification and hand washing, and is sometimes used in herbal medicine (Lewis *et al*., 2000).

*Moringa oleifera* is a fast-growing, deciduous tree. It can reach a height of 10–12 m (32–40 ft) and the trunk can reach a diameter of 45 cm (1.5 ft). The bark has a whitish-grey colour and is surrounded by thick cork. Young shoots have purplish or greenish-white, hairy bark. The tree has an open crown of drooping, fragile branches and the leaves build up a feathery foliage of tripinnate leaves (Tedradovich and Elevitch, 2011). The flowers are fragrant and bisexual, surrounded by five unequal, thinly veined, yellowish-white petals. The flowers are about 1.0-1.5 cm (1/2") long and 2.0 cm (3/4") broad. They grow on slender, hairy stalks in spreading or drooping later flower clusters which have a length of 10–25 cm (Parotta and John,1993). Flowering begins within the first six months after planting. In seasonally cool regions, flowering only occurs once a year between April and June. In more constant seasonal temperatures and with constant rainfall, flowering can happen twice or even all year-round (Parotta and John, 1993).

The fruit is a hanging, three-sided brown capsule of 20–45 cm size which holds dark brown, globular seeds with a diameter around 1 cm. The seeds have three whitish papery wings and are dispersed by wind and water . In cultivation, it is often cut back annually to 1–2 m (3–6 ft) and allowed to regrow so the pods and leaves remain within arm's reach. The moringa tree is grown mainly in semiarid, tropical, and subtropical areas, corresponding in the United States to USDA hardiness zones 9 and 10. It tolerates a wide range of soil conditions, but prefers a neutral to slightly acidic (pH 6.3 to 7.0), well-drained sandy or loamy soil. In waterlogged soil, the roots have a tendency to rot (Tedradovich and Elevitch, 2011). Moringa is a sun- and heat-loving plant, thus does not

tolerate freezing or frost. Moringa is particularly suitable for dry regions, as it can be grown using rainwater without expensive irrigation techniques (Tedradovich and Elevitch, 2011).

India is the largest producer of moringa, with an annual production of 1.1 to 1.3 million tonnes of fruits from an area of 380 km². Among Indian states, Andhra Pradesh leads in both area and production (156.65 km²) followed by Karnataka (102.8 km²) and Tamil Nadu (74.08 km²), a pioneering state having varied genotypes from diversified geographical areas and introductions from Sri Lanka.

Moringa is grown in home gardens in Odisha and as living fences in southern India and Thailand, where it is commonly sold in local markets. In the Philippines and Indonesia, it is commonly grown for its leaves which are used as food. Moringa is also actively cultivated by the World Vegetable Center in Taiwan, a center for vegetable research. In Haiti, it is grown as windbreaks and to help reduce soil erosion (Tedradovich,and Elevitch, 2011). More generally, moringa grows in the wild or is cultivated in Central America and the Caribbean, northern countries of South America, Africa, Southeast Asia and various countries of Oceania.

L

**PlateIV: Whole plant of *Moringa oleifera***

**S: Stem bark sample** **PlateV: Leave of *Moringa oleifera***
**L: Leave sample**

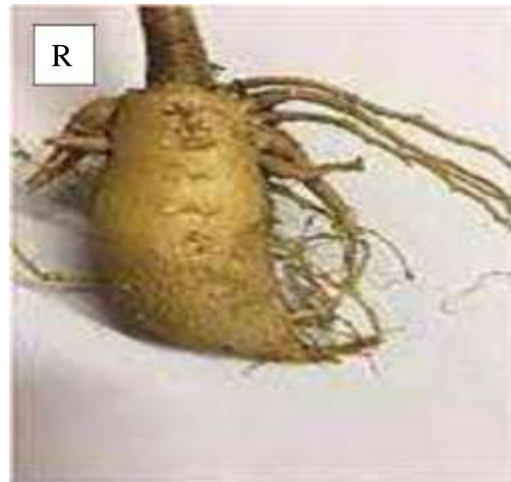



**Plate VI : *Moringa oleifera* root**
**R:Root sample**
**Source: Field photographs**

### 2.2.3 *Azadirachta indica*

*Azadirachta indica*, also known as Indian Lilac is a tree in the mahogany family Meliaceae (Nwanchukwu and Nweala, 2009). It is commonly called as Neem, Nim tree, and It is one of two species in the genus *Azadirachta*. In Nigeria, it is also known as Wonder leaves, Alhough, it is mostly found in the Northern region where it grows tall and produces fruit. It is called by the native name Dogoyaro, it is typically grown in tropical and semi-tropical regions, is a herb known to have numerous health benefits (Nwanchukwu and Nweala, 2009). Neem is a fast-growing tree that can reach a height of 15–20 metres (49–66 ft), and rarely 35–40 metres (115–131 ft). It is evergreen, but in severe drought it may shed most or nearly all of its leaves. The neem tree is very similar in appearance to its relative, the Chinaberry (*Melia azedarach*) (Devmurari and Jivani, 2010).

The neem tree is noted for its drought resistance. It thrives in areas with sub-arid to subhumid conditions, with an annual rainfall of 400–1,200 millimetres (16–47 in). (Devmurari and Jivani, 2010). It can grow in regions with an annual rainfall below 400

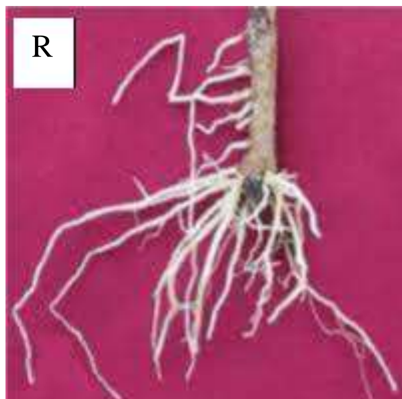
mm, but in such cases it depends largely on ground water levels (Devmurari and Jivani, 2010). Neem can grow in many different types of soil, but it thrives best on well drained deep and sandy soils. It is a typical tropical to subtropical tree and exists at annual mean temperatures of 21–32 °C (70– 90 °F). It can tolerate high to very high temperatures and does not tolerate temperature below 4 °C (39 °F). Neem is one of a very few shade-giving trees that thrive in drought-prone areas e.g. the dry coastal, southern districts of India and Pakistan (Tiwari *et al*., 2014). The trees are not at all delicate about water quality and thrive on the merest trickle of water, whatever the quality. In India and tropical countries where the Indian diaspora has reached, it is very common to see neem trees used for shade lining streets, around temples, schools and other such public buildings or in most people's back yards. In very dry areas the trees are planted on large areas of land (Saleem *et al*., 2018).

Neem has great medical benefits through its various biological properties (Salem *et al*., 2018). Almost all parts of the neem plant can be used to treat various diseases (Chen *et al*., 2018). Based on the previous reports, the neem leaves extract have numerous biological and pharmacological activities including antipyretic, analgesic, antihepatotoxic (Ogbuewu *et al*., 2011). Spermicide, anti-implantation, antihyperglycemic, antiulcer, antifungal, antibacterial,anti-inflammatory, immunomodulatory, antimutagenic, anticancer, antimalarial, antiviral, antioxidant, antifertility, and contraception (Tiwari *et al*., 2014). Furthermore, the bark of neem tree is known to possess tannins, phenolic compounds (salicylic acid and gallocatechin) which have been reported to be the anti-inflammatory principles (Devmurari and Jivani, 2010). Various parts of the neem plant have been successfully isolated. It contain more than 140 hemical compounds (Subapriya and Nagini, 2005) and have been used as herbal medicinesfor thousands of years (Saleem *et al*., 2018). Neem contains various primary compounds including fat derivatives,

carbohydrates, and proteins and secondary compounds such a flavonoids, steroids, saponins, terpenoids, alkaloids, glycosides, and tannins (Al-Hashemi and Hossain, 2016).

**Plate VII: Whole plant of *Azadirachta indica***

**Plate VIII: Leaf of *Azadirachta indica***
**L: Leaf sample**




S

**Plate IX: Root of *Azadirachta indica***
**R: Root sample**

**Plate X: Stembark of *Azadirachta indica***
**S: Stembark sample**

**Source: Field photographs**

### 2.2.4 *Vitellaria paradoxa*

*Vitellaria paradoxa* (formerly *Butyrospermum parkii*), commonly known as shea tree, shi tree, or vitellaria, is a tree of the Sapotaceae family. It is the only species in genus *Vitellaria* and is indigenous to Africa. The shea fruit consists of a thin, tart, nutritious pulp that surrounds a relatively large, oilrich seed from which shea butter is extracted (Byakagba, 2011). The shea tree is a traditional African food plant. It has been claimed to have potential to improve nutrition, boost food supply in the "annual hungry season" foster rural development, and support sustainable land care (National Research Council, 2006).

The tree starts bearing its first fruit when it is 10 to 15 years old; full production is attained when the tree is about 20 to 30 years old. It then produces nuts for up to 200 years. The fruits resemble large plums and take 4 to 6 months to ripen. The average yield is 15 to 20 kilograms of fresh fruit per tree, with optimum yields up to 45 kilograms. Each kilogram of fruit gives approximately 400 grams of dry seeds (NRC, 2006).

Shea butter is composed of five principal fatty acids: palmitic, stearic, oleic, linoleic, and arachidic . About 85 to 90 % of the fatty acid composition is stearic and oleic acids. The relative proportion of these two fatty acids affects shea butter consistency. The stearic acid gives it a solid consistency, while the oleic acid influences how soft or hard the shea butter is depending on ambient temperature. The proportions of stearic and oleic acids in the shea kernels and butter differ across the distribution range of the species. Ugandan shea butter has consistently high oleic acid content and is liquid at warm ambient temperatures. It fractionizes into liquid and solid phases, and is the source of liquid shea oil. The fatty acid proportion of West African shea butter is much more variable than Ugandan shea butter, with an oleic content of 37 to 55 %. Variability can be high even locally, and a tree that produces hard butter can grow with one that produces soft butter. Nuts are gathered

from a wide area for local production, so shea butter consistency is determined by the average fatty acid profile of the population. Within West Africa, shea butter from the Mossi Plateau region of Burkina Faso has a higher average stearic acid content, and so is usually harder than shea butter from other West African regions (Maranz *et al*., 2004).

Phenolic compounds are known to have antioxidant properties. A recent study characterized and quantified the most important phenolic compounds in shea butter. This study identified 10 phenolic compounds, eight of which are catechins, a family of compounds being studied for their antioxidant properties. The phenolic profile is similar to that of green tea, and the total phenolic content of shea butter is comparable to virgin olive oil. Also, this study was performed on shea butter that had been extracted with hexane, and the authors note that traditional extraction methods may result in higher phenolic levels. Furthermore, they note that the catechin content alone of shea kernels is higher than the total phenolic content of ripe olives. This study also found that the overall concentration and relative percentages of different phenolic content in shea kernels varied from region to region. The authors hypothesized that the overall concentration of phenols in shea kernels is linked to the level of environmental stress that the trees endure (Maranz *et al*., 2004).

The shea tree grows naturally in the wild in the dry savannah belt of West Africa from Senegal in the west to Sudan in the east, and onto the foothills of the Ethiopian highlands. It occurs in 19 countries across the African continent, namely Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Ethiopia, Ghana, Guinea Bissau, Ivory Coast, Mali, Niger, Nigeria, Senegal, Sierra Leone, South Sudan, Sudan, Togo, Uganda, Democratic Republic of the Congo, and Guinea. A testa found at the site of the medieval village of Saouga is evidence of shea butter production by the 14th century (Maranz *et al*., 2004).

Shea butter has many uses and may or may not be refined. In the West it is mostly used for cosmetics as emollient. Throughout Africa it is used extensively for food, is a major source of dietary fat, and for medicinal purposes (Maranz *et al*., 2004). In Nigeria ethno medicine the seed of *Vitellaria paradoxa* have been used to treat ring worm infections. It is also considered as an excellent moisturizer for eczema, psoriasis, and dermatitis (Maranz *et al*. 2004). The antifungal efficacies of this plant have as also be shown against *Aspergillus niger* and *Aspergillus fumigatus* as reported by Ahmad *et al*. (2009).

**PlateXI : Whole plant of *Vitellaria paradoxa paradox*a**

**PlateXII: Leaf of *Vitellaria***

**L: Leaf sample**

**Plate XIII: Stembark of *Vitellaria paradoxa* Plate XIV: Root of *Vitellaria paradoxa***
**S: Stembark R: Root sample**

**Source: Field photographs**

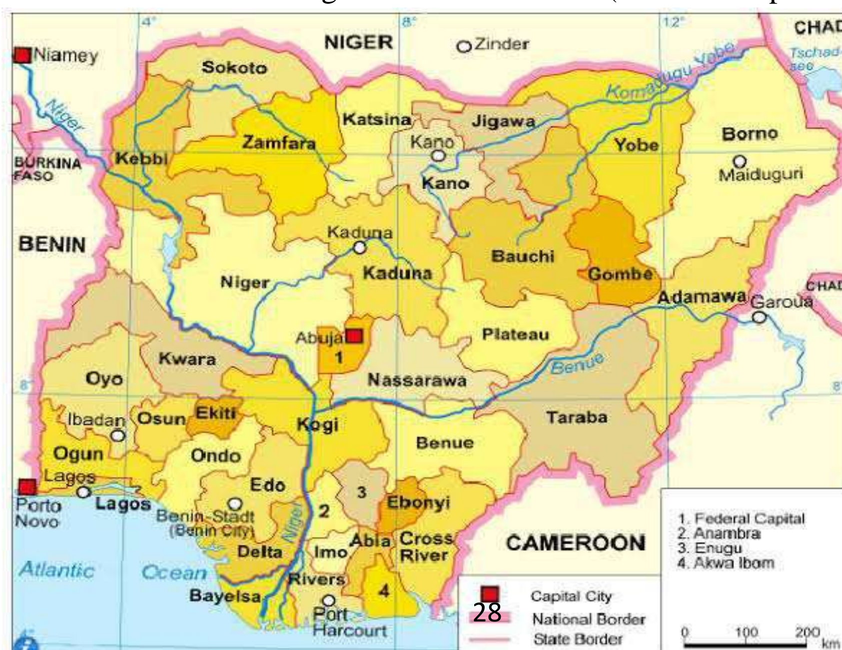
# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Study Area

The study was carried out in Bosso Local Government, Niger State Nigeria (Figure 3.1). Niger State is a state in Central Nigeria and the largest State in the country. Niger State is between Longitude 6° 32‟ 51.94” and Latitude 9 ° 331 and 9 ° 40 North and 6 ° 351 East. It is in the North Central Zone and occupies an area of approximately 29,484 square area in the present political zoning system. It's cover the distance 76,363 $km^2$ from Jebba to Abuja, Nigeria's Federal Capital . The State Capital is Minna, and other major cities are Bida, Kontagora, and Suleja. It was formed in 1976 when the then North-Western State was bifurcated into Niger State and Sokoto State. It is home to Ibrahim Babangida and Abdulsalami Abubakar, two of Nigeria's former military rulers. The Nupe, Gbagyi, Kamuku, Kambari, Dukawa, Hausa and Koro form the majority of numerous indigenous tribes of Niger State (United Nation, 2010).

The State is named after the River Niger. Two of Nigeria's major hydroelectric power stations, the Kainji Dam and Shiroro Dam, are located in Niger State, and the newly Zungeru Dam is also located there, the Jebba Dam is half Niger State and Kwara Sharing boundaries. The famous Gurara Falls is in Niger State, and Gurara Local Government Area is named after the Gurara River, on whose course the fall is situated. Also situated there is Kainji National Park, the largest National Park of Nigeria, which contains Kainji Lake, the Borgu Game Reserve and the Zugurma Game Reserve (National Population Commission, 2017).



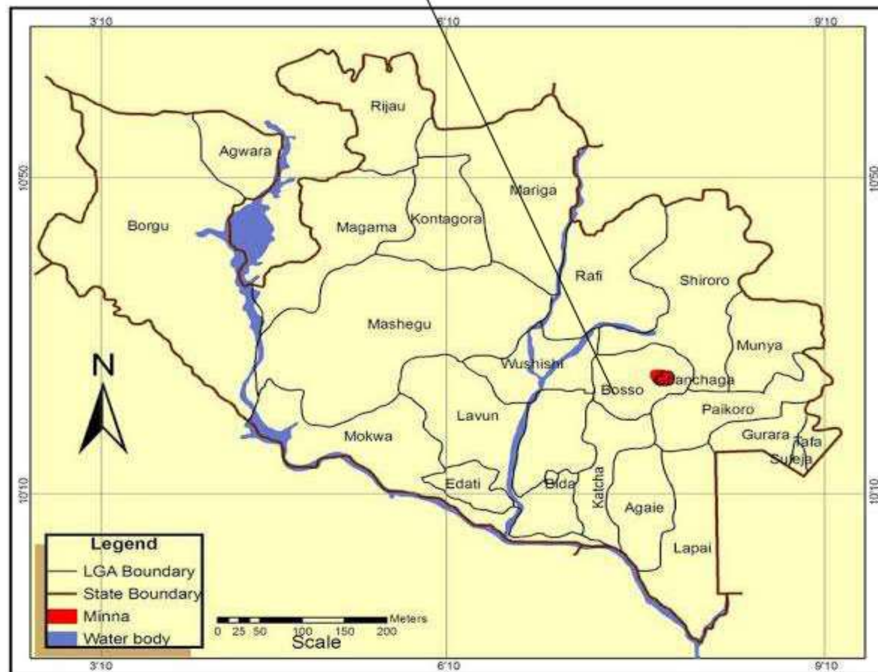



**Figure 3.1: Map of Niger State showing the study area**
**Source: National Research Council, 2006**

### 3.2 Collection of the Selected Medicinal Plants

The plant samples for this study were : *Vernonia* amygdalina (bitter leaf) *Azardirachta indica* (neem)*, Moringa oleifera* (moringa) and *Vitellaria paradoxa* (shea tree). The leaves, stem bark and root of these plants were all collected from Maikunkele in Bosso Local

Government *Vernonia amygdalina* and *Moringa oleifera* parts were collected during the raining season from May 2015 to August 2015 while *Azadirachta indica* and *Vitellaria paradoxa* parts were collected during the dry season from October 2015 to April 2016.

### 3.3 Identification, Authentication and Processing of the Selected Medicinal Plants

Fresh samples of the plant materials for this study (leaves, stembark and root) were identified by Dr. Dauda Yusuf Abdullazeez of the Department of Biological Science, Federal University of Technology, Minna while authentication of the plant samples was done by Mr. Lateef Akeem of the Herbarium Department of National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja with the following voucher numbers: *Vernonia amygdalina* (NIPRD/H/6872), *Moringa oleifera* (NIPRD/H/6873), *Azadirachta indica* (NIPRD/H/6879) and *Vitellaria paradoxa* (NIPRD/H/6880) and the voucher specimens were deposited in the Herbarium. The identified and authenticated selected medicinal plants parts were washed with distilled water to get rid of dirt. The washed leaves, stembark and root was air dried under shady environment (away from sunlight). The air-dried leaves, stembark and root of the medicinal plant was separately ground with the aid of a mortar and pestle and subsequently pulverized into powdered form using an electric blender. The pulverized samples was further sieved with a 150 µm pore size filter to obtain a fine powdered-like texture, stored in amber bottles and kept in a cool dried environment under room temperature until it is required for usage.

### 3.4 Extraction Procedure

The ground plant samples (Pulverized samples) were subjected to reflux extraction according to Hymete (2004) to obtain the crude extracts. The extraction was carried out

beginning from solvent with very low polarity to high polarity (n-hexane, ethyl acetate and methanol). 100 g of the of the plant samples was weighed and dissolve in 400 mL of the extracting solvent in a round bottom flask of 500 mL capacity.

Starting with n-hexane (polarity index= 0.1p‘‘) was gradually added until a ratio of 1:4 of the pulverized samples to the extracting solvent was attain. The flask containing the mixture was then placed on the heating mantle and the opening of the flask was connected to the condenser. The power source was then switched on to supply heat and the temperature was controlled (adjusted) to 30 $^{O}$C. The mixture was allowed to reflux for 2 hours. After refluxing, the mixture was filtered using muslin cloth and later with whatman No 1 filter paper with pore size 20 um to obtain a clear filtrate and further concentrated to a semi solid substance with the use of a rotary evaporator at a temperature of 40 $^{O}$C and then dried using water bath at 60 $^{O}$C. The extract was then stored in an air tight amber bottle and kept in the refrigerator at a temperature of 37 $^{O}$F for further analysis. The Marc(residue) was dried at room temperature for 45 minutes and was extracted with the next solvent in increasing polarity (further in succession using ethyl acetate with polarity index=4.4p‘‘). This procedure was repeated using the last solvent and the weight of the extracts for all the solvents used were measured and recorded accordingly. Percentage yield of each of the crude extract was calculated using the formulae below:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of sample (dry plant material)}} \times 100$$

## 3.5 Quantitative Phytochemical Estimation of the Crude Extracts

### 3.5.1 Determination of flavonoid

Aluminium chloride colorimetric method was used for flavonoid determination. A 0.5 mL (1 mg/mL) of the plant crude extract was mixed with 1.5 mL of methanol, 0.1 mL of 10 % aluminium chloride, 0.1 mL of 1 M sodium acetate and 2.8mL of distilled water and kept at room temperature for 30 minutes. The absorbance of the reaction mixture was taken at 415 nm with a double beam Shimadzu UV spectrophotometer, UV-1800. The calibration curve was prepared by using quercetin solutions at concentration of 12.5 to 100 g/ml in methanol (Chang *et al*., 2002).

### 3.5.2 Determination of total phenol

The total phenol content of the crude extract was determined was determine according to the method described by Singleton *et al*. (1999). A 0.5 mL(1mg/mL) was oxidized with 2.5 ml of 10 % Folin- Ciocalteau"s reIagent (v/v) and neutralized by 2 mL of 7.5 % sodium carbonate. The reaction mixture was incubated for 40 minutes at 45 $^{O}$C and the absorbance was taken at 765 nm using the double beam Shimadzu UV spectrophotometer, UV-1800. The total phenol content was subsequently calculated using Gallic acid as standard.

### 3.5.3 Determination of total alkaloid

A 0.5 g of the crude extract was mixed with 5 mL of 96 % ethanol- 20 % $H_2S0_4$ in ratio (1:1) and filter. 1 mL of the filtrate was added to 5 mL of 60 % $H_2SO_4$ the mixture was allowed to stand for 15 minutes and 5 ml of 0.5 % of formaldehyde solution was added and allowed to stand for 3 hours. The absorbance was taken at a wave length of 565 nm using Shimadzu UV spectrophotometer, UV-180. The concentration of alkaloid in the sample was calculated using the molar extinction coefficient of vincristine,Ɛ = 15136 mol/cm (Oloyed, 2005).

### 3.5.4 Determination of saponin

A 0.5 g of the crude extract was mixed with 20 mL of 1 MHCL and the mixture was boiled for 4 hours and allowed to cool. After cooling and filtered, 50 mL of petroleum ether was added filtrate, for ether layer and evaporated to dryness. 5 mL of acetone- ethanol (1:1) were added to the residue, 6 mL ferrous sulphate reagent and 2 mL of concentrated $H_2SO_4$. The mixture was homogenized and allowed to stand for 10 minutes before the absorbance was taken at 490 nm using Shimadzu UV spectrophotometer UV- 1800 (Oloyed, 2005).

### 3.5.5 Determination of tannin

A 0.2 g of the extract was weighed into a 50 mL beaker, 20 ml of 50 % methanol was added, covered with para film and placed in a water at 80 $^{O}C$ for one hour. The mixture was shaken thoroughly and the content was transferred into a 100 mL volumetric flask. 20mL of water, 2.5 ml of 10 % Folin Denis reagent and 10 mL of 17 % $Na_2 CO_3$ were added and mixed thoroughly. The mixture was allowed to stand for 20 minutes. Observation for bluish green colouration was done at the end of range 12.5100 μg/mL of Tannic acid. The absorbance of tannic acid standard solution as well as sample was taken after colour development on a spectrophotometer at wave length of 760 nm using Shimadzu UV spectrophotometer, UV-1800 (Emmanuel *et al.*, 2014).

### 3.5.6 Phytic acid content

The phytic acid content was determined using a modified indirect colorimetric method of Wheeler and Ferrel (1971). The method depends on an iron phosphorous ratio of 4:6 and is based on the ability of standard ferric chloride to precipitate phytate in dilute HCL extract of the sample. 5 g of the sample was extracted with 20 ml of 3 % trichloroacetic acid and filtered. 5 ml of the filtrate was used for the analysis; the phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding 5 mL of 1mL NaOH. The precipitate was dissolved with hot 3.2 M $HNO_3$ and the absorbance and immediately at 48 nm. Preparation of standard curve for phytic acid was done as follows:

standard curve of different Fe $(NO_3)_3$ concentration was plotted against the corresponding absorbance of spectrophotometer to calculate the ferric iron concentration. The phytate phosphorous was calculated from the concentration of ferric iron assuming 4:6 iron: phosphorous molar ratio.

### 3.5.7 Determination of oxalate

Oxalate was determine by permanganate titrimetric method as described by Oke (1996). Two gram (2 g) of the crude extract was suspended in 190 mL of distilled water in 250mL volumetric Flask, 10 mL of 6 M HCL was added and the suspension digested at 100 $^0$C for 1hour, cooled, then made to the mark before titration. Duplicate portion of 125 of the filtrate were measured into beakers and four drops of methyl red indicator added. This is followed by the addition of concentrated $NH_4OH$ solution drop wise until the test solution changes from salmon pink colour to a faint yellow colour (pH 4-4.5). Each portion is then heated to 90 $^0$C and 10ml of 5 % $CaCl_2$ solution added while being stirred constantly. After heating, it was cooled and left overnight at 5 $^o$C. The solution was then centrifuged in 10 ml of 20 % (v/v) $H_2SO_4$ solution. The total filtrate resulting from the digestion was made up to 300 ml. Aliquots of 125 mL of the filtrate was heated until near boiling and then titrated against 0.05 M standardized $KMno_4$ solution to a pink colour which persist for 30 minutes. The calcium oxalate content was calculated using the formula.

$$\frac{T \times (Vme)(Df) \times 10^5}{(ME) \times Mf} \text{ (mg/100g)} \qquad (3.1)$$

Where T is the titre of $KMnO_4$ (mL), Vme is the volume- mass equivalent (1 cm$^3$ of 0.05 M $KNnO_4$ solution is equivalent to 0.00225 anhydrous oxalic acid), Df is the dilution factor $V_T/A$ (2.5 where $V_T$ is the total volume of titrate (300 mL) and A is the aliquot used

(125 mL), ME is the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction) and Mf is the mass of extract used.

### 3.6 Ethical Consideration and Collection of Microbial Samples used in the Investigation

The study was reviewed and approved by the Ethical, Research and Publication Committee of General Hospital in Minna, Niger State recruited for this study (Appendix A). Thirty- clinical samples of high vagina swab (HVS) (25 samples) and urine (5 samples) were collected from patients at the General Hospital in Minna, Niger State. High vagina swab was collected using a sterile swab stick while urine samples was collected in sterile universal bottle by the assistance of the Medical Laboratory Scientist and Technologist of the Hospital. The samples were brought to the Microbiology laboratory with the aid of an ice pack for further analysis.

### 3.7 Isolation of *Candida albicans*

#### 3.7.1 Culturing on sabouraud dextrose broth (SDB)

The high vaginal Swab (HVS) and urine samples were cultured each onto sabouraud dextrose broth supplemented with chloramphenicol to suppress the growth of bacteria and increase selectivity. The cultures were then incubated at room temperature for 72 hours (Bhavan *et al.,* 2010).

#### 3.7.2 Culturing on sabouraud dextrose agar (SDA)

Isolates from sabouraud dextrose broth were then sub cultured onto SDA containing chloramphenicol at room temperature for 72 hours. Mixed growth were further sub cultured onto freshly prepared SDA (Bhavan *et al*., 2010).

### 3.8 Identification of Isolate

#### 3.8.1 Macroscopic examination

Macroscopic identification of the culture plates were based on colony colour, and consistency of their colonies. This was then compared with known taxa of Candida species (Pfaller *et al*., 1996).

#### 3.8.2 Microscopic examination

Microscopic examination was carried out to determine the morphology of the isolates.

##### 3.8.2.1 Gram staining procedure

A sterile wire loop was used to transfer a small portion of the isolate onto a clean grease free slide containing a loopful of normal saline and a thin smear was made by emulsification. The smear was allowed to air dry and then heat fixed by passing the slide through a bursen flame two – three times. The smear was then flooded with crystal violet dye and allowed to stand for one minute. The slide was washed briefly with water. Grams iodine was added and allowed to stand for one minute. The slide was also washed with water and decolourized using 95 % alcohol until no further colour came off. The alcohol was rinsed off immediately. The slide was finally stained using Safranin for two minutes before it was washed off with water and allowed to air dry. After it was airdried, oil immersion was added and then viewed under the objective lens (×100) of the compound microscope. When viewed the cells appeared purple/blue which was indicative of gram positive organisms and their morphology revealed were oval in shape (Cheesbrough, 2000).

#### 3.8.3 Biochemical test

**Composition:**Tengram(10gram)ofsugar, 15gram of peptone water, 0.4 gram of phenol red and 1000 mL of distilled water.

The suspected yeast culture were subjected to sugar fermentation test (glucose, fructose, D-mannitol, Mannose, sucrose, galactose, maltose, arabinose, lactose and sorbitol). Ten gram (10 gram) of each sugar was weighed together with 15 gram of peptone water, 0.4 gram of phenol red and dissolved into 1000 mL of distilled water. 10 mL of the solution was dispensed into tubes containing inverted vials known as Durham tubes. The test tubes were autoclaved at 121 °C for 15 minutes. After cooling the tubes were inoculated with the test organism (yeast isolate). A control test was also prepared (uninoculated) both the inoculated and uninoculated tubes were incubated at 28±2 °C for 72 hours. The result was observed and recorded as positive or negative. Positive result indicated fermentation shown by colour change (acid production) and (gas production) which was determined by its accumulation in the Durham tubes while negative result showed the absence of acid and gas production (John *et al*., 2010).

### 3.8.4 Germ tube test

Germ tube test was carried out to differentiate the suspected yeast cells (*Candida albicans*) from other yeast cells . A Small inoculum of suspected yeast cultures was inoculated into 500 μL (0.5 mL) of human serum in a small test tube and incubated at 37 °C for 3 hours. After incubation, a Pasteur pipette was used to transfer a drop of the serum yeast culture to a glass slide, overlaid with a cover slip and examined microscopically for the presence or absence of germ tubes (Cheesbrough, 2000).

### 3.8.5 Molecular identification of test organisms

The molecular confirmation of the test organisms (*Candida albicans*) was carried out according to the method stated in Promega Technical Manual (Promega Corporation, 2014).

#### 3.8.5.1 Preparation of yeast lysate

One millilitre (1 mL) of yeast culture (broth) was pipetted into a sterile test tube and centrifuged at 15000 x g for 2 minutes and the supernatant was decanted leaving the pellet cells. 293 µL of 50 mM ethylene diamine tetra acetic acid (EDTA) was added to the pellet cells to facilitate the lysing process and it was allowed to suspend. 7.5 µL of 20 mM/mL lyticase was then added and the mixture was gently mixed. The mixture was next incubated for 60 minutes at 37 $^{O}$C and it was allowed to attain room temperature. The mixture was centrifuged for 2 minutes at 13,000 xg and the supernatant was decanted. 300 µL of nuclei Lysate solution was added and the mixture was subjected to protein precipitation and DNA rehydration (Wawrik *et al*., 2005).

#### 3.8.5.2 Protein precipitation

One hundred microliter of protein precipitation solution was added to the mixture above and vortexed for 5 seconds after which it was incubated for 5 minutes. The mixture was centrifuged at 15000 xg for 3 minutes. The supernatant was transferred into a clean tube containing 300 µL of isopropanol and mixed gently. The mixture was centrifuged for 2 minute at 15000 xg and the supernatant was decanted. 300 µL of 70 % ethanol was added and it was mixed and centrifuged for another 2 minutes at 15000 xg. The supernatant was kept for DNA precipitation and rehydration (Wawrik *et al*., 2005).

#### 3.8.5.3 DNA precipitation and rehydration

The ethanol was aspirated and the DNA pallet was air-dried. 1.5 µL of RNase enzyme was added and incubated at 37 $^{O}$C for 15 minutes and the DNA pallet was rehydrated in 50 µL rehydration solution for 24 hours at 4 $^{O}$C.

#### 3.8.5.4 Polymerase chain reaction (PCR) reaction cocktail

Ten microliter (10 µL) of 5x GoTaq reagent, 3 µL of $MgCl_2$, 1 µL of 10 pmol each 27F 5‟-AGA GTT TGA TCM TGG CTC AG-3‟ and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′

primers and 0.3units of Taq DNA polymerase (Promega, USA) was made up to 42 µL with sterile distilled water and 8 µL DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) PCR profile, an initial denaturation of 30 cycles at 94 °C for 5 minutes; 50 °C for 60 seconds and 72 °C for 1 minute 30 seconds and a final extension at 72 °C for 10 minutes. It was then allowed to cool down to 4 $^{O}$C (Wawrik *et al*., 2005; Frank *et al*., 2008).

**3.8.5.5 Integrity check on agarose gel**

The integrity of the amplified gene fragment was checked on a 1 % Agarose gel to confirm amplification. This was carried out by mixing 8 µL of amplified product to 4 µL of loading dye and ran on the solidified Agarose gel at 110 V for about 1 hour. Also the amplified product was checked on a nanodrop of model 2000 from thermo scientific to quantify the concentration of the amplified product (Wawrik *et al*., 2005; Frank *et al*., 2008).

**3.8.5.6 Purification of amplified product**

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µL of sodium acetate 3 M and 240 µL of 95 % ethanol were added to each fragments of the PCR amplified products in a new sterile Eppendorf tube, it was vortexed for 5 seconds and kept at 20 °C for 30 minutes. The mixture was centrifuged for 10 minutes at 13000 xg and 4°C followed by removal of the supernatant (by inverting the tube on trash once) after which the pellets were washed by adding 150 µL of 70 % ethanol, mixed and then centrifuged for 15 minutes at 7500 xg and 4 °C. Again the supernatant was decanted and the tube was inverted on blotting paper and was allowed to dry in the fume hood at room temperature for 10-15 minutes. It was then suspended with 20 µL of sterile distilled water and kept in a refrigerator at -20 $^{O}$C prior to sequencing. The purified fragment was checked on a 1.5 % Agarose gel, ran on a voltage of 110 V for

about 1hour as described above, to confirm the presence of the purified product before sequencing (Wawrik *et al*., 2005; Frank *et al*., 2008).

#### 3.8.5.7 Sequencing and BLAST

The amplified fragments were sequenced using a Genetic Analyser 3130 xl sequencer from Applied Biosystems using manufacturers‟ manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis. Identities and accession numbers of the test organisms were determined by BLAST from the GENE bank through the National Centre for Biotechnology and Information (Wawrik *et al*., 2005).

## 3.9 Antifungal Activity of the Crude Extracts and Fractions

### 3.9.1 Preparation of 0.5 Mcfarland standard

The population of the clinical isolate were determined from the McFarland turbidity standard (Mcfarland, 1907). Exactly 0.05 mL of 1.175 % $Bacl_2$ was mixed thoroughly with 9.95 mL of 1 % $H_2SO_4$ in a test tube. The absorbance of the mixture (white precipitate) was determined at 530 nm (Mcfarland, 1907).

### 3.9.2 Standardization of the test organisms

The Standardization of the test organisms was carried out as described by Magaidi *et al.* (2000) with slight modification. The test organisms was cultured on sabouraud dextrose broth at room temperature for 72 hours. 1 mL of the cultured organism (*Candida albicans*) was transferred into 9 mL of sterile sabouraud dextrose broth. Serial dilutions of $10^{-1}$ – $10^{-7}$ was made and the absorbance was determined at 530 nm using spectrophotometer. The optical density (absorbance) was compared with the optical density (absorbance) of 0.5Mcfarland standard previously obtained. The dilution corresponding to that of the 0.5Mcfarland standard was used as the standard organism (Magaidi *et al*., 2000)

## 3.10 Preparation of Extracts and Fractions Concentration

Two hundred milligram (200mg), 300, 400 and 500mg of the normal hexane, ethyl acetate and methanol extract were weighed and dissolved in 5 mL each of 10 % Dimethyl sulfoxide (DMSO) to give concentrations of 40, 60, 80 and 100 mg/mL respectively. The concentration of the fractions was determined by measuring 10 and 20 mg of each fractions obtained and dissolved in 2 mL of 10 % DMSO to give a concentration of 5 and 10 mg/mL (Ewansiha *et al*., 2016).

### 3.11 Determination of the Antifungal Activity of the Crude Extracts

The susceptibility test was carried out using Agar Well Diffusion Method as described by Magaidi *et al*. (2000). Sabouraud dextrose agar (SDA) was prepared according to the manufacturer"s instruction. The prepared SDA was then inoculated with a loop full of the standardized test organism by the spread plate method using a sterile rod spreader to obtain uniform growth. Wells were made using 6 mm sterile cork borer and labelled. Accordingly. 100 µL (0.1 mL) of the prepared crude extract of varying concentration(40, 60, 80 and 100 mg/mL) was transferred into each of the wells with a sterile micropipette and allowed to stand for 30 minutes for pre- diffusion and then incubated at room temperature for 24-72 hours. The experiment was done in triplicates and the mean values with the corresponding standard deviation of the inhibition zone diameter (IZD) 100 µL of 10 % DMSO (free from extract) was transferred into a freshly prepared SDA (containing the test organism) to serve as negative control. Fluconazole (1 mg/mL) was used as the positive control. This was achieved by transferring 100 µL of the prepared standard antibiotics into wells and cultures were allowed to stand for 30 minutes after which they were incubated at room temperature for 24-72 hours. The zone of inhibition (ZOI) was measured using a meter scale rule. The experiment was done in triplicates and the mean values with the corresponding standard deviation of the inhibition zone diameter (IZD was recorded. Crude extract that gave zone of inhibition ≤10 mm were recorded as resistant while zone of inhibition >10 mm were recorded for sensitivity ( Edeoga *et al.,*2005).

### 3.12 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the Active Crude Extract

The MIC was determined using the Tube Dilution Method as described by Ewansiha *et al*. (2016). A four fold serial dilution of the most active plant extract was carried to give a decrease in concentration from 100, 50, 25, 12.5, 6.25, 3.125, 1.25 and 0.78 mg/mL. The stock solution was prepared by dissolving 800 mg of the extract in 4 mL of 10 % dimethylsulphoxide (200 mg/mL). Two millilitres (2 mL) of the stock concentration was transferred to a test tube labeled A containing fresh 2 mL Sabouraud Dextrose Broth (SDB) to give a concentration of 100 mg/ml. From tube A, 2 mL was transferred into a second tube labeled B containing 2 mL SDB to give a concentration of 50 mg/mL. This procedure continued until a concentration of 0.78 mg/mL was obtained in the last test tube labeled H. They were properly shaken to obtain a homogenous mixture and all test tubes were inoculated with 0.1 mL of the standardized test organism. Two separate test tubes containing sterile broth plus 10 % DMSO and sterile broth plus test organism were prepared for negative and positive controls respectively. All test tubes were incubated at room temperature for 24 to 72 hours, after which the test tubes were compared with each control tubes. The concentration/tube without visible turbidity was taken as the MIC. The MFC was determined by subculturing from the MIC tube and other tubes that showed no turbidity onto freshly prepared SDA and incubated at room temperature for 72 hours. The concentration that showed no visible growth after incubation was taken as the MFC.

### 3.13 Fractionation of the Extracts by Chromatography

The crude extracts with higher activity was separated using the column and thin layer chromatography as described by Fair and korsmas (2008).

### 3.13.1 Column chromatography of the active crude extracts

Silical gel (0.015-0.04mesh size) was used as the stationary phase. It was packed in a column (40 mm diameter width and 150 mm length) with a stop cock near the bottom. A plug of cotton wool was placed at the bottom of the column to support the adsorbent. One fourth of the tube was left empty. Slurry of the stationary phase material (silical gel) was prepared by moistening with n-hexane and packed sufficiently in the column with a cotton pad at the bottom. A loose plug of cotton wool was placed at the top of the adsorbent column. The extract to be separated was adsorbed unto the silical gel by disolving in appropriate solvent and adding silical gel, then allowed to dry; this was done to increase the surface area. The adsorbed extract was then gently poured on the top of the column. The mobile phase was poured into the column over the sample and allowed to pass slowly through it. As it passes through the column, the different constituents of the extract were adsorbed differently on the basis of their polarity, forming bands in different parts of the column. The components were then eluted out. A collection tube was placed at the bottom of the column near the end to collect the elute. The weakly adsorbed component was eluted more rapidly than a more strongly adsorbed component. Different components of the extract were collected in the form of different fractions in separate conical flasks which was based on their retension factor(RF values).

The different fractions collected were spotted on a Thin Layer Chromatographic (TLC) plate developed in an appropriate solvent system, and viewed under the UV – lamp and iodine tank to detect fractions with similar components. All fractions with similar retension factor (RF) were bulk together and properly labelled. The resulting fractions were subjected to antifungal susceptibility test and their MIC and MFC were also determined.

Rf is equal to the distance traveled by the substance divided by the distance traveled by the solvent. Its value is always less than one.

$$R_f = \frac{\text{Migration distance of the substance (cm)}}{\text{Migration distance of the solvent (cm)}} \quad (3.2)$$

**3.14 Determination of the Antifungal Activity of the Fractions**

The susceptibility test was carried out using Agar Well Diffusion Method as described by Magaidi *et al*. (2000). Sabouraud Dextrose Agar (SDA) was prepared according to the manufacturer‟s instruction. The prepared SDA was then inoculated with a loop full of the standardized test organism by the spread plate method using a sterile rod spreader to obtain uniform growth. Wells were made using 6 mm sterile cork borer and labelled. Accordingly. 100 μL (0.1 mL) of the prepared fractions of varying concentration (5 and 10 mg/mL) was transferred into each of the wells with a sterile micropipette and allowed to stand for 30 minutes for pre- diffusion and then incubated at room temperature for 24-72 hours. The experiment was done in triplicates and the mean values with the corresponding standard deviation of the inhibition zone diameter (IZD). 100 μL of 10 % DMSO (free from extract) was transferred into a freshly prepared SDA (containing the test organism) to serve as negative control. Fluconazole (1 mg/mL) was used as the positive control. This was achieved by transferring 100 μL of the prepared standard antibiotics into wells and cultures were allowed to stand for 30 minutes after which they were incubated at room temperature for 24-72 hours. The zone of inhibition (ZOI) was measured using a meter scale rule. The experiment was done in triplicates and the mean values with the corresponding standard deviation of the inhibition zone diameter (IZD was recorded. Fractions that gave zone of inhibition ≤10 mm were recorded as resistant while zone of inhibition >10 mm were recorded for sensitivity (Edeoga *et al.,*2005).

### 3.15 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the Active Plant Fractions

The MIC was determined using the Tube Dilution Method as described by Ewansiha *et al*. (2016). A four fold serial dilution of the most active plant extract was carried to give a decrease in concentration from 100, 50, 25, 12.5, 6.25, 3.125, 1.25 and 0.78 mg/mL. The stock solution was prepared by dissolving 400 mg of the fraction in 2 mL of 10 % dimethylsulphoxide (200 mg/mL). Two millilitres (2 mL) of the stock concentration was transferred to a test tube labeled A containing fresh 2 mL Sabouraud Dextrose Broth (SDB) to give a concentration of 100 mg/mL. From tube A, 2 mL will be transferred into a second tube labeled B containing 2 mL SDB to give a concentration of 50 mg/mL. This procedure continues until a concentration of 0.78 mg/mL was obtained in the last test tube labeled H. They were properly shaken to obtain a homogenous mixture and all test tubes were inoculated with 0.1 mL of the standardized test organism. Two separate test tubes containing sterile broth plus 10 % DMSO and sterile broth plus test organism was prepared for negative and positive controls respectively. All test tubes were incubated at room temperature for 72 hours, after which the test tubes were compared with each control tubes. The concentration/tube without visible turbidity was taken as the MIC. The MFC was determined by subculturing from the MIC tube and other tubes that showed no turbidity onto freshly prepared SDA and incubated at room temperature for 72 hours. The concentration that showed no visible growth after incubation was taken as the MFC.

### 3.16 Quantitative Analysis and Identification of Compounds by GC-MS

The determination of the identity of active components in the partially purified fractions was done by GC-MS analysis using GC-MS-QP 2010 Plus Shimadzu system (SHIMADZU, JAPAN). The gas chromatograph interface to a mass spectrometer (GC-

MS) instrument was used while the Column elite-1 was fused with silica capillary column (30 m x 0.25 mm 1D x μL df, composed of 100 % dimethyl polysiloxane). An electronic ionization system with ionization energy of 60 eV was used for the GC-MS detection while Helium gas (99.99 %) was used as the carrier gas at a flow rate of 1 mL/minute and injection size of the fraction was made 2 μL (0.002 mL with split ratio of 1:40 and film thickness of 0.20 μm). The GC oven temperature was set at 60 °C for 3 minutes and then programmed to rise from 60 to 200 °C at a rate of 3 °C $min^{-1}$ and held isothermally for 3 minutes at 200 °C (Isothermal for 2 minutes.) with an increase of 10 $^{O}$C/min to 200 $^{O}$C then 5 $^{O}$C/min to 280 $^{O}$C/min, ending with a 9 minutes isothermal at 280 $^{O}$C. Mass spectra was be taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. Total GC running time was 27 minutes. Relative percentages and amount of each components was deduced by comparing individual average peaks area to the total areas. Turbomass was used for the mass spectra and chromatogram while the detection of compounds was done using the database of National Institute of Standard and Technology (NIST) NIST Ver. 2.0 year 2009 (Sarswati *et al*., 2013).

### 3.17 Toxicity Assessment of Properties of the Active Extracts of the Plants Sample

This was done to determine the toxicity of the active crude extracts and fractions. Acute and subacute pharmacological properties were evaluated by the method of Lorkes, (1983) and Organization for Economic Corporation and Development (OECD,2008) respectively.

#### 3.17.1 Experimental animals

Wistar Albino rats of mixed sex weighing between 150 – 180 g each was obtained from the National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. The rats were housed under suitable environmental conditions (12 h day light/night cycles). All the rats were allowed free access to food and water *ad libitum* throughout the experimental period. Good hygiene were maintained by constant cleaning

and removal of feces from cages daily. The toxicity study was carried out in strict compliance with internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review (CCAC, 1997).

### 3.17.2 Acute toxicity evaluation and determination of $LD_{50}$ of crude extract of the plants.

$LD_{50}$ of both *Vernonia amygdalina* (bitter leaf) and *Azardirachta indica* (neem) leaf crude extract was determined using the method of Lorke, (1983). In the phase one method nine animals were divided into three groups of three animals each. They were administered with doses of 10, 100 and 1000 mg/Kg bodyweight of both *Vernonia amygdalina (*bitter leaf) and *Azardichta indica* (neem) leaf crude extracts independently. In the absence of mortality in both extracts in phase I, phase II of Lorke"s method was conducted for both extracts.

One animal served as control which was used to observe the experiments. In the phase II method, nine animals were also divided into three groups of three animals each in which they were administered with 1900, 2900 and 5000 mg/Kgbodyweight of both extracts respectively. The animals were placed under observation for any sign of toxicity, mortality or behavioural changes which included coma, pawlicking, salivation, stretching of the entire body, weakness, sleep and respiratory distress for 24 hours. Likewise, One animal was also used as control to observe the experiments.

### 3.17.3 Subacute toxicity study of the crude extracts of *Vernonia amygdalina* (bitter leaf) and *Azardirachta indica* (neem)

The subacute toxicity study of the crude extracts was conducted in accordance to OECD, (2008).

#### 3.17.3.1 Grouping and extract administration

Twelves (12) wistar albino rats were distributed into four groups of three rats each. Group 4 rats served as the control and were fed with 1 ml of normal saline while Groups 1, 2 and 3 were administered with 100, 300 and 600 mg/kgbw doses of the crude extract of bitter leaf and neem leaf respectively for 28 days. The animals were fasted overnight but given free access to water before the administration of the extract throughout the experimental days. The administration was through the oral route. The animals were given access to feed 4-6hours after the administration. The weight variations of the experimental animals were also determined on a weekly basis throughout the experimental days. The experimental animals were fasted overnight after administration on the $28^{th}$ day and were sacrificed on the $29^{th}$ day and their blood sample were collected for biochemical and haematological analysis.

### 3.17.4 Sub-acute toxicity study of *Vernonia amygdalina*(bitterleaf) fraction (F4) of n – hexane extract and *Azardirachta indica* Fraction (F6) of methanol extract

The sub-acute toxicity study of the active fraction (F4) of n- hexane extract of *Vernonia amygdalina* was conducted in accordance to Organization for Economic Co-operation and Development(OECD) guidelines (OECD, 2008). The same procedure was repeated for fraction (F6) of methanolic extract of *Azardirachta indica*.

#### 3.17.4.1 Grouping and extract administration

Twelves(12) wistar albino rats were distributed into four groups of three rats each. Group 4 rats served as the control and were fed with 1ml of normal saline while Groups 1, 2 and 3 were administered with 10, 20 and 40 mg/kgbw doses of the crude extract of bitter leaf and neem leaf respectively for 28 days. The animals were fasted overnight but given free access to water before the administration of the extract throughout the experimental days. The administration was through the oral route. The animals were given access to feed 4-6

hours after the administration. The weight variations of the experimental animals were also determined on a weekly basis throughout the experimental days. The experimental animals were fasted overnight after administration on the 28$^{th}$ day and were sacrificed on the 29$^{th}$ day and their blood samples were collected for biochemical and haematological analysis.

#### 3.17.4.2 Observation of feeding pattern, mortality and clinical signs

During the four-week dosing period, all the animals were observed on daily basis for likely clinical signs, and mortality patterns once before dosing, immediately after dosing, and up to 4 hours after dosing. Also, rate of food consumption and water intake were monitored at least trice per week (Sofowora *et al*., 2013).

#### 3.17.4.3 Collection of blood and preparation of sera samples

Blood were collected from the heart of the animals into plain bottles and ethylene di-amine tetra acetic acid (EDTA) coated tubes. Serum were prepared by blood centrifugation at 3000 rpm for 15 minutes in a Laboratory bench. The clear supernatant was used for estimating biochemical parameter while blood was used for haematological parameters (Sofowora *et al*., 2013).

#### 3.17.4.4 Haematological analysis

The prepared blood samples were used to determine the total white blood cells (TWBC), total red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) neutrophils (N), lymphocytes (L),monocytes(M), basophil, eosinophil and platelet count (PLC) using an automated haematological blood analyzer Mythic 18_ 100829 (Sofowora *et al*., 2013).

#### 3.17.4.5 Serum biochemical analysis

The biochemical analysis were focused on Liver function, Lipid profile and Kidney function parameters based on research report that sub-acute toxicity of various plant

extracts usually affect the liver and kidneys as the liver acts as the main detoxifying organ for chemical substances, while the kidney is a principal route of excretion for many chemical substances in their active and/or inactive forms. The sellectral machine 108033 were used to determine the liver functions parameters like: total protein (TP), albumin,(A), total bilirubin(TB), Direct bilirubin(DB) and even liver enzymes that are aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum total proteins (TP). The same method were also employed to determine the lipid profile such as cholesterol (CHOL), triglycerides (TRIG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) and the kidney function such as urea, creatinine and serum electrolytes were all determined (Oh, 2006).

### 3.17.5 Gross pathology and microscopic examination

Histopathological analysis of the animal‟s tissues was carried out in the histopathology laboratory of the Teaching Hospital Gwagwalada, Abuja, Nigeria. The heart, lungs, kidneys and liver for each experimental and control rats were been fixed and preserved in 10 % formaldehyde before subjection to tissue processing (Sofowora, 2013).

### 3.17.6 Tissue processing

The Automatic tissue processing using auto tissue processor involve four process which are:

Stage 1: Fixation: using formalin two changes 1hours in each

Stage 2: Dehydration: using alcohol five changes 1hours in each

Stage 3: clearing :using xylene three changes 1hours in each

Stage4: Impregnation: using wax two changes 1hours in each. All processes attained 12 hours circle on the machine. Followed by the embedding process using embedding machine, tissues pieces are buried in molting paraffin wax using embedding mulds and tissue cassettes and allow to solidify. This is done to get a solid support for the tissue so as

to enable it to be cut into thin sections. The sectioning stage was carried out using microtome machine were by the solidified tissue block was cut into thin sections of about 5microns(5 µ) thick. The cut section was placed on water bath which was maintained at a temperature of 2-3 $^{O}$C below the melting point of the wax. Section were picked on a glass slide. Tissue section on the slide was placed on hot plate which was maintained at a temperature 2-3 $^{O}$C above the melting point of the wax and were allowed to fix properly on the slide for a minimum of 20 minutes before ready for staining. The staining process was carried out using haematoxylene and Eosin (Hand E). The staining process involved dewaxing section in three changes of xylene for 1minutes in each, followed by clearing in descending grade of alcohol(100, 100, 90 and 70 %) for 1 minute in each, then washed section in water. It was further stained in Haris haematoxylene for 5 minutes and washed in water, after washing, it was then differentiated in 1 % acid alcohol for 30 seconds and washed in water. The process continued by blueing in scott"s tap water for three minutes and washed in water, It was counter stained in 1% eosin for 3 minutes and subsequently washed off in water. It was further dehydrate in ascending grades of alcohol(70, 90 and 100 % ) for 1 minute in each followed by clearing in three changes of xylene for 1minutes each and then finally mount in dextrose phosphate xylene (DPX) using cover slips for proper examination,

Result: Nucleus- blue to blue black

Cytoplasm: pink

Red blood cells: Mauve pink

Other structures- Various shades of pink (Sofowora, 2013; Oh, 2006).

**3.18 Data Analysis**

All data were presented as mean± standard deviation, data were analysed using one way Anaslysis of Varienvce (ANOVA) to check for significant difference. Differences were

considered significant at $P \leq 0.05$. The analysis were carried out using Statistical Package for Social Sciences (SPSS) version 20.

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

### 4.1 Results

#### 4.1.1 Biochemical characteristics of yeast isolates from clinical samples (test organisms)

The total numbers of yeast isolated from clinical samples is shown in Table 4.1. Many of the isolates were able to ferments glucose, fructose and galactose and utilize these sugars. Out of the thirty (30) yeast isolates, eighteen were positive for Germ tube test which was an indication for *Candida albicans.* Eight (8) out of the eighteen samples that was screened for germ tube test were subjected to molecular analysis to reveal their various strains. All the eight (8) strains were tested for their susceptibility.

#### 4.1.2 Molecular characteristics of the yeast isolate

The results from the molecular analysis revealed the identity of various strains as shown in Table 4.2. The polymerase chain reaction (PCR), sequencing and subsequent blast revealed the following strains with their corresponding ascension numbers:strain1161 (AF075293.1)sample(S5),SC5314(CP025163.1)sample(B7),SC5314(CP025160.1)sample (C1),SC5314(CP0176251.1)sample(C2),SC5314(XM_712981.2)sample(S3),P37005(AP023893.1)sample(B4), RM1000(AB_017634.2) sample (B2) and SC5314(XM_ 707553.2).

**Table 4.1: Biochemical Characteristics of Yeast isolates from Clinical Samples (Test Organisms)**

| | | | | SUGAR FERMENTATION TEST | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S/N | CODE ISOLATE | REACTION GRAM | SHAPE | GLUCOSE | FRUCTOSE | SORBITOL | D-MANNITO | LACTOSE | SUCROSE | MANNOSE | ARABINOSE | GALACTOSE | GERMTUBETEST | INFERENCE |
| 1 | B1 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |
| 2 | B2 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |
| 3 | B3 | + | OVAL | + | + | - | - | - | - | - | - | - | + | *Candida albicans* |
| 4 | B4 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |
| 5 | B5 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |
| 6 | B6 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |
| 7 | B7 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |
| 8 | B8 | + | OVAL | + | + | - | - | - | - | - | - | - | - | *Candida glabrata* |
| 9 | B9 | + | OVAL | + | + | - | - | - | - | - | - | - | - | *Candida glabrata* |
| 10 | B10 | + | OVAL | + | + | - | - | - | - | - | - | - | - | *Candida glabrat* |
| 11 | S1 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |
| 12 | S2 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |
| 13 | S3 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |
| 14 | S4 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |
| 15 | S5 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |
| 16 | S6 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |
| 17 | S7 | + | OVAL | + | + | - | - | - | - | - | - | - | - | *Candida tropicalis* |
| 18 | S8 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |

**Table 4.1: Biochemical Characteristics of Yeast isolates from Clinical Samples (Test Organisms) (Cont'd)**

| S/N | ISOLATE CODE | GRAM REACTION | SHAPE | GLUCOSE | FRUCTOSE | SORBITOL | D-MANNITO | LACTOSE | SUCROSE | MANNOSE | ARABINOSE | GALACTOSE | GERMTUBETEST | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | SUGAR FERMENTATION TEST | | | | | | | | | | |
| 19 | S9 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |
| 20 | S10 | + | OVAL | + | + | - | - | - | - | - | - | + | - | *Candida albicans* |
| 21 | G1 | + | OVAL | + | + | - | - | - | - | - | - | - | + | *Candida albicans* |
| 22 | G2 | + | OVAL | + | + | - | - | - | - | - | - | + | - | *Candida albicans* |
| 23 | G3 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |
| 24 | G4 | + | OVAL | + | + | - | - | - | - | - | - | + | + | *Candida albicans* |
| 25 | G5 | + | OVAL | + | + | - | - | - | - | - | - | + | - | *Candida albicans* |
| 26 | G6 | + | OVAL | + | + | - | - | - | - | - | - | - | - | *Candida glabrata* |
| 27 | G7 | + | OVAL | + | + | - | - | - | - | - | - | - | - | *Candida glabrata* |
| 28 | G8 | + | OVAL | + | + | - | - | - | - | - | - | - | - | *Candida glabrat* |
| 29 | G9 | + | OVAL | + | + | - | - | - | - | - | - | + | - | *Candida albicans* |
| 30 | G10 | + | OVAL | + | + | - | - | - | - | - | - | + | - | *Candida albicans* |

**Table 4.2: Molecular Characteristics of the Yeast Isolate**

| Isolate code | Strains | Maxscore | Total score | Query cover | Expected value | Identity | Ascension number |
|---|---|---|---|---|---|---|---|
| S5 | STRAIN1161 | 8000 | 9822 | 100% | 0.0 | 100% | AF075293.1 |
| B7 | SC5314 | 238 | 5243 | 100% | 0.0 | 100% | CP025163.1 |
| C1 | SC5314 | 200 | 4680 | 91% | 0.0 | 97% | CP025160.1 |
| C2 | SC5314 | 8543 | 11950 | 100% | 0.0 | 100% | CP017625.1 |
| S3 | SC5314 | 7468 | 8080 | 100% | 0.0 | 100% | XM_712981.2 |
| B4 | P37005 | 7542 | 5850 | 100% | 0.0 | 100% | APO23893.1 |
| B2 | RM1000 | 700 | 8811 | 100% | 0.0 | 100% | AB_017634.2 |
| S1 | SC5314 | 444 | 444 | 100% | 0.0 | 100% | XM_707553.2 |

### 4.1.3 Percentage yield for the crude extracts of *Vernonia amygdalina*

Percentage yields of the crude extracts of *Vernonia amygdalina* plant for the leaves, stembark and root is shown in Table 4.3. The milled plant samples were extracted with n – hexane, ethyl acetate and methanol.The leaves of *Vernonia amygdalina* had a percentage yield of 11.42, 0.39 and 8.28 % which were obtained in n- hexane leaf extract (NHLE), ethyl acetate leaf extract (EALE) and methanol leaf extract (MLE) respectively. NHLE had the highest percentage yield of 11.42 % and the lowest yield was obtained in EALE 0.39 %. The percentage yield of methanol stembark extract (MSBE) 6.77 % was the highest in percentage for the stembark followed by n- hexane stembark extract (NHSBE) with a percentage of 3.11 % ethyl acetate stembark extract (EASBE) had the lowest percentage of 1.82 %. On the other hand the root of *Vernonia amygdaina* had a percentage of 10.16, 12.19 and 8.33 % obtained for n- hexane root extract (NHRE), ethyl acetate root extract (EARE) and methanol root extract (MRE) respectively.

### 4.1.4 Percentage yield for the crude extracts of *Moringa oleifera*

The percentage yields of the crude extract of *Moringa oleifera* for the leaves, stembark and root is shown in Table 4.4. The leaves of *Moringa oleifera* had a percentage yield of 3.3, 9.24 and 4.51 % which were obtained in NHLE, EALE and MLE respectively. EALE had the highest percentage yield of 9.24 % and the lowest yield was NHLE 3.39 %. The stem bark had a percentage yield of 3.79, 5.56 and 3.49 % for NHSBE EASBE and MSBE respectively. The EASBE had the highest percentage yield of 5.56 % and the lowest was MSBE with a percentage yield of 3.49 %. The root of *Moringa oleifera* had a percentage of 4.59, 7.14 and 5.33% which was obtained for NHRE, EARE and MRE respectively.

### 4.1.5 Percentage yield for the crude extracts of *Azardirachta indica*

The percentage yields of the crude extract of *Azardirachta indica* for the leaves, stem bark and root is presented in Table 4.5. The leaves of *Azardirachta indica* had a percentage yield of 7.12, 2.61 and 6.29 % which were obtained in NHLE, EALE and MLE respectively. NHLE had the highest percentage yield of 7.12 % and the lowest yield was obtained in EALE 2.61 %. The stembark had a percentage yield of 8.14 , 11.34 and 3.4 % for NHSBE, EASBE and MSBE respectively . The EASBE was the highest with a percentage yield of 11.34 % while MSBE had the lowest percentage yield of 3.4 %. The root of *Azardirachta indica* had a percentage of 3.39, 4.41 and 5.22 % for NHRE, EARE and MRE respectively.

### 4.1.6 Percentage yield for the crude extracts of *Vitellaria paradoxa*

The values for the the percentage yields of the crude extracts of *Vitellaria paradoxa* for the leaves, stem bark and root is shown in Table 4.6 .The leaves of *Vitellaria paradoxa* had a percentage of 9.22, 3.6 and 15.5 % which were obtained from NHLE , EALE and

MLE respectively. MLE had the highest percentage yield of 15.5 % and the lowest was EALE 3.6 %. The stembark of *Vitellaria paradoxa* had a percentage yield of 3.55, 10.72 and 8.52 % which were obtained from NHSBE, EASBE and MSBE respectively. EASBE had the highest percentage yield of 10.72 % while NHSBE was the lowest in percentage yield of 3.55 % .The root of *Vitellaria paradoxa* had a percentage yield of 5.42, 4.38 and 4.62 % for NHRE, EARE and MRE respectively.

**Table 4.3: Percentage Yield of *Vernonia amygdalina* Plant for the Leaves, Stembark and Root**

| | | Leaves | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Plant sample | WS/DP(g) | NHLEg(%) | EALEg(%) | MLEg(%) | NHSBEg(%) | EASBEg(%) | MSBEg(%) | NHREg(%) | EAREg(%) | MREg(%) |
| *Vernonia Amygdalina* | 100 | 11.42(11.42) | 0.39(0.39) | 8.28(8.28) | 3.11(3.11) | 1.82(1.82) | 6.77(6.77) | 10.16(10.16) | 12.19(12.19) | 8.33(8.33) |

**Table 4.4: Percentage Yield of *Moringa oleifera* Plant for the Leaves, Stem bark and Root**

| | | Leaves | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Plant sample | WS/DP(g) | NHLEg(%) | EALEg(%) | MLEg(%) | NHSBEg(%) | EASBEg(% ) | MSBEg(%) | NHREg(%) | EAREg(%) | MREg(% |
| *Moringa oleifera* | 100 | 3.39(3.39) | 9.24(9.24) | 4.51(4.51) | 3.79(3.79) | 5.56(5.56) | 3.49(3.49) | 4.59(4.59) | 7.14(7.14) | 5.33(5.33) |

Key: WS/DP: Weight of sample/ weight of dried powered material, NHLE: n-hexane leaf extract, EALE: Ethyl acetate leaf extract, MLE: Methanol leaf extract, NHSBE: n-hexane stembark extract, EASBE: Ethyl acetate stembark extract, MSBE: Methanol stembark extract, NHRE: n-hexane root extract , EARE: Ethyl acetate root extract MRE: Methanol root extract

**Table 4.5: Percentage Yield of *Azardirachta indica* Plant for the Leaves, Stembark and Root**

| | | Leaves | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Plant sample | WS/DP(g) | NHLEg(%) | EALEg(%) | MLEg(%) | NHSBEg(%) | EASBEg(%) | MSBEg(%) | NHREg(%) | EAREg(%) | MREg(%) |
| *Azardirachta Indica* | 100 | 7.12(7.12) | 2.61(2.61) | 6.29(6.29) | 8.14(8.14) | 11.34(11.34) | 3.4(3.4) | 3.39(3.39) | 4.41(4.41) | 5.22(5.22) |

**Table 4.6: Percentage Yield of *Vitellaria paradoxa* Plant for the Leaves, Stem bark and Root**

| | | Leaves | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Plant sample | WS/DP(g) | NHLEg(%) | EALEg(%) | MLEg(%) | NHSBEg(%) | EASBEg(%) | MSBEg(%) | NHREg(%) | EAREg(%) | MREg(%) |
| *Vitellaria Paradoxa* | 100 | 9.22(9.22) | 3.6(3.60) | 15.5(15.5) | 3.55(3.55) | 10.72(10.72) | 8.52(8.52) | 5.42(5.42) | 4.38(4.38) | 4.62(4.62) |

Key: WS/DP: Weight of sample/ weight of dried powered material, NHLE :n-hexane leaf extract, EALE: Ethyl acetate leaf extract, MLE: Methanol leaf extract, NHSBE: n-hexane stembark extract, EASBE: Ethyl acetate stembark extract, MSBE: Methanol stembark extract, NHRE: n-hexane root extract , EARE: Ethyl acetate root extract MRE: Methanol root extract

### 4.1.7 Phytochemical composition of *Vernonia amygdalina* leaf crude extracts obtained using three different solvents

The quantitative phytochemical result of n- hexane leaf extract(NHLE), ethyl acetate leaf extract (EALE) and Methanol leaf extract (MLE) of *Vernonia amygdalina* is shown in Table 4.7. The n- hexane leaf extract (NHLE) of *Vernonia amygdalina* had phytic acid (160.78±0.58 mg/100g) as the highest in composition while oxalate (5.65± 0.58 mg/100g) was the lowest. The composition of others were flavonoid (60.88±0.58 mg/100g), phenols (113.46±0.58 mg/100g), tannin (68.32±0.58 mg/100g), alkaloid (132.11±0.58 mg/100g) and saponins (40.22±0.58 mg/100g). The ethyl acetate leaf extract (EALE) of *Vernonia amygdalina* also had phytic acid (45.63±0.58 mg/100g) as the highest in composition while oxalate (4.55±0.58 mg/100g) was the lowest. The composition of others were flavonoid (24.96±0.58 mg/100g), phenols (38.69±0.58 mg/100g), tannin (19.88±0.58 mg/100g), alkaloid (18.66.±0.58 mg/100g) and saponins (20.44±0.58 mg/100g). Methanol leaf extract had phenols (178.92±0.58 mg/100g) as the highest in amount while oxalate (5.30±0.58 mg/100g) was the lowest. The composition of others were flavonoid (79.28±0.58 mg/100g), tannin (98.92±0.58 mg/100g), alkaloid (135.65±0.58 mg/100g), saponins (49.16±0.58 mg/100g) and phytic acid (70.59±0.58 mg/100g).

### 4.1.8 Phytochemical composition of *Vernonia amygdalina* Stembark crude extracts obtained using three different solvents

The results obtained from the quantitative phytochemical determination of *Vernonia amygdalina* is shown in Table 4.8. The n- hexane stembark extract (NHSBE) of *Verninia amygdalina* had phytic acid (149.66±0.58 mg/100g) as the highest in amount while oxalate (5.13± 0.58 mg/100g) was the lowest. The composition of others were flavonoid (49.11±0.58 mg/100g), phenols(60.99±0.58 mg/100g), tannin (40.11±0.58

mg/100g), alkaloid (139.66±0.58 mg/100g) and saponins (38.12.±0.58 mg/100g). The ethyl acetate stem bark extract (EASBE) of *Vernonia amygdalina* also had phytic acid (50.11±0.58 mg/100g) as the highest in while oxalate (4.99±0.58 mg/100g) was the lowest. The composition of others were flavonoid (44.98±0.58 mg/100g), phenols (33.88.±0.58 mg/100g), tannin (14.11±0.58 mg/100g), alkaloid (24.88±0.58 mg/100g) and saponins (29.11.±0.58 mg/100g). In methanol stembark extract (MSBE) alkaloid had the highest composition (133.44±0.58 mg/100g) while oxalate (5.48±0.58 mg/100g) was the lowest. The composition of others were flavonoid (50.88±0.58 mg/100g), phenols (89.66±0.58 mg/100g), tannin (44.44±0.58 mg/100g), saponins (38.88.±0.58 mg/100g) and phytic acid (66.39±0.58 mg/100g).

### 4.1.9 Phytochemical composition of *Vernonia amygdalina* root crude extracts obtained from three different solvents

The values obtained from the quantitative phytochemical determination of *Vernonia amygdalina* is shown in Table 4.9. The n- hexane root extract of (NHRE) of *Vernonia amygalina* had phytic acid (147.22±0.58 mg/100g) as the highest in composition while oxalate (5.21± 0.51 mg/100g) was the lowest. The composition of others were flavonoid (56.98±0.58 mg/100g), phenols (129.34±4.58 mg/100g), tannin (76.13.±0.58 mg/100g), alkaloid (130.86±0.69 mg/100g) and saponins (39.99±0.58 mg/100g). The ethyl acetate root extract of (EARE) also had phytic acid (39.33±0.58 mg/100g) as the highest in composition while oxalate (3.99±0.51 mg/100g) was the lowest. The composition of others were flavonoid (36.88±0.58 mg/100g), phenols (45.96±4.58 mg/100g), tannin (12.32.±0.58 mg/100g), alkaloid (18.96±0.69 mg/100g) and saponins (21.66±0.58 mg/100g). Methanol root extract(MRE) had alkaloid (138.98±0.69 mg/100g) as the highest in amount while oxalate (3.88±0.51 mg/100g) was the lowest. The composition of others were flavonoid (60.83±0.58 mg/100g), phenols (99.17±4.58 mg/100g), tannin

(91.29.±0.58 mg/100g), alkaloid (138.98±0.69 mg/100g), saponins (43.8±0.58 mg/100g) and phytic acid (53.88±0.58 mg/100g).

**Table 4.7: Phytochemical Composition of *Vernonia amygdalina* Leaf Crude Extracts Using Three Different Solvents**

| | Phytochemicals(mg/100g) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | **Flavonoids** | **Phenols** | **Tannins** | **Alkaloids** | **Saponins** | **Phytic acid** | **Oxalate** |
| n- hexane | 60.88±0.58$^{b}$ | 113.46±0.58$^{b}$ | 68.32±0.58$^{b}$ | 132.11±0.58$^{b}$ | 40.22±0.58$^{b}$ | 160.78±0.58$^{a}$ | 5.65±0.58$^{a}$ |
| Ethyl acetate | 24.96±0.58$^{c}$ | 38.69±0.58$^{c}$ | 19.88±0.58$^{c}$ | 18.66±0.58$^{c}$ | 20.44±0.58$^{c}$ | 45.63±0.58$^{c}$ | 4.55±0.58$^{a}$ |
| Methanol | 79.28±0.58$^{a}$ | 178.92±0.58$^{a}$ | 98.92±0.58$^{a}$ | 135.65±0.58$^{a}$ | 49.16±0.58$^{a}$ | 70.59±0.58$^{b}$ | 5.30±0.58$^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at p≤0.05

**Table 4.8 Quantitative Phytochemical Composition of *Vernonia amygdalina* Stembark Crude Extracts Using Three Different Solvents**

| | Phytochemicals (mg/100g) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | **Flavonoids** | **Phenols** | **Tannins** | **Alkaloids** | **Saponins** | **Phytic acid** | **Oxalate** |
| n- hexane | 49.11±0.58$^{a}$ | 60.99±0.58$^{b}$ | 40.11±0.58$^{b}$ | 139.66±0.58$^{a}$ | 38.12±0.58$^{a}$ | 149.66±0.58$^{a}$ | 5.13±0.58$^{a}$ |
| Ethyl acetate | 44.98±0.58$^{b}$ | 33.88±0.58$^{c}$ | 14.11±0.58$^{c}$ | 24.88±0.58$^{c}$ | 29.11±0.58$^{b}$ | 50.11±0.58$^{c}$ | 4.99±0.58$^{b}$ |
| Methanol | 50.88±0.58$^{a}$ | 89.66±0.58$^{a}$ | 44.44±0.58$^{a}$ | 133.44±0.58$^{b}$ | 38.88±0.58$^{a}$ | 66.39±0.58$^{b}$ | 5.48±0.58$^{c}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at p≤0.05

**Table 4.9: Phytochemical Composition of *Vernonia amygdalina* Root Crude Extracts Using Three Different Solvents**

| | Phytochemicals(mg/100g) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | **Flavonoids** | **Phenols** | **Tannins** | **Alkaloids** | **Saponins** | **Phytic acid** | **Oxalate** |
| n- hexane | 56.98±0.58$^{b}$ | 129.34±4.58$^{a}$ | 76.13±0.58$^{b}$ | 130.86±0.69$^{a}$ | 39.99±0.58$^{b}$ | 147.22±0.58$^{a}$ | 5.21±0.51$^{a}$ |
| Ethyl acetate | 36.88±0.58$^{c}$ | 45.96±4.58$^{c}$ | 12.32±0.58$^{c}$ | 18.96±0.69$^{c}$ | 21.66±0.58$^{c}$ | 39.33±0.58$^{c}$ | 3.99±0.51$^{b}$ |
| Methanol | 60.83±0.58$^{a}$ | 99.17±4.58$^{b}$ | 91.29±0.58$^{a}$ | 138.98±0.69$^{a}$ | 43.8±0.58$^{a}$ | 53.88±0.58$^{b}$ | 3.88±0.51$^{b}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at p≤0.05

### 4.1.10 Phytochemical composition of *Moringa oleifera* leaf crude extracts obtained from three different solvents

The results obtained from the quantitative phytochemical determination of *Moringa oleifera* leaf is shown in Table 4.10. The n- hexane leaf extract of *Moringa oleifera* (NHLE) had phytic acid (143.42±0.60 mg/100g) as the highest in amount while oxalate (2.17± 0.58 mg/100g) was the lowest. The composition of others were flavonoid (56.55±0.58 mg/100g), phenols (79.88±0.58 mg/100g), tannin (30.89±0.58 mg/100g), alkaloid (121.98±0.58 mg/100g) and saponins (18.69±0.58 mg/100g). The ethyl acetate leaf extract (EALE) of *Moringa oleifera* also had phytic acid (60.11±0.60 mg/100g) as the highest while oxalate (1.21±0.58 mg/100g) was the lowest. The composition of others were flavonoid (17.55±0.58 mg/100g), phenols (19.68±0.58 mg/100g), tannin (12.89±0.58 mg/100g), alkaloid (11.11±0.58 mg/100g) and saponins (14.83±0.58 mg/100g). Methanol leaf extract (MLE) had phenols (178.11±0.58 mg/100g) as the highest in composition while oxalate (2.19±0.58 mg/100g) was the lowest. The composition of others were flavonoid (153.98±0.58 mg/100g), tannin (98.77±0.58 mg/100g), alkaloid (128.66±0.58 mg/100g), saponins (22.22.±0.58 mg/100g) and phytic acid (120.57±0.60 mg/100g).

### 4.1.11 Phytochemical composition of *Moringa oleifera* stembark crude extracts obtained from three different solvents

The quantitative phytochemical results of *Moringa oleifera* stembark is shown in Table 4.11. The n- hexane stembark extract of *Moringa oleifera* (NHSBE) had phytic acid (130.19±0.58 mg/100g) as the highest in composition while oxalate (2.10± 0.58 mg/100g) was the lowest in composition. The composition of others were flavonoid (60.23±0.58 mg/100g), phenols (97.78±0.51 mg/100g), tannin (40.13±0.58 mg/100g), alkaloid (139.32.±0.51 mg/100g) and saponins (30.98.±0.58 mg/100g). The ethyl acetate leaf extract (EALE) of *Moringa oleifera* also had phytic acid (55.13±0.58

mg/100g) as the highest in composition while oxalate (2.99±0.58 mg/100g) was the lowest. The composition of others were flavonoid (19.11±0.58 mg/100g), phenols(25.98±0.51 mg/100g), tannin (15.13±0.58 mg/100g), alkaloid (40.98±0.51 mg/100g) and saponins (18.16±0.58 mg/100g). Methanol leaf extract (MLE) had phenols (160.81±0.51 mg/100g) as the highest in composition while oxalate (2.00±0.58 mg/100g) was the lowest in composition. The composition of others were flavonoid (143.88.±0.58 mg/100g), tannin (82.95±0.58 mg/100g), alkaloid (134.90±0.51 mg/100g), saponins (44.00±0.58 mg/100g) and Phytic acid (138.88±0.58 mg/100g).

**4.1.12 Phytochemical composition of *Moringa oleifera* root crude extracts obtained from three different solvents**

The quantitative phytochemical results of n- hexane root extract of *Moringa oleifera* (NHRE) is shown in Table 4.12. Phenols (142.81±0.58 mg/100g) was the highest in composition while oxalate (2.21± 0.69 mg/100g) was the lowest. The composition of others were flavonoid (110.00±0.58 mg/100g), tannin (43.00.±0.58 mg/100g), alkaloids (132.11±0.58 mg/100g), saponins (19.00±6.08 mg/100g) and phytic acid (139.50± 0.58 mg/100g). The ethyl acetate root extract (EARE) had phytic acid (40.23±0.58 mg/100g) as the highest in amount while oxalate (1.21±0.69 mg/100g) was the lowest. The composition of others were flavonoid (14.00±0.58 mg/100g), phenols (23.50.±0.58 mg/100g), tannins (12.00±0.58 mg/100g), alkaloids(10.00.±0.58 mg/100g) and saponins (14.99±6.08 mg/100g). Methanol root extract (MRE) had phenols (162.91.±0.58 mg/100g) as the highest in amount while oxalate (1.79±0.69 mg/100g) was the lowest. The composition of others were flavonoids (148.88± 0.58 mg/100g), tannins (53.13.±0.58 mg/100g), alkaloids (128.17±0.58 mg/100g) and saponins (21.44±6.08 mg/100g)

**Table 4.10: Phytochemical Composition of *Moringa oleifera* Leaf Crude Extracts Using Three Different Solvents**

| | Phytochemicals (mg/100g) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | **Flavonoids** | **Phenols** | **Tannins** | **Alkaloids** | **Saponins** | **Phytic acid** | **Oxalate** |
| n- hexane | $56.55 \pm 0.58^b$ | $79.88 \pm 0.58^b$ | $30.89 \pm 0.58^b$ | $121.98 \pm 0.58^b$ | $18.69 \pm 0.58^b$ | $143.42 \pm 0.60^b$ | $2.17 \pm 0.58^a$ |
| Ethyl acetate | $17.55 \pm 0.58^c$ | $19.68 \pm 0.58^c$ | $12.89 \pm 0.58^c$ | $11.11 \pm 0.58^c$ | $14.83 \pm 0.58^c$ | $60.11 \pm 0.60^c$ | $1.21 \pm 0.58^a$ |
| Methanol | $153.98 \pm 0.58^a$ | $178.11 \pm 0.58^a$ | $98.77 \pm 0.58^a$ | $128.66 \pm 0.58^a$ | $22.22 \pm 0.58^a$ | $120.57 \pm 0.60^a$ | $2.19 \pm 0.58^a$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column

do not differ significantly at $p \leq 0.05$

**Table 4.11: Phytochemical Composition of *Moringa oleifera* Stembark Crude Extracts Using Three Different Solvents**

| | Phytochemicals (mg/100g) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | **Flavonoids** | **Phenols** | **Tannins** | **Alkaloids** | **Saponins** | **Phytic acid** | **Oxalate** |
| n- hexane | $60.23 \pm 0.58^b$ | $97.78 \pm 0.51^b$ | $40.13 \pm 0.58^b$ | $139.32 \pm 0.51^a$ | $30.98 \pm 0.58^b$ | $130.19 \pm 0.58^b$ | $2.10 \pm 0.58^a$ |
| Ethyl acetate | $19.11 \pm 0.58^c$ | $25.98 \pm 0.51^c$ | $15.13 \pm 0.58^c$ | $40.98 \pm 0.51^c$ | $18.16 \pm 0.58^c$ | $55.13 \pm 0.58^c$ | $2.99 \pm 0.58^a$ |
| Methanol | $143.88 \pm 0.58^a$ | $160.81 \pm 0.51^a$ | $82.95 \pm 0.58^a$ | $134.90 \pm 0.51^b$ | $44.00 \pm 0.58^a$ | $138.88 \pm 0.58^a$ | $2.00 \pm 0.58^b$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.12: Phytochemical Composition of *Moringa oleifera* Root Crude Extracts Using Three Different Solvents**

| | Phytochemicals (mg/100g) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | **Flavonoids** | **Phenols** | **Tannins** | **Alkaloids** | **Saponins** | **Phytic acid** | **Oxalate** |
| n- hexane | 110.00±0.58[b] | 142.81±0.58[b] | 43.00±0.58[a] | 132.11±0.58[a] | 19.00±6.08[b] | 139.50±0.58[a] | 2.21±0.69[a] |
| Ethyl acetate | 14.00±0.58[c] | 23.50±0.58[c] | 12.00±0.58[c] | 10.00±0.58[c] | 14.99±6.08[b] | 40.23±0.58[c] | 1.21±0.69[a] |
| Methanol | 148.88±0.58[a] | 162.91±0.58[a] | 53.13±0.58[a] | 128.17±0.58[b] | 21.44±6.08[a] | 125.98±0.58[b] | 1.79±0.69[a] |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

### 4.1.13 Phytochemical composition of *Azadirachta indica* leaf crude extracts obtained from three different solvents

The quantitative phytochemical result of n- hexane leaf extract (NHLE) of *Azadirachta indica* is shown in Table 4.13. Alkaloid (183.44±0.64 mg/100g) was the highest in composition while oxalate (2.46± 0.58 mg/100g) was the lowest. The composition of others were flavonoid (61.66±0.58 mg/100g), phenols (130.81±0.58 mg/100g), tannins (56.66±0.58 mg/100g), and saponins (90.00±0.58 mg/100g). The ethyl acetate leaf extract (EALE) of *Azadirachta indica* had phytic acid (80.11±0.58 mg/100g) as the highest in composition while oxalate (2.39±0.58 mg/100g) was the lowest. The composition of others were flavonoid (30.32±0.58 mg/100g), phenols (39.96±0.58 mg/100g), tannins (13.68±0.58 mg/100g), alkaloids (17.50±0.64 mg/100g) and saponins (19.11±0.58 mg/100g). Methanol leaf extract (MLE) had alkaloids (169.80±0.64 mg/100g) as the highest in composition while oxalate (2.87±0.58 mg/100g) was the lowest. The composition of others were flavonoid (68.61±0.58 mg/100g), phenols (118.51±0.58 mg/100g), tannins (86.80±0.58 mg/100g) and saponins (40.20±0.58 mg/100g).

### 4.1.14 Phytochemical composition of *Azadirachta indica* stembark crude extracts obtained from three different solvents

The quantitative phytochemical result for the n- hexane stembark extract (NHSBE) of *Azadirachta indica* is shown in Table 4.14. Alkaloid (133.33±0.58 mg/100g) was the highest in composition while oxalate (2.29± 0.69 mg/100g) was the lowest. The composition of others were flavonoid (59.66±2.08 mg/100g), phenols (120.68±0.58 mg/100g), tannin (38.61±0.58 mg/100g), saponins (43.33±0.58 mg/100g) and phytic acid (2.90±0.58 mg/100g). The ethyl acetate stembark extract(EASBE) of *Azadirachta indica* had phenols (40.19±0.58 mg/100g) as the highest in composition while oxalate

(1.61±0.69 mg/100g) was the lowest. The composition of others were flavonoid (38.11±2.08 mg/100g), tannin (31.23.±0.58 mg/100g), alkaloids (24.66 ±0.58 mg/100g), saponins (18.19±0.58 mg/100g) and phytic acid (1.10±0.58 mg/100g). Methanol stembark extract (MSBE) had alkaloids (168.00±0.58 mg/100g) as the highest in composition while oxalate (2.99±0.69 mg/100g) was the lowest. The composition of others were flavonoid (63.56±2.08 mg/100g), phenols (131.11.±0.58 mg/100g), tannins (76.50±0.58 mg/100g) saponins (31.11±0.58 mg/100g) and phytic acid (2.60±0.58 mg/100g).

**4.1.15 Phytochemical composition of *Azadirachta indica* root crude extracts obtained from three different solvents**

The values obtained from the quantitative phytochemical result of n- hexane root extract (NHRE) of *Azadirachta indica* is shown in Table 4.15. Phytic acid (199.53±0.52 mg/100g) was the highest in composition while oxalate (2.33± 0.58 mg/100g) was the lowest. The composition of others were flavonoid (80.89±0.58 mg/100g), phenols (151.51±0.58 mg/100g), tannin (96.00±0.58 mg/100g), alkaloid (166.66.±0.58 mg/100g) and saponins (70.77±0.69 mg/100g). The ethyl acetate root extract (EARE) of *Azadirachta indica* had phytic acid (60.99±0.52 mg/100g) as the highest in composition while oxalate (2.17±0.58 mg/100g) was the lowest . The composition of others were flavonoid (19.19.±0.58 mg/100g), phenols (40.13±0.58 mg/100g), tannin (24.23±0.58 mg/100g), alkaloid (33.33..±0.58 mg/100g) and saponins (21.11±0.69 mg/100g). Methanol root extract (MRE) had phytic acid (201.20.±0.52 mg/100g) as the highest in compostion while oxalate (2.50±0.58 mg/100g) was the lowest . Others were flavonoid (59.66.±0.58 mg/100g), phenols (121.21±0.58 mg/100g), tannin (70.00±0.58 mg/100g), alkaloid (138.46..±0.58 mg/100g) and saponins (133.77±0.69 mg/100g).

**Table 4.13: Phytochemical Composition of *Azadirachta indica* Leaf Crude Extracts Using Three Different Solvents**

| | Phytochemicals (mg/100g) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | **Flavonoids** | **Phenols** | **Tannins** | **Alkaloids** | **Saponins** | **Phytic acid** | **Oxalate** |
| n- hexane | 61.66±0.58[b] | 130.81 ±0.58[a] | 56.66 ±0.58[b] | 183.44±0.64[b] | 90.00±0.58[a] | 2.14±0.58[c] | 2.46±0.58[b] |
| Ethylacetae | 30.32±0.58[c] | 39.96 ±0.58[c] | 13.68 ±0.58[c] | 17.50±0.64[b] | 19.11±0.58[c] | 80.11±0.58[b] | 2.39±0.58[c] |
| Methanol | 68.61±0.5[a] | 118.51 ±0.58[b] | 86.80 ±0.58[a] | 169.80 ±0.64[b] | 40.20±0.58[b] | 89.36±0.58[a] | 2.87±0.58[a] |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.14: Phytochemical Composition of *Azadirachta indica* Stem bark Crude Extracts using Three Different Solvents**

| | Phytochemicals (mg100/g) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | **Flavonoids** | **Phenols** | **Tannins** | **Alkaloids** | **Saponins** | **Phytic acid** | **Oxalate** |
| n- hexane | 59.66±2.08[ab] | 120.68±0.58[b] | 38.61±0.58[a] | 133.33±0.58[b] | 43.33±0.58[a] | 2.90±0.58[a] | 2.29±0.69[b] |
| Ethyl acetate | 38.11±2.08[c] | 40.19±0.58[c] | 31.23±0.58[c] | 24.66±0.58[c] | 18.19±0.58[c] | 1.10±0.58[c] | 1.61±0.69[c] |
| Methanol | 63.56±2.08[a] | 131.11±0.58[a] | 76.50±0.58[a] | 168.00±0.58[a] | 31.11±0.58[b] | 2.60±0.58[b] | 2.99±0.69[a] |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.15: Phytochemical Composition of *Azadirachta indica* Root Crude Extracts Using Three Different Solvents**

| | Phytochemicals (mg/100g) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | **Flavonoids** | **Phenols** | **Tannins** | **Alkaloids** | **Saponins** | **Phytic acid** | **Oxalate** |
| n- hexane | 80.89±0.58[a] | 151.51±0.58[a] | 96.00±0.58[a] | 166.66±0.58[a] | 70.77±0.69[b] | 199.53±0.52[b] | 2.33±0.58[b] |
| Ethylacetate | 19.19±0.58[c] | 40.13 ±0.58[c] | 24.23±0.58[b] | 33.33 ±0.58[b] | 21.11±0.69[c] | 60.99±0.52[c] | 2.17±0.58[c] |
| Methanol | 59.66±0.58[b] | 121.21±0.58[b] | 70.66±0.58[c] | 138.46±0.58[b] | 133.77±0.69[a] | 201.20±0.52[a] | 2.50±0.58[a] |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

### 4.1.16 Phytochemical composition of *Vitellaria paradoxa* leaf crude extracts obtained from three different solvents

The result of the quantitative phytochemical determination of the n- hexane leaf extract(NHLE) of *Vitellaria paradoxa* is shown in Table 4.16. Phytic acid (136.00±0.58 mg/100g) was the highest in composition while oxalate (4.65± 0.58 mg/100g) was the lowest . Others were flavonoid (38.99±0.58 mg/100g), phenols (50.11±0.52 mg/100g), tannin (38.00±0.52 mg/100g), alkaloid (93.75±0.58 mg/100g), and saponins (14.91±0.58 mg/100g). The ethyl acetate leaf extract (EALE) of *Vitellaria paradoxa* had phytic acid (90.78±0.58 mg/100g) as the highest in amount while oxalate (4.55±0.58 mg/100g) was the lowest. The composition of others were flavonoid (21.64±0.58 mg/100g), phenols (38.66±0.52 mg/100g), tannins (20.55.±0.52 mg/100g), alkaloid (18.96.±0.58 mg/100g) and saponins (15.66±0.58 mg/100g). Methanol leaf extract (MLE) had phenols (208.58± 0.52 mg/100g) as the highest in composition while oxalate (4.99±0.58 mg/100g) was the lowest. The composition of others were flavonoid (40.44±0.58 mg/100g), tannins (95.37 ±0.52 mg/100g), alkaloid (49.57.±0.58 mg/100g), saponins (51.40±0.58 mg/100g) and phytic acid (132.22±0.58 mg/100g).

### 4.1.17 Phytochemical composition of *Vitellaria paradoxa* stembark crude extracts obtained from three different solvents

The result of the quantitative phytochemical determination of *Vitellaria paradoxa* stembark extract is shown in Table 4.17. The n- hexane stembark extract (NHSBE) of *Vitellaria paradoxa* had Phytic acid (128.24±0.58 mg/100g) as the highest in amount while oxalate (4.25± 0.58 mg/100g) was the lowest. The composition of others were flavonoid (98.99±0.58 mg/100g), phenols (110.11±0.58 mg/100g), tannin (66.81±0.58 mg/100g), alkaloid (114.86.±0.58 mg/100g) and saponins (30.88±0.58 mg/100g). The ethyl acetate leaf extract of *Vitellaria paradoxa* (EASBE) had phytic acid (70.28±0.58

mg/100g) as the highest in composition while oxalate (3.97±0.58 mg/100g) was the lowest. The composition of others were flavonoid (38.13±0.58 mg/100g), phenols (40.98±0.58 mg/100g), tannin (38.11±0.58 mg/100g), alkaloid (31.23.±0.58 mg/100g) and saponins (34.98±0.58 mg/100g). Methanol stembark extract(MSBE) had phytic acid (130.19±0.58 mg/100g) as the highest in amount while oxalate (4.69±0.58 mg/100g) was the lowest. The composition of others were flavonoid (116.38±0.58 mg/100g), phenols (117.56.±0.58 mg/100g), tannin (79.33±0.58 mg/100g), alkaloid (128.66±0.58 mg/100g) and saponins (53.65±0.58 mg/100g).

#### 4.1.18 Phytochemical composition of *Vitellaria paradoxa* root crude extracts obtained from three different solvents

The n- hexane root extract (NHRE) of *Vitellaria paradoza* is shown in Table 4.18. Phytic acid (129.67±2.26 mg/100g) showed the highest in composition while oxalate (4.22± 0.58 mg/100g) was the lowest. The composition of others were flavonoid (62.99±15.31 mg/100g), phenols (201.11±0.58 mg/100g), tannin (50.44±0.58 mg/100g), alkaloid (140.28±0.58 mg/100g) and saponins (70.82±0.58 mg/100g). The ethyl acetate root extract (EARE) of *Vitellaria paradoxa* had phytic acid (56.11±2.26 mg/100g) as the highest in composition while oxalate (3.53±0.58 mg/100g) was the lowest . The composition of others were flavonoid (30.36±15.31 mg/100g), phenols (28.12±0.58 mg/100g), tannin (18.63±0.58 mg/100g), alkaloid (17.11±0.58 mg/100g) and saponins (20.96±0.58 mg/100g). Methanol root extract(MRE) had alkaloid (151.5±0.58 mg/100g) as the highest in composition while oxalate (4.23±0.58 mg/100g) was the lowest . The composition of others were flavonoid (59.50±15.31 mg/100g), phenols (78.11.±0.58 mg/100g), tannin (69.4±0.58 mg/100g) and saponins (48.96±0.58 mg/100g).

**Table 4.16: Phytochemical Composition of *Vitellaria paradoxa* Leaf Crude Extracts Using Three Different Solvents**

| Extracts | Phytochemicals (mg/100g) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Flavonoids | Phenols | Tannins | Alkaloids | Saponins | Phytic acid | Oxalate |
| n- hexane | 38.99±0.58[b] | 50.11±0.52[b] | 38.00±0.52[b] | 93.75±0.58[a] | 14.91±0.58[b] | 136.00±0.58[a] | 4.65±0.58[a] |
| Ethyl acetate | 21.64±0.58[c] | 38.66±0.52[c] | 20.55±0.52[c] | 18.96±0.58[c] | 15.66±0.58[b] | 90.78±0.58[c] | 4.55±0.58[a] |
| Methanol | 40.44±0.58[a] | 208.58±0.52[a] | 95.37±0.52[a] | 49.57±0.58[b] | 51.40±0.58[a] | 132.22±0.58[b] | 4.99±0.58[a] |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.17: Phytochemical Composition of *Vitellaria paradoxa* Stembark Crude Extracts Using Three Different Solvents**

| | Phytochemicals (mg/100g) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | **Flavonoids** | **Phenols** | **Tannins** | **Alkaloids** | **Saponins** | **Phytic acid** | **Oxalate** |
| n- hexane | 98.99±0.58[b] | 110.11±0.58[a] | 66.81±0.58[b] | 114.86±0.58[b] | 30.88±0.58[b] | 128.24±0.58[a] | 4.25±0.58[a] |
| Ethyl acetate | 38.13±0.58[c] | 40.98±0.58[b] | 38.11±0.58[c] | 31.23±0.58[c] | 34.98±0.58[c] | 70.28±0.58[c] | 3.97±0.58[a] |
| Methanol | 116.38±0.58[a] | 117.56±0.58[c] | 79.33±0.58[a] | 128.66±0.58[a] | 53.65±0.58[a] | 130.19±0.58[a] | 4.69±0.58[a] |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.18: Phytochemical Composition of *Vitellaria paradoxa* Root Crude Extracts Using Three Different Solvents**

| | Phytochemicals (mg/100g) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | **Flavonoids** | **Phenols** | **Tannins** | **Alkaloids** | **Saponins** | **Phytic acid** | **Oxalate** |
| n- hexane | 62.99±15.31$^{a}$ | 201.11±0.58$^{b}$ | 50.44±0.58$^{b}$ | 140.28±0.58$^{b}$ | 70.82±0.58$^{a}$ | 129.67±2.26$^{a}$ | 4.22±0.58$^{a}$ |
| Ethyl acetate | 30.36±15.31$^{b}$ | 28.12±0.58$^{b}$ | 18.63±0.58$^{b}$ | 17.11±0.58$^{c}$ | 20.96±0.58$^{c}$ | 56.11±2.26$^{b}$ | 3.53±0.58$^{a}$ |
| Methanol | 59.50±15.31$^{b}$ | 78.11±0.58$^{b}$ | 69.4±0.58$^{b}$ | 151.5±0.58$^{a}$ | 48.96±0.58$^{b}$ | 134.93±2.26$^{a}$ | 4.23±0.58$^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

### 4.1.19. Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* (mm)

The antifungal activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* against strain 1161 (Isolate :S5) is presented in Table 4.19. The n-hexane leaf extract (NHLE) showed no antifungal activity at a concentrations of 40 and 60 mg/mL but at 80 and 100 mg/mL concentrations there was inhibitory activity and the zones of inhibition (ZOI) recorded was 8.00±0.20 and 13.00±0.70 mm respectively. Ethyl acetate leaf extracts (EALE) showed no inhibitory activity for all the varying concentrations used at 40, 60, 80 and 100 mg/mL. The methanol leaf extract (MLE) had inhibitory activity at a concentrations of 40, 60, 80 and 100 mg/mL. The zones of inhibitions were 7.00±0.10, 8.00±0.10, 10.00±0.70 and 15.33±1.23 mm respectively. There was no inhibitory activity for n-hexane, ethylacetate and methanol crude extracts for the stembark and root of *Vernonia amygdalina* at 40, 60, 80 and 100 mg/mL concentrations.

### 4.1.20. Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Moringa oleifera* (mm)

The antifungal activity of the leaf, stem bark and root of *Moringa oleifera* against strain 1161 (Isolate: S5) is shown in Table 4.20. There was no inhibitory activity for n-hexane, ethylacetate and methanol crude extracts of the leaf, stembark and root of *Moringa oleifera* against strain 1161 (Isolate: S5) at the various concentrations of 40, 60, 80 and 100 mg/mL.

### 4.1.21 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Azadirachta indica* (mm)

The antifungal activity of the leaf, stembark and root of *Azadirachta indica* against strain1161 (Isolate: S5) is shown in Table 4.21 .The n-hexane leaf extract (NHLE) showed no inhibitory activity at 40 and 60 mg/mL concentrations but there was inhibitory activity 16.00±0.20 and 20.33±1.04 mm at 80 and 100 mg/mL concentrations. Ethyl acetate leaf extract (EALE) showed no inhibitory activity at 40, 60, 80 and 100 mg/mL concentrations. The methanol leaf extract (MLE) also showed no activity at 40 and 60 mg/ml but there was activity 13.00±0.20 and 16.00± 0.20 mm at 80 and 100 mg/mL concentrations respectively. The n-hexane stem bark extract (NHSBE) showed no inhibitory activity at 40 and 60 mg/mL but there was inhibitory activity 7.00±0.20 and 9.00±0.70 mm at 80 and 100 mg/mL concentrations while the ethyl acetate and methanol extract of the stem bark had no activity . The root of n-hexane, ethyacetate and methanol extracts of *Azardirachta indica* showed no inhibitory activity at 40, 60, 80 and 100 mg/mL concentrations.

### 4.1.22 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vitellaria paradoxa* (mm)

The inhibitory activity of the leaf, stembark and root of *Vitellaria paradoxa* against strain1161 (Isolate: S5) is shown in Table 4.22. There was no inhibitory activity for n-hexane, ethylacetate and methanol crude extracts for the leaf, stembark and root of *Vitellaria paradoxa* against strain 1161 (Isolate: S5) at the various concentrations of 40, 60, 80 and 100 mg/mL concentrations.

**Table 4.19: Antifungal Inhibitory Activity of the Leaf, Stembark and Root of *Vernonia amygdalina* Extract (40-100mg/mL)Against Strain1161(Isolate:S5)(mm)**

| | **Leaf** | | | **Stembark** | | | **Root** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $7.00\pm0.10^{b}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |
| 60 | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $8.00\pm0.10^{c}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |
| 80 | $8.00\pm0.20^{b}$ | $0.00\pm0.00^{a}$ | $10.00\pm0.70^{d}$ | $0.00\pm0.00^{a}$ | 0.00±0.00a | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |
| 100 | $13.00\pm0.70^{c}$ | $0.00\pm0.00^{a}$ | $15.33\pm1.23^{e}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |
| Fluconazole(1mg/ml) | $42.00\pm0.40^{d}$ | $42.00\pm0.40^{b}$ | $42.00\pm0.40^{f}$ | $42.00\pm0.40^{b}$ | 42.00±0.40b | $42.00\pm0.40^{b}$ | $42.00\pm0.40^{b}$ | $42.00\pm0.40^{b}$ | $42.00\pm0.40^{b}$ |
| DMSO(100ul) | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | 0.00±0.00a |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p\leq0.05$

**Table 4.20: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Moringa oleifera* Extract (40-100mg/mL) Against Strain1161(Isolate:S5)(mm)**

| | **Leaf** | | | **Stembark** | | | **Root** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |
| 60 | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |
| 80 | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |
| 100 | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | 0.00±0.00a |
| Fluconazole(1mg/ml) | $42.00\pm0.40^{b}$ | $42.00\pm0.40^{b}$ | $42.00\pm0.40^{b}$ | $42.00\pm0.40^{b}$ | $42.00\pm0.40^{b}$ | $42.00\pm0.40^{b}$ | $42.00\pm0.40^{b}$ | $42.00\pm0.40^{b}$ | $42.00\pm0.40^{b}$ |
| DMSO(100ul) | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{b}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | 0.00±0.00a |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p\leq0.05$

**Table 4.21: Antifungal Inhibiroty Activity of the Leaf, Stem bark and Root of *Azadirachta indica* Extract (40-100mg/mL) Against Strain 1161(Isolate:S5) (mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| 60 | 0.00±0.20[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| 80 | 16.00±0.20[b] | 0.00±0.00[a] | 13.00±0.20[b] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| 100 | 20.33±1.04[c] | 0.00±0.00[a] | 16.00±0.20[c] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| Fluconazole(1mg/ml) | 42.00±0.40[d] | 42.00±0.40[b] | 42.00±0.40[d] | 42.00±0.40[b] | 42.00±0.40[b] | 42.00±0.40[b] | 42.00±0.40b | 42.00±0.40[b] | 42.00±0.40[b] |
| DMSO(100ul) | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.22: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Vitellaria paradoxa* Extract (40-100mg/mL) Against Strain 1161(Isolate: S5) (mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ |
| 60 | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ |
| 80 | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ |
| 100 | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ |
| Fluconazole(1mg/ml) | $42.00{\pm}0.40^{b}$ | $42.00{\pm}0.40^{b}$ | 42.00±0.40b | $42.00{\pm}0.40^{b}$ | $42.00{\pm}0.40^{b}$ | $42.00{\pm}0.40^{b}$ | $42.00{\pm}0.40^{b}$ | $42.00{\pm}0.40^{b}$ | $42.00{\pm}0.40^{b}$ |
| DMSO(100ul) | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | 0.00±0.00a | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ | $0.00{\pm}0.00^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

### 4.1.23 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* (mm)

Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* against strain RM1000 (Isolate :B2) is shown in Table 4.23. The n-hexane leaf extract (NHLE) showed no activity at 40 mg/mL concentration but at 60, 80 and 100 mg/mL there was inhibitory activity 6.00±0.20, 8.67±0.67 and 10.00±0.60 mm respectively. Ethyl acetate leaf extracts (EALE) showed no inhibitory activity for all the varying concentrations of 40, 60, 80 and 100 mg/mL. The methanol leaf extract (MLE) had no inhibitory activity at 40 and 60 mg/mL concentrations but there was inhibitory activity 6.00±0.20 and 8.00±0.20 mm at 80 and 100mg/mL. There was no inhibitory activity for n-hexane, ethylacetate and methanol crude extracts for the stembark and the root of *Vernonia amygdalina* at varying concentrations of 40, 60, 80 and 100 mg/mL against strain RM1000 (Isolate:B2).

### 4.1.24 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Moringa oleifera* (mm)

The antifungal inhibitory activity of the leaf, stembark and root of *Moringa oleifera* against strain RM1000 (Isolate: B2) is shown in Table 4.24 There was no inhibitory activity for n-hexane, ethylacetate and methanol crude extracts of the leaf, stembark and root of *moringa oleifera* against strain RM1000 (Isolate:B2) at the various concentrations of 40, 60, 80 and 100 mg/mL.

### 4.1.25 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Azadirachta indica* (mm)

Antifungal inhibitory activity of the leaf, stembark and root of *Azadirachta indica* against strain RM1000 (Isolate: B2) is shown in Table 4.25. The n-hexane leaf extract (NHLE) showed no activity at 40 mg/mL concentration but there was inhibitory activity 7.00±0.20, 11.00.±1.00 and 15.00.±1.00 mm, at 60, 80 and 100 mg/mL respectively. Ethyl acetate leaf extract (EALE) showed no inhibitory activity at 40, 60, 80 and 100 mg/mL concentrations. The methanol leaf extract showed no activity at 40 and 60 mg/mL but there was inhibitory activity 6.00±0.20 and 9.00± 0.70 mm at 80 and 100 mg/mL respectively. The ethyl acetate stembark extract (EASBE) showed no inhibitory activity at 40 and 60 mg/mL but there was inhibitory activity 7.00±0.20 and 9.00±0.70 mm at 80 and 100 mg/mL respectively while the ethyl acetate and methanol extracts had no inhibitory activity. The root of n-hexane, ethyacetate and methanol extracts of *Azardirachta indica* showed no inhibitory activity.

### 4.1.26 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vitellaria paradoxa* (mm)

The antifungal inhibitory activity of the leaf, stembark and root of *Vitellaria paradoxa* against strain RM1000 (Isolate: B2) is shown in Table 4.26. There was no activity for n-hexane, ethylacetate and methanol crude extracts of the leaf, stembark and root of *Vitellaria paradoxa* against strain RM1000 (Isolate: B2) at the various concentrations of 40, 60, 80 and 100 mg/mL.

**Table 4.23: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Vernonia amygdalina* Extract (40-100mg/mL) Against StranRM1000 (Isolate: B2) (mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ |
| 60 | $6.00{\pm}0.20^b$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ |
| 80 | $8.67{\pm}0.67^c$ | $0.00{\pm}0.00^a$ | $6.00{\pm}0.20^b$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ |
| 100 | $10.00{\pm}0.60^d$ | $0.00{\pm}0.00^a$ | $8.00{\pm}0.20^c$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ |
| Fluconazole(1mg/ml) | $37.00{\pm}0.80^e$ | $37.00{\pm}0.80^b$ | $37.00{\pm}0.80^d$ | $37.00{\pm}0.80^b$ | $37.00{\pm}0.80^b$ | $37.00{\pm}0.80^b$ | $37.00{\pm}0.80^b$ | $37.00{\pm}0.80^b$ | $37.00{\pm}0.80^b$ |
| DMSO(100ul) | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.24: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Moringa oleifera* Extract (40-100mg/mL) Against Strain RM1000(Isolate: B2) (mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ |
| 60 | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ |
| 80 | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ |
| 100 | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ | $0.00{\pm}0.00^b$ |
| Fluconazole(1mg/ml) | $37.00{\pm}0.80^a$ | $37.00{\pm}0.80^a$ | $37.00{\pm}0.80^a$ | $37.00{\pm}0.80^a$ | $37.00{\pm}0.80^a$ | $37.00{\pm}0.80^a$ | $37.00{\pm}0.80^a$ | $37.00{\pm}0.80^a$ | $37.00{\pm}0.80^a$ |
| DMSO(100 ul) | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ | $0.00{\pm}0.00^a$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.25: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Azadirachta indica* Extract (40-100mg/mL) Against Strain RM1000 (Isolate: B2) (mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 60 | 7.00±0.20$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 80 | 11.00±1.00$^{c}$ | 0.00±0.00$^{a}$ | 6.00±0.20$^{b}$ | 7.00±0.20$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 100 | 16.00±1.00$^{d}$ | 0.00±0.00$^{a}$ | 9.00±0.70$^{c}$ | 9.00±0.70$^{c}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| Fluconazole(1mg/ml) | 37.00±0.80$^{e}$ | 37.00±0.80$^{b}$ | 37.00±0.80$^{d}$ | 37.00±0.80$^{d}$ | 37.00±0.80$^{b}$ | 37.00±0.80$^{b}$ | 37.00±0.80$^{b}$ | 37.00±0.80$^{b}$ | 37.00±0.80$^{b}$ |
| DMSO(100ul) | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.26: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Vitellaria paradoxa* Extract (40-100mg/mL) Against Strain RM1000 (Isolate: B2) (mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **n-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 60 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 80 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 100 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| Fluconazole(1mg/ml) | 37.00±0.80$^{b}$ | 37.00±0.80$^{b}$ | 37.00±0.80$^{b}$ | 37.00±0.80$^{b}$ | 37.00±0.80$^{b}$ | 37.00±0.80$^{b}$ | 37.00±0.80$^{b}$ | 37.00±0.80$^{b}$ | 37.00±0.80$^{b}$ |
| DMSO(100ul) | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00a | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$.

### 4.1.27 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* (mm)

Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* against strain P37005 (Isolate :B4) is shown in Table 4.27. The n-hexane leaf extract (NHLE) showed no inhibitory activity at 40 mg/mL concentration but at 60, 80 and 100 mg/mL concentrations there was inhibitory activity and the zone of inhibition (ZOI) were 8.00±0.20, 9.33±0.06 and 13.00±0.70 mm respectively. Ethyl acetate leaf extracts (EALE) showed no inhibitory activity for all the varying concentration used at 40 60, 80, and 100 mg/mL respectively. The methanol leaf extract (MLE) had no inhibitory activity at 40 and 60 mg/mL concentrations but there was inhibitory activity 7.00±0.20 and 9.00±0.70 mm at 80 and 100 mg/mL. There was no inhibitory activity for n-hexane, ethylacetate and methanol extracts for the stembark and root of *Vernonia amygdalina* against strain P37005 at the varying concentrations of 40, 60, 80 and 100 mg/mL.

### 4.1.28 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Moringa oleifera* (mm)

The antifungal activity of the leaf, stembark and root of *Moringa oleifera* against strain P37005 (Isolate: B4) is shown in Table 4.28. There was no inhibitory activity for n-hexane, ethylacetate and methanol crude extracts for the leaf, stembark and root of *Moringa oleifera* against strain P37005 (Isolate:B4) at the various concentrations of 40, 60, 80 and 100 mg/mL.

### 4.1.29 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Azardiracta indica* (mm)

The antifungal activity of the leaf, stembark and root of *Azardirachta indica* against strain P37005 (Isolate: B4) is shown in Table 4.29. The n-hexane leaf extract (NHLE) showed no inhibitory activity at 40 and 60 mg/mL concentrations but there was inhibitory activity 8.00±0.20 and 12.67±2.08mm at 80 and 100 mg/mL. Ethyl acetate leaf extract(EALE) showed no inhibitory activity at 40, 60, 80 and 100 mg/mL concentrations. The methanol leaf extract (MLE) showed no inhibitory activity at 40 and 60 mg/ml but there was inhibitory activity 6.67±1..52 and 8.27± 0.50 mm at 80 and 100 mg/mL concentrations respectively. The n-hexane, stembark extract (NHSBE) showed no inhibitory activity at 40 , 60 and 80 mg/mL but there was inhibitory activity 8.00±0.20 mm at 100 mg/mL concenentation while the ethyl acetate and methanol extracts had no inhibitory activity. The root of n-hexane, ethyacetate and methanol extracts showed no inhibitory activity.

### 4.1.30 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vitellaria paradoxa* (mm)

The antifungal activity of the leaf, stembark and root crude extracts of *Vitellaria paradoxa* is shown in Table 4.30. There was no inhibitory activity for n-hexane, ethylacetate and methanol crude extracts of the leaf, stembark and root of *Vitellaria paradoxa* against strain P37005 (Isolate:B4) at the various concentrations of 40, 60, 80 and 100 mg/mL.

**Table 4.27: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Vernonia amygdalina* Extract (40-100mg/mL) Against Strain P37005(Isolate: B4) (mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| Conc. (mg/ml) | N-hexane | Ethylacetate | Methanol | N-hexane | Ethylacetate | Methanol | N-hexane | Ethylacetate | Methanol |
| 40 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 60 | 8.00±0.20$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 80 | 9.33±0.08$^{c}$ | 0.00±0.00$^{a}$ | 7.00±0.20$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 100 | 13.00±0.70$^{d}$ | 0.00±0.00$^{a}$ | 9.00±0.70$^{c}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| Fluconazole(1mg/ml) | 40.00±0.80$^{e}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{d}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ |
| DMSO(100ul) | 0.00±0.00$^{a}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.28: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Moringa oleifera* Extract (40-100mg/mL)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| Conc. (mg/ml) | N-hexane | Ethylacetate | Methanol | N-hexane | Ethylacetate | Methanol | N-hexane | Ethylacetate | Methanol |
| 40 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 60 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 80 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 100 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| Fluconazole(1mg/ml) | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ |
| DMSO(100ul) | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |

**Against Strain P37005 (Isolate: B4) (mm)**

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.29: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Azadirachta indica* Extract (40-100mg/mL) Against Strain P37005(Isolate:B4)(mm)**

| Conc. (mg/ml) | Leaf<br>N-hexane | Ethylacetate | Methanol | Stemback<br>N-hexane | Ethylacetate | Methanol | Root<br>N-hexane | Ethylacetate | Methanol |
|---|---|---|---|---|---|---|---|---|---|
| 40 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 60 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 80 | 8.00±0.20$^{b}$ | 0.00±0.00$^{a}$ | 6.67±1.52$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 100 | 12.67±2.08$^{c}$ | 0.00±0.00$^{a}$ | 8.27±0.50$^{c}$ | 8.00±0.20$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| Fluconazole(1mg/ml) | 40.00±0.80$^{d}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{d}$ | 40.00±0.80$^{c}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ | 40.00±0.80$^{b}$ |
| DMSO(100ul) | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{c}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.30: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Vitellaria paradoxa* Extract (40-100mg/mL) Against Strain P37005 (Isolate: B4)(mm)**

| Conc. (mg/ml) | Leaf<br>N-hexane | Ethylacetate | Methanol | Stembark<br>N-hexane | Ethylacetate | Methanol | Root<br>N-hexane | Ethylacetate | Methanol |
|---|---|---|---|---|---|---|---|---|---|
| 40 | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a |
| 60 | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a |
| 80 | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a |
| 100 | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a |
| Fluconazole(1mg/ml) | 40.00±0.80b | 40.00±0.80b | 40.00±0.80b | 40.00±0.80b | 40.00±0.80b | 40.00±0.80b | 40.00±0.80b | 40.00±0.80b | 40.00±0.80b |
| DMSO(100ul) | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a | 0.00±0.00a |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0$.

### 4.1.31 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* (mm)

Antifungal activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* against strain SC5314 (Isolate :S3) is shown in Table 4.31. The n-hexane leaf extract (NHLE) showed no inhibitory activity at 40 and 60 mg/mL concentrations but at 80 and 100 mg/mL there was inhibitory activity and the zone of inhibition (ZOI) was 8.00±0.20 and 10.00±0.60 mm respectively. Ethyl acetate leaf extracts (EALE) showed no inhibitory activity for all the varying concentrations used at 40, 60, 80 and 100 mg/mL. The methanol leaf extract (MLE) had no inhibitory activity at 40 and 60 mg/mL concentrations but there was inhibitory activity 6.67± 2.08 and 9.00±0.07 mm at 80 and 100 mg/mL. There was no inhibitory activity for n-hexane, ethyacetate and methanol extracts for the stembark and the root of *Vernonia amygdalina* at varying concentrations of 40, 60, 80 and 100 mg/mL.

### 4.1.32 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Moringa oleifera* (mm)

The antifungal activity of the leaf, stembark and root crude extracts of *Moringa oleifera* is shown in Table 4.32. There was no inhibitory activity for n-hexane, ethylacetate and methanol crude extracts of the leaf, stembark and root of *Moringa oleifera* against strain SC5314 (Isolate: S3) at the various concentrations of 40, 60, 80 and 100 mg/mL.

### 4.1.33 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Azadirachta indica* (mm)

The antifungal activity of the leaf, stembark and root of *Azardirachta indica* against SC5314 (Isolate: S3) is shown in Table 4.33 . The n-hexane leaf extract (NHLE) showed no inhibitory activity at 40 and 60 mg/mL concentrations but there was inhibitory activity 8.00±0.20 and 10.00±0.60 mm at 80 and100 mg/mL. Ethyl acetate leaf extract (EALE) showed no inhibitory activity at 40, 60, 80 and 100 mg/mL . The methanol leaf extract (MLE) showed no inhibitory activity at 40 and 60mg/ml concentrations but there was inhibitory activity 6.67±2.08 and 9.00± 0.07mm at 80 and 100 mg/mL respectively. The n-hexane, ethyl acetate and methanol extract for the stembark and root of *Azardirachta indica* showed no inhibitory activity at a varying concentrations of 40, 60, 80 and 100 mg/mL.

### 4.1.34 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vitellaria paradoxa* (mm)

The antifungal activity of the leaf, stembark and root of *Vitellaria paradoxa* against strain SC5314 (Isolate: S3) is shown in Table 4.34. There was no activity for n-hexane, ethylacetate and methanol crude extracts of the leaf, stembark and root of *Vitellaria paradoxa* against strain SC5314 (Isolate: S3) at the various concentrations of 40, 60, 80 and 100 mg/mL.

**Table 4.31: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Vernonia amygdalina* Extract (40-100mg/mL) Against Strain SC5314 (Isolate: S3) (mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | 00.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| 60 | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| 80 | 8.00±0.20[b] | 0.00±0.00[a] | 6.67±2.08[b] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| 100 | 10.00±0.60[c] | 0.00±0.00[a] | 9.00±0.07[c] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| Fluconazole(1mg/ml) | 34.00±0.91[d] | 34.00±0.91[b] | 34.00±0.91[d] | 34.00±0.91[b] | 34.00±0.91[b] | 34.00±0.91[b] | 34.00±0.91[b] | 34.00±0.91[b] | 34.00±0.91[b] |
| DMSO(100ul) | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4:32: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Moringa oleifera* Extract (40-100mg/mL) Against StrainSC5314(Isolate: S3) (mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| 60 | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| 80 | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| 100 | 0.00±0.00a | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| Fluconazole(1mg/ml) | 34.00±0.50[b] | 34.00±0.50[b] | 34.00±0.50[b] | 34.00±0.50[b] | 34.00±0.50b | 34.00±0.50[b] | 34.00±0.50[b] | 34.00±0.50[b] | 34.00±0.50[b] |
| DMSO(100ul) | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.33: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Azadirachta indica* Extract (40-100mg/mL) Against Strain SC5314(Isolate: S3) (mm)**

| | Leaf | | | Stemback | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 60 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 80 | 8.00±0.20$^{b}$ | 0.00±0.00$^{a}$ | 6.67±0.08$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 100 | 10.00±0.60$^{c}$ | 0.00±0.00$^{a}$ | 9.00±0.70$^{c}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| Fluconazole(1mg/ml) | 34.00±0.92$^{d}$ | 34.00±0.92$^{b}$ | 34.00±0.92$^{d}$ | 34.00±0.92$^{b}$ | 34.00±0.92$^{b}$ | 34.00±0.92$^{b}$ | 34.00±0.92$^{b}$ | 34.00±0.92$^{b}$ | 34.00±0.92b |
| DMSO(100ul) | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{b}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at p≤0.05

**Table 4.34 : Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Vitellaria paradoxa* Extract(40-**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 60 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 80 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 100 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| Fluconazole(1mg/ml) | 34.00±0.92$^{b}$ | 34.00±0.92$^{b}$ | 34.00±0.92$^{b}$ | 34.00±0.92$^{b}$ | 34.00±0.92$^{b}$ | 34.00±0.92$^{b}$ | 34.00±0.92$^{b}$ | 34.00±0.92$^{b}$ | 34.00±0.92$^{b}$ |
| DMSO(100ul) | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |

**100mg/mL) Against Strain SC5314 (Isolate:S3) (mm)**

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at p≤0.05

### 4.1.35 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* (mm)

Antifungal activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* against strain SC5314 (Isolate :B7) is shown in Table 4.35 . The n-hexane leaf extract (NHLE) showed no activity at 40 mg/mL concentration but at 60, 80 and 100 mg/mL concentrations there was inhibitory activity and the zone of inhibition (ZOI) was 8.00±0.76, 12.67±0.76 and 14.00±0.60 mm respectively. Ethyl acetate leaf extracts (EALE) showed no inhibitory activity for all the varying concentration used at 40, 60, 80 and 100 mg/mL

.The methanol leaf extract (MLE) had no inhibitory activity at 40 and 60 mg/mL but there was inhibitory activity 16.00± 0.70 and 21.00±0.30 mm at 80 and 100mg/mL concentrations. There was no inhibitory activity for the n-hexane, ethyacetate and methanol extracts for the stem bark and root of *Vernonia amygdalina at* varying concentrations of 40, 60, 80 and 100 mg/mL.

### 4.1.36 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Moringa oleifera* (mm)

The antifungal activity of the leaf, stembark and root of *Moringa oleifera* against strain SC5314 (Isolate: B7) is shown in Table 4.36. There was no inhibitory activity for n-hexane, ethylacetate and methanol crude extracts of the leaf, stembark and root of *Moringa oleifera* against strain SC5314 (Isolate: B7) at the various concentrations of 40, 60, 80 and 100 mg/mL.

### 4.1.37 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Azadirachta indica* (mm)

The antifungal activity of the leaf, stembark and root of *Azadirachta indica* against strain SC5314 (Isolate: B7) is shown in Table 4.37. The n-hexane leaf extract (NHLE) showed no inhibitory activity at 40 mg/mL concentration but there was inhibitory activity 7.67.±0.09, 8.00±0.76 and 9.00±0.70 mm at 60, 80 and 100 mg/mL respectively. Ethyl acetate leaf extract (EALE) showed no inhibitory activity at 40, 60, 80 and 100 mg/mL concentrations. The methanol leaf extract showed inhibitory activity at all the varying concentratons used at 40, 60, 80 and 100 mg/mL the zones of inhibition (ZOI) recorded were 7.00±0.20, 8.00±0.76, 10.00±0.60 and 13.00± 0.70 mm respectively (plate XV). There was no inhibitory activity for the n-hexane, ethylacetate and methanol crude extracts for the stem bark and root at varying concentrations of 40, 60, 80, and 100 mg/mL.

### 4.1.38 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vitellaria paradoxa* (mm)

Antifungal activity of the leaf, stembark and root of *Vitellaria paradoxa* against strain SC5314 (Isolate: B7) is shown in Table 4.38. There was no activity for n-hexane, ethylacetate and methanol crude extracts for the leaf, stembark and root of *Vitellaria paradoxa* against strain SC5314 (Isolate:B7) at the various concentrations of 40, 60, 80 and 100 mg/mL.

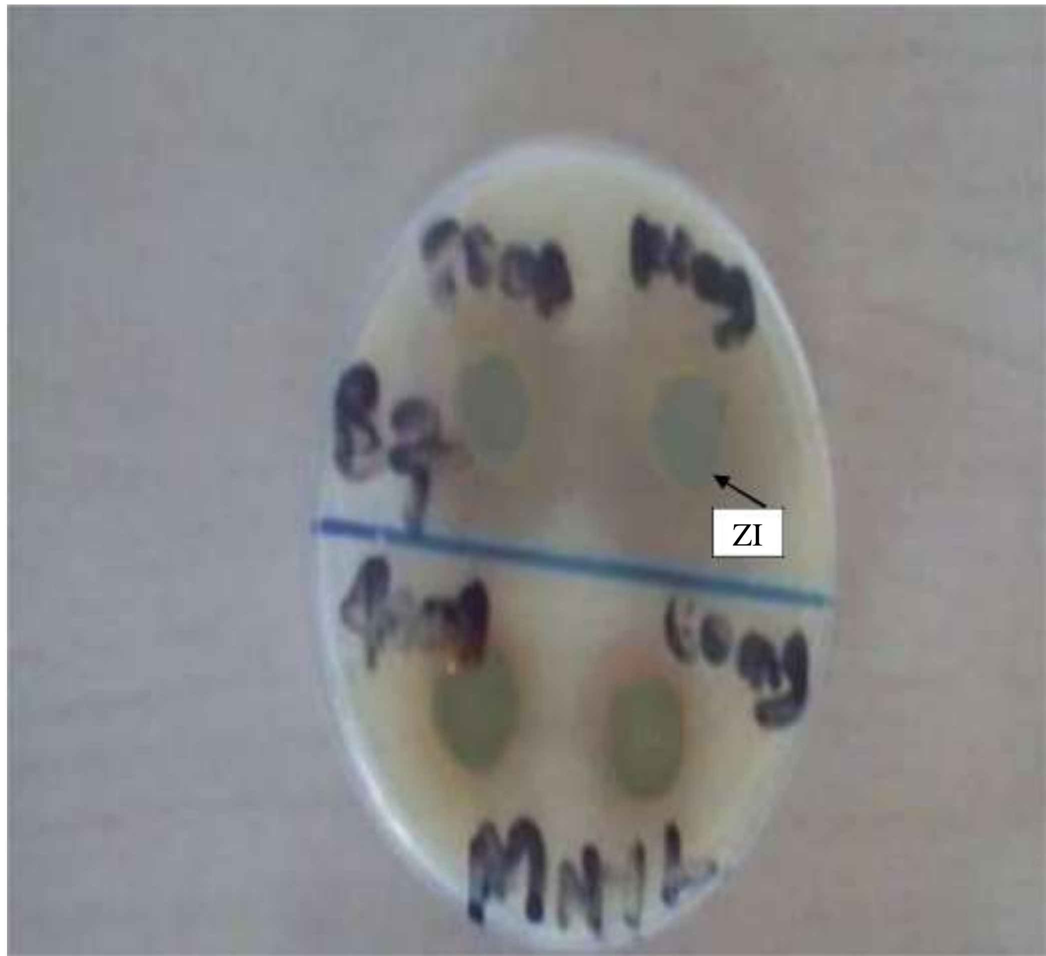



**Plate XV: Zones of Inhibition of Methanol *Azadirachta indica* (Neem) Leaf Extract at Varying Concentrations**

**ZI: Zone of Inhibition**

**Table 4.35 : Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Vernonia amygdalina* Extract(40-100mg/mL) Against Strain SC5314(Isolate: B7)(mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $00.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |
| 60 | $8.00 \pm 0.76^{b}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |
| 80 | $12.67 \pm 0.76^{c}$ | $0.00 \pm 0.00^{a}$ | $16.00 \pm 0.70^{b}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |
| 100 | $14.00 \pm 0.60^{d}$ | $0.00 \pm 0.00^{a}$ | $21.00 \pm 0.30^{b}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |
| Fluconazole(1mg/ml) | $38.00 \pm 0.92^{e}$ | $38.00 \pm 0.92^{b}$ | $38.00 \pm 0.92^{c}$ | $38.00 \pm 0.92^{b}$ | $38.00 \pm 0.92^{b}$ | $38.0 \pm 0.92^{b}$ | $38.00 \pm 0.92^{b}$ | $38.00 \pm 0.92^{b}$ | $38.00 \pm 0.92^{b}$ |
| DMSO(100ul) | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.36: Antifungal Inihibitory Activity of the Leaf, Stem bark and Root of *Moringa oleifera* Extract (40-100mg/mL) Against Strain SC5314(Isolate: B7) (mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |
| 60 | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |
| 80 | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |
| 100 | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |
| Fluconazole(1mg/ml) | $38.00 \pm 0.92^{b}$ | $38.00 \pm 0.92^{b}$ | $38.00 \pm 0.92^{b}$ | $38.00 \pm 0.92^{b}$ | $38.00 \pm 0.92^{b}$ | $38.00 \pm 0.92^{b}$ | $38.00 \pm 0.92^{b}$ | $38.00 \pm 0.92^{b}$ | $38.00 \pm 0.92^{b}$ |
| DMSO(100ul) | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ | $0.00 \pm 0.00^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.37: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Azadirachta indica* Extracts (40-100mg/mL) Against Strain SC5314(Isolate: B7)(mm)**

| Conc.(mg/ml) | Leaf | | | Stembark | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | N-hexane | Ethylacetate | Methanol | N-hexane | Ethylacetate | Methanol | N-hexane | Ethylacetate | Methanol |
| 40 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 7.00±0.20$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 60 | 7.67±0.09$^{b}$ | 0.00±0.00$^{a}$ | 8.00±0.76$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 80 | 8.00±0.76$^{b}$ | 0.00±0.00$^{a}$ | 10.00±0.60$^{c}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 100 | 9.00±0.70$^{b}$ | 0.00±0.00$^{a}$ | 13.00±0.70$^{d}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| Fluconazole(1mg/ml) | 38.00±0.92$^{c}$ | 38.00±0.92$^{b}$ | 38.00±0.92e | 38.00±0.92$^{b}$ | 38.00±0.92$^{b}$ | 38.00±0.92$^{b}$ | 38.00±0.92$^{b}$ | 38.00±0.92$^{b}$ | 38.00±0.92$^{b}$ |
| DMSO(100ul) | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at p≤0.05

**Table 4.38: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Vitellaria paradoxa* Extract (40-100mg/mL) Against Strain SC5314(Isolate: B7) (mm)**

| | Leaf | | | Stemback | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| Conc. (mg/ml) | N-hexane | Ethylacetate | Methanol | N-hexane | Ethylacetate | Methanol | N-hexane | Ethylacetate | Methanol |
| 40 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 60 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 80 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 100 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.60$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| Fluconazole(1mg/ml) | 38.00±0.92$^{b}$ | 38.00±0.92$^{b}$ | 38.00±0.92$^{b}$ | 38.00±0.92$^{b}$ | 38.00±0.92$^{b}$ | 38.00±0.92$^{b}$ | 38.00±0.92$^{b}$ | 38.00±0.92$^{b}$ | 38.00±0.92$^{b}$ |
| DMSO(100ul) | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at p≤0.05.

### 4.1.39 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* (mm)

Antifungal activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* against strain SC5314 (Isolate: C1) is shown in Table 4.39. The n-hexane leaf extract (NHLE) showed no inhibitory activity at 40 and 60 mg/mL concentrations but at 80 and 100 mg/mL there was inhibitory activity and the zone of inhibition (ZOI) was 8.00±0.20 and 12.67±0.77 mm respectively. Ethyl acetate leaf extracts (EALE) showed no inhibitory activity for all the varying concentrations used at 40, 60, 80 and 100 mg/mL. The methanol leaf extract (MLE) showed no inhibitory activity at 40, 60, and 80 mg/mL but there was inhibitory activity 9.00± 0.70 mm at 100 mg/mL. There was no inhibitory activity for n-hexane, ethyacetate and methanol crude extracts for the stem bark and root of *Vernonia amygdalina* at varying concentrations of 40, 60, 80 and 100 mg/mL.

### 4.1.40 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *moringa oleifera* (mm)

Antifungal activity of the leaf, stembark and root of *Moringa oleifera* against strain SC5314 (Isolate: C1) is shown in Table 4.40. There was no inhibitory activity for n-hexane, ethylacetate and methanol crude extracts of the leaf, stembark and root of *Moringa oleifera* against strain SC5314 (Isolate : C3) at the various concentrations of 40, 60, 80 and 100 mg/mL.

### 4.1.41 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Azadirachta indica* (mm)

The antifungal activity of the leaf, stembark and root of *Azadirachta indica* against strain SC5314 (Isolate: C1) is shown in Table 4.41. The n-hexane leaf extract(NHLE) showed no activity at 40 and 60 mg/mL concentrations but there was inhibitory activity 8.00±0.20 and

10.10±0.45mm at 80 and 100 mg/mL. Ethyl acetate leaf extract (EALE) showed no inhibitory activity at 40, 60, 80 and 100 mg/mL concentrations. The methanol leaf extract (MLE) showed no inhibitory activity at 40 and 60 mg/mL but there was inhibitory activity 7..00±0.20 and 9.00±0.70 mm at 80 and 100 mg/mL respectively. There was no inhibitory activity for n-hexane, ethyacetate and methanol crude extract for the stembark and root of *Azardirachta indica* at the varying concentrations of 40, 60, 80 and 100 mg/mL.

### 4.1.42 Antifungal inhibitory activity of the Leaf, Stembark and Root Crude Extracts of *Vitellaria paradoxa* (mm)

The antifungal activity of the leaf, stembark and root of *Vitellaria paradoxa* against strain SC5314 (Isolate: C1) is shown in Table 4.42. There was no inhibitory activity for n-hexane, ethyl acetate and methanol crude extracts of the leaf, stembark and root of *Vitellaria paradoxa* against strain SC5314 (Isolate : C1) at the various concentrations of 40, 60, 80 and 100 mg/mL.

**Table 4.39: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Vernonia amygdalina* Extract(40-100mg/mL) Against Strain SC5314 (Isolate: C1) (mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |
| 60 | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |
| 80 | $8.00\pm0.20^{b}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |
| 100 | $12.67\pm0.77^{c}$ | 0.00±0.00a | $9.00\pm0.70^{b}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |
| Fluconazole(1mg/ml) | $34.33\pm1.00^{d}$ | $34.33\pm1.00^{e}$ | $34.33\pm1.00^{c}$ | $34.33\pm1.00^{b}$ | $34.33\pm1.00^{b}$ | $34.33\pm1.00^{b}$ | $34.33\pm1.00^{b}$ | $34.33\pm1.00^{b}$ | $34.33\pm1.00^{b}$ |
| DMSO(100ul) | $0.00\pm0.00^{e}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p\leq0.05$

**Table 4.40: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Moringa oleifera* Extract (40-100mg/mL) Against Strain SC5314 (Isolate:C1) (mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |
| 60 | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |
| 80 | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |
| 100 | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |
| Fluconazole(1mg/ml) | $34.33\pm1.00^{b}$ | $34.33\pm1.00^{b}$ | $34.33\pm1.00^{b}$ | $34.33\pm1.00^{b}$ | $34.33\pm1.00^{b}$ | $34.33\pm1.00^{b}$ | $34.33\pm1.00^{b}$ | $34.33\pm1.00^{b}$ | $34.33\pm1.00^{b}$ |
| DMSO(100ul) | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ | $0.00\pm0.00^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p\leq0.05$

**Table 4.41: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Azadirachta indica* Extract (40-100mg/mL) Against Strain SC5314 (Isolate: C1) (mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ |
| 60 | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ |
| 80 | $8.00±0.20^{b}$ | $0.00±0.00^{a}$ | $7.00±0.20^{b}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ |
| 100 | $10.10±0.45^{c}$ | $0.00±0.00^{a}$ | $9.00±0.70^{c}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ |
| Fluconazole(1mg/ml) | $34.33±1.00^{d}$ | $34.33±1.00^{b}$ | $34.33±1.00^{d}$ | $34.33±1.00^{b}$ | $34.33±1.00^{b}$ | $34.33±1.00^{b}$ | $34.33±1.00^{b}$ | $34.33±1.00^{b}$ | $34.33±1.00^{b}$ |
| DMSO(100ul) | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{e}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.42: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Vitellaria paradoxa* Extract (40-100mg/mL) Against Strain SC5314(Isolate: C1)(mm)**

| | Leaf | | | Stemback | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ |
| 60 | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ |
| 80 | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ |
| 100 | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ |
| Fluconazole(1mg/ml) | $34.33±1.00^{b}$ | $34.33±1.00^{b}$ | $34.33±1.00^{b}$ | $34.33±1.00^{b}$ | $34.33±1.00^{b}$ | $34.33±1.00^{b}$ | $34.33±1.00^{b}$ | $34.33±1.00^{b}$ | $34.33±1.00^{b}$ |
| DMSO(100ul) | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ | $0.00±0.00^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

### 4.1.43 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* (mm)

Antifungal activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* against strain SC5314 (Isolate :C2) is presented in Table 4.43. The n-hexane leaf extract (NHLE) showed no inhibitory activity at 40 and 60 mg/mL concentrations but at 80 and 100 mg/mL concentrations there was inhibitory activity and the zone of inhibition (ZOI) recorded was 8.00±0.20 and 9.67±0.77 mm respectively. Ethyl acetate leaf extracts (EALE) showed no inhibitory activity for all the varying concentrations used at 40, 60, 80 and 100 mg/mL. The methanol leaf extract (MLE) showed inhibitory activity at 40, 60 80 and 100 mg/mL concentrations and their zones of inhibition recorded were 8.00±0.20, 9.67±0.77, 11.00±1.00 and 14.00. ±1.00 mm respectively. There was no inhibitory activity for n-hexane, ethyacetate and methanol crude extracts for the stembark and root of *Vernonia amygdalina* at varying concentrations of 40, 60, 80 and 100 mg/mL.

### 4.1.44 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Moringa oleifera* (mm)

Antifungal activity of the leaf, stembark and root of *Moringa oleifera* against strain SC5314 (Isolate: C2 ) is shown in Table 4.44. There was no inhibitory activity for n-hexane, ethylacetate and methanol crude extracts of the leaf, stembark and root of *Moringa oleifera* against strain SC5314 (Isolate:C2) at the various concentrations of 40, 60, 80 and 100 mg/mL.

### 4.1.45 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Azadirachta indica* (mm)

The antifungal activity of the leaf, stembark and root of *Azadirachta indica* against SC5314 (Isolate: C2) is shown in Table 4.45. The n-hexane leaf extract (NHLE) showed no inhibitory activity at 40 and 60 mg/mL concentrations but there was inhibitory activity 8.00±0.20 and 10.00±60 mm at 80 and 100 mg/mL. Ethyl acetate leaf extract (EALE) showed no inhibitory activity at 40, 60, 80 and 100 mg/mL concentrations. The methanol leaf extract (MLE) showed no inhibitory activity at 40 and 60 mg/mL but there was inhibitory activity 6.6.00±2.08 and 9.00± 0.70 mm at 80 and 100 mg/mL respectively. The n-hexane, ethyl acetate and methanol crude extract for the stem bark and root of *Azadirachta indica* showed no inhibitory activity at the varying concentrations of 40, 60,80 and 100 mg/mL.

### 4.1.46 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vitellaria paradoxa* (mm)

Antifungal activity of the leaf, stembark and root of *Vitellaria paradoxa* against strain1161 (Isolate: C2) is shown in Table 4.46. There was no activity for the n-hexane, ethyacetate and methanol crude extracts of the leaf, stembark and root of *Vitellaria paradoxa* against strain SC5314 (Isolate C2) at the various concentrations of 40, 60, 80 and 100 mg/mL.

**Table 4.43: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Vernonia amygdalina* Extract (40-100mg/mL) Against Strain SC5314(Isolate:C2)(mm)**

| | Leaf | | | Stemback | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | 0.00±0.00[a] | 0.00±0.00[a] | 8.00±0.20[b] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| 60 | 0.00±0.00[a] | 0.00±0.00[a] | 9.67±0.77[c] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| 80 | 8.00±0.20[b] | 0.00±0.00[a] | 11.00±1.00[d] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| 100 | 9.67±0.77[b] | 0.00±0.00[a] | 14.00±1.00[e] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| Fluconazole(1mg/ml) | 33.66±1.00[c] | 33.66±1.00[b] | 33.66±1.00[f] | 33.66±1.00[b] | 33.66±1.00[b] | 33.66±1.00[b] | 33.66±1.00[b] | 33.66±1.00[b] | 33.66±1.00[b] |
| DMSO(100ul) | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[b] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at p≤0.05

**Table 4.44: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Moringa oleifera* Extract (40-100mg/mL) Against Strain SC5314 (Isolate: C2) (mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| 60 | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| 80 | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| 100 | 0.00±0.00[a+] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |
| Fluconazole(1mg/ml) | 33.66±1.00[b] | 33.66±1.00[b] | 33.66±1.00[b] | 33.66±1.00[b] | 33.66±1.00[b] | 33.66±1.00[b] | 33.66±1.00[b] | 33.66±1.00[b] | 33.66±1.00[b] |
| DMSO(100ul) | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] | 0.00±0.00[a] |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at p≤0.05

**Table 4.45: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Azadirachta indica* Extract (40-100mg/mL) Against Strain SC5314 (Isolate: C2) (mm)**

| | Leaf | | | Stemback | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ |
| 60 | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ |
| 80 | $8.00±0.20^b$ | $0.00±0.00^a$ | $6.6±2.08^b$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ |
| 100 | $10.00±0.60^c$ | $0.00±0.00^a$ | $9.00±0.70^c$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ |
| Fluconazole(1mg/ml) | $33.00±0.92^d$ | $33.00±0.92^b$ | $33.00±0.92^d$ | $33.00±0.92^b$ | $33.00±0.92^b$ | $33.00±0.92^b$ | $33.00±0.92^b$ | $33.00±0.92^b$ | $33.00±0.92^b$ |
| DMSO(100ul) | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at p≤0.05

**Table 4.46 : Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Vitellaria paradoxa* Extract (40-100mg/mL) Against Strain SC5314 (Isolate: C2)(mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ |
| 60 | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ |
| 80 | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ |
| 100 | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ |
| Fluconazole(1mg/ml) | $33.00±0.92^b$ | $33.00±0.92^b$ | $33.00±0.92^b$ | $33.00±0.92^b$ | $33.00±0.92^b$ | $33.00±0.92^b$ | $33.00±0.92^b$ | $33.00±0.92^b$ | $33.00±0.92^b$ |
| DMSO(100ul) | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ | $0.00±0.00^a$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at p≤0.05

### 4.1.47 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* (mm)

Antifungal activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* against strain SC5314(Isolate :S1) is presented in Table 4.47. The n-hexane leaf extract (NHLE) showed no inhibitory activity at 40 and 60 mg/mL concentrations but at 80 and 100 mg/mL concentrations there was activity and the zone of inhibition (ZOI) was 8.00±1.00 and 12.67±2.08 mm respectively. Ethyl acetate leaf extracts (EALE) showed no activity for all the varying concentrations of 40, 60, 80 and 100 mg/mL. The methanol leaf extract (MLE) had inhibitory activity 5.00±0.00, 7.00±1.00, 8.00±1.00 and 10.00±0.60 mm at the varying concentrations of 40, 60, 80 and 100 mg/mL respectively (Plate XVI). There was no inhibitory activity for n-hexane, ethyacetate and methanol crude extracts for the stembark and the root of *Vernonia amygdalina* at the varying concentrations of 40, 60, 80 and 100 mg/mL.

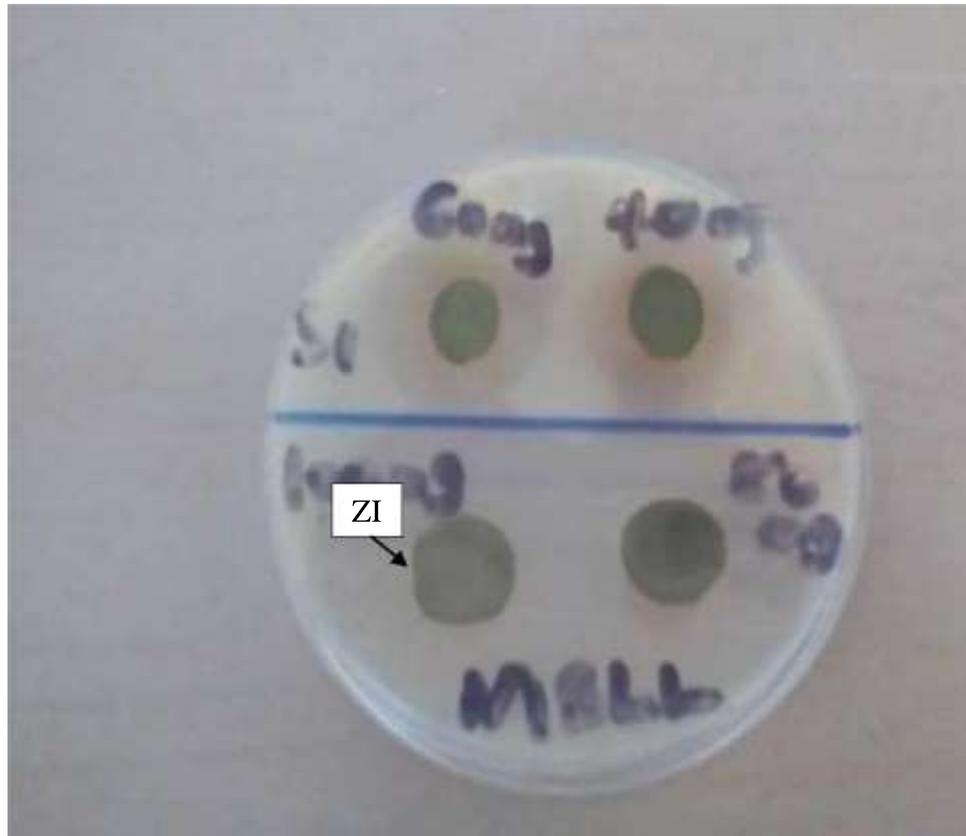



**Plate XVI : Zones of Inhibition of Methanol Leaf Crude Extract of *Vernonia amygdalina* (Bitter leaf) at Varying Concentrations. ZI: Zone of Inhibition**

### 4.1.48 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Moringa oleifera* (mm)

The antifungal activity of the leaf, stembark and root of *Moringa oleifera* against strain SC5314 (Isolate: S1) is shown in Table 4.48. There was no activity for n-hexane, ethylacetate and methanol crude extracts of the leaf, stembark and root of *Moringa oleifera* against strain SC5314 (Isolate:S1) at the various concentrations of 40, 60, 80 and 100 mg/mL.

### 4.1.49 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Azadirachta indica* (mm)

The antifungal activity of the leaf, stembark and root of *Azadirachta indica* against strain SC5314 (Isolate: S1) is shown in Table 4.49. The n-hexane leaf extract (NHLE) showed no inhibitory activity at 40 and 60 mg/mL concentrations but there was inhibitory activity 7.00±0.20 and 9.00±0.70 mm at 80 and 100 mg/mL. Ethyl acetate leaf extract (EALE) showed no inhibitory activity at 40, 60, 80 and 100 mg/mL. The methanol leaf extract (MLE) showed inhibitory activity at all the various concentrations of 40, 60, 80 and 100 mg/mL and there zones of inhibition (ZOI) recorded were 7.00±0.20, 9.0±0.7, 12.00±1.00 and 15.00±1.00 mm respectively. There was no inhibitory activity for the n-hexane, ethyl acetate and methanol crude extracts for the stembark and root of *Azadirachta indica* at the varying concentrations of 40,60, 80 and 100 mg/mL.

### 4.1.50 Antifungal inhibitory activity of the leaf, stembark and root crude extracts of *Vitellaria paradoxa* (mm)

The antifungal activity of the leaf, stembark and root of *Vitellaria paradoxa* against strain SC5314 (Isolate: S1) is shown in Table 4.50. There was no activity for n-hexane, ethylacetate and methanol crude extracts of the leaf, stembark and root of *Vitellaria paradoxa* against strain SC5314 (Isolate:S1) at the various concentrations of 40, 60, 80 and 100 mg/mL.

**Table 4.47: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Vernonia amygdalina* Extract (40-100mg/mL) Against Strain SC5314(Isolate:S1)(mm)**

| | **Leaf** | | | **Stembark** | | | **Root** | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | Methanol |
| 40 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 5.00±0.00$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 60 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 7.00±1.00$^{c}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 80 | 8.00±1.00$^{b}$ | 0.00±0.00$^{a}$ | 8.00±1.00$^{d}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 100 | 12.67±2.08$^{c}$ | 0.00±0.00$^{a}$ | 10.00±0.60$^{e}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| Fluconazole(1mg/ml) | 34.66±1.00$^{d}$ | 34.66±1.00$^{b}$ | 34.66±1.00$^{f}$ | 34.66±1.00$^{b}$ | 31.66±1.00$^{b}$ | 31.66±1.00$^{b}$ | 31.66±1.00$^{b}$ | 31.66±1.00$^{b}$ | 31.66±1.00$^{b}$ |
| DMSO(100ul) | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.48: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Moringa oleifera* Extract (40-100mg/mL) Against Strain SC5314(Isolate:S1)(mm)**

| | Leaf | | | Stemback | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| Conc. (mg/ml) | N-hexane | Ethylacetate | Methanol | N-hexane | Ethylacetate | Methanol | N-hexane | Ethylacetate | Methanol |
| 40 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ |
| 60 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ |
| 80 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ |
| 100 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ |
| Fluconazole(1mg/ml) | 34.66±1.00$^{b}$ | 34.66±1.00$^{b}$ | 34.66±1.00$^{b}$ | 34.66±1.00$^{b}$ | 34.66±1.00$^{a}$ | 34.66±1.00$^{b}$ | 34.66±1.00$^{a}$ | 34.66±1.00$^{a}$ | 34.66±1.00$^{a}$ |
| DMSO(100ul) | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ | 0.00±0.00$^{b}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.49: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Azadirachta indica* Extract (40-100mg/mL) Against Strain SC5314 (Isolate: S1) (mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 7.00±0.20$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 60 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 9.00±0.70$^{c}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 80 | 7.00±0.20$^{b}$ | 0.00±0.00$^{a}$ | 12.00±1.00$^{d}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 100 | 9.00±0.70$^{c}$ | 0.00±0.00$^{a}$ | 15.00±1.00$^{e}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| Fluconazole(1mg/ml) | 34.66±1.00$^{d}$ | 34.66±1.00$^{b}$ | 34.66±1.00$^{f}$ | 34.66±1.00$^{b}$ | 34.66±1.00$^{b}$ | 34.66±1.00$^{b}$ | 34.66±1.00$^{b}$ | 34.66±1.00$^{b}$ | 34.66±1.00$^{b}$ |
| DMSO(100ul) | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**Table 4.50: Antifungal Inhibitory Activity of the Leaf, Stem bark and Root of *Vitellaria paradoxa* Extract (40-100mg/mL) Against Strain SC5314(Isolate: S1)(mm)**

| | Leaf | | | Stembark | | | Root | | |
|---|---|---|---|---|---|---|---|---|---|
| **Conc. (mg/ml)** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** | **N-hexane** | **Ethylacetate** | **Methanol** |
| 40 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 60 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 80 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| 100 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00a | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |
| Fluconazole(1mg/ml) | 34.66±1.00$^{b}$ | 34.66±1.00$^{b}$ | 34.66±1.00b | 34.66±1.00$^{b}$ | 34.66±1.00$^{b}$ | 34.66±1.00$^{b}$ | 34.66±1.00$^{b}$ | 34.66±1.00$^{b}$ | 34.66±1.00$^{b}$ |
| DMSO(100ul) | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00a | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

### 4.1.51 Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of active crude extracts

The result of the Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of the active crude extracts is shown in Table 4.51. *Vernonia amygdalina* crude extracts of n- hexane and methanol had a value of 12.5 mg/mL as the MIC value against strain 1161 (Isolate: S5) where as the MFC value was 50 mg/mL. The *Azadirachta indica* crude extracts of n-hexane and methanol had a value of 6.25 mg/mLwhile the MFC value recorded was 50 mg/mL against strain 1161 (IsolateS5). Likewise, n-hexane crude extract of *Vernonia amygdalina* against strain RM1000 (Isolate: B2) recorded a value of 25 mg/mL as the MIC while the MFC was 100 mg/mL similarly, the n-hexane crude extract of *Azardirachta indica* against RM1000 (Isolate B2) recorded a value of 6.25 and 100 mg/mL for the MIC and MFC value respectively. On the other hand the n-hexane crude extract of *Vernonia amygdalina* against strain SC5314 (Isolate: S3 and B7) had a value of 12.5 mg/mL and 100 mg/mL as the value for MIC and MFC respectively. The Methanol crude extract of *Vernonia amygdalina* against strain SC5314 (B7) had a value of 12.5 and 100 mg/mL for the MIC and MFC respectively. The MIC and MFC values for n- hexane crude extract of *Vernonia amygdalina* against strain SC5314 (Isolate : C1) was 25 and 100 mg/mL. The methanol crude extract of *Azardirachta indica* recorded a value of 6.25 and 50 mg/mL against strain SC5314 (Isolate: C1). However, the methanol crude extract of *Vernonia amygdalina* against strain SC5314 (Isolate: C2) had values of 6.25 and 25 mg/mL for the MIC and MFC respectively. The n-hexane crude exstract of *Azardirachta indica* showed values of 6.25 and 50 mg/mL for their respective MIC and MFC against strain SC5314 (Isolate C2). The n-hexane crude extract of *Vernonia amygdalina* had values of 6.25 and 25 mg/mL as the MIC and MFC against SC5314

(Isolate S1), were as the methanol crude extract of *Azardirachta indica* was 6.25 and 50 mg/mL for their respective MIC and MFC.

**Table 4.51: Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of the Active Crude Extracts**

| S/NO | Isolate code | Strain | Medicinal plant | Plant part | Crude extract | MIC | MFC |
|---|---|---|---|---|---|---|---|
| 1. | S5 | 1161 | *Vernonia amygdalina* | Leaf | n-hexane | 12.5mg/ml | 50mg/ml |
| 2 | S5 | 1161 | *Vernonia amygdalina* | Leaf | methanol | 12.5mg/ml | 50mg/ml |
| 3 | S5 | 1161 | *Azardirachta indica* | Leaf | n-hexane | 6.25mg/ml | 50mg/ml |
| 4 | S5 | 1161 | *Azardirachta indica* | Leaf | Methanol | 6.25mg/ml | 50mg/ml |
| 5. | B2 | RM1000 | *Vernonia amygdalina* | Leaf | n-hexane | 25mg/ml | 100mg/ml |
| 6 | B2 | RM1000 | *Azardirachta indica* | Leaf | n-hexane | 6.25mg/ml | 100mg/ml |
| 7 | B4 | P37005 | *Vernonia amygdalina* | Leaf | n-hexane | 12.5mg/ml | 100mg/ml |
| 8 | B4 | P37005 | *Azardirachta indica* | Leaf | n-hexane | 12.5mg/ml | 50mg/ml |
| 9 | S3 | SC5314 | *Vernonia amygdalina* | Leaf | n-hexane | 12.5mg/ml | 100mg/ml |
| 10 | B7 | SC5314 | *Vernonia amygdalina* | Leaf | n-hexane | 12.5mg/ml | 100mg/ml |
| 11 | B7 | SC5314 | *Vernonia amygdalina* | Leaf | Methanol | 12.5mg/ml | 100mg/ml |
| 12 | C1 | SC5314 | *Vernonia amygdalina* | Leaf | n-hexane | 25mg/ml | 100mg/ml |
| 13 | C1 | SC5314 | *Azardirachta indica* | Leaf | n-hexane | 6.25mg/ml | 50mg/ml |
| 14 | C2 | SC5314 | *Vernonia amygdalina* | Leaf | Methanol | 6.25mg/ml | 25mg/ml |
| 15 | C2 | SC5314 | *Azardirachta indica* | Leaf | n-hexane | 6.25mg/ml | 50mg/ml |
| 16 | S1 | SC5314 | *Vernonia amygdalina* | Leaf | n-hexane | 6.25mg/ml | 25mg/ml |
| 17 | S1 | SC5314 | *Azardirachta indica* | Leaf | Methanol | 6.25mg/ml | 50mg/ml |

### 4.1.52 Solvent system and number of fractions obtained from *Vernonia amygdalina* leaf of n- hexane extract eluted from column chromatography

The results obtained from the column chromatography as presented in Table 4.52 shows the solvent system, number of partially purified fractions obtained from the crude extracts of n- hexane *Vernonia amygdalina* leaf and their various colour descriptions. A total number of 4 fractions were eluted from the extract. Fraction (F4) had the highest yield of 1.5 g (6.0 %) followed by fraction F2 with 1.3 g (5.2 %) while the lowest was fraction F1 and F3 with a percentage yield of 1.1 g ( 4.4 %) respectively.

**4.1.53 Solvent system and number of fractions obtained from *Vernonia amygdalina* leaf of ethyl acetate extract eluted from column chromatography**

The results obtained from the column chromatography as presented in Table 4.53 shows the solvent system, number of partially purified fractions obtained from the crude extracts of ethyl acetate *Vernonia amygdalina* leaf and their various colour descriptions. A total number of 6 fractions were eluted from the extract. Fraction (F6) had the highest yield of 1.2 g (4.8 %) followed by fraction F2 with 1.1 g (4.4 %) while the lowest was fraction F1 and with a percentage yield of 0.8 g ( 3.2 %).

**4.1.54 Solvent system and number of fractions obtained from *Vernonia amygdalina* leaf of methanol extract eluted from column chromatography**

The results obtained from the column chromatography as presented in Table 4.54 shows the solvent system, number of partially purified fractions obtained from the crude extracts of methanol *Vernonia amygdalina* leaf and their various colour descriptions. A total number of 6 fractions were eluted from the extract. Fraction (F6) had the highest yield of 1.8 g (6.6 %) followed by fraction F4 with 1.6 g (5.92 %) while the lowest was fraction F1 with a percentag yield of 0.9 g (3.3 %)

**Table 4.52 : Solvent System and Number of Fractions Obtained from *Vernonia***

| Solvent system | No.offraction | Description | Percentage yield g(%) |
|---|---|---|---|
| 100% n-hexane | F1 | Dark green | 1.1(4.4) |
| 9:1n-hexane:ethylacetate | F2 | Dark green | 1.3(5.2) |
| 7:3n-hexane:ethylacetate | F3 | Dark green | 1.1(4.4) |
| 6:4n-hexane:ethylacetate | F4 | Light green | 1.5(6.0) |

***amygdalina* Leaf of n- hexane Extract (25 g) Eluted from Column Chromatography**

**Table 4.53: Solvent System and Number of Fractions of *Vernonia amygdalina* Leaf of Ethylacetate Extract (25 g) Eluted from Column Chromatography**

| Solvent system | No of fraction | Description | Percentage Yield g(%) |
|---|---|---|---|
| 100% ethylacate | F1 | Dark green | 0.8(3.2) |
| 9: 1 ethyl acetate: methanol | F2 | Dark green | 1.1(4.4) |
| 7:3 ethyl acetate: methanol | F3 | Dark green | 0.91(3.64) |
| 6:4 ethyl acetate: methanol | F4 | Dark green | 0.86(3.44) |
| 5:5 ethyl acetate : methanol | F5 | Light green | 0.95(3.8) |
| 4:6 ethylacetate: methanol | F6 | Lght green | 1.2(4.8) |

**Table 4.54: Solvent System and Number of Fractions of *Vernonia amygdalina* Leaf of n- hexane Extract Eluted (27 g) from Column Chromatography**

| Solvent system | No. of fraction | Description | Percentage Yield g(%) |
|---|---|---|---|
| 100% methanol | F1 | Dark green | 0.9(3.3) |
| 100% methanol | F2 | Dark green | 1.2(4.4) |
| 100% methanol | F3 | Dark green | 1.3(4.8) |
| 100% methanol | F4 | Dark green | 1.6(5.92) |
| 100% methanol | F5 | Dark green | 1.4(5.18) |
| 100% methanol | F6 | Dark green | 1.8(6.6) |

### 4.1.55 Solvent system and number of fractions obtained from *Azardirachta indica* leaf of n-hexane extract eluted from column chromatography

The results obtained from the column chromatography as presented in Table 4.55 shows the solvent system, number of partially purified fractions obtained from the crude extracts of n-hexane *Vernonia amygdalina* leaf and their various colour descriptions. A

total number of 7 fractions were eluted from the extract. Fraction(F6) had the highest yield of 1.8 g (6.0 %)followed by fraction F4 with 1.7 g (5.7 %) while the lowest was fraction F7 with a percentag yield of 0.8 g (2.7 %).

#### 4.1.56 Solvent system and number of fractions obtained from *Azardirachta indica* leaf of ethylacetate extract eluted from column chromatography

The results obtained from the column chromatography is presented in Table 4.56 shows the solvent system, number of partially purified fractions obtained from the crude extracts of ethylacetate *Vernonia amygdalina* leaf and their various colour descriptions. A total number of 7 fractions were eluted from the extract. Fraction(F6) had the highest yield of 1.8 g ( 6.7 %) followed by fraction F1 with 1.6 g (5.9 %) while the lowest was fraction F3 with a percentage yield of 0.6 g (2.2 %).

#### 4.1.57 Solvent system and number of fractions obtained from *Azardirachta indica* leaf of methanol extract eluted from column chromatography

The results obtained from the column chromatography is presented in Table 4.57 shows the solvent system, number of partially purified fractions obtained from the crude extracts of methanol *Vernonia amygdalina* leaf and their various colour descriptions. A total number of 7 fractions were eluted from the extract. Fraction(F6) had the highest yield of 1.8 g (7.2 %) followed by fraction F3 with 1.7 g (6.8 %) while the lowest was fraction F1 with a percentage yield of 1.3 g (5.2 %).

**Table 4.55: Solvent System and Number of Fractions of *Azardirachta indica* Leaf of n- hexane Extract ( 30 g) Eluted from Column Chromatography**

| Solvent system | No of fraction | Description | Percentage yieldg(%) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 100% ethylacate | F1 | Dark green | 1.4(4.6) |
| 9: 1 ethyl acetate: methanol | F2 | Dark green | 1.5(5.0) |
| 7:3 ethyl acetate: methanol | F3 | Light yellow | 0.9(3.0 |
| 6:4 ethyl acetate: methanol | F4 | Dark green | 1.7(5.7) |
| 5:5 ethyl acetate : methanol | F5 | Light green | 1.3(4.3) |
| 4:6 ethylacetate: methanol | F6 | Lght green | 1.8(6.0) |
| 3: 7 ethyl acetate: methanol | F7 | light green | 0.8(2.7) |

**Table 4.56: Solvent System and Number of fractions of *Azardirachta indica* Leaf of Ethyl acetate Extract (27 g) Eluted from Column Chromatography**

| Solvent system | No of fraction | Description | Percentage yield g(%) |
|---|---|---|---|
| 100% ethylacate | F1 | Dark green | 1.6(5.9) |
| 9: 1 ethyl acetate: methanol | F2 | Dark green | 0.7(2.6) |
| 7:3 ethyl acetate: methanol | F3 | Dark green | 0.6(2.2) |
| 6:4 ethyl acetate: methanol | F4 | Dark green | 0.8(2.10) |
| 5:5 ethyl acetate : methanol | F5 | Light green | 0.5(1.9) |
| 4:6 ethylacetate: methanol | F6 | Lght green | 1.8(6.7) |
| 3: 7 ethylacetate : methnol | F7 | Light green | 1.2(4.4) |

**Table 4.57: Solvent System and Number of fractions of *Azardirachta indica* Leaf of Methanol Extract (25 g) Eluted from Column Chromatography**

| Solvent system | No of fraction | Description | Percentage yield g(%) |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 100% ethylacate | F1 | Dark green | 1.3(5.2) |
| 100 % methanol | F2 | Dark green | 1.4(5.6) |
| 100% methanol | F3 | Dark green | 1.7(6.8) |
| 100% methanol | F4 | Dark green | 1.5(6.0) |
| 100% methanol | F5 | Light green | 1.6(6.4) |
| 100% methanol | F6 | Lght green | 1.8(7.2) |
| 100% methanol | F7 | Light green | 1.5(6.0) |

#### 4.1.58 Thin layer chromatography results of n-hexane fractions of *Vernonia amygdalina* leaf

The results of the thin layer chromatography of the n-hexane fraction of *Vernonia amygdalina* leaf is presented in Table 4.58. The highest number of spots (4) was recorded for 70:30 n- hexane : ethylacetate with RF values of 0.62,0.50,0.44 and 0.36 while the lowest number of spots was recorded for the non-polar solvents as compared to the polar solvent.

**Table 4.58: Solvent System, Number of spots, Distance Moved by Solvent, Distance Moved by Fraction and Retention Factor of n- hexane Fractions of *Vernonia amygdalina* Leaf**

| Solvent system | NS | DMS(cm) | DMF(cm) | RF |
|---|---|---|---|---|
| 100% n-hexane | 1 | 5 | 2.1 | 0.42 |
| 90:10 n-hexane: ethylacetate | 3 | 5 | 1.9, 2.3, 2.5 | 0.38,0.46, 0.50 |
| 80:20 n- hexane: ethylacetate | 2 | 5 | 2,2, 3.0 | 0.44, 0.60 |
| 70:30 n- hexane: ethylacetate | 4 | 5 | 3.1, 2.5, 2.2, 1.8 | 0.62,0.50,0.44.0.36 |

**KEY: DMS =** Distance moved by the solvent (mobile phase), DMF= distance moved by fraction, RF= retention factor, NS: number of spot

#### 4.1.59 Thin layer chromatography results of ethylacetate fractions of *Vernonia amygdalina* leaf

The results of the thin layer chromatography of the ethylacetate fractions of *Vernonia amygdalina* leaf is presented in Table 4.59. The highest spots (3) was recorded for 50: 50 n- hexane : ethylacetate with RF values of 0.42, 0.56 and 0.64. The lowest number of spots were recorded for the non-polar solvents as compared to the polar solvent.

**Table 4.59: Solvent System, Number of spot, Distance Moved by Solvent, Distance Moved by Fraction, and Retention Factor of Ethylacetate Fractions of *Vernonia amygdalina* Leaf**

| Solvent system | NS | DMS(cm) | DMF(cm) | RF |
|---|---|---|---|---|
| 90: 10 n-hexane :ethylacetate | 2 | 5.0 | 1.0, 1.6 | 0.20, 0.32 |
| 80:20 n-hexane: ethylacetate | 1 | 5.0 | 1.8 | 0.36 |
| 60:40 n-hexane: ethylacetate | 1 | 5.0 | 2.4 | 0.48 |
| 50:50 n-hexane: ethylacetate | 3 | 5.0 | 2.1, 2.8 3.2 | 0.42, 0.56,0.64 |
| 40: 60 n-hexane: ethylacetate | 2 | 5.0 | 2.4, 1.9 | 0.48, 0.38 |
| 20:80 n-hexane: ethylacetate | 1 | 5.0 | 3.0, 2.7 | 0.60, 0.54 |

**Key:** DMS = Distance moved by the solvent (mobile phase), DMF= distance moved by fraction, RF= retention factor, NS: number of spot

### 4.1.60 Thin layer chromatography results of methanol fractions of *Vernonia amygdalina* leaf

The results of the thin layer chromatography of the methanol fractions of *Vernonia amygdalina* leaf is presented in Table 4.60. The highest spots (4) was recorded for 80: 20 ethylacetate: methanol with RF values of 0.70, 0.56, 0.50 and 0.48. The lowest number of spots were recorded for the non-polar solvents as compared to the polar solvent.

**Table 4.60: Solvent System, Number of spots Distance Moved by Solvent, Distance Moved by Fraction and Retention Factor of Methanol Fractions *of Vernonia amygdalina* Leaf**

| Solvent system | NS | DMS(cm) | DMF(cm) | RF |
|---|---|---|---|---|
| 90:10 ethyl acetate: n-hexane | 1 | 5.0 | 2.8 | 0.56 |
| 100% ethylacetate | 3 | 5.0 | 1.8, 2.4,2.2 | 0.36, 0.48, 0.44 |

| | | | | |
|---|---|---|---|---|
| 90:10 ethylacetate: methanol | 2 | 5.0 | 3.4, 2.6 | 0.68, 0.52 |
| 80:20 ethylaceate: methanol | 4 | 5.0 | 3.5, 2.8 2.5 2.4 | 0.70, 0.56, 0.50, 0.48 |
| 70:30 ethylacetate: methanol | 1 | 5.0 | 3.8 | 0.76 |
| 60:40 ethylacetate: methanol | 1 | 5.0 | 3.0 | 0.60 |

**KEY: DMS =** Distance moved by the solvent (mobile phase), DMF= distance moved by fraction, RF= retention factor, NS: number of spot

### 4.1.61 Thin layer chromatography results of n- hexane fractions of *Azardirachta indica* leaf

The results of the thin layer chromatography of the n-hexane fractions of *Azardirachta indica* is presented in Table 4.61. A total of 4 spots was recorded for 60:40 n-hexane: ethylacetate as the highest value with corresponding RF values of 0.64, 0.72 and 0.52. The lowest number of spots was recorded for the non-polar solvents as compared to the polar solvent.

**Table 4.61: Solvent System, Number of spots, Distance Moved by Solvent, Distance Moved by Fraction and Retention Factor of n- hexane Fractions of *Azardirachta indica* Leaf**

| Solvent system | NS | DMS(cm) | DMF(cm) | RF |
|---|---|---|---|---|
| 100% n-hexane | 2 | 5.0 | 3.4, 2.8 | 0.68, 0.56 |
| 90:10 n-hexane: ethylacetate | 1 | 5.0 | 2.5 | 0.50 |
| 80:20 n- hexane: ethylacetate | 2 | 5.0 | 3.2, 3.5 | 0.64, 0.70 |
| 70:30 n- hexane: ethylacetate | 3 | 5.0 | 2.8, 2.4, 2.3 | 0.56, 0.48, 0.46 |
| 60:40 n-hexane: ethylacetate: | 4 | 5.0 | 3.2, 3.6, 2.6 | 0.64, 0.72, 0.52 |
| 50:50 n-hexane: ethylacetate | 1 | 5.0 | 3.8 | 0.76 |
| 40: 60 n-hexane: ethylacetate | 2 | 5.0 | 3.8, 4.4 | 0.76, 0.88 |

**KEY: DMS** = distance moved by the solvent (mobile phase), DMF= distance moved by fraction, RF= retention factor, NS: number of spot

### 4.1.62 Thin layer chromatography results of ethylacetate fractions of *Azardirachta indica* leaf

The results of the thin layer chromatography of the ethylacetate fractions of *Azardirachta indica* leaf is presented in Table 4.62. The highest spots (4) was recorded for 80:20 ethylacetate: methanol with corresponding RF values of 0.38, 0.56, 0.72, and 0.84. The lowest numbers of spots was recorded for the 90:10 ethyl acetate :methanol with RF value of 0.72.

**Table 4.62: Solvent system, Number of spots, Distance Moved by Solvent, Distance Moved by Fraction and Retention Factor of Ethylacetate Fractions of *Azardirachta indica* Leaf**

| Solvent system | NS | DMS(cm) | DMF(cm) | RF |
|---|---|---|---|---|
| 80:20 n-hexane: ethylacetate | 2 | 5.0 | 1.2, 1.4 | 0.24, 0.28 |
| 50:50 n-hexane: ethylacetate | 2 | 5.0 | 1.5, 1.8 | 0.30, 0.36 |
| 100% ethylacetate | 3 | 5.0 | 3.1, 4.0,3.4 | 0.62, 0.80, 0.68 |
| 90:10 ethylacetate: methanol | 1 | 5.0 | 3.6 | 0.72 |
| 80:20 Ethylacetate: methanol | 4 | 5.0 | 1.9, 2.8 3.6, 4.2 | 0.38, 0.56, 0.72, 0.84 |
| 60:40 Ethylacetate: methanol | 2 | 5.0 | 3.0, 3.4 | 0.60, 0.68 |
| 60: 40 n-hexane: ethylacetate | 3 | 5.0 | 2.8, 3.1 | 0.56, 0.62 |

**Key:** DMS = Distance moved by the solvent (mobile phase), DMF= distance moved by fraction, RF= retention factor, NS: number of spot

### 4.1.63 Thin layer chromatography results of methanol fractions of *Azardirachta indica* leaf

The results of the thin layer chromatography of the methanol fractions of *Azardirachta indica* leaf is presented in Table 4.63. The highest spots (4) was recorded for 50:50 ethylacetate : methanol with corresponding RF values of 0.38, 0.52, 0.48 and 0.60. The lowest number of spot one (1) was recorded for the 80: 20 ethylacetate : methanol, 40: 60 ethylacetate: methanol with RF values of 0.48 and 0.56 respectively.

**Table 4.63: Solvent System, Number of spots, Distance Moved by Solvents, Distance Moved by Fraction and Retention Factor of Methanol Fractions of *Azardirachta indica* Leaf**

| Solvent System | NS | DMS(cm) | DMF(cm) | RF |
|---|---|---|---|---|
| 80: 20 Ethyl acetate: n-hexane | 2 | 5.0 | 1.9, 2.3 | 0.38, 0.46 |
| 90:20 Ethylacetate: n-hexane | 3 | 5.0 | 3.0, 2.5, 1.7 | 0.60,0.50,0.34 |
| 80:20 ethylacxate: methanol | 1 | 5.0 | 2.4 | 0.48 |
| 60:40 ethyl acetate: methanol | 2 | 5.0 | 2.9,2.6 | 0.58,0.52 |
| 50:50 ethylacte: methanol | 4 | 5.0 | 1.9,2.6, 2.4 3.0 | 0.38, 0.52,0.48, 0.60 |
| 40:60 ethylacate: methanol | 1 | 5.0 | 2.8 | 0.56 |
| 20:80 Ethylacate: methanol | 2 | 5.0 | 3.6, 3.9 | 0.32, 0.38 |

**Key:** DMS = Distance moved by the solvent (mobile phase), DMF= distance moved by fraction, RF= retention factor, NS: number of spot

**4.1.64 Antifungal inhibitory activity of *Vernonia amygdalina* fractions of n- hexane leaf extract(mm)**

Antifungal activity of *Vernonia amygdalina* fractions of n- hexane extract is presented in Table 4.64. The activity of fraction F1 showed zone of inhibition 11.00±0.40 and 15.00±0.60 mm at concentrations of 5 and 10 mg/mL against strain P37005. Fraction F4 showed inhibitory activity of 10.00±0.10 and 16.00±0.90 mm at concentrations of 5 and 10 mg/mL against strain 1161 respectively.

The activity of Fraction F4 was also seen against P37005 at the same concentration which gave zones of inhibition of 12.00±30 and 16.00±0.80 mm respectively. Other fractions showed no inhibitory activity against the tested strains, the positive control (fluconazole 1 mg/mL) tested against the strains showed varying zones of inhibition. The negative control (DMSO) used against the tested strains showed no inhibitory activity. The activity of the fraction F1 and F4 were less compared to the activity of the positive control.

**Table: 4.64 Antifungal Inhibitory Activity of *Vernonia amygdalina* Fractions of n- hexane Leaf Extract (mm)**

| S/N | ISOLATE | STRAIN | F1 5 mg/ml | F1 10 mg/ml | F2 5 mg/ml | F2 10 mg/ml | F3 5 mg/ml | F3 10 mg/ml | F4 5 mg/ml | F4 10 mg/ml | fluconazole (1mg/ml) | DMSO (100µl) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | S5 | strain1161 | 0.00± 0.00$^{a}$ | 0.00 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 10.00 0.10b | 16.00 0.90$^{c}$ | 42.00±0.40$^{d}$ | 0.00±0.00$^{a}$ |
| 2 | B7 | SC5314 | 0.00± 0.00$^{a}$ | 0.00± 0.00a | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 38.00±0.94$^{b}$ | 0.00±0.00$^{a}$ |
| 3 | C1 | SC5314 | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 34.33±1.00$^{b}$ | 0.00±0.00$^{a}$ |
| 4 | C2 | SC1354 | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.0$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 33.66±1.00b | 0.00±0.00a |
| 5 | S3 | SC5314 | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00a | 34.00±0.52$^{b}$ | 0.00±0.00$^{a}$ |
| 6 | B4 | P37005 | 11.00± 0.40$^{b}$ | 15.00± 0.60$^{c}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 12.00± 0.30$^{d}$ | 16.00± 0.80$^{e}$ | 40.00±0.70$^{f}$ | 0.00±0.00$^{a}$ |
| 7 | B2 | RM1000 | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 37.00±0.91b | 0 .00±0.00a |
| 8 | S1 | SC5314 | 0.00± 0.00$^{a}$ | 0.00± 0.00a | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 34.33±1.00b | 0.00±0.00a |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same row do not differ significantly at $p \leq 0.05$

### 4.1.65 Antifungal inhibitory activity of *Vernonia amygdalina* fractions of ethylacetate leaf extract(mm)

Antifungal activity of *Vernonia amygdalina* fractions of ethylacetate extract is presented in Table 4.65. The activity of fraction F3 showed zone of inhibition 8.00±0.10 and 9.00±0.10 mm at a concentrations of 5 and 10 mg/mL respectively against strain RM1000. Other fractions showed no inhibitory activity against the tested strains, the positive control (fluconazole 1 mg/mL) tested against the strains showed varying zones of inhibition. Although, the negative control (DMSO) tested showed no activity.

### 4.1.66 Antifungal inhibitory activity of *Vernonia amygdalina* fractions of methanol leaf extract(mm)

Antifungal activity of *Vernonia amygdalina* fractions of methanol extract is presented in Table 4.66. The activity of Fraction F1 showed zone of inhibition 7.00±0.20 and 9.00±0.60 mm at concentrations of 5 and 10 mg/mL respectively against strain P37005. Fraction F6 also showed inhibitory activity 8.00±0.30 and 12.00±0.70 mm at concentrations of 5 and 10 mg/mL respectively against strain P37005. The activity of fraction F1 was also recorded against RM1000 at a concentration of 5 and 10 mg/mL which gave inhibitory zones of 9.00±0.30 and 14.00±0.67 mm respectively .

Other fraction showed no inhibitory activity against the tested strains, although, the positive control (fluconazole 1 mg/mL) tested against the strains showed a varying zone of inhibition. The activity of the fraction F1 and F6 were less compared to the activity of the positive control. The negative control (DMSO) used against the tested strains showed no inhibitory activity.

### 4.1.67 Antifungal inhibitory activity of *Azardirachta indica* fractions of n-hexane leaf extract(mm)

Antifungal activity of *Azardirachta indica* fractions of n- hexane extract is presented in Table 4.67 . The activity of fraction F3 showed zone of inhibition 9.00±0.40 and 11.00±0.78 mm at a concentration of 5 and 10 mg/mL respectively against strain P37005, fraction F3 also showed inhibitory activity at the same concentrations against strain RM1000, the zones of inhibition were 6.00±0.20 and 7.00±0.20 mm respectively (Plate XVII). Fraction F7 showed inhibitory activity

6.00±0.2 mm and 9.00±0.40 mm at concentrations of 5 and 10 mg/mL respectively against strain 1161, there was also activity 7.00±0.10 and 8.00±0.10 mm against strain P37005 at the concentrations of 5 and 10 mg/mL respectively. The activity of fraction F7 also showed inhibitory activity 7.00±.0.30 and 9.50±0.50 mm against strain RM1000 at concentrations of 5 and 10mg/mL. Other fractions showed no inhibitory activity against the tested strains although, the positive control (fluconazole 1mg/mL) tested against the strains showed a varying zone of inhibition. The activity of the fraction F3 and F7 were less compared to the activity of the positive control . The negative control (DMSO) used against the tested strains showed no inhibitory activity.

### 4.1.68 Antifungal inhibitory activity of *Azardirachta indica* fractions of ethylacetate leaf extract(mm)

Antifungal activity of *Azardirachta indica* fractions of ethylacetate is presented in Table 4.68. The activity of fraction F1 showed zone of inhibition 8.00±0.30 and 9.00± 0.30 mm at concentrations of 5 and 10 mg/ml respectively against strain 1161, fraction F6 showed inhibitory activity at the same concentrations against strain P37005, the zones of inhibition were 7.00±0.20 and 10.00 ± 0.60 mm respectively. Fraction F7 showed inhibitory activity 6.00±0.20 and 9.00±0.56 mm at a concentration of 5 and 10 mg/mL respectively against strain 1161. There was also activity 6.00±0.40

and 8.00±0.50 mm against strain RM1000 at the concentrations of 5 and 10 mg/mL respectively. The activity of fraction F7 also showed inhibitory activity 7.00±0.10 and 9.00±0.30 mm against strain RM1000 at concentration of 5 and 10 mg/mL .Other fractions showed no inhibitory activity against the tested strains although, the positive control (fluconazole 1mg/mL) tested against the strains showed a varying zones of inhibition. The activity of fraction F1, F6 and F7 were less compared to the activity of the positive control . The negative control (DMSO) used against the tested strains showed no inhibitory activity.

### 4.1.69 Antifungal inhibitory activity of *Azardirachta indica* fractions of methanol leaf extract(mm)

Antifungal activity of *Azardirachta indica* fractions of methanol extract is shown in Table 4.69. The activity of fraction F3 showed zone of inhibition 9.00 ± 0.30 and 11.00±0.60 mm at a concentration of 5 and 10 mg/mL against strain P37005, fraction F3 also showed inhibitory activity 6.00±0.50 and 7.00±0.80 mm against strain RM1000. Fraction F6 showed inhibitory activity 8.00±0.40 and 12.00±0.60 mm at the same concentrations of 5 and 10 mg/mL against strain 1161. Other fractions showed no inhibitory activity against the tested strains, The positive control (fluconazole 1mg/mL) used against the tested strain showed a varying zone of inhibition. Activity of fraction F3 and F6 were less compared to the activity of the positive control . The negative control (DMSO) used against the tested strains showed no inhibitory activity.

**Table: 4.65 Antifungal Inhibitory Activity of *Vernonia amygdalina* Fractions of Ethylacetate leaf Extract (mm)**

| ISOLATE | STRAIN | F1 | | F2 | | F3 | | F4 | | F5 | | F6 | | Fluconazole (1mg/ml) | DMSO (100µl) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 5 mg/ml | 10 mg/ml | 5 mg/ml | 10 mg/ml | 5 mg/ml | 10 mg/ml | 5 mg/ml | 10 mg/ml | 5 mg/ml | 10 mg/ml | 5 mg/ml | 10 mg/ml | | |
| S5 | strain1161 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00 0.00a | 0.00 0.00a | 0.00± 0.00$^{a}$ | 0.00± 0.00$^{a}$ | 0.00 0.00$^{a}$ | 0.00 0.00$^{a}$ | 42.00±0.40$^{b}$ | 0.00±0.00$^{a}$ |
| B7 | SC5314 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 38.00±0.93$^{b}$ | 0.00±0.00$^{a}$ |
| C1 | SC5314 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 34.33±1.00b | 0.00±0.00a |
| C2 | SC1354 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 33.66±2.00 | 0.00±0.00a |
| S3 | SC5314 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 34.00±0.91b | 0.00±0.00a |
| B4 | P37005 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00 0.00a | 0.00 0.00a | 40.00±0.82b | 0.00±0.00a |
| B2 | RM1000 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 8.00± 0.10a | 9.00± 0.10a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 37.00±0.11b | 0.00±0.00aa |
| S1 | SC5314 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 34.33±1.00b | 0.00±0.00a |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same row do not differ significantly at $p \leq 0.05$

**Table 4.66 : Antifungal Inhibitory Activity of *Vernonia amygdalina* Fractions of Methanol Leaf Extract (mm)**

| | | F1 | | F2 | | F3 | | F4 | | F5 | | F6 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **ISOLATE** | **STRAIN** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **fluconazole (1mg/ml)** | **DMSO (100µl)** |
| S5 | strain1161 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 42.00±0.48b | 0.00±0.00 |
| B7 | SC5314 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 38.00±0.91b | 0.00±0.00 |
| C1 | SC5314 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 | 0.00± 0.00 | 34.33±1.00 | 0.00±0.00 |
| C2 | SC1354 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 33.66±2.00b | 0.00±0.00a |
| S3 | SC5314 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 34.00±0.94b | 0.00±0.00a |
| B4 | P37005 | 7.00 0.20a | 9.00 0.60a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 8.00 0.30a | 12.00 0.70a | 40.00±0.84b | 0.00±0.00 |
| B2 | RM1000 | 9.00± 0.30c | 14.00± 0.67b | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 37.00±0.94d | 0.00±0.00a |
| S1 | SC5314 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 34.33±1.00b | 0.00±0.00a |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same row do not differ significantly at $p \leq 0.05$

**Table 4.67: Antifungal Inhibitory Activity of *Azardirachta indica* Fractions of n- hexane Leaf Extract (mm)**

| | | F1 | | F2 | | F3 | | F4 | | F5 | | F6 | | F7 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **ISOLATE** | STRAIN | 5 mg/ml | 10 mg/ml | 5 mg/ml | 10 mg/ml | 5 mg/ml | 10 mg/ml | 5 mg/ml | 10 mg/ml | 5 mg/ml | 10 mg/ml | 5 mg/ml | 10 mg/ml | 5 mg/ml | 10 mg/ml | fluconazole (1mg/ml) | DMSO (100µl) |
| **S5** | strain116 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 6.00± 0.00b | 9.00± 0.00c | 42.00±0.49d | 0.00±0.00 |
| **B7** | SC5314 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 38.00±0.92b | 0.00±0.00 |
| **C1** | SC5314 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 34.33±1.00b | 0.00±0.00 |
| **C2** | SC1354 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 33.66±1.00b | 0.00±0.00 |
| **S3** | SC5314 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 34.00±0.97b | 0.00±0.00 |
| **B4** | P37005 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 9.00± 0.00c | 11.00± 0.00d | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 7.00± 0.00b | 8. 00 0.00b | 40.00±0.80e | 0.00±0.00a |
| **B2** | RM1000 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 6.00± 0.00b | 7.00± 0.00b | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 7.00± 0.00c | 9.00± 0.00d | 37.00±0.92e | 0.00±0.00a |
| **S1** | SC5314 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 34.33±1.00b | 0.00±0.00a |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same row do not differ significantly at p≤0.05

**Table 4.68: Antifungal Inhibitory Activity of *Azardirachta indica* Fractions of Ethylacetate Leaf Extract(mm)**

| | | F1 | | F2 | | F3 | | F4 | | F5 | | F6 | | F7 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **ISOLATE** | **STRAIN** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **Fluconazole (1mg/ml)** | **DMSO (100µl)** |
| **S5** | strain116 | 8.00± | 900± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 6.00± | 9.00± | 42.00±0.49c | 0.00±0.00a |
| | | 0.30b | 0.30b | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.20a | 0.56a | | |
| **B7** | SC5314 | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 38.00±0.92b | 0.00±0.00a |
| **C1** | SC5314 | 0.00 | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | | |
| | | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 34.33±1.00b | 0.00±0.00a |
| **C2** | SC1354 | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | | |
| | | 0.0± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.0± | 0.00± | 0.0± | 0.00± | 33.66±1.00b | 0.00±0.00a |
| **S3** | SC5314 | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | | |
| | | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 34.00±0.91b | 0.00±0.00a |
| **B4** | P37005 | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | | |
| | | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 7.00± | 10.00± | 0.00 | 0.00 | 40.00±0.80d | 0.00±0.00a |
| **B2** | RM1000 | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.20b | 0.60c | 0.00a | 0.00a | | |
| | | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 6.00± | 800± | 7.00± | 9.00± | 37.00±0.91f | 0.00±0.00a |
| **S1** | SC5314 | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.40b | 0.50c | 0.10d | 0.30e | | |
| | | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 0.00± | 34.33±1.00b | 0.00±0.00a |
| | | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | 0.00a | | |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same row do not differ significantly at $p \leq 0.05$

**Table 4.69 Antifungal Inhibitory Activity of *Azardirachta indica* Fractions of Methanol Leaf Extract (mm)**

| | | F1 | | F2 | | F3 | | F4 | | F5 | | F6 | | F7 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **ISOLATE** | **STRAIN** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **5 mg/ml** | **10 mg/ml** | **Fluconazole (1mg/ml)** | **DMSO (100µl)** |
| **S5** | Strain1161 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 8.00± 0.40b | 12.00± 0.60c | 0.00± 0.00a | 0.00± 0.00a | 42.00±0.42d | 0.00±0.00a |
| **B7** | SC5314 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 38.00±0.92b | 0.00±0.00a |
| **C1** | SC5314 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 34.33±1.00b | 0.00±0.00a |
| **C2** | SC1354 | 0.0± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.0± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.0± 0.00a | 0.00± 0.00a | 0.0± 0.00a | 0.00± 0.00a | 33.66±1.00b | 0.00±0.00a |
| **S3** | SC5314 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 34.00±0.92b | 0.00±0.00a |
| **B4** | P37005 | 0.00± 0.00a | .00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 9.00 0.30b | 11.00 0.60c | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00 0.00a | 0 .00 0.00a | 40.00±0.85d | 0.00±0.00a |
| **B2** | RM1000 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 6.00± 0.50b | 7.00± 0.80b | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 37.00±0.95c | 0.00±0.00a |
| **S1** | SC5314 | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 0.00± 0.00a | 34.33±1.00b | 0.00±0.00a |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same row do not differ significantly at $p \leq 0.05$

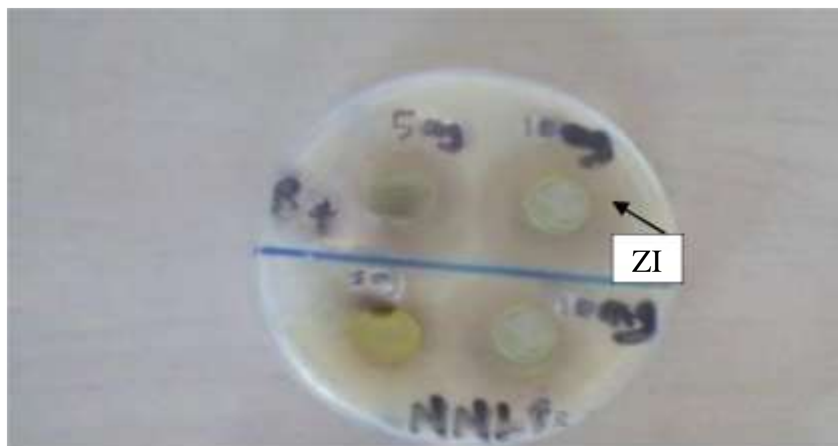



**Plate XVII: Zones of Inhibition of *Azadirachta indica* (neem) Leaf Fraction(F3) of n-hexane Extract ZI: Zone of Inhibition**

### 4.1.70 Minimum inhibitory and Minimum fungicidal concentrations of active fractions

The Minimum Inhibitory Concentration and Minimum Fungicidal Concentration of the active fractions is shown in Table 4. 70. *Vernonia amygdalina* fraction F4 showed an MIC of 12.5 and 25 mg/mL for the MFC against strain 1161(Isolate:S5). Fraction (F1) had an MIC and MFC reading of 3.125 and 6.25 mg/mL against strain P37005. Fraction (F4) had values of 6.25 and 12.5 mg/mL for both the MIC and MFC. The *Azadirachta indica* fraction F6 showed values of 1.25 and 3.125 mg/mL for the MIC and MFC respectively. Fraction F3 and F1 of *Azadirachta indica* showed the same MIC and MFC of 3.125 and 6.25 mg/mL respectively.

**Table 4.70: Minimum Inhibitory and Minimum Fungicidal Concentrations of Active Fractions (mg/mL)**

| SN | Isolate | Strain | Medicinal plant | Plant part | Fractions | MIC (mg/mL) | MFC (mg/mL) |
|---|---|---|---|---|---|---|---|
| 1 | S5 | Strain 1161 | *Vernonia amygdalina* | Leaf | F4 | 12.5 | 25 |
| 2 | B4 | P37005 | *Vernonia amygdalina* | Leaf | F1 | 3.125 | 6.25 |
| 3 | B4 | P37005 | *Vernonia amygdalina* | Leaf | F4 | 6.25 | 12.5 |
| 4 | B2 | RM1000 | *Vernonia amygdalina* | Leaf | F1 | 3.125 | 6.25 |
| 5 | B4 | P37005 | *Vernonia amygdalina* | Leaf | F6 | 1.25 | 3.125 |
| 6 | B4 | P37005 | *Azadirachta indica* | Leaf | F3 | 3.125 | 6.25 |
| 7 | S5 | Strain 1161 | *Azardirachta indica* | Leaf | F6 | 3.125 | 6.25 |

### 4.1.71 Phytoconstituents of *Vernonia amygdalina* of fraction (F4) of n- hexane leaf extract revealed by gas chromatography mass spectrophotometer(GC-MS)

The result of the gas chromatography mass spectrophotometry of *Vernonia amygdalina* fraction (F4) of n- hexane extract is presented in Table 4.71. While the chromatogram is presented in Appendix B. Twenty (20) probable compounds were identified in the fraction. The result showed the peak number, retension time and their percentage concentrations in the fraction.

**Table: 4.71 Probable Compounds and Percentage Concentrations of *Vernonia amygdalina* Fraction(F4) of n-hexane Leaf Extract Revealed by Gas Chromatography Mass Spectrophotometer (GC-MS)**

| Peak No/serial No | Retention time(min) | Percentage Concentration (%) | Probable Compound |
|---|---|---|---|
| 1 | 10.544 | 100.00 | 2,4-Di-tert-butylphenol |
| 2 | 13.502 | 10.18 | E-15-Heptadecenal |
| 3 | 13.866 | 22.82 | 2-Pentadecanone, 6,10,14-trimethyl |
| 4 | 13.927 | 57.53 | Neophytadiene |
| 5 | 14.135 | 15.74 | Hexadecanal |
| 6 | 14.299 | 34.12 | 1,E-11,Z-13-Hexadecatriene |
| 7 | 14.567 | 25.76 | Hexadecanoic acid, methyl ester |
| 8 | 14.897 | 84.79 | n-Hexadecanoic acid |
| 9 | 15.105 | 52.12 | Phenol, 4,4',4''-ethylidynetris- |
| 10 | 15.160 | 23.05 | Hexadecanoic acid, ethyl ester |
| 11 | 15.265 | 14.58 | Cycloeicosane |
| 12 | 16.160 | 44.18 | Phytol |
| 13 | 16.245 | 14.18 | Methyl stearate |
| 14 | 16.356 | 33.67 | 1,19-Eicosadiene |
| 15 | 16.477 | 20.02 | Linoleic acid ethyl ester |
| 16 | 16.545 | 32.34 | Ethyl Oleate |
| 17 | 16.872 | 11.46 | 1-Docosene |
| 18 | 18.872 | 89. 30 | Bis(2-ethylhexyl) phthalate |
| 19 | 20.427 | 21.34 | .alpha.-Tocospiro B |
| 20 | 20.527 | 20.64 | .delta.-Tocopherol |

### 4.1.72 Phytoconstituents of *Vernonia amygdalina*(leaf) fraction(F1) of methanol leaf extract revealed by gas chromatography mass spectrophotometer (GC-MS)

The result of the gas chromatography mass spectrophotometry of *Vernonia amygdalina* fraction (F1) of methanol extract is presented in Table 4.72 while the chromatogram is presented in Appendix C . Nineteen (19) probable compounds were identified in fraction (F1). The result showed the peak number, retension time, percentage concentrations and probable compounds in the fraction.

**Table: 4.72 Probable Compounds and Percentage Concentrations of *Vernonia amygdalina* Fraction(F1) of Methanol Leaf Extract Revealed by Gas Chromatography Mass Spectrophotometer (GC-MS)**

| Peak /serial No | Retention time(min) | Percentage Concentration (%) | Probable Compound |
|---|---|---|---|
| 1 | 4.275 | 18.29 | 3-Ethoxy-1,2-propanediol |
| 2 | 13.921 | 11.56 | Neophytadiene |
| 3 | 14.566 | 28.68 | Hexadecanoic acid, methyl ester |
| 4 | 14.875 | 68.88 | n-Hexadecanoic acid |
| 5 | 15.155 | 13.75 | Hexadecanoic acid, ethyl ester |
| 6 | 16.004 | 8.41 | 2-Propenamide, N-(1-cyclohexylethy 48750 1000142-14-6 53l)- |
| 7 | 16.154 | 18.57 | Phytol |
| 8 | 16.237 | 12.85 | Methyl stearate |
| 9 | 16.512 | 10.26 | N-Serylserine |
| 10 | 16.539 | 17.78 | Dihydroxymaleic acid |
| 11 | 18.967 | 16.77 | Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate |
| 12 | 19.612 | 30.58 | Di(E)-but-2-enyl phthalate |
| 13 | 19.963 | 29.25 | Stigmasterol |
| 14 | 20.105 | 100 | MDMA methylene homolog |
| 15 | 20.275 | 2.51 | .gamma.-Sitosterol |
| 16 | 20.363 | 17.65 | Benzo[h]quinoline, 2,4-dimethyl- |
| 17 | 20.45 | 11.72 | Tetrasiloxane, decamethyl- |
| 18 | 20.621 | 12.03 | 1H-Indole, 5-methyl-2-phenyl |
| 19 | 20.675 | 12.11 | Benzaldehyde2-nitro-, diaminomethylidenhydrazone |

### 4.1.73 Phytoconstituents of *Azardirachta indica* fraction (F3) of n-hexane leaf extract revealed by by gas chromatography mass spectrophotometer(GC-MS)

The result of the gas chromatography mass spectrophotometry of *Azardirachta indica* fraction (F3) of n-hexane extract is presented in Table 4.73 While the chromatogram is presented in Appendix D. Thirty four (34) probable compounds were identified in the fraction. The result showed the peak number, retention time, and as well their percentage concentration in the fraction.

**Table: 4.73 Probable Compounds and Percentage Compositions Revealed in *Azardirachta indica* Fraction (F3) of n-hexane Leaf Extract by Gas chromatography Mass Spectrophotometer**

| Peak No | N/S | Retention time(min) | Percentage Concentration (%) | Probable Compound |
|---|---|---|---|---|
| 1 | | 10.541 | 5.12 | 2,4-Di-tert-butylphenol |
| 2 | | 11.159 | 5.27 | Dodecanoic acid |
| 3 | | 12.733 | 5.09 | Methyl tetradecanoate |
| 4 | | 13.101 | 5.71 | Tetradecanoic acid |
| 5 | | 13.502 | 5.36 | Bromoacetic acid, hexadecyl ester |
| 6 | | 13.867 | 10.77 | 2-Pentadecanone, 6,10,14-trimethyl |
| 7 | | 13.926 | 16.44 | Phytol, acetate |
| 8 | | 14.136 | 4.56 | Neophytadiene |
| 9 | | 14.298 | 6.88 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol |
| 10 | | 14.494 | 7.12 | Nonadecane |
| 11 | | 14.586 | 81.73 | Hexadecanoic acid, methyl ester |
| 12 | | 14.664 | 23.27 | Dibutyl phthalate |
| 13 | | 14.958 | 100.00 | n-Hexadecanoic acid |
| 14 | | 15.167 | 42.74 | Hexadecanoic acid, ethyl ester |
| 15 | | 15.268 | 9.01 | Trifluoroacetoxy hexadecane |
| 16 | | 15.343 | 4.47 | Heptadecane |
| 17 | | 15.432 | 5.83 | Heptadecanoic acid, methyl ester |
| 18 | | 15.942 | 25.25 | 9,12-Octadecadienoic acid, methyl |
| 19 | | 15.974 | 14.58 | 9,12,15-Octadecatrienoic acid, met hyl ester, (Z,Z,Z)- |
| 20 | | 16.018 | 42.99 | 11-Octadecenoic acid, methyl ester |
| 21 | | 16.164 | 26.61 | Phytol |
| 22 | | 16.246 | 23.43 | Methyl stearate |
| 23 | | 16.325 | 22.51 | cis-13-Octadecenoic acid |
| 24 | | 16.355 | 12.62 | cis-13-Octadecenoic acid |
| 25 | | 16.479 | 7.15 | Linoleic acid ethyl ester |
| 26 | | 16.544 | 37.77 | Ethyl Oleate |
| 27 | | 16.776 | 13.45 | Octadecanoic acid, ethyl ester |
| 28 | | 16.875 | 6.46 | 1-Octadecene |
| 29 | | 16.967 | 36.37 | Neophytadiene |
| 30 | | 17.713 | 7.44 | cis-10-Nonadecenoic acid |
| 31 | | 17.772 | 3.68 | Methyl 18-methylnonadecanoate |
| 32 | | 18.973 | 38.14 | Bis(2-ethylhexyl) phthalate |
| 33 | | 20.240 | 8.94 | 7,22-Ergostadienone |
| 34 | | 20.447 | 8.96 | 4,4,6a,6b,8a,11,12,14b-Octamethyl |

**4.1.74 Phytoconstituents of *Azardirachta Indica* fraction (F6) of methanol leaf extract revealed by gas chromatography mass spectrophotometer(GC-MS)**

The result of the gas chromatography mass spectrophotometry of *Azardirachta indica* fraction(F6) of methanol extract is presented in Table 4.74 .While the chromatogram is presented in Appendix E . Twenty (20) probable compounds were identified in the fraction. The result showed the peak number, retention time, and as well their percentage concentrations in the fraction.

**Table 4.74: Probable Compounds and Percentage Compositions Identified in *Azardirachta indica* Fraction (F6) of Methanol Leaf Extract by Gas chromatography Mass Spectrophotometer**

| Peak No/serial No | Retention time(min) | Percentage Concentration (%) | Probable Compound |
|---|---|---|---|
| 1 | 3.553 | 100.00 | 2,4-Di-tert-butylphenol |
| 2 | 3.618 | 11.18 | cetene |
| 3 | 6.213 | 21.82 | 2-Pentadecanone, 6,10,14-trimethyl |
| 4 | 6.865 | 57.53 | Neophytadiene |
| 5 | 8.911 | 14.74 | Hexadecanal |
| 6 | 9.040 | 33.12 | Trans- 2- Dodecen-1. |
| 7 | 9.040 | 24.76 | Hexadecanoic acid, methyl ester |
| 8 | 9.351 | 85.79 | n-Hexadecanoic acid |
| 9 | 9.696 | 51.12 | Furo[2,3-H] coumarine, 6-methyl-1-p |
| 10 | 10.061 | 22.05 | Hexadecanoic acid, ethyl ester |
| 11 | 10.291 | 13.58 | Trifluoroacetoxyhexadecane |
| 12 | 10.345 | 43.18 | Phytol |
| 13 | 10.405 | 13.18 | Methyl stearate |
| 14 | 10.450 | 32.67 | 1,19-Eicosadiene |
| 15 | 10.482 | 22.02 | Linoleic acid ethyl ester |
| 16 | 10.512 | 33.34 | Ethyl Oleate |
| 17 | 10.689 | 14.46 | 9- nonadecene |
| 18 | 10.766 | 88. 30 | Diisococtylphthalate |
| 19 | 11.292 | 22.34 | 4- fluorophenyl-5-methyl |
| 20 | 11.349 | 21.64 | delta.-Tocopherol B |

### 4.1.75 The results of acute toxicity of methanol crude extract of *Vernonia amygdalina* (Bitter leaf) leaf on experimental rats (phase one)

The result on Table 4.75 showed that the animals received doses of 10, 100 and 1000 mg/kgbw of the plant extracts of *Vernonia amygdalina* for phase one. There was no mortality or death from all the groups that were exposed to the extract, as well no group showed signs of toxicity.

**Table 4.75: Acute Toxicity of Methanol Crude Extract of *Vernonia amygdalina*(Bitter leaf) Leaf on Experimental Rats (phase one)**

| Extract | No. of rats | Dose(mg/kgbw) | Mortality/survival | Toxicity reaction |
|---|---|---|---|---|
| MBLE | 3 | 10 | 0/3 | No sign of toxicity |
| | 3 | 100 | 0/3 | No sign of toxicity |
| | 3 | 1000 | 0/3 | No sign of toxicity |
| (Control)NS | 1 | 1mL | 0 | No sign of toxicity |

**Key: MBLE: Methanol bitter leaf extract, NS: normal saline**

**4.1.76 The results of acute toxicity of methanol crude extract of *Vernonia amygdalina* (bitter leaf) leaf on experimental rats (phase two)**

The result on Table 4.76 showed that the animals received doses of 1600, 2900 and 5000 mg/kgbw of the plant extracts of *Vernonia amygdalina* for phase two. There was no mortality or death from all the groups that were exposed to the extract, as well no group showed signs of toxicity.

**Table 4.76: Acute Toxicity of Methanol Crude Extract of *Vernonia amygdalina*(Bitter leaf) Leaf on Experimental Rats (phase two)**

| Extract | No. of rats | Dose(mg/kgbw) | Mortality/survival | Toxicity reaction |
|---|---|---|---|---|
| MBLE | 3 | 1600 | 0/3 | No sign of toxicity |
| | 3 | 2900 | 0/3 | No sign of toxicity |
| | 3 | 5000 | 0/3 | No sign of toxicity |
| NS (Control) | 1 | ImL | 0 | No sign of toxicity |

**Key: MBL: Methanol bitter leaf extract, NS: normal saline**

**4.1.77 The result of acute toxicity of n- hexane crude extract of *Azadirachta indica*(neem) leaf on experimental rats(phase one)**

The result on Table 4.77 showed that the animals received doses of 10, 100 and 1000 mg/kgbw of the plant extracts of *Azadirachta indica* each in phase one. The results revealed that the rats in phase one showed no signs of toxicity and there was no mortality or death from all the groups that were exposed to the extract, as well no group showed signs of toxicity.

**Table 4.77: Acute Toxicity of n- hexane Crude Extract of *Azardirachta indica*(Neem) Leaf on Experimental Rats (phase one)**

| Extract | No. of rats | Dose(mg/kgbw) | Mortality/survival | Toxicity reaction |
|---|---|---|---|---|
| NHNLE | 3 | 10 | 0/3 | No sign of toxicity |
| | 3 | 100 | 0/3 | No sign of toxicity |
| | 3 | 1000 | 0/3 | No sign of toxicity |
| NS(control) | 1 | 1mL | 0 | No sign of toxicity |

**Key :NHNLE: n- hexane neem leaf extract, NS: normal saline**

### 4.1.78 The result of acute toxicity of n- hexane crude extract of *Azadirachta indica* (neem)leaf on experimental rats (phase two)

The result on Table 4.78 showed that the animals received doses of 1600, 2900 and 5000 mg/kgbw of the plant extracts of *Azadirachta indica* each in phase two . The results showed that the rats in phase two showed no signs of toxicity and there was no mortality or death from all the groups that were exposed to the extract, as well no group showed signs of toxicity.

| Extract | No. of Animal | DOSE(mg/kgbw) | Mortality/survival | Toxicity reaction |
|---|---|---|---|---|

| NHNLE | 3 | 1600 | 0/3 | No sign of toxicity |
|---|---|---|---|---|
| | 3 | 2900 | 0/3 | No sign of toxicity |
| | 3 | 5000 | 0/3 | No sign of toxicity |
| NS(control) | 1 | ImL | 0 | No sign of toxicity |

**Table 4.78: Acute Toxicity of n- hexane Crude Extract of *Azadirachta indica* (Neem) Leaf on Experimental Rats (phase two)**

**Key :NHNLE : n- hexane neem leaf extract , NS: normal saline**

**4.1.79 Pharmacological effect of methanol leaf crude extract of *Vernonia amygdalina* on the body weight(g) of experimental rats after 28 days of adminstration**

The effect of *Vernonia amygdalina* crude extract on the body weight of experimental rats is presented in Table 4.79. The weight of the various treated groups increased as the experiment proceeds from week one to week four.There was significant differences P<0.05 between the treated groups and the control.The significant increase was observed continuously from week one to week four.

**Table 4.79: Pharmacological Effect of Methanol Leaf Crude Extract on the Bodyweight(g) of Experimental Rats after 28 Days of Administration**

| Crude extract | Week 1 | Week 2 | Week3 | Week 4 |
|---|---|---|---|---|
| Group1 (100mg/kgbw) | $167.67^{c}$ | $174.81^{c}$ | $179.27^{c}$ | $182.25^{c}$ |
| Group2 (300mg/kgbw) | $158.54^{b}$ | $160.99^{a}$ | $163.97^{a}$ | $170.36^{b}$ |
| Group3 (600mg/kgbw) | $154.79^{a}$ | $162.16^{b}$ | $166.4^{b}$ | $171.32^{a}$ |
| Control | $183.66^{d}$ | $191.28^{d}$ | $194.10^{d}$ | $203.49^{d}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at p≤0.05.

**4.1.80 Haematological parameters of experimental rats administered with methanol leaf crude extract of *Vernonia amygdalina*(bitter leaf) after 28days of administration**

The results of the haematological parameters for white blood cells, (WBC), red blood cell (RBC) Haemoglobin (HB), Pack cell volume (PCV), mean cell volume(MCV), Mean cell haemoglobin(MCH) Mean cell haemoglobin concentration(MCHC), Platelet count, neutrophils,(N) lymphocytes,(L), monocytes(M), eosinophil(E) and Basophils (B) for both the experimental and control rats is presented in Table 4.80. The WBC($\times 10^9$/L) for group1 (100 mg/kgbw), group 2(300 mg/kgbw) and group 3(600 mg/kgbw) showed a value of 3.35±0.78, 5.15±0.14 & 5.10 ± 0.02 respectively. Although, there was significant difference P<0.05 between group 1 and the control groups while group 2 and group 3 showed no significance difference with the control group. The RBC($\times 10^{12}$/L) showed a value of 5.15±0.14,' 5.23±0.45 and 5.70±0.30 for group 1 group 2 and group 3 there was no significant difference (P>0.05) for all treated groups as compared with the control group. However, the value of the HB (g/dL) recorded was 10.55±0.43, 11.5±0.01 & 11.60±0.19 for group 1, group2 & group 3 respectively. There was no significant difference p≤ 0.05 between all the group as compared with the control groups. The PCV(%) value recorded was 33.50±0.91, 35±0.56 and 35.50±1.09 for group 1group 2 and group 3 respectively. Group 1 showed significant difference (P< 0.05) between the control groups while the value of group 2 and group 3 showed no significant difference as compared with the control group.

The result for the MCV, MCH, MCHC showed no significant difference between all the groups as compared with the control group. The result of the differential white blood cells counts: neutrophil lymphocytes and monocytes showed significant difference

(P<0.05) for all the groups. The result of the eosinophil and basophil showed no significant difference (P>0.05) for all the groups

**Table 4.80: Effect of Methanol Leaf Crude Extract of *Vernonia amygdalina*(Bitter leaf) on Haematological Parameters of Experimental Rats after 28Days of Administration**

| Parameter | Dosage | | | |
|---|---|---|---|---|
| | **100mg/Kgbw (Group 1)** | **300mg/Kgbw (Group2)** | **600 mg/Kgbw (Group3)** | **Control** |
| WBC($\times 10^{9}$/L) | 3.35±0.78$^{a}$ | 5.15±0.14$^{b}$ | 5.10±0.02$^{b}$ | 6.00 ±0.21$^{b}$ |
| RBC($\times 10^{12}$/L) | 5.15±0.14$^{a}$ | 5.23 ±0.45$^{a}$ | 5.70±0.30$^{a}$ | 5.70±0.32$^{a}$ |
| HB (g/dL) | 10.55±0.43$^{a}$ | 11.5±0.01$^{a}$ | 11.60±0.19$^{a}$ | 11.70±0.34$^{a}$ |
| PCV (%) | 33.50±0.91$^{a}$ | 35±0.56$^{b}$ | 35.50±1.09$^{b}$ | 36.00±0.31$^{b}$ |
| MCV ( fl) | 61.15±0.21$^{a}$ | 69.75±0.25$^{a}$ | 62.85±0.32$^{a}$ | 63.70±0.09$^{a}$ |
| MCH ( Pg) | 20.35±0.89$^{a}$ | 22.05±0.08$^{a}$ | 20.3±0.98$^{a}$ | 20.40±0.11$^{a}$ |
| MCHC( g/dL) | 31.05±0.11$^{a}$ | 31.6±0.65$^{a}$ | 32.25±0.11$^{a}$ | 32.00±0.34$^{a}$ |
| PLT $\times 10^{9}$/L | 361±0.43$^{a}$ | 382±0.41$^{b}$ | 385.5±0.01$^{c}$ | 476.00±0.39ab |
| Neu (%) | 7±0.17$^{b}$ | 7.5±0.01$^{c}$ | 2.5±0.01$^{a}$ | 08.00±0.34$^{d}$ |
| Lymp (%) | 84±0.11$^{a}$ | 88±0.32$^{c}$ | 94±0.67$^{d}$ | 85±0.72$^{b}$ |
| Mono (%) | 4.5±0.47$^{c}$ | 4.5±0.34$^{c}$ | 2.5±0.01$^{b}$ | 02±0.32$^{a}$ |
| Eosi (%) | 2.0±0.18$^{a}$ | 2.6±0.14$^{a}$ | 2.5±0.78$^{a}$ | 02±0.11$^{a}$ |
| Baso (%) | 2.5±0.19$^{ab}$ | 2.0±0.11$^{b}$ | 2.4±0.16$^{ab}$ | 03±0.18$^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same row do not differ significantly at p≤0.05

### 4.1.81 Pharmacological effect of n- hexane leaf crude extract of *Azardirachta indica*(neem) on the body weight (g) of experimental rats after 28 days of administration

The effect of *Azardirachta indica* crude extract on the body weight of experimental rats is presented in Table 4.81. The weight of the various treated groups increased as the experiment proceeds from week one to week four.There was significant differences p≤0.05 between the treated groups and the control.The significant increase was observed continuously from week one to week four.

**Table 4.81:Pharmacological Effect of n- hexane Leaf Crude Extract of *Azardirachta indica*(neem) on the Body weight (g) of Experimental Rats after 28 Days of Administration**

| Crude extracts | Week 1 | Week2 | Week 3 | Week4 |
|---|---|---|---|---|
| Group 1(100mg/kgbw) | 157.71[a] | 162.68[b] | 168.11[c] | 171.45[c] |
| Group2(300mg/kgbw) | 158.14[b] | 162.36[b] | 165.20[b] | 171.25[b] |
| Group 3(600mg/kgbw) | 158.42[c] | 159.91[a] | 163.52[a] | 169.86[a] |
| Control | 192.11[d] | 201.52[c] | 206.45[d] | 212.01[d] |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at $p \leq 0.05$

**4.1.82 Haematological parameters of experimental rats administered with n- hexane leaf crude extract of *Azardirachta indica* (neem) after 28days of administration**

The results of the haematological parameters for white blood cells, (WBC), red blood cell(RBC) Haemoglobin(HB), Pack cell volume(PCV), mean cell volume(MCV), Mean cell haemoglobin, mean cell haemoglobin concentration(MCHC) , Platelet count , neutrophils,(N) lymphocytes,(L), monocytes(M), eosinophil(E) and Basophils (B) for both the experimental and control rats is presented in Table 4.82. The WBC($\times 10^9$/L) for group 1(100mg/kgbw), group 2(300mg/kgbw) and group 3(600mg/kgbw) showed a values of 5.2 ± 0.91, 4.05±0.45 & 7.65±0.12 respectively. There was significant different between all treated groups and the control groups. The RBC($\times 10^{12}$/L) for the treated groups showed values of 5.65±0.21' 5.10±0.21 and 4.40±0.90 for group 1, group 2 and group 3 respectively .There was no significant difference for group 1 and group2 when compared with the control group. The values of the HB(g/dL) recorded was 11.35±0.38, 11.0±0.32 and 9.6±0.12 for group 1, group 2 & group3 respectively. There was no significant difference($P>0.05$) between the treated groups and the control groups. The PCV(%) for the treated groups showed values of 35.5±0.78, 34.5±0.11 and 30.0±0.29 for group 1 group 2 and group 3 respectively. There was no significant difference ($P>0.05$)for all the treated groups.The result for the MCV, MCH and MCHC showed

significant difference for all the groups except MCH that had no significant difference (P>0.05) .The platelet count showed no significant difference (P>0.05) between the treated groups and the control groups. The results of the differential white blood cells count: neutrophils, lymphocytes, monocytes eosinophil and basophils of all the treated groups showed no significant differences when compared with their various control groups.

**Table 4.82: Effect of n- hexane Leaf Crude Extract of *Azardirachta indica* (neem) on Haematological Parameters of Experimental Rats after 28 Days of Administration**

| Parameter | Dosage | | | |
|---|---|---|---|---|
| | **100 mg/Kgbw** | **300 mg/Kgbw** | **600 mg/Kgbw** | **Control** |
| WBC ($\times 10^9$/L) | $5.20\pm0.91^{b}$ | $4.05\pm0.45^{a}$ | $7.65\pm0.12^{c}$ | $6.2\pm 0.34^{d}$ |
| RBC($\times 10^{12}$/L) | $5.65\pm0.21^{b}$ | $5.10\pm0.21^{b}$ | $4.40\pm0.90^{a}$ | $6.3\pm0.21^{c}$ |
| HB (g/dL) | $11.35\pm0.38^{b}$ | $11.0\pm0.32^{b}$ | $9.6\pm0.12^{a}$ | $12.5\pm0.01^{c}$ |
| PCV (%) | 35.5±0.78c | 34.5±0.11b | 30.0±0.29a | 38±0.18d |
| MCV ( fL) | 62.7±0.23a | 67.7±0.24d | 67.5±0.22c | 60.3±0.10b |
| MCH ( Pg) | $20.0\pm0.21^{a}$ | $21.7\pm0.23^{a}$ | $21.6\pm0.32^{a}$ | $20.0\pm0.19^{a}$ |
| MCHC ( g/dL) | 31.85±0.32c | 32.05±0.91b | 32±0.45b | 33.2±0.10a |
| PLT $\times 10^9$/L | $472.5\pm0.76^{d}$ | $402\pm0.25b^{b}$ | $377\pm0.23^{a}$ | 441±0.11c |
| Neu (%) | $03\pm0.27^{a}$ | 04±0.32b | $06\pm0.46^{c}$ | $09\pm0.90^{d}$ |
| Lymp (%) | $91.5\pm0.24^{a}$ | $90\pm0.12^{a}$ | $89.5\pm0.21^{b}$ | $84\pm0.11^{c}$ |
| Mono (%) | $02\pm0.21^{a}$ | $02\pm0.67^{a}$ | $04\pm0.11^{b}$ | $01\pm0.12^{c}$ |
| Eosi (%) | 01±0.29a | 02±0.46b | 02±0.32c | $03\pm0.23^{d}$ |
| Baso (%) | $2.5\pm0.34^{ab}$ | $02\pm0.21^{ab}$ | $1.5\pm0.11^{b}$ | $03\pm0.11^{a}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same row do not differ significantly at p≤0.05

### 4.1.83 Effect of methanol leaf crude extract of *Vernonia amygdalina* (bitterleaf) on the Liver function and lipid test parameters of experimental rats after 28days of administration

The result of the liver function test for the experimental rats is presented in Table 4.83. The determined parameters were: total protein (TP), Albumin (Alb). Cholesterol (chol), Triglyceride(TG), Highdensity lipoprotein(HDL),low density lipoprotein,(LDL), Alkaline phosphate(ALP), Aspartate aminotransferase(AST), Alanin aminotransferase(ALT), total bilirubin (Tbil) and Direct bilirubin (Db).

The result showed that the liver parameters increase with increase in the dosage. The highest parameters values were 126.5±0.67 g/L, 41.0±0.34 g/L, 3.6±0.14 mmol/L, 2.5±1.00 mmol/L, 0.31±0.34 mmol/L 3.69±0.67 mmol/L, 60.0±0.35 U/L, 180.0±0.51 U/L, 120.0±0.25 U/L, 39.5±0.43 µmol/L and 6.0±0.32 µmol/L at 600 mg/kgbw of *Vernonia amygdalina* were recorded for total protein(TP), Albumin (Alb).cholesterol (chol), Triglyceride (TG), Highdensity lipoprotein (HDL),low density lipoprotein (LDL), Alkaline phosphate (ALP),Aspartate aminotransferase (AST),Alanin aminotransferase (ALT), total bilirubin (Tbil) and Direct bilirubin (Db) respectively. There was significant differences ($P<0.05$) for all other parameters recorded but the values for Albumin, cholesterol, HDL, LDL and Direct bilirubin showed no significant differences .While the lowest parameters values were 72.0±0.16 g/L, 33.5±0.21 g/L 2.5±0.22 mmol/L, 1.1± 0.34 mmol/L, 0.27± 0.16 mmol/L, 3.51±0.67 mmol/L, 16.0± 0.23 U/L, 78.0± 0.10 U/L, 25.0±1.09 U/L 13.5±0.23 µmol/L and 1.65±0.19 µmol/L at 100 mg/kgbw were seen for TP, ALB, Chol, TG, HDL, LDL, ALP, AST, ALT, Tbil and Dbil respectively. There was significant differences for the values of the parameters analysed but there was no significant difference($P>0.05$) for TP, HDL, LDL and Total bilirubin.

**Table 4.83 : Effect of Methanol Leaf Extract of *Vernonia amygdalina* (Bitter leaf) on Liver Function and Lipid Test Parameters of Experimental Rats after 28 Days of Administration**

| Parameter | Dosage | | | |
|---|---|---|---|---|
| | 100mg/Kgbw Group1 | 300mg/Kgbw Group 2 | 600mg/Kgbw Group3 | Control |
| Total protein(g/L) | 72.0±0.16$^{b}$ | 73.0±0.12$^{b}$ | 126.5±0.67$^{a}$ | 70.0±0.12$^{b}$ |
| Albumin(g/L) | 33.5±0.21$^{b}$ | 36.5±0.19$^{b}$ | 41.0±0.34$^{a}$ | 43.0±0.45$^{a}$ |
| Cholesterol(mmol/L) | 2.5±0.22$^{b}$ | 2.7±0.12$^{b}$ | 3.6±0.14$^{a}$ | 3.42±0.08$^{a}$ |
| Triglyceride(mmol/L) | 1.1±0.34$^{b}$ | 1.2±0.34$^{b}$ | 2.5±1.00$^{a}$ | 0.40±0.01$^{c}$ |
| HDL(mmol/L) | 0.27±0.16$^{a}$ | 0.29±0.09$^{a}$ | 0.31±0.34$^{a}$ | 0.31±0.31$^{a}$ |
| LDL(mmol/L) | 3.51±0.67$^{a}$ | 3.52±0.45$^{a}$ | 3.69±0.67$^{a}$ | 3.93±0.46$^{a}$ |
| ALP(U/L) | 16.0±0.23$^{d}$ | 25.0±0.13$^{b}$ | 60.0±0.35$^{a}$ | 20.0±0.23$^{c}$ |
| AST(U/L) | 78.0±0.10$^{c}$ | 92.0±1.23$^{bc}$ | 180.0±0.51$^{a}$ | 100.0±0.18$^{b}$ |
| ALT(U/L) | 25.0±1.09$^{c}$ | 36.0±0.22$^{b}$ | 120.0±0.25$^{a}$ | 40.0±0.09$^{b}$ |
| Total bilirubin(µmol/L) | 13.5±0.23$^{c}$ | 23.0±0.12$^{b}$ | 39.5±0.43$^{a}$ | 15.0±0.30$^{c}$ |
| Direct bilirubin(µmol/L) | 1.65±0.19$^{c}$ | 1.65±0.34$^{c}$ | 6.0±0.32$^{b}$ | 4.0±0.16$^{b}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same row do not differ significantly at $p \leq 0.05$

### 4.1.84 Effect of n- hexane leaf crude extract of *Azardirachta indica* (neem) on the liver function and lipid test parameters of experimental rats after 28days of administration

The values of the liver function parameters for the experimental rats after 28days of administration of n-hexane leaf extract of *Azardirachta indica* is presented in Table 4.84. The determined parameters were: total protein (TP), Albumin (Alb).cholesterol (chol), Triglyceride (TG), Highdensity lipoprotein (HDL), low density lipoprotein (LDL), Alkaline phosphate (ALP), Aspartate aminotransferase (AST) Alanin aminotransferase (ALT) total bilirubin (Tbil) and Direct bilirubin (Dbil). The results showed that the values for liver function increased as the dosage increased. The highest values were 114.1±1.08 g/L, 45.0±0.89 g/L, 3.79±0.34 mmol/L, 1.36±0.13 mmol/L, 0.30±0.11 mmol/L, 3.53±0.03 mmol/L, 76.0±0.56 U/L, 205.1±0.21 U/L, 95.0±0.32 U/L, 40.0±0.28 µmol/L and 10.3±0.89 µmol/l at 600 mg/kgbw as recorded for total protein (TP), Albumin (Alb), cholesterol(chol), Triglyceride (TG), Highdensity lipoprotein (HDL), low density

lipoprotein (LDL), Alkaline phosphate (ALP), Aspartate aminotransferase (AST) Alanin aminotransferase (ALT) total bilirubin (Tbil) and Direct bilirubin (Dbil).

There was significant difference(P<0.05) between the experimental and control groups but the values for the Cholesterol and LDL showed no significant differences (P>0.05). The lowest values were 59±0.89 g/L, 41.5±0.21 g/L, 3.14±0.19 mmol/L, 0.12±0.34 mmol/L, 0.21±0.16 mmol/L, 3.36±0.12 mmol/L, 11.5±0.1 U/L, 56±0.18 U/L and 20±0.23 U/L at 100mg/kgbw which were recorded for TP, ALB, Chol, TG, HDL, LDL, ALP, AST, ALT, Tbil and Dbil respectively. There was significant difference for all parameters between the experimental and control groups. Although, the values for the HDL and LDL showed no significant differences. The group 2(300 mg/kgbw), there was significant difference between the experimental and control groups (P<0.05) except for TP, HDL and LDL that showed no significant differences (P>0.05).

**Table 4.84 Effect of n- hexane Leaf Extract of *Azardirachta indica* (Neem) on Liver Function and Lipid Test Parameters of Experimental Rats after 28 Days of Administraion**

| Parameters | Dosage | | | |
|---|---|---|---|---|
| | **100mg/Kgbw Group 1** | **300mg/Kgbw Group 2** | **600mg/Kgbw Group 3** | **Control** |
| Total protein(g/L) | 59.0±0.89$^{c}$ | 66.5±0.11$^{b}$ | 114.1±1.08a | 68.0±0.11$^{b}$ |
| Albumin (g/L) | 41.5±0.21$^{a}$ | 43.0±0.09$^{a}$ | 45.0±0.89$^{a}$ | 38.0±0.39$^{b}$ |
| Cholesterol(mmol/L) | 3.14±0.19$^{c}$ | 3.27±0.14$^{b}$ | 3.79±0.34$^{a}$ | 4.0 ±0.08$^{a}$ |
| Triglyceride(mmol/L) | 0.12±0.34$^{d}$ | 1.11±0.08$^{b}$ | 1.36±0.13$^{a}$ | 3.21±0.19$^{c}$ |
| HDL(mmol/L) | 0.21±0.16$^{b}$ | 0.25±0.11$^{b}$ | 0.30±0.11$^{a}$ | 0.21±0.04$^{b}$ |
| LDL(mmol/L) | 3.36±0.12$^{a}$ | 3.43±0.23$^{a}$ | 3.53±0.03$^{a}$ | 3.69±1.07$^{a}$ |
| ALP(U/L) | 11.5±0.10$^{d}$ | 15.1±0.24$^{c}$ | 76.0±0.56$^{a}$ | 18.0±0.10$^{b}$ |
| AST(U/L) | 56±0.18$^{d}$ | 60.0±0.45$^{c}$ | 205.1±0.21$^{a}$ | 110.0±0.21$^{b}$ |
| ALT(U/L) | 20±0.23$^{d}$ | 35.0±0.32$^{c}$ | 95.0±0.32$^{a}$ | 45.0±0.34$^{b}$ |
| Totalbilirubin (µmol/L) | 12±0.19$^{c}$ | 16.0±0.24$^{b}$ | 40.0±0.28$^{a}$ | 14.0±0.39$^{bc}$ |
| Direct bilirubin(µmol/L) | 2.5±0.08$^{c}$ | 4.5±0.01$^{b}$ | 10.3±0.89$^{a}$ | 3.2±0.11$^{bc}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same row do not differ significantly at p≤0.05

### 4.1.85 Effect of methanol leaf crude extract of *Vernonia amygdalina*(bitter leaf) on kidney function parameters of the experimental rats after 28days of administration

The effect of methanol leaf crude extract of *Vernonia amygdalina* on kidney function parameters of the experimental rats after 28days of administration is presented in Table 4.85. The determined parameters were: Urea, potassium, creatinine, chloride sodium and biocarbonate. The highest values of 6.1±0.32, 6.15±0.12, 58.5±0.03, 96.0±0.78 , 121±0.21 and 38.0±0.11 mmol/L at 600 mg/kgbw of *Vernonia amygdalina* crude extract were recorded for : Sodium, potassium, biocarbonate, Chloride, creatinine and urea respectively. There was significant differences (P<0.05) between all the values of the parameters tested and the control groups. However, the lowest parameters was observe in group 1(100mg/kgbw) with value of 5.0±0.10, 5.15±0.21, 46.5± 0.40, 94.5±0.10, 118.50.12 and 25.5± 0.25 mmol/L. They were all recorded for: Sodium, potassium, biocarbonate, chloride, creatinine and urea. There was significant difference (P<0.05) between the various parameters analysed and the control except for potassium that showed no significant difference. The values of group 2(300 mg/kgbw) showed significant difference (P<0.05) for all the parameters analyzed as compared to the control groups.

**Table 4.85: Effect of methanol leaf crude extract of *Vernonia amygdalina* (bitter leaf) on Kidney Function Test Parameters of Experimental Rats after 28Days of Administration**

| Parameter | Dosage | | | |
|---|---|---|---|---|
| | **100 mg/Kg bw Group1** | **300mg/Kgbw Group2** | **600mg/Kgbw Group3** | **Control** |
| Sodium (mmol/L) | $5.0 \pm 0.10^{b}$ | $5.8 \pm 0.11^{c}$ | $6.1 \pm 0.32^{d}$ | $4.8 \pm 1.21^{a}$ |
| Potassium (mmol/L) | $5.15 \pm 0.21^{a}$ | $5.8 \pm 0.89^{c}$ | $6.15 \pm 0.12^{d}$ | $5.5 \pm 0.56^{a}$ |
| Bicarbonate (mmol/L) | $46.5 \pm 0.40^{a}$ | $48.0 \pm 0.34^{b}$ | $58.5 \pm 0.03^{c}$ | $80 \pm 0.32^{d}$ |
| Chloride (mmol/L) | 94.5±0.10b | 91.0±0.23a | 96.0±0.78c | 100±0.21d |
| Creatinine (mmol/L) | $118.5 \pm 0.12^{a}$ | $120 \pm 0.28^{b}$ | $121 \pm 0.21^{c}$ | $138 \pm 0.34^{d}$ |
| Urea (mmol/L) | $25.5 \pm 0.25^{a}$ | $26.0 \pm 0.22^{b}$ | $38.0 \pm 0.11^{c}$ | $31 \pm 0.01^{d}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same row do not differ significantly at $p \leq 0.05$

### 4.1.86 Effect of n- hexane leaf crude extract of *Azardirachta indica* on kidney function parameters of experimental rats after 28days of administration

The effect of n – nexane leaf crude extract of *Azardirachta indica* on kidney function parameters of experimental rats after 28days of administration is presented in Table 4.86 .The determined parameters were: sodium, potassium, biocarbonate, chloride, creatinine and urea .The highest parameters of 136.5± 0.90, 6.15±0.07, 28.0±0.11, 97.3 ±1.05, 69.0± 0.26, and 6.95±0.09 mmol/L at 600 mg/kgbw of *Azadirachta indica* crude extract were recorded for: sodium, potassium, biocarbonate, chloride, creatinine and urea respectively. There was significant difference ($P<0.05$) for all the values of the kidney function parameters between the experimental and the control groups. The lowest values observe in group 1(100 mg/kgbw) were: 123.5±0.02, 4.50±0.19, 24.0±0.19, 96±0.11, 52.5±0.24, and 6.1± 0.34 mmol/L. They were recorded for: sodium, potassium, biocarbonate, chloride, creatinine and urea respectively. There was significant difference for all the values of the kidney function parameters between the experimental and the control groups. The value of group 2(300 mg/kgbw) showed

significant difference (P<0.05) for all the values of the kidney function parameters between the experimental and control groups.

**Table 4.86: Effect of n- hexane Leaf Crude Extract of *Azardirachta indica* (neem) on Kidney Function Test Parameters of Experimental Rats after 28Days of Administration**

| Parameter | Dosage | | | |
|---|---|---|---|---|
| | 100mg/Kgbw Group 1 | 300mg/Kgbw Group2 | 600mg/Kgbw Group3 | Control |
| Sodium (mmol/L) | 123.5±0.02[a] | 132.5±0.35[b] | 136.5±0.90[c] | 140±0.32[d] |
| Potassium (mmol/L) | 4.50±0.19[a] | 5.85±0.34[c] | 6.15±0.07[d] | 5.1±0.08[b] |
| Bicarbonate(mmol/L) | 24.0±0.19[b] | 26.5±0.22[c] | 28.0±0.11[a] | 33±0.21[d] |
| Chloride (mmol/L) | 96±0.11[a] | 97.0±0.09[b] | 97.3±1.05[b] | 103±0.45[c] |
| Creatinine (mmol/L) | 52.5±0.24[a] | 85.0±0.04[c] | 69.0±0.26[b] | 95±0.02[d] |
| Urea (mmol/L) | 6.1±0.34[b] | 6.65±0.12[c] | 6.95±0.09[d] | 5.0±0.23[a] |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same row do not differ significantly at p≤0.05

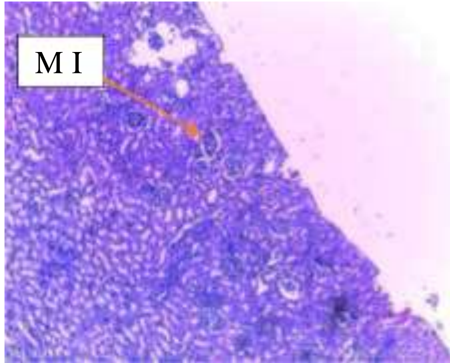
### 4.1.87 Histopathological results for the crude extracts

#### 4.1.87.1 Histology of the heart

The heart of the treated groups (experimental groups) and the control groups showed normal histological features. The appearance of the heart showed no pathological signs for both the bitter leaf and neem leaf crude extract. There was no disaggregation, vaculation and distortional changes in tissue which could affect the normal functions of the heart. The absence of abnormal changes in the heart, impaired cardiovascular functions and as well ultimately tissue necrosis shows that the extract might not have shown significant effect on some serum parameters which are indicative evidence of cardiovascular toxicity.

#### 4.1.87.2 Histology of the kidney

The effect of the bitter leaf and neem leaf crude extract on the kidney histology showed no signs of tissue vacuolation, distortion of collecting ducts and degenerative changes which was observed for both the treated groups (experimental groups) and the control groups. Although, at 600 mg/kgbw of bitterleaf extract there was mild infiltration of the tubulo interstitial space with lymphocytes. All other architectures like the glomeruli were free of inflammatory cells. The neem extract also showed mild infiltration of the tubulo insterestial space with lymphocytes. No other specific features were observed. The mild infiltration may be possible due to toxic agents present in the plant extracts. Although, it‟s obvious that the changes were not significant even at a higher doses to induce kidney damage and impair its physiological functions.



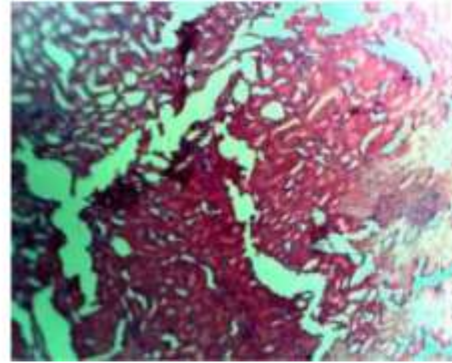
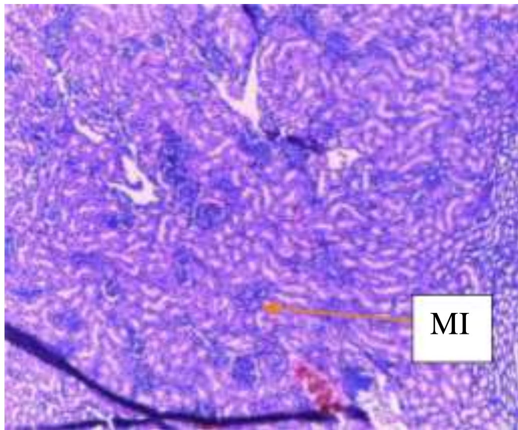


**Plate XVIII: Photomicrograph of the Kidney**
**Treated with Bitterleaf Crude Extract at 600mg/kgbw PlateXIX: Control**
**Showing Mild Inflammation (MI)**



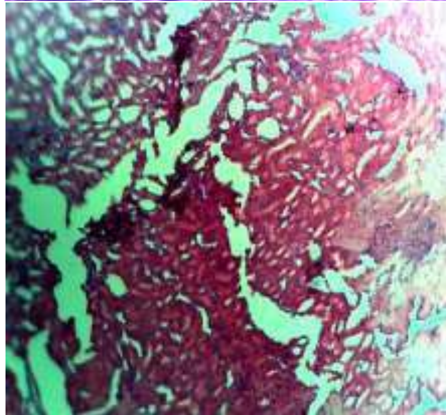


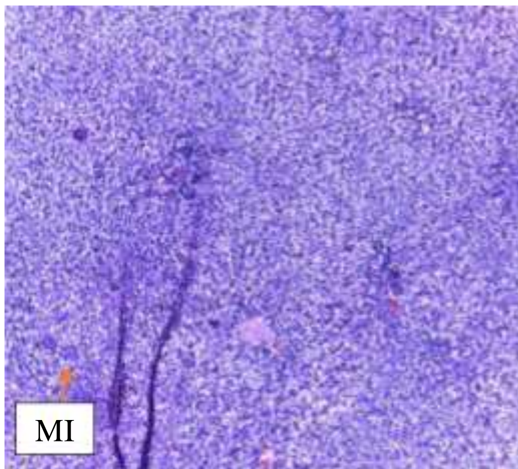
**Plate XX: Photomicrograph of the kidney**
**Treated with Neem Crude Extract at 600mg/kgbw Plate XXI : Control Showing Mild Inflammation(MI)**

**Stain: Haematoxylene and Eosin(H and E) Magnification× 40**

**Sources:** (Field Photographs)

### 4.1.87.3 Histology of the liver

Hepatocytes distortion and degenerative changes were not observed in the treated groups and the control groups. Although, at 600 mg/kgbw(group3) of the bitterleaf and neem extract there was mild infiltration of the portal tract with lymphocytes. There was focal infiltration of the liver parenchyma with sprineles of lymphocytes.



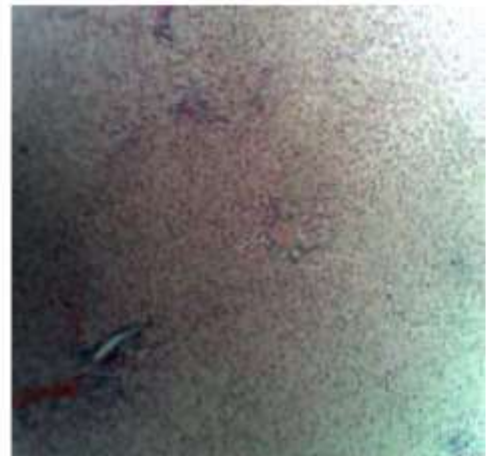
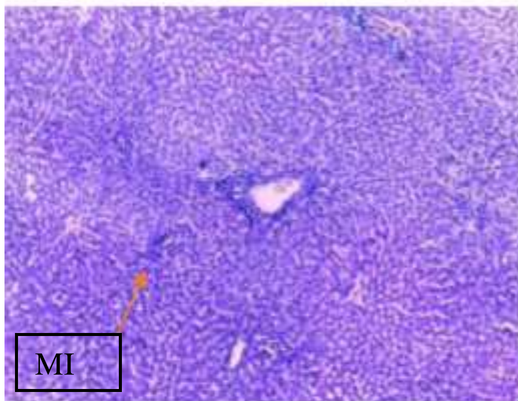



**Plate XXII: Photomicrograph of the Liver**
**Treated with Bitter Leaf Crude Extract at 600 mg/kgbw** **Plate XXIII : Control**
**Showing Mild Inflammation(MI)**



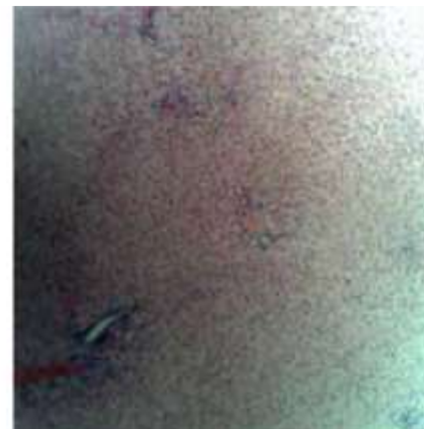



**Plate XXIV: Photomicrograph of the Liver Treated with Neem Crude Extract at 600 mg/kgbw Showing Mild Inflammation(MI)**
**Stain: Haematoxylene and Eosin(Hand E)**
**Magnification: × 40**

**Plate XXV: Control**

**Sources:** (Field Photographs)

**4.1.87.4 Histology of the lungs**

The lungs part from this study showed normal histological features of pulmonary alveoli and normal distribution of collagen fibres even at high concentration. Sections of the lungs tissue from the rat administered with bitterleaf and neem leaf at doses of 600 mg/kgbw showed normal histology.

**4.1.88 Pharmacological effect of *Vernonia amygdalina*(bitterleaf) fraction F4 of n-hexane leaf extract on the body weight(g) of experimental rats after 28 days**

The effect of *Vernonia amygdalina* fraction F4 of n- hexane leaf extract on the body weight of experimental rats is presented in Table 4.88. The weight of the various treated groups increased as the experiment proceeds from week one to week four.There was significant differences p≤0.05 between the treated groups and the control.The significant increase was seen continuously from week one to week four.

**Table 4.87: Pharmacological Effect of *Vernonia amygdalina*(bitterleaf) Fraction F4 of n-hexane Leaf Extract on the Bodyweight(g) of Experimental Rats after 28 Days**

| Bitterleaf | Week 1 | Week 2 | Week3 | Week 4 |
|---|---|---|---|---|

| Fraction(F4)of n-hexane extract | | | | |
|---|---|---|---|---|
| Group 1(10mg/kgbw) | 150.93[b] | 162.03[b] | 172.88[b] | 185.21[b] |
| Group 2(20mg/kgbw | 144.45[a] | 154.87[a] | 166.37[a] | 176.82[a] |
| Group 3(40mg/kgbw) | 165.21[c] | 178.23[c] | 188.70[c] | 198.49[c] |
| Control | 170.13[d] | 184.84[d] | 193.49[d] | 204.38[d] |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscript letter on the same column do not differ significantly at p≤0.05

**4.1.89 Haematological parameters of rats administered with *Vernonia amygdalina*(bitter leaf) fraction F4 of methanol leaf extract after 28days of administration**

The result for white blood cells, (WBC), red blood cell (RBC) Haemoglobin (HB), Pack cell volume (PCV), mean cell volume(MCV), Mean cell haemoglobin(MCH), Mean cell haemoglobin concentration(MCHC), Platelet count, neutrophils,(N) lymphocytes,(L), monocytes (M), eosinophil (E) and Basophils (B) for both the experimental and control rats is presented in Table 4.89 . The values for the WBC($\times 10^{9}$/L) for group1 (100 mg/kgbw), group 2(300 mg/kgbw) and group 3(600 mg/kgbw) showed a value of 5.10±0.78, 5.13±0.14 & 5.22±0.02 respectively. There was no significant difference (P> 0.05) for all the groups. The RBC($\times 10^{12}$/L) value recorded were 3.15±0.14' 4.23±0.45 and 4.80±0.32 for all the treated groups, there was no significant difference between the treated groups and the control groups (P> 0.05). The value of the HB(g/dL) recorded was 10.55±0.43, 11.50±0.01 & 11.60±0.19 for group 1, group2 & group 3 respectively. There was no significant difference (P> 0.05) between the treated groups and the control groups . The PCV(%) value recorded were: 32.50±0.91, 33±0.56 and 33.50±1.0 for group 1, group 2 and group 3 respectively. There was no significant differences (P>0.05) between the treated groups and the control groups.

The result for the values of the MCV, MCH, MCHC and platelet count showed no significant difference (P>0.05) between all the treated groups and the control groups . Likewise, The result of the differential white blood cells counts: neutrophils, lymphocyes, monocytes, basophils and eosinophil showed no significant difference (P>0.05) between all the treated groups and the control groups.

**Table 4.88 : Effect of *Vernonia amygdalina*(Bitter leaf) Fraction F4 of n-hexane Leaf Extract on Haematological Parameters after 28Days of Administration**

| Parameter | Dosage | | | |
|---|---|---|---|---|
| | **10mg/Kgbw (Group 1)** | **20 mg/Kgbw (Group2)** | **40 mg/Kgbw (Group3)** | **Control** |
| WBC ($\times 10^{9}$/L) | $5.10 \pm 0.78^{a}$ | $5.13 \pm 0.14^{b}$ | $5.22 \pm 0.02^{c}$ | $6.20 \pm 0.21^{d}$ |
| RBC($\times 10^{12}$/L) | $3.15 \pm 0.14^{a}$ | 4.23±0.45b | $4.70 \pm 0.30^{c}$ | $4.80 \pm 0.32^{d}$ |
| HB (g/dL) | $10.70 \pm 0.43^{a}$ | $11.70 \pm 0.01_{b}$ | $11.30 \pm 0.19^{b}$ | $12.33 \pm 0.34^{c}$ |
| PCV (%) | $32.50 \pm 0.91^{a}$ | $33 \pm 0.56^{b}$ | $33.50 \pm 1.0^{b}$ | $37.90 \pm 0.31^{c}$ |
| MCV ( fL) | $50 .43 \pm 0.21^{a}$ | $52.75 \pm 0.25^{b}$ | $54.85 \pm 0.32^{c}$ | $59.50 \pm 0.09^{d}$ |
| MCH ( Pg) | $18.35 \pm 0.89^{a}$ | $21.03 \pm 0.08^{b}$ | $21.4 \pm 0.98^{b}$ | 20.40±0.11c |
| MCHC ( g/dL) | $26.04 \pm 0.11^{a}$ | $27.08 \pm 0.65^{b}$ | $29.30 \pm 0.11^{c}$ | $32.00 \pm 0.34^{d}$ |
| PLT $\times 10^{9}$/L | $321 \pm 0.33^{a}$ | $362 \pm 0.39^{b}$ | $365.5 \pm 0.41^{c}$ | $476.00 \pm 0.39^{d}$ |
| Neu (%) | 5.9±0.17a | $6.2 \pm 0.01^{b}$ | $6.5 \pm 0.01^{c}$ | $07.00 \pm 0.34^{d}$ |
| Lymp (%) | $67 \pm 0.11^{a}$ | $68 \pm 0.32^{b}$ | $71 \pm 0.67^{c}$ | $82 \pm 0.72^{d}$ |
| Mono (%) | 2.1±0.47a | $2.2 \pm 0.34^{b}$ | $2.3 \pm 0.01^{b}$ | $02 \pm 0.32^{c}$ |
| Eosi (%) | $1.8 \pm 0.18^{a}$ | $1.9 \pm 0.14^{a}$ | $2.5 \pm 0.78^{b}$ | $02 \pm 0.11^{c}$ |
| Baso (%) | $2.3 \pm 0.19^{a}$ | $2.6 \pm 0.11^{b}$ | $2.7 \pm 0.16^{c}$ | $03 \pm 0.18^{d}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscripts letter on the same row do not differ significantly at (P<0.05).

**4.1.90 Pharmacological effect of *Azardirachta indica*(neem) leaf fraction(F6) of methanol extract on the Body weight(g) of experimental rats after 28 days of administration**

The effect of *Azardirachata indica*(neem) leaf fraction(F6) of methanol extract on the body weight of experimental rats is presented in Table 4.90 The weight of the various treated groups increased as the experiment proceeds from week one to week four.There was significant differences (P<0.05) between the treated groups and the control.The significant increase was seen continuously from week one to week four.

**Table 4.89 : Pharmacological Effect of *Azardirachta indica*(neem) Leaf Fraction(F6) of Methanol Extract on the Bodyweight(g) of Experimental Rats after 28days of Administration**

| Neemleaf fraction(F6)of methanol extract | Week 1 | Week 2 | Week3 | Week 4 |
|---|---|---|---|---|
| Group1(10mg/kgbw) | $172.38^{c}$ | $184.42^{c}$ | $191.62^{c}$ | $204.18^{c}$ |
| Group 2(20mg/kgbw | $153.38^{b}$ | $168.54^{b}$ | $173.44^{b}$ | $185.55^{b}$ |
| Group3(40mg/kgbw) | $142.01^{a}$ | $159.38^{a}$ | $169.85^{a}$ | $176.49^{a}$ |
| Control | $174.13^{d}$ | $186.84^{d}$ | $193.49^{d}$ | $210.38^{d}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscripts letter on the same column do not differ significantly at (P<0.05).

**4.1.91 Haematological parameters of rats administered with *Azardirachta indica* leaf fraction(F6) of methanol extract after 28days of administration**

The hematological parameters of rats administered with *Azardirachta indica* leaf fraction F6 of methanol extract is shown in Table 4.91; which showed the result of the values for white blood cells, (WBC), red blood cell (RBC), Haemoglobin (HB), Pack cell volume (PCV), mean cell volume (MCV), Mean cell haemoglobin, mean cell haemoglobin

concentration(MCHC), Platelet count , neutrophils,(N) lymphocytes,(L), monocytes(M), eosinophil (E) and Basophils (B) for both the experimental and control rats. The result for WBC($\times$ $10^9$/L) for group 1(100 mg/kgbw), group 2(300 mg/kgbw) and group 3(600 mg/kgbw) had a value of 3.2±0.56, 4.05±0.25 and 5.56±0.22 respectively. There was significant difference (P< 0.05) between the treated groups and the control groups. The RBC($\times$ $10^{12}$/L) value recorded were: 4.65±0.21, 5.10±0.21 and 5.45±0.90 presented for group 1, group2 and group 3 respectively. There was no significant differences (P >0.05) between the treated groups and the control group. The value of the HB (g/dL) recorded was 10.35±0.38, 11.3±0.32 and 11.6±0.12 presented for group 1 ,group 2 and group 3 respectively. There was no significant difference (P>0.005) between the treated groups and the control groups. The PCV(%) values record ed were: 30.5±0.78, 34.5±0.11 and 35.0±0.29 presented for group1 group 2 and group 3 respectively. There was no significant difference (P >0.05) between the treated groups and the control groups. The result for the values of the MCV ,MCH, MCHC and platelet count showed no significant difference (P>0.05) between all the treated groups and the control groups. Where as, the result of the differential white blood cells counts: neutrophils, lymphocyes, monocytes, basophils and eosinophil showed no significant difference (P>0.05) between all the treated groups and the control groups.

**Table 4.90`: Effect of *Azardirachta indica* Leaf Fraction(F6) of Methanol Extract on Haematological Parameters after 28 Days of Administration**

| Parameter | Dosage | | | |
|---|---|---|---|---|
| | **10mg/Kgbw Group1** | **20 mg/Kgbw Group2** | **40 mg/Kg bw Group3** | **Control** |
| WBC ($\times$ $10^9$/L) | 3.2±0.56a | 4.05±0.25b | 5.56±0.22c | 6.5± 0.67d |
| RBC($\times$ $10^{12}$/L) | 4.65±0.21a | 5.10±0.21b | 5.45±0.90c | 6.7±0.11d |
| HB (g/dL) | 10.35±0.38a | 11.3±0.32b | 11.6±0.12b | 12.6±0.01c |
| PCV (%) | 30.5±0.78a | 34.5±0.11b | 35.0±0.29b | 38.0±0.18c |
| MCV ( fl) | 52.6±0.23a | 56.9±0.26b | 58.4±0.21c | 60.1±0.19d |

| | | | | |
|---|---|---|---|---|
| MCH ( Pg) | 17.0±0.31[a] | 18.2±0.21b | 18.4±0.52b | 20.0±0.34c |
| MCHC ( g/dL) | 27.2±0.78a | 28.8±0.91b | 29.±0.45b | 33.2±0.10c |
| PLT × $10^9$/L | 342.5±0.66[a] | 344±0.75b | 389±0.33c | 441±0.91d |
| Neu (%) | 5.0±0.44a | 06±0.39b | 07±0.46c | 09±0.90d |
| Lymp (%) | 71.5±0.21[a] | 76.0±0.21b | 79.5±0.31c | 82±0.18d |
| Mono (%) | 0.1±0.11a | 0.1±0.22[b] | 0.2±0.33c | 02±0.98d |
| Eosi (%) | 2.6±0.34[a] | 2.7±0.89b | 2.9±0.32c | 03±0.83d |
| Baso (%) | 2.4±0.34[a] | 2.8±0.21b | 2.9±0.11c | 03±0.91d |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscripts letter on the same row do not differ significantly at (P<0.05).

**4.1.92 Effect of *Vernonia amygdalina* (bitterleaf) fraction(F4) of n- hexane extract on the liver function and lipid test parameters of rats after 28days of administration**

The values of the liver function parameter for the experimental and control rats is presented in Table 4.92. The determined parameters were: total protein (TP), Albumin (Alb) cholesterol (chol), Triglyceride (TG), Highdensity lipoprotein (HDL),low density lipoprotein,(LDL), Alkaline phosphate (ALP),Aspartate aminotransferase (AST) Alanin aminotransferase (ALT) total bilirubin (Tbil) and Direct bilirubin (Dbil). The result showed that the liver parameters increase with increase in the dosage. The highest values were 65.5±0.67 g/L, 36.0±0.34 g/L, 3.1±0.14 mmol/L, 2.5±1.00 mmol/L, 0.29±0.21 mmol/L, 3.82±0.98 mmol/L, 19.0±0.45 U/L, 88.0±0.59 U/L, 41.0±0.15 U/L 17.5±0.34 µmol/L and 3.0±0.32 µmol/L at 40 mg/kgbw(group3) of n- hexane fraction of *Vernonia amygdalina* were recorded for total protein (TP), Albumin (ALb), Cholesterol (Chol), Triglyceride (TG), Highdensity, lipoprotein (HDL), lowdensitylipoprotein (LDL), Alkaline phosphate (ALP), Aspartate aminotransferase (AST), Alanin aminotransferase (ALT), total bilirubin (TBIL) and Direct bilirubin (DBIL) respectively. There was no significant differences (P>0.05) between the treated group and the control. The lowest parameters of 62.0±0.16 g/L, 31.5± 0.21 g/L, 2.1±0.22 mmol/L, 1.7± 0.34 mmol/L, 0.26±0.11 mmol/L, 3.41±0.45 mmol/L. 16.0±0.32 U/L, 78.0±0.30 U/L, 23.0±1.07 U/L, 12.5±0.78 µmol/L and 2.56±0.19 µmol/L at 10 mg/kgbw were seen for TP, ALB, CHOL,

TG, HDL, LDL, ALP, AST, ALT, TBIL AND DBIL respectively. There was no significant differences (P>0.05) between the treated group (group1) and the control group. However group 2 also showed no significant differnces (P>0.05) with the control group.

**Table 4.91 : Effect of *Vernonia amygdalina* (bitter leaf) Fraction F4 of n- hexane Extract on Liver Function and Lipid Test Parameters**

| Parameter | Dosage | | | |
|---|---|---|---|---|
| | 10mg/Kgbw Group 1 | 20mg/Kgbw Group 2 | 40mg/Kgbw Group 3 | Control |
| Total protein(g/L) | 62.0±0.16$^{a}$ | 63.0±0.11$^{b}$ | 65.5±0.67$^{c}$ | 69.0±0.12$^{d}$ |
| Albumin(g/L) | 31.5±0.21$^{a}$ | 34.5±0.19$^{b}$ | 36.0±0.34$^{c}$ | 40.0±0.45$^{d}$ |
| Cholesterol(mmol/L) | 2.1±0.22$^{a}$ | 2.2±0.12$^{a}$ | 3.1±0.14$^{b}$ | 3.44±0.08$^{c}$ |
| Triglyceridel(mmol/L) | 1.7±0.34$^{a}$ | 1.9±0.34$^{b}$ | 2.5±1.00$^{c}$ | 2.61±0.01$^{d}$ |
| HDL(mmol/L) | 0.26±0.11$^{a}$ | 0.27±0.19$^{b}$ | 0.29±0.21$^{c}$ | 0.39±0.21$^{d}$ |
| LDL(mmol/L) | 3.41±0.45$^{a}$ | 3.68±0.76$^{b}$ | 3.82±0. 98$^{c}$ | 3.96±0.64$^{d}$ |
| ALP(U/L) | 16.0±0.32$^{a}$ | 17.0±0.31$^{a}$ | 19.0±0.45$^{b}$ | 21.0±0.89$^{c}$ |
| AST(U/L) | 78.0±0.30$^{a}$ | 80.0±1.26$^{b}$ | 88.0±0.59$^{c}$ | 102.0±0.18$^{d}$ |
| ALT(U/L) | 23.0±1.07$^{a}$ | 38.0±0.12$^{b}$ | 41.0±0.15$^{c}$ | 45.0±0.09$^{d}$ |
| Total bilirubin(µmol/L) | 12.5±0.78$^{a}$ | 14.0±0.21$^{b}$ | 17.5±0.34$^{c}$ | 35.5±0.45$^{d}$ |
| Direct bilirubin(µmol/L) | 2.56±0.19$^{a}$ | 2. 85±0.34$^{b}$ | 3.0±0.32$^{c}$ | 4.1±0.16$^{d}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscripts letter on the same row do not differ significantly at ($P<0.05$).

**4.1.93 Effect of *Azardirachta indica* (neem)leaf fraction F6 of methanol extract on the liver function and lipid test parameters of rats after 28 days of administration** The values of the liver function parameter for the experimental and control rats is

presented in Table 4.93 The determined parameters were: total protein(TP), Albumin (Alb).cholesterol(chol), Triaglycerol(Trig), Highdensity lipoprotein(HDL),low density lipoprotein,(LDL), Alkaline phosphate(ALP),Aspartate aminotransferase(AST) Alanin aminotransferase(ALT) total bilirubin (Tbil) and Direct bilirubin (Dbil). The result showed that the liver parameters increase with increase in the dosage. The highest parameters values of 57.1±2.80 g/L, 37.2±0.80 g/L, 3.19±0.67 mmol/L, 1.36±0.21 mmol/L, 0.34±0.15 mmol/L, 3.89±0.43 mmol/L, 17.0±0.16 U/L, 67.1±0.22 U/L, 32.0±0.42 U/L, 13.0±0.17 µmol/L and 2.93±0.89 µmol/L of 40 mg/kgbw(group3) of methanol fraction of *Azardirachta indica* were recorded for total protein(TP), Albumin (ALb)Cholesterol(Chol), Triglyceride(TG), Highdensity lipoprotein(HDL),low density lipoprotein,(LDL), Alkaline phosphate(ALP),Aspartate aminotransferase(AST) Alanin

aminotransferase(ALT) total bilirubin (TBIL) and Direct bilirubin (DBIL) respectively. There was no significant difference ($P>0.05$) between the treated group(group3) and the control. The lowest values were 52±0.98 g/L, 29.3±0.21 g/L, 2.19±0.19 mmol/L, 0.12±0.43 mmol/L, 0.21±0.18 mmol/L, 2.54±0.14, 12.5±0.12, 48±0.19, 25±0.21, 9.0±0.11 and 2.5±0.01 at 10 mg/kgbw (group1) which were seen for TP, ALB, CHOL, TG, HDL, LDL, ALP, AST, ALT, TBIL AND DBIL respectively. There was no significant difference ($P>0.05$) between the treated group(group1) and the control group. However group 2 also showed no significant difference($P>0.05$) with the control group.

**Table 4:92 Effect of *Azardirachta indica* (neem)Leaf Fraction(F6) of Methanol Extract on the Liver Function and Lipid Test Parameters of Experimental Rats after 28 days of Administration**

| Parameters | Dosage | | | |
|---|---|---|---|---|
| | **10mg/Kgbw Group1** | **20mg/Kgbw Group2** | **40mg/Kgbw Group 3** | **Control** |
| Total protein(g/L) | 52.0±0.98[a] | 54.5±0.21[b] | 57.1±2.80[c] | 62.0±0.22[d] |
| Albumin(g/L) | 29.3±0.21[a] | 27.1±0.04b | 37.2±0.80[c] | 39.0±0.87[d] |
| Cholesterol(mmol/L) | 2.19±0.19[a] | 2.45±0.13[b] | 3.19±0.67[c] | 4.5 ±0.08[d] |
| Triglyceride(mmol/L) | 0.12±0.43[a] | 1.11±0.71[b] | 1.36±0.21[c] | 3.31±0.29[d] |
| HDL(mmol/L) | 0.21±0.18[a] | 0.23±0.19[b] | 0.34±0.15[c] | 0.86±0.55[d] |
| LDL(mmol/L) | 2. 54±0.14[a] | 2.56±0.33b | 2.89±0.43[c] | 3.89±1.70[d] |
| ALP(U/L) | 12.5±0.12[a] | 14.1±0.23b | 17.0±0.16[c] | 18.0±0.10b |
| AST(U/L) | 48±0.19[a] | 59.0±0.15b | 67.1±0.22[c] | 112.0±0.24[d] |
| ALT(U/L) | 25±0.21[a] | 28.0±0.22b | 32.0±0.42[c] | 45.0±0.34[d] |
| Total bilirubin(μmol/L) | 9.0±0.11[a] | 12.0±0.15b | 13.0±0.17[c] | 14.0±0.38[d] |
| Direct bilirubin(μmol/L) | 2.5±0.01[a] | 2.7±0.71[b] | 2.93±0.89[c] | 3.2±0.91[d] |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscripts letter on the same row do not differ significantly at ($P<0.05$).

### 4.1.94 Effect of *Vernonia amygdalina*(bitter leaf) fraction F4 of n- hexane extract on kidney function parameters of experimental rats after 28days of administration

The effect of *Vernonia amygdalina* (Bitter leaf) fraction F4 of n – hexane extract on kidney function parameters of the experimental rats after 28days of extract administration is presented in Table 4.94. The determined parameters were : sodium, potassium biocarbonate chloride creatinine and urea .The highest parameters values of 3.5± 0.87, 6.57±0.26, 58.5±0.43, 64.40.87, 131±0.67 and 25.0±0.90 mmol/L at 40 mg/kgbw of n-hexane fraction of *Vernonia amygdalina* crude extract were recorded for sodium, potassium, biocarbonate, chloride, creatinine and urea respectively. There was no significant differences(P>0.05) between the treated group(group3) and the control groups. However, the lowest parameters values was seen in group 1(10mg/kgbw) with value of 3.0±0.70, 5.15±0.90, 52.5±0.49, 60.5±0.18, 102.5 and 21.5±0.85 mmol/L.There was no significant difference (P>0.05)between the treated group(group1) analysed and the control. Group 2 showed no significant differences(P>0.05).

**Table 4.93: Effect of *Vernonia amygdalina* (bitterleaf) Fraction(F4) of n- hexane Extract on Kidney Function Parameters of the Experimental Rats after 28 Days of Administration**

| Parameter | Dosage | | | |
|---|---|---|---|---|
| | 10 mg/Kgbw Group1 | 20 mg/Kgbw Group2 | 40 mg/Kgbw Group3 | Control |
| Sodium (mmol/L) | $3.0 \pm 0.70^{a}$ | $3.4 \pm 0.19^{b}$ | $3.5 \pm 0.87^{c}$ | $5.8 \pm 1.81^{d}$ |
| Potassium (mmol/L) | $5.15 \pm 0.90^{a}$ | $5.8 \pm 0.78^{b}$ | $6.57 \pm 0.26^{c}$ | $7.5 \pm 0.86^{d}$ |
| Bicarbonate (mmol/L) | $52.5 \pm 0.49^{a}$ | $56.0 \pm 0.40^{b}$ | $58.5 \pm 0.43^{c}$ | $84 \pm 0.39^{d}$ |
| Chloride (mmol/L) | $60.5 \pm 0.18^{a}$ | $61.0 \pm 0.93^{b}$ | $64.0 \pm 0.87^{b}$ | $100 \pm 0.81^{c}$ |
| Creatinine (mmol/L) | $102.5 \pm 0.92^{a}$ | $123 \pm 0.80^{b}$ | $131 \pm 0.67^{c}$ | $140 \pm 0.44^{d}$ |
| Urea (mmol/L) | $21.5 \pm 0.85^{a}$ | $24.0 \pm 0.80^{b}$ | $25.0 \pm 0.90^{c}$ | $39 \pm 0.10^{d}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscripts letter on the same row do not differ significantly at (P<0.05).

### 4.1.95 Effect of *Azardirachta indica*(neem)leaf fraction(F6) of methanol extract on kidney function parameters of the experimental rats after 28days of administration

The effect of *Azardirachta indica*(neem)leaf fraction F6 of methanol extract on kidney function parameters of the experimental rats after 28days of administration is presented in Table 4.95. The determined parameters were: sodium, potassium, biocarbonate, chloride, creatinine and urea. The highest parameters values of 124±0.90 mmol/L, 5.54±0.90 mmol/L, 25.0±0.18 mmol/L 79.3±1.05 mmol/L, 65.0±0.26 mmol/L and

4.35±0.09 mmol/L at 40 mg/kgbw of methanol fraction of *Azardirachta indica* crude extract were recorded for sodium, potassium, biocarbonate chloride creatinine and urea respectively. There was no significant difference in the kidney function parameters between the experimental(group3) and the control groups, The lowest parameters was seen in group 1(10 mg/kgbw), with values of : 115.5 mmol/L, 3.50±0.17 mmol/L, 20.0±0.16 mmol/L, 74±0.11 mmol/L 52.5±0.24 mmol/L and 4.1±0.34 mmol/L. They were recorded for: urea, potassium, creatine, chloride, sodium and biocarboante respectively. There was no significant difference in the kidney function parameters between the experimental and the control groups. The value of group 2 (20 mg/kgbw) showed no significant difference ($P>0.05$) in the kidney function parameters between the experimental and control groups.

**Table 4.94: Effect of *Azardirachta indica*(neem)Leaf Fraction F6 of Methanol Extract on Kidney Function Parameters of the Experimental Rats after 28days of Administration**

| Parameter | Dosage | | | |
|---|---|---|---|---|
| | 10 mg/Kgbw Group1 | 20 mg/Kgbw Group2 | 40 mg/Kgbw Group3 | Control |
| Sodium (mmol/L) | $115.5\pm0.02^{a}$ | $122.5\pm0.35^{b}$ | $124\pm0.90^{c}$ | $140\pm0.32^{d}$ |
| Potassium (mmol/L) | $3.50\pm0.17^{a}$ | 5.65±0.60b | $5.54\pm0.09^{c}$ | $6.12\pm0.07^{d}$ |
| Bicarbonate (mmol/L) | $20.0\pm0.16^{a}$ | $21.5\pm0.32^{b}$ | $25.0\pm0.18^{c}$ | $33\pm0.91^{d}$ |
| Chloride (mmol/L) | $74\pm0.11^{a}$ | $76.0\pm0.09^{b}$ | $79.3\pm1.05^{c}$ | $103\pm0.45^{d}$ |
| Creatinine (mmol/L) | $52.5\pm0.24^{a}$ | $63.0\pm0.04^{b}$ | $65.0\pm0.26^{c}$ | $95\pm0.02^{d}$ |
| Urea (mmol/L) | $4.1\pm0.34^{a}$ | $4.25\pm0.12^{b}$ | 4.35±0.09c | $6.0\pm0.73^{d}$ |

Results are expressed as mean ± standard deviation for triplicate determination. Values with the same superscripts letter on the same row do not differ significantly at (P<0.05).

### 4.1.95 Histopathology result for the fractions

#### 4.1.95.1 Histology of the heart

The heart of the treated groups (experimental groups) and the control groups showed normal histological features. The appearance of the heart showed no pathological signs for both the bitterleaf and neemleaf crude extract. There was no disaggregation, vaculation and distortional changes in tissue which could affect the normal functions of the heart. The absence of abnormal changes in the heart, impaired cardiovascular functions and as well ultimately tissue necrosis shows that the extract might not have shown significant effect on some serum parameters which are indicative evidence of cardiovascular toxicity.

#### 4.1.95.2 Histology of the kidney

The effect of the bitter leaf and neem leaf crude extract on the kidney histology showed no sign of tissue vacuolation, distortion of collecting ducts and degenerative changes which was observed for both the treated groups (experimental groups) and the control groups. Although, at 40 mg/kgbw of the fractions of bitterleaf and neem leaf there was mild

tubulo interstitial inflammation of the kidney. All other architectures like the glomeruli and collecting duct were free of inflammatory cells. No other specific features were observed.

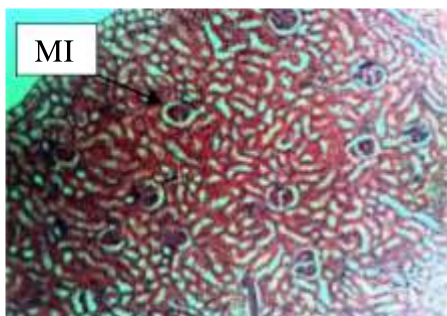



**Plate XXVI: Photomicrography of the Kidney Treated with n- hexane Fraction(F4) of Bitter leaf at 40 mg/kgbw Showing Mild Inflammation(MI)**

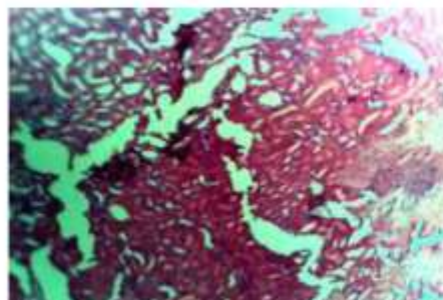
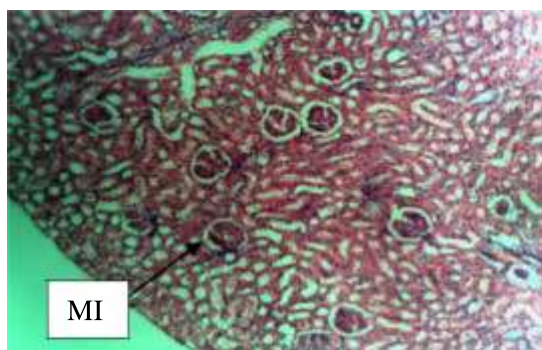


**Plate XXVII: Control**



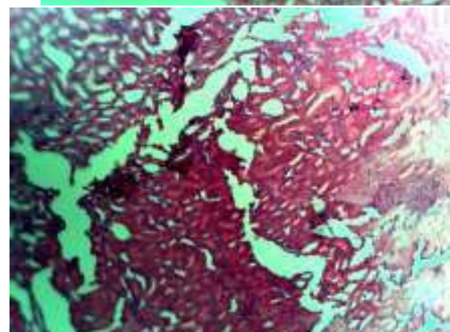



**Plate XXVIII: Photomicrography of the Kidney Treated with Methanol Fraction(F6) of Neem at 40 mg/kgbw Showing Mild Inflammation (MI) Stain: Haematoxylene and Eosin(Hand E)**

**Plate XXIX: Control**

**Magnification:** × **40**

**Sources:** (Field Photographs)

#### 4.1.95.3 Histology of the liver

The liver is a large vital organ that is found in the animal system that functions in the detoxification of chemicals and metabolizes drugs and as well serves in the release of bile into the intestine(Treadway1998). The liver produces protein and blood clothing factors, it also synthesis glycogen, tryglycerides and cholesterol. Hepatocytes distortion and degenerative changes were not obverved in the treated groups. Although, at 40 mg/kgbw of the bitterleaf fractions there was mild infiltration of the portal tract with lymphocytes. The neem leaf fraction also showed mild infiltration of the portal tract with lymphocytes. There was focal infiltration of the liver parenchyma with sprineles of lymphocytes.

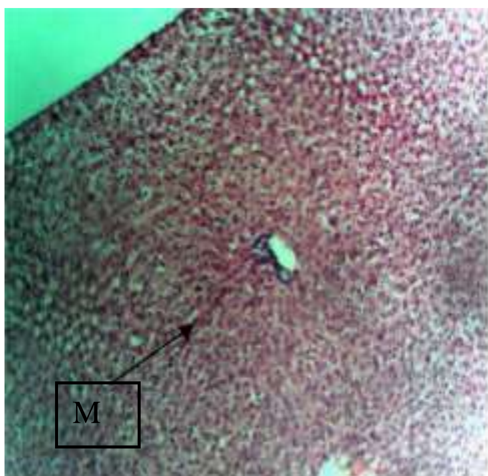



**Plate XXX: Photomicrography of the Liver Treated with n- hexane Fraction(F4) of Bitter leaf at 40mg/kgbw Showing Mild Inflammation(MI)**

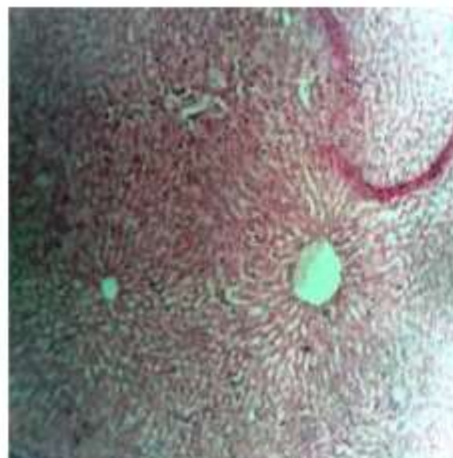
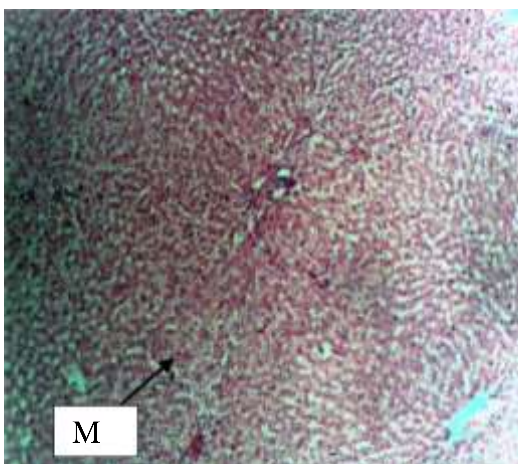


**Plate XXXI: Control**



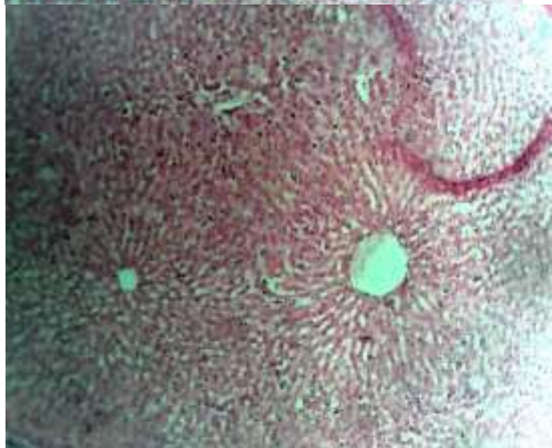



**Plate XXXII: Photomicrography of the Liver Treated with Methanol Fraction(F6) of Neem at 40mg/kgbw Showing Mild**

**Plate XXXIII: Control**

**Inflammation(MI)**
**Stain: Haematoxylene and Eosin(Hand E)**
**Magnification: × 40**

**Sources:** (Field Photographs)

#### 4.1.95.4 Histology of the lungs

The lungs are the primary organs of the respiratory system in animals and even humans. The respiratory system together with the lungs takes oxygen from the air and transport it to the red blood cell which in response carries it to other parts of the body. Th lungs functions in getting out of carbondioxide (Co2) gas when breathe is exhaled out of the body. The lungs part from this study showed normal histological features of pulmonary alveoli and normal distribution of collagen fibres even at high concentration. Sections of the lungs tissue from the rat administered with bitterleaf and neem leaf at a dose of 10 mg/kgbw, 20 mg/kgbw and even at a higher dose of 40 mg/kgbw showed normal histology.

## 4.2 Discussion

### 4.2.1 Extraction and phytochemical compositions

The percentage yield of extract is a reflection of the relationship between the extracting solvents, method of extraction and solubility of the desired components. Three different solvents namely (n- hexane, ethyl acetate and methanol) with different polarity index obtained the leaves, stembark, and root of *Vernonia amygdalina*, *Moringa oleifera*, *Azardirachta indica and Vitellaria paradoxa*. The percentage yields of these plants is shown in Table 4.3-4.6. The ethyl acetate extract showed the highest yield compared to

other extract observed from the various percentage yields of the plants, Based on this result ethylacetate was more suitable for extraction owing to the fact that higher percentage extracts were observed especially in *Moringa oleifera* leaves, stem bark and root Table 4.4 . The differences in percentage yield could be due to solvent type , solubility of the different extractable components in them and as well availability of the different extractable components in them( Ewansiha *et al*., 2016 ; Oke and Aslim 2010). The extraction process was carried out in increasing order of polarity and yet more yields were obtained with ethylacetate although a moderately polar solvent. This explains the fact that polar constituents might be more than the non polar constituents.

The result of the phytochemical analysis revealed the presence of seven phytochemical which were flavonoids, alkaloids, saponins, phenols, tannin, phytic acid and oxalate content as shown in Table 4.7-4.18. The presence of these constituents may be responsible for the plant to be medicinal in nature oweing to the fact that most of the phytochemical constituents present are reported to be used for the prevention, treatment of one ailment or the other as reported by Kenner and Requena, (1996).

This phytochemical components are responsible for both pharmacological and toxic activities in plants. These medicinally bioactive components exert antimicrobial action through different mechanisms. Tannins cause inhibition in the cell wall synthesis by forming irreversible complexes with proline rich protein (Mamtha *et al*., 2004).

The saponins have the ability to cause leakage of proteins and certain enzymes from the cell (Zablotowicz *et al*., 1996). Terpenoids are responsible for dissolution of the cell wall of microorganism by weakening the membranous tissue (Hemandez *et al*., 2000).

Flavonoids which have been found to be effective antimicrobial substances against a wide array of microorganisms invitro are known to be synthesized in response to microbial infection by plants.They have the ability to bind with extracellular and soluble proteins and complexes with bacterial cell walls (Hemandez *et al*., 2000).

The differences that occurred from the results obtained in the various quantitative phytochemical of the selected medicinal plant extract might have been as a result of the differences in solvent use (solvent type) and as well the geographical location were the plants were collected. As reported by Mann and Kuta (2014) these phytochemicals constituents have shown their effectiveness against many microorganisms also they have enable plant parts to function as herbs or drugs by producing biological activity in animals and humans . Abalaka *et al.* (2016) also described these phytochemicals to possess antimicrobial effects and in which they can serve or be used for the treatment of disease. The results of the phytochemicals were in agreement with the work of Imaga and Banigbetan (2013) which also detected the presence of saponins, alkaloids, tannins and Flavonoids. Flavonoids generally are also reported to be present in glycosylated forms in plants and the sugar moiety has been found to be an important factor in determining their bioactivity. Flavonoids have antioxidants potentials hence could offer protection against heart disease and cancer probably by enhancing the body defense against pathology induced free –radicals (Alhumaid *et al*., 2010). As reported by Tapas *et al.* (2008) flavonoids also possess activities such as anti allergic, antitumor, vascular and cytotoxic activities. Jones *et al*. (1994) reported that phenolic compounds are some of the most widespread molecules among plant secondary metabolites which are known to act as natural antioxidants, antiulcer, anti–inflammatory, antispasmodic and antidepressant

activities (Silva *et al*., 2007). The tannins containing plant extracts can be used against stomach and duodenal tumors and as anti-inflammatory, antiseptic, antioxidant and homeostatic pharmaceuticals (Mamta *et al*., 2013).

Furthermore, it has been reported by Kenner and Requenna (1996) that alkaloids potentials which tend to be organic and natural ingredients that have nitrogen and are also physiological active together with sedative and analgesic roles. Although they are generally found in the form of salt with organic acids, among plant substances, they are considered to be the most efficient therapeutic agent. As a result of their analgesic and antibacterial properties, they can be used for medicinal agents when they are purified and synthesized. Saponins on the other hand have been reported to have immune boosting capacity and anti inflammatory properties (Kenner and Requena, 1996). Oxalates and phytic acid are said to be antinutrients but they have proven potentials especially phytic acid which was found to be anticancer against bone, prostate, ovarian, breast, liver, cholesterol, leukemia, sarcoma and skin cancers. They are also important in protection of the gut from tozxins and may have a positive impact on cholesterol and blood sugar.

**4.2.2 Antifungal activity of crude extracts**

The antifungal activity of the leaf, stembark and root crude extracts of *Vernonia amygdalina* against the selected *Candida albicans* strain: 1161 (isolateS5), RM1000 (isolate B2), P37005(isolate B4) and SC5314(isolate S3, B7, C1, C2 and S1) showed that n-hexane leaf extract (NHLE) of *Vernonia amygdalina* at 40 and 60 mg/mL concentration had no inhibitory activity against strain 1161 (isolate S5). However, at concentration of 80 and 100 mg/mL there was inhibitory activity 8.00±0.20 and 13.00±0.70 mm respectively (Table 4.19). This may imply that the crude extracts which serve as an antimicrobial

agent is concentration dependent. This finding was is in line with Mann *et al*. (2008) who reported that the antimicrobial activity of plant extracts is concentration dependent. The NHLE gave the highest zone of inhibition (21.00± 0.30 mm) at a concentration of 100 mg/mL against strain SC5314 (isolate B7)as shown in Table 4.35. This may implies that strain SC5314 were more susceptible to the extract than others . The activity of *Vernonia amygdalina* leaf against strain RM1000, P37005 and SC5314 (isolate S3, C1, C2 and S1) showed that there zones of inhibition increased as the concentration were increased such that, increase in the concentration gives a direct increase in the inhibition zones which cut across all the test organisms.

Prescott (2002) also reported that the activity of antimicrobial agent is concentration dependent Futhermore, This agrees with the similar work reported by Agah *et al*. (2011) and also in line with the work reported by Abayomi (1993) in addition, Edeoga *et al*. (2005) reported that the inhibitory zones varies with the type of solvents used for extraction. This result is in agreement with the work of Gamba *et al*. (2014) and also in conformity with a similar work of Okigbo and Mmeka (2008) who reported inhibitory activity of the leaf of *Vernonia amygdalina* against *Candida albicans*. The Ethyl acetate leaf extracts (EALE) showed no inhibitory activity for all the varying concentrations used at 40, 60, 80 and 100 mg/mL. The absence of activity may be due to the solvent type which may depict that ethyl acetate may not be a good solvent for the extraction of *Vernonia amygdalina* bioactive substance and a solvent that support its activity. The methanol leaf extract(MLE) had inhibitory activity at all concentrations of 40,60, 80 and 100 mg/mL. Their zones of inhibitions were 7.00±0.10, 8.00±0.10, 10.00±0.70 and 15.33±1.23 mm. respectively. This may suggest that methanol could be a good solvent for extraction aand probably specific bioactive substance may be present for bioactivity. There

was no inhibitory activity for n-hexane, ethylacetate and methanol crude extracts for the stembark and root of *Vernonia amygdalina* .The absence in inhibitory activity could be due to a decrease in the concentration of phytochemical constituents and probably the specific bioactive substance may not be present in this extract.

The activity of the n- hexane and methanol crude extracts of *Vernonia amygdalina* was the best compared to ethyl acetate crude extract that showed no activity. This could be due to the presences of higher concentration of bioactive substances that were found in them. The concentration of this specific bioactive substance eg flavonoids, saponin, Tanin and alkaloids might have been present for both the n-hexane and methanol crude extracts compared to that of the ethyl acetate. This bioactive substance are in no doubt the major element that carry out bioactivity. Ijeh and Adedokun (2006) reported that the concentration of bioactive substance is one of the factors that affect microbial susceptibility, the higher the concentrations the higher the activity of the extract (chemical substance). These bioactive substance are mostly the antimicrobial agents that carry out inhibitory activity for both bacterial and fungi, there importance cannot be overemphasized .The result of the antifungal activity of the standard drug (fluconazole1mg/mL) used in this study showed a better zone of inhibition as compared to the crude with a range of values from 33.66±1.00 - 42.00±0.49 mm. The purity of the standard drug may be responsible for its activity. However, The negative control dimethylsulphoxide (DMSO) showed no inhibitory activity against the standard test strain . This suggests that DMSO does not contain any antimicrobial agent.

Antifungal activity of the leaf, stem bark and root of *Moringa oleifera* against strain 1161 (Isolate: S5) as shown in Table 4.20 showed no inhibitory activity for n-hexane,

ethylacetate and methanol crude extracts for the leaf, stembark and root of *Moringa oleifera* against strain 1161 (Isolate: S5) at the various concentrations of 40, 60, 80 and 100 mg/mL. The absence of activity was also observed in strain RM1000, P37005 and SC5314 (isolate S3, B7,C1, C2 and S1) as shown in Table 4.23, 4.28, 4.32, 4.36, 4.40, 4.44 and 4.48. This finding agrees with a similar work of Onuoha *et al*. (2020) who had no activity in the extract of *Moringa oleifera* agaisnt *Candida albicans*. However, this findings disagrees with a similar work reported by El mohamedy and Abdalla (2014) in which antifungal activity was recorded at 40 mg/mL of the concentration used. The differences in results could be due to solvent types used for extraction, differences in polarity and the test organisms used (Obeidat *et al*., 2012).

The inhibitory activity of n-hexane leaf extract(NHLE) of *Azardirachta indica* against strain 1161 is shown in Table 4.21. The NHLE at a concentration of 40 and 60 mg/mL showed no concentration. There was inhibitory activity at a concentration of 80 and 100 mg/mL which gave zones inhibition of 16.00±0.20 and 20.33±1.04 mm respectively. This may suggest that the antifungal activity of this extract is concentration dependent. This result is in agreement with a similar work of Mahmoud *et al*. (2011) and also in conformity with the work of Simhadri *et al*.(2017) who reported the inhibitory activity of the leaf of *Azardirachta indica* against *Candida albicans*. The NHLE against strain RM1000, P37005 and SC5314(isolate S3,B7 C1,C2 and S1) showed concentration dependency as shown in Table 4.25, 4.29, 4.37, 4.41, 4.45 and 4.49 respectively. Most activity were observed at 80 and 100mg/ml. Ethyl acetate leaf extract (EALE) had no inhibitory activity at the concentration of 40, 60, 80 and 100 mg/mL against all strains. This may be due to solvent type and inadequacy of specific bioactive constituents. The

methanol leaf extract (MLE) showed inhibitory activity only at 80 and 100 mg/mL for most of the strains except strain SC5314 (isolate B7) which had inhibitory activity for all the concentrations used (Table 4.27). This result is indicative that methanol may be good for the extraction of the bioactive substance of this plant, the method of extraction support the availability of specific bioactive substance that are capable of bioactivity.

The result of the inhibitoy activity of n-hexane stem bark extract (NHSBE) in Table 4.25 showed that strain RM1000 was the only strain that had inhibitory activity at a concentration of 80 and 100 mg/mL. The ethyl acetate and methanol extract for all strains tested had no inhibitory activity. This may also be due to solvent type, method of extraction which may affect bioactive substance and the presence of specific phytochemicals that can confer activity. The root of n-hexane, ethyl acetate and methanol extracts of *Azardirachta indica* against strain 1161, P37005, RM1000 and SC5314 (S3,B7, C1, C2 and S1) showed no inhibitory activity at 40, 60, 80 and 100mg/mL concentration In contrast, Mahmoud *et al*. (2011) reported the activity of ethyl acetate extract at concentration of 20

% against *Candida albicans*. The differences could be due to the laboratory procedure and solvent type.

The result of the antifungal activity of the leaf, stembark and root of n- hexane, ethylacetate and methanol extracts of *Vitellaria paradoxa* against strain 1161(Isolate:S5),RM1000(isolateB2),P37005(B4) and SC5314(IsolateS3, B7, CI, C2 and S1) had no activity at all concentrations of 40, 60, 80 and 100 mg/Ml. Table 4.34, 4.38, 4.42, 4.46 and 4.50. The result disagrees with the report of John *et al*. (2017) who observed inhibitory activity at a concentration of 25 and 100 mg/mL against *Candida albicans.*

The low value of the MIC and MFC recorded for both n- hexane and methanol crude extracts against the strain indicates that it has a good antifungal activity(Table 4.51). The lower the MIC and MFC value the greater the activity of the crude extract. Futher more, this low MIC and MFC values by the crude extracts indicates its great importance in health care delivery The MIC and MFC values were in agreement with the work of Mahmoud *et al.* (2011) and also in conformity with the similar work of Edeoga *et al*. (2005) who reported low values of MIC and MFC against *Candida albicans*.

### 4.2.3 Antifungal activity of the fractions

The result obtained with 5 and 10 mg/mL of n-hexane fractions of *Vernonia amygdalina* is shown in Table 4.64. Fraction F1 had inhibitory activity against strain P37005 whereas Fraction F4 showed inhibitory activity at the same concentration of 5 and 10 mg/mL against strain 1161 and P37005. There activity were concentration dependent which was in line with ugwachukwu *et al.*(2014). Other fractions showed no inhibitory activity against the tested strains.

The presence of activity by this fraction may be due to the availability of specific bioactive substance and their partial purification.

The positive control (fluconazole 1mg/mL) tested against the strains showed varying zones of inhibition. The negative control (DMSO) used against the tested strains showed no inhibitory activity. Activity of fraction F1 and F4 were less compared to the activity of the positive control. This could be due to the purity of the drug. Antifungal activity of ethyl

acetate fractions of *Vernonia amygdalina* in Table 4.65. Revealed that fraction F3 showed inhibitory activity at a concentration of 5 and 10 mg/mL against strain RM1000. Other fractions tested showed no activity. This may indicate that fraction F3 could have bioactive substance in them that led to bioactivity.

Table 4.66 revealed the methanol fractions of *Vernonia amygdalina* which showed that Fraction F1 at a concentration of 5 and 10 mg/mL had inhibitory activity against strain P37005. Further more, Fraction F6 also showed inhibitory activity at the same concentration of 5 and 10 mg/mL respectively against strain P37005. The activity of fraction F1 was also seen against RM1000 at a concentration of 5 and 10 mg/mL. .Other fraction showed no inhibitory activity against the tested strains.

Result of n- hexane fractions of *Azardirachta indica* in Table 4.67 showed that fraction F3 had inhibitory activity against strain P37005, RM1000 at a concentration of 5 and 10 mg/mL. Fraction F7 showed inhibitory activity at a concentration of 5 and 10 mg/mL against strain 1161 and P37005. Other fractions showed no inhibitory activity against the tested strains. It is obvious that the activity of this fractions were also concentration dependent (ugwachukwu *et al*., 2014) which was also observed in the crude extract. Most of the activity of n- hexane fractions were observed for fractions (F3 and F7) which were the two fractions that showed better activity compared to the activity of other fractions.

Ethyl actetate fraction of *Azardirachta indica* (Table 4.68) showed that fraction F1 had inhibitory activity agaisnt strain 1161 at a concentration of 5 and 10 mg/mL. Fraction F6 and F7 also showed inhibitory activity against strain P37005 and strain 1161 respectively. Fraction F7 on the otherhand had inhitory activity against RM1000 a varying zones of

inhibition. The activity of fraction F1, F6 and F7 were less compared to the activity of the positive control.The negative control (DMSO) used against the tested strains showed no inhibitory activity.

Antifungal activity of methanol fraction of *Azardirachta indica* in Table 4.69 showed that Fraction F3 at a concentration of 5 and 10 mg/mL had inhibitory against strain P37005 and RM1000. Fraction F6 also showed inhibitory activity at the same concentration of 5 and 10 mg/mL against strain 1161. Other fractions showed had no inhibitory activity against the strains.

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the active fractions in Table 4.70 showed a low MIC and MFC values which suggest that the fractions might be highly effective against the tested strains.

The antifungal activity of the fractions showed that the n- hexane fraction (F4) and methanol fraction(F1) of *Vernonia amygdalina* were the two most active fractions that were active against the test strains. Likewise the n- hexane and methanol fraction (F3 and F6) respectively of *Azardirachta indica* were the two most active fractions that were active against the test strains, There activity were >10 mm at a concentration of 10 mg/mL which were in line with Edeoga *et al*. (2005). They were analyzed by Gas Chromatography Mass Spectrophotometer (GCMS) to know the compounds present in them. Fractions that were ≤10 mm were not considered for GCMS analysis.

The n-hexane fraction (F4) of *Vernonia amygdalina* leaf showed 20 probable compounds that were revealed by the GCMS, this findings were similar with the work reported by Jarvis (1999). Some of the compounds present were 2, 4- diter-butylphenol (100%), E-15-

heptadecenol (10.18%), Neophytadiene (57.53%), n- hexadecenoic acid (84.79%), phenol, 4,4-4-ethylidynetris (52.12%), Bis (2-ethylhexyl) phthalate (89.30%), phytol (44.18%), (Table 4.71). Some of these compounds were the Fatty acids, phenol group of compounds,Terpenes and Terpeniods. Terpene"s have being classified as monoterpene ($C_{10}$ ), Sesquiterpene $C_{15}$ Diterpenes (C20) and sesterpenes ($C_{25}$ ) (Guangyi *et al*., 2005). However, the terpenes and terpeniods, phenols and even the saturated and unsaturated fatty acids were more of the compounds identified in the n-hexane fraction(F4). The presence of these compounds were also similar to to the findings of Erasto *et al*.(2009). Several of these compounds possess antifungal activity, the phenolic compounds like the 2, 4- diter-butylphenol with functional hydroxyl group possess antifungal activity (Park *et al*., 2001). The phenolic compounds found in this fractions were in trace amount but might also contribute to the activity of the fractions against the test strains. The activity of these fractions and as well as the crude extract activity could also be due to the presences of this terpenes and terpeniods, phenolic and fatty acids. Fatty acids that include : n-hexadecanioc acid and decanoic acid. This is in line with the report of Lambert *et al*. (2001). This could be the reason why this fraction(F4) had a better activity compare to the other fractions found in this study.

Furthermore the methanol fraction (F1) of *Vernonia amygdalina* leaf showed 19 probable compounds (Table 4.72). This compounds were less compared to the report of Jisika *et al*.(1992) but conforms with the finding of Erastos *et al*.(2009). The compounds present still showed the availability of the terpenes and terpeniods, phenolics and a lot of fatty acids. The presence of these notable compounds like the fatty acids, which have therapeutic antimicrobial agent owing to their potency, braod spectrum of activity and absence of resistance mechanisms by micoorganisms against the action of these compounds

is becoming well acknowledged as a viable means of drug development. The presence of this antimicrobial compounds agrees with the finding of Andrew *et al*. (2013). It has being reported that fatty acids are part of the components responsible for the first line of defence in breast milk for sucking infant against invading pathogenic micoorganisms which have being reported by Isaac (2005). Research findings by Petshow *et al*. (1996) and Ruzin and Novick (2000) shows that fatty acids exert their antimicrobial activity by surpressing antibiotics resistance genes in microorganism especially seen in bacteria and provoke a relative low frequency of spontaneous development of resistance in bacteria while other reports describes it activity to direct penetration of the cell walls and cytoplasmic membranes of bacteria, where it gain access to enter and disrupt the cytoplasm while Petshow *et al*. (1996) reported that terpenes and terpeniods compounds on the other hand act by increasing membrane permeability.

The n-hexane fraction (F3) of *Azardirachta indica* leaf had a total number of 34 probable compounds as shown in Table 4.73. The presence of this compounds agrees with the research findings of Desbois and smith (2010) that found out that the leave of *Azardirachta indica* contain chemical comopounds. The presence of this compounds like the tetradecanioc, neophytadiene and 2, 4-Di- tert- butylphenol were similar to the compounds identified by Andrew *et al*. (2013) from the leaf of *Azardirachta indica*. Some of this compounds have being reported to have antifungal activity which was reported by Andrew *et al*., (2013) . The activity of this fraction(F3) could be traced to the presence of these compound. This finding agrees with the work of Ruzin and Novick (2000) who reported on the presence of some chemical compounds that could confer antifungal activity. The methanol fraction(F6) of *Azardirachta indica* leaf showed a total of 20

probable compounds that were identified Table 4.74. These compounds were similar to the compounds in Fraction F3 which have antifungal potency. Some of them include the Neophytadiene(57.53%), Hexadecanal (14.74%), trans -2- dodecen -1(33.12%), hexadecenoic acid, methyl ester(24.76%), n-hexadecanoic acid (85.79%) furo[2,3-H] coumarine, 6-methyl-1-p(51.12%), phytol(43.18), 1,19-eicosadiene(32.67%), ethyloleate(33.34), disococtylphthalate(88.30%), 4-fluorophenyl-5- methyl (22.34%) and delta-tocophenol B(21.64%). The activity of this fraction(f6) could also be traced to the presence of these compounds especially fatty acids which are known to have an inhibitory effect on fungal germination and sporulation (Desbois and Smith, 2010). This finding is in agreement with the work of Ruzin and Novick, (2000) who reported the presence of fatty acids compounds on *Azardirachta indica* leaf that could confer antifungal activity. Although, this study showed higher number of compounds compare to the findings of other researchers and the reasons could be traced to geographical location, solvent use, time of harvest and processing methods of this plants.

### 4.2.4 Acute and subacute toxicity indices of the extracts in rat

#### 4.2.4.1 Acute toxicity

The toxicity result of the two most active extracts (*Vernonia amygdalina* leaf and *Azardirachta indica* leaf ) in this research work showed a safe dose of the extract even when they were administered at both phases (phase 1 and 2) as shown in Table 4.75-4.78. Determination of $LD_{50}$ is usually the first step in the toxicological evaluation of medicinal plants. The absence of mortality at the dose of 5000 mg/kg bodyweight of both *Vernonia amygdalina* (bitter leaf) and *Azardirachta indica* (neem) used in the experimental rats showed that the extracts are non-toxic acutely. Plant extracts or drugs with $LD_{50} > 5000$ mg/kg bodyweight, are placed in class 5, which is the lowest toxicity class by Guidance

Document on Acute Oral Toxicity Testing based on oral $LD_{50}$ value recommended by Organization for Economic Cooperation and Development (OECD, 2008). Therefore, the crude extracts of bitter leaf and neem with $LD_{50}$ > 5000 mg/kg bodyweight are placed in class 5 of oral acute toxicity testing put forward by OECD. However, the non toxic effect of *Vernonia amygdalina* acutely is in line with the similar work reported by Yusmazura *et al*. (2006) in which all the treated rats survived and no toxicity sign were observed acutely. *Azardirachta indica* non toxic effect have also been reported in the similar study of Kanagasanthosh *et al*. (2015) were no significant changes were observed in the behavioural or autonomic responses in mice after treatment with different dosses of *Azadirachta indica* leaf extract. There was no mortality in these animals during the observational period .

Table 4.79 and 4.81 shows the effect of the dose of *Vernonia amygdalina* crude extract and *Azardirachta indica* crude extract on the body of the experimental animals. The weight of the treated groups increased as the experiment proceeds from the first week to the end of the experiment. There was significant differences ($P<0.05$) between the treated groups and the control. The significant increase was seen continuously from week one to week four. The increase in weight might implied that water was readily consumed by the animals since it is one of the most important nutrient for growth and development. Also, increased weight by these animals may be due to the fact that the doses of the extract enhanced the sense of taste and appetite of the animals after their consumption which could have led to a significant rise in their body weight and as well certain biochemical processes might influence and even stimulate high intake of food comsumption and metabolism. This results resembles similar report of Anofi -Omotayo *et al*. (2012) on the increase in

weight of male wister rats with ethanolic extract of *Azardirachta indica* stem for four weeks of administration which was attributed to the dose of the extract that enhanced sense of taste and appetite of the animals.

**4.2.5 Subacute toxicity indices of extract in rats**

**4.2.5.1 Hematological indices**

Evaluation of haematological parameters reflects level of positive or negative effect of foreign compounds on blood, which may include compounds contained in medicinal plants (Agbaje *et al*., 2009; Ibrahim *et al*., 2016). This may be useful in determining the haematological relating functions of plant products (Agbaje *et al*., 2009). Furthermore, such investigation is necessary for risk evaluation of toxicity of medicinal plants products as changes in haematological status of the body have higher predictive value in human toxicity when data are translated in human (Olson *et al*., 2000). Ajayi and Raji (2012) defined haematological parameters as those factors in the blood whose levels are assessed for determining the body physiological status. Thus, haematological parameters reflect the physiological state of an animal. In this studies, haematological parameters that include: white blood cells, (WBC), red blood cell (RBC), Haemoglobin (HB), pack cell volume (PCV), mean cell volume(MCV), mean cell haemoglobin(MCH), mean cell haemoglobin concentration(MCHC), platelet count, neutrophils (N) lymphocytes (L), monocytes (M), eosinophil(E) and Basophils (B)were all analysed for *Vernonia amygdalina*(bitter leaf *and Azardirachta indica* (neem). The white blood cells (WBCs) and its differentials represent the body immune machineries. Animals with low WBCs count are susceptible to disease infections, where as those with high WBCs are not due to their ability to produce antibodies during phagocytosis and have high degree of disease resistance (Soetan *et al*., 2013). Adaptability to local environmental and disease prevalence conditions are enhanced by

high WBC count (NseAbasi *et al*., 2014). Shah *et al*. (2016) reported low levels of WBCs differentials especially low eosinophils and lymphocytes in patient with heart failure.

In this result, there was significant decrease (P< 0.05) in White Blood Cells Count at all test of bitter leaf crude extract when compared to the normal control which suggests that the extracts do not stimulate synthesis of White Blood Cells and this could lead to reduced immune sensitivity/capacity of the rats and thus can easily contract infections (Table 4.80). Nevertheless, Significant increase in white blood cells differentials suggest that the extract to some extent can awaken immune system to fight certain infections which was seen in group 2( 300mg/kgbw) and group 3(600mg/kgbw). The RBC showed significant

increase (P>0.05) which was observed at the highest tested dose when compared to the control group. This is indicative of the hematinic effect of the bitter leaf crude extract at higher doses, and can be used in treating anemia. The significant decrease in haemoglobin level in all tested doses of bitter leaf crude extract may be as a result of continuous haemolysis of the red blood cells thus leading to reduced haemoglobin content. There was non-significant difference (P>0.05) in MCH and MCHC levels at all tested doses of the bitter leaf crude extract when compared to the control groups respectively This indicates that even with reduced RBCs at doses of 100 and 300 mg/Kg bodyweight of bitter leaf crude extract, the extracts still show positive effects on erythropoiesis by keeping these parameters constant. Significant increase (P>0.05) in platelets levels in all group treated with 300 and 600 mg/Kg bodyweight respectively of bitter leaf crude extract further attest the positive effect of the bitter leaf crude extract on erythropoiesis but also on wound healing rate at higher doses. Packed Cell Volume (PCV) also known as hematocrit (Ht or Hct) or Erythrocyte Volume Fraction (EVF) is the percentage of red blood cells in the total

blood volume (Purves *et al*., 2003). Guenter and Lawrence (2005) reported that PCV measures the percentage volume of red blood cells in the blood; low production of red blood cells or increased hemolysis is associated with anemia. Oxygen and absorbed nutrients transport are also measured by PCV. Increased PCV level is an indication of better transportation and thus result in primary and secondary polycythemia (Isacc *et al*., 2013). Hence, significant increase in PCV levels in groups treated with 300 and 600 mg/Kg bodyweight of bitter leaf crude extract implies that the extract was able to increase the rate of erythropoiesis thus increasing red blood cells levels at higher doses, further supporting the eryhropoietic stimulating effect of the extract at higher doses and can be used in the management of anemia.

The hematological parameters of rats administered with *Azardirachta indica(neem)* shows a significant increase ($P>0.05$) in White Blood Cells at dose of 600 mg/Kgbody weight(group 3) of neem leaf crude extract as compared to the control group (Table 4.82). This portrays that the extract was able to stimulate the rate of White Blood Cells formation and thus may result in enhanced immune system and thus prevent animals from contracting some diseases. Also, the increased levels of lymphocytes and monocytes (though at higher dose of 600 mg/Kg bodyweight of neem leaf crude extract) further shows the role of the extract in disease prevention. According to Guyton and Hall (2006) reported that RBCs, Hb, MHC, MCHC, and Platelets are associated with erythropoiesis and osmotic fragility of red blood cells. Thus, the significant decrease ($P<0.05$) in RBCs and haemoglobin at lower tested doses that is 100 and 300 mg/Kg bodyweight portrays that the neem leaf crude extract reduced the rate at which erythropoiesis occurs and thus can be an advantageous property which can be used to combat polycythemia. The significant decrease in

haemoglobin level in all tested doses of neem leaf crude extract could arise as a result of continuous haemolysis of the red blood cells thus leading to reduced haemoglobin content. Non-significant difference (P>0.05) in MCH and MCHC levels at all tested doses of neem leaf crude extract as compared to the control groups respectively. This may implies that even with reduced RBCs at doses of 100 and 300 mg/Kg bodyweight of bitter leaf crude extract and 600 mg/Kg bodyweight of neem crude extract, the extracts still had positive effects on erythropoiesis by keeping these parameters constant. There was significant increase (P>0.05) in platelets levels in all groups treated with 300 and 600 mg/Kg bodyweight respectively of neem crude extract further attest the positive effect of the neem crude extract on erythropoiesis but also on wound healing rate at higher doses but on contrarily, significant increase in platelets in group treated with 100 mg/Kg bodyweight of neem leaf crude extract (Table 4.82) buttress the point that the exhibit positive effect on erythropoiesis and rate of wound healing at lower doses. At all tested doses of neem leaf crude extract, significant decrease in PCV levels was observed and this could be linked to the reduced RBCs found in the groups treated with this extract.

#### 4.2.5.2 Biochemical findings

Assessment of liver and kidneys function tests is an important factor when evaluating toxicity of drugs and plant extracts since they are both necessary for the animal survival (Olorunnisola *et al.,* 2012).

Nair *et al*. (2010) reported the serum protein level as an important marker relating to liver synthetic functions and it is a very helpful guide in assessing the degree of liver damage (George, 2009) also reported decrease in the level of serum total protein in hepatotoxicity state to simply indicate the presence of para proteins or deficient production of antibodies. Thus, non-significant difference observed in groups treated with 100 and 300 mg/Kg

bodyweight of bitter leaf crude extract (Table 4.83) shows that the extract was able to produce antibodies at constant rate as the control group while at 600 mg/Kg bodyweight, the extract stimulated rate of serum total protein formation, which also implies that extract can boost immune system at higher doses. Also, significant increase (P>0.05) in serum total protein level in group treated with 600 mg/Kg bodyweight of the neem crude extract (Table 4.84) further support the immune-stimulatory effect of the extract at higher doses. Albumin is a serum protein which functions in the maintenance of osmotic pressure of the body fluids and transport hormones, fatty acids and drugs (George, 2009). Hence, the significant decrease (P<0.05) in albumin levels of groups treated with 100 and 300 mg/Kg bodyweight of the bitter leaf crude extract when compared to the control group implies that there was no efficient transportation of nutrients and hormones at lower doses and can thus lead to malnutrition, but significant increase (P>0.005) was observed in group treated with 600 mg/Kg bodyweight of bitter leaf crude extract (Table 4.83) indicating the efficient transportation of nutrients and hormones and oncotic pressure regulatory effect of the extract at higher doses of the extract.

Lipid profile of an animal compromise four important indices; total cholesterol, Triglycerol, high density lipoprotein-cholesterol (HDL-cholesterol) and low density lipoprotein (LDL-cholesterol). Low serum HDL-cholesterol level reflects poor cholesterol transport by HDL particles from peripheral tissues to liver for its metabolism (Kwiterovich, 2000). Meikle *et al.* (2015) also reported that increased levels of total cholesterol, triglycerol and LDL-cholesterol and decrease in the level of HDL-cholesterol contribute the increased risk for the development of cardiovascular diseases in patient with diabetes. Thus, increased levels of Cholesterol at 600 mg/Kg bodyweight of bitter leaf crude extract and triglycerides at all

tested doses as shown in (Table 4.83) may be an indication of the extract having the ability of stimulate lipogenesis, which too much of it may leads to cardiovascular disorders, however unaltered level of HDL and LDL had proved that the consumption of the extract will not definitely not lead to cardiovascular diseases since there is efficient transportation of cholesterol from peripheral tissues to the liver for metabolism. The significant decrease in cholesterol (at 100 and 300 mg/Kg bodyweight) and triglycerides at 100 mg/Kg bodyweight when compared to the control group reflect the ability of the extract to retard the rate at which lipogenesis takes place thus preventing unnecessary accumulation of lipids in the body. In addition, the significant increase ($P>0.05$) in HDL level at 600 mg/Kg bodyweight of neem leaf crude extract (Table 4.84) implies that there is efficient transportation of cholesterol in the body and thus may be able to prevent cardiovascular diseases at higher doses by transporting excess cholesterol to the liver for metabolizing which could have accumulated in the body.

Increased levels of liver enzymes have been attributed to the leakage and malfunctioning of the liver cell membrane (Moore *et al*., 1985 and Imo *et al*., 2013). AST and ALT are transaminases found in the liver, but ALT is more liver specific than AST as AST can be found in other tissues like muscles, kidney, brain to mention a few as ALP. (Palmer, 2009). This however means that elevated level of AST may not be due to the liver damage, but elevated level of ALT is mostly due to the liver damage. Hence, the significant increase ($P>0.05$) in AST and ALT levels observed at 600 mg/Kg bodyweight of bitter leaf crude extract (Table 4.83) shows the extract is hepatotoxic at higher doses but with no adverse effect on liver at lower doses. The same observation was seen with animals treated with

600 mg/Kg bodyweight of the neem leaf crude extract as shown in (Table 4.84) also indicating hepatotoxic effect of neem leaf crude extract at higher doses.

Elevation in the level of indirect bilirubin majorly indicates liver damage and haemolysis of RBCs. In this condition, liver can no longer conjugate bilirubin with glucoronic acid and therefore indirect bilirubin re-enters the circulation. This happens in conditions like severe hemolytic anemia, where excessive indirect bilirubin overwhelms the liver conjugating mechanisms. Increased direct bilirubin level on the other hand indicates biliary obstruction (Ofem *et al*., 2008).

Elevated levels of total bilirubin, indirect bilirubin and direct bilirubin can also be caused by increased rate of RBCs formation (polycythemia), hemolysis, as seen in ineffective erythropoiesis, or from deficient bilirubin transport across the liver as presented in Gilbert"s syndrome ((Edwards *et al*., 2008). The significant increase (P>0.05) found in groups treated with 600 mg/kg bodyweight of bitter leaf and neem leaf crude extracts (Tables 4.85 and 4.86) implies that the extracts are toxic at higher doses causing haemolysis.

The primary roles played by renal system include electrolyte/fluid regulation, buffering effect and in the elimination of waste products (Dixon, 1991) . Sodium is the major extracellular cation which serves to regulate the total amount of water into and out of individual cells, it also plays role in critical body function (Ezekwesili *et al*., 2008). Therefore, higer sodium in blood otherwise called Hypernatremia have reported to be due to an inappropriate production of anti-diuretic hormone (vasopressin) by the leukemic cells (Imo *et al*., 2013). According to the Centers for Disease Control and Prevention (CDCP, 2016), increased sodium level can cause increase blood pressure and the risk for heart

diseases and stroke in some individuals (CDCP, 2016). Thus, significant decrease (P<0.05) in sodium levels in all treated groups for the two extracts portrays that the extracts may reduce the risk of developing high blood pressure i.e possess antihypertensive effect (Tables 4.85 and4.86 ). Potassium ion is the major intracellular cation which protects against hypertension (Henry, 2015). Hence, the significant increase (P>0.05) at higher doses for both bitter leaf and neem leaf crude extracts (Tables 4.85and 4.86) further buttress the point that both extracts possess antihypertensive by not reducing the level of potassium. Bicarbonate acts as a buffering system that prevents significant change in physiological pH (Ali and Ibiam, 2014). Bicarbonate test helps to evaluate and keep track of the conditions that affect blood bicarbonate level including liver, kidney and metabolic conditions (Tiku, 2007). Therefore, the significant decrease (P>0.05) observed at all tested doses for both the extracts ( Tables 4.85 and 4.86 ) means that there may be a slight change in the physiological pH which could be of an advantage in a cases like alkalosis. Were as, Serum creatinine being an easily measured byproduct of muscle metabolism that is excreted unchanged by the kidneys, it is regarded as an important indicator of renal health. Biological system involving creatine, phosphocreatine and adenosine triphosphate (ATP) gives rise to creatinine. Creatinine is chiefly removed from the blood by the kidneys, primarily by glomerular filtration, but by proximal tubular secretion. There is little or no tubular reabsorption of creatinine. In kidney dysfunction, there is surge in the level of serum creatinine. Hence, creatinine level in the blood and urine may be used to calculate creatinine clearance time, which is direct proportional approximately to glomerular filtration rate (GFR). Blood creatinine may also be used to calculate estimated GFR (eGFR). The significant decrease (P<0.05) in creatinine levels observed at all tested doses for both bitter leaf and neem leaf crude extracts (Table 4.85 and 4.86) implies that both

extracts facilitate creatinine excretion by kidneys thus can be said to be renal-protective in nature since creatinine is the most important parameter that measures the excretive capacity of kidneys.

Urea is formed by the liver by combining two molecules of ammonia and a molecule of carbon (IV) oxide in the urea cycle. Urea is a highly soluble compound and plays an important role in the metabolism of nitrogen-containing compounds in animals. It is also the chief nitrogen-containing compound in the urine of mammals. Body uses urea for nitrogen excretion preventing physiological toxicity of ammonia resulting from deamination of amino acids. Hence, the significant increase ($P>0.05$) found at higher doses tested for both extracts ( Table4.85 and 4.86) reveals that the extracts could be nephrotoxic at these doses since they slow down the rate of urea excretion which could even lead to ammonia intoxication, which is a life-threatening condition. Although, some kidney parameters values were within the control which may not really justify the toxicity of the extracts on the kidney.

### 4.2.6 Histopathology of the effect of crude extracts on the selected organs

#### 4.2.6.1 Histology of the heart

The heart is a muscular organ, that functions or serves as a pump for the movement of blood via the body through the circulatory system, giving nutrients to the tissues and removing carbon dioxide and other wastes (Ajani *et al*., 2008). The heart of the treated groups and the control groups showed normal histological features which was observed for

both the bitterleaf and neem leaf extract. There was no disaggregation, vacuolation and distortional changes in tissues that could affect the normal functions of the heart. There was no changes that could affect the heart tissues and impair cardiovascular fucntions and untimately tissue necrosis. This finding is indicative that the extract from bitter leaf and neem leaf might not have any considerable effect on the cardiovascular system of the experimental rats even at a higher dose of 600 mg/kgbw. Although, in contrast cardiovascular disease have being reported by Ogbonna *et al.*(2010) that degenerative changes were observed in the heart tissue of mice following subchronic administration of high doses of plant extract. This report is also in line with the work of Ajani *et al*. (2008) that reported on drug administration which could increase some serum parameters and as such could serve an indicative evidence of cardiovascular toxicity. The differences in result may be due to method of administration of the extracts and the toxicity potency of the extracts.

**4.2.6.2 Histology of the kidney**

The kidneys are two shaped identical organs of the body that functions in the removal of waste from the blood, forms urine, balances body fluids and also help in other important activities of the body (Maghrani *et al.,* 2005). The kidney principally filter extra water and toxins from the blood, make hormones which help to regulate blood pressure. The tissues involve are the nephron and the collecting duct (collecting tubule). The effect of the bitter leaf and neem leaf crude extract on the kidney histology showed no signs of tissue vacuolation, distortion of collecting ducts and degenerative changes which was observed for both the treated groups (experimental groups) and the control groups. Although, at 600

mg/kgbw of the bitterleaf and neem leaf extract there was mild infiltration of the tubulo interstitial space with lymphocytes.

The mild infiltration may be possible due to toxic agents present in the extracts Although, it"s obvious that the changes were not significant even at a higher doses to induce kidney damage and impair its physiological functions. Although, in contrast Berndit and Devis, (1995) have established that physiological changes in kidney histology might not be sensitive enough in detecting renal toxicity or damage. Other researchers have also observed kidney weight to be a relatively sensitive indicator of nephrotoxicity for nephrotoxicants ( Simon, 1993). The effect of the extracts on the kidney histology observed for both the bitter leaf and neem leaf were mainly on the collecting tubule at a concentration of 600 mg/kgbw of the extracts without much effect on other vital parts of the kidney. Plate XVIII and PlateXX respectively This findings corroborate with the findings of Maghrani *et al.*(2005) that reported on medicinal plants to exerts therapeutic effects on animal tissues which could be toxic due to the presence of phytochemical components such as flavonoids, saponins and some organic acids present.

**4.2.6.3 Histology of the the liver**

The liver is a large vital organ that is found in the animal system that functions in the detoxification of chemicals and metabolizes drugs and as well serves in the release of bile into the intestine (Treadway1998). The liver produces protein and blood clothing factors, it also synthesis glycogen, tryglycerides,cholesterol and break down of drugs and medication There was no hepatocytes distortion and degenerative changes observed in the liver of the treated groups except at a higer concentration of 600 mg/kgbw were mild inflammation

was observed for both the bitterleaf and neem leaf extracts (plate XXII and XXIV) respectively. Although some report regarding hepatocytes have being reported by Abdelgadir *et al*. (2010) who observed severe distortion and degeneration of hepatocytes of whistar rats with *Lawsonia intermis* extract. The severe toxicities and side effects of medicinal plants extracts on hepatocytes include hepatotoxicity. Though, the exact principle for the induced toxicity is not well known, several studies have reported that herbal medicines are taken up and accumulated in the liver cells, resulting in the enhancing production of reactive oxygen (Treadway1998).

#### 4.2.6.4 Histology of the lungs

The lungs are the primary organs of the respiratory system in animals and even humans. The respiratory system together with the lungs takes oxygen from the air and transport it to the red blood cell which in response carries it to other parts of the body. Th lungs functions in getting out of carbondioxide ($Co_2$) gas when breathe is exhaled out of the body (Abdelgadir *et al*., 2010). The lungs section from the treated rats and the control rats showed normal histology. This reaserch finding revealed normal histological feature of pulmonary tissue such as normal shape of alveoli, thin septate between alveoli and normal distribution of collagen fibre even at higher concentration.

Table 4.88 and 4.90 shows the weight of the the effect of n- hexane fraction of *Vernonia amygdalina* extract and methanol fraction of *Azardirachta indica* on experimental rats. There was consecutive increase in the weight of the treated groups as compared to the control. The increase during the course of the experiment started from the first to the last week of the experiment. However, there was significant differences $p \leq 0.05$ between the

treated groups and the control.The significant increase was seen continuously from week one to week four. This increase in weight might be due to the fact that this extract could be able to induce appetite in this animals which lead to a significant rise in their body weight. As well certain biochemical processes could influence and stimulate high intake of food comsumption and metabolism.

**4.2.7 Subacute toxicity findings for active fractions**

**4.2.7.1 Haematology toxicity implications**

The results of the subacute toxicity study of the fractions after 28days of experiment showed that all of the haematological parameters analysed for both *Vernonia amygdalina* fraction (F4) of n- hexane extract and *Azardirachta indica* fraction (F6) of methanol extract of the administered groups were within the normal range as compared with the control groups. There was no significant difference ($P>0.05$) when compared with the control (Table 4.89and 4.91). This might indicates that the fraction may not be toxic to circulating red cells, nor interferences with their normal production, knowing the fact the haematologcal system is one aspect that is a sensitive targets of toxic compounds and is an important guide of physiological and pathological status in animal and man (Adeneye *et al*., 2006).

**4.2.7.2 Subacute toxicity on biochemical parameters**

The parameters for the liver function and kidney function test analyzed in this study showed that the groups administered with the fractions of both bitter leaf and neem respectively were within the normal range of the control values .All important parameters

that are indicators for healthiness of the organs like the liver enzymes such as aminotransferase (ALT) aspartate aminotransferase (AST) and alkaline phosphate were all within the normal range as compared with the control value. The tolerating value were good indicators of liver functionality (Table 4.92and4.93). The values showed by the kidney parameters were also within normal range. Parametrs that are major indicaors of kidney impairment were within the control values (Table 4.94 and 4.95).This is indicative that the fractions showed no or less toxicity to the kidney and the liver which are two principal organs responsible for metabolic processes (kokdil *et al*., 2006).

### 4.2.8 Histopathology of the effect of fractions on the selected organs

#### 4.2.8.1 Histology of the heart

The heart of the treated groups and the control groups showed normal histological features which was observed for both the bitterleaf and neem leaf fractions. There was no disaggregation, vacuolation and distortional changes in tissues that could affect the normal functions of the heart. Moreso no change occurred that could impair cardiovascular function. This results is indicative that the fractions for both the bitter leaf and neem leaf might not have have any significant effect on the heart. This findings agrees with the work reported by John *et al*. (1999) that observed normal histology of the heart when administered with *Vernonia amygdalina extract* on experimental mice.

#### 4.2.8.2 Histology of the kidney

The effect of the bitter leaf and neem leaf crude extract on the kidney histology showed no signs of tissue vacuolation, distortion of collecting ducts and degenerative changes which was observed for both the treated groups (experimental groups) and the control groups. At a

higher concentration of 40mg/kgbw of the bitterleaf and neem leaf fractions there was mild inflammation (Plate XXVI and XXVIII). The mild inflamation may be due to toxic agents present in the fractions which could have brought about the inflammation on the kidney. it‟s is clear that the changes were not significant even at a higher doses to completely induce kidney damage and impair its physiological functions. This findings agrees with a similar work reported by Onyenmechi *et al*.(2005) that observed mild inflammation on the kidney of exerperimental rats.

**4.2.8.3 Histology of the liver**

There was no hepatocytes distortion and degenerative changes observed in the liver of the treated groups. Although, at a higer concentration of 40 mg/kgbw mild inflammation was observed for both the bitterleaf and neem leaf fractions (Plate XXX and XXXII). This is indicative that the bitter leaf and neem leaf fractions showed no significant effect at doses of 10 mg/kgbw and 20 mg/kgbw on the treated groups. It is suggestive that low concentration of this fractions should be used even in treating diseases casused by *Candida albicans*.This findings agrees with the similar work documented by Mora *et al*. (2003) that observe mild inflammation of the liver section of experimental rats and a decrease in antioxidant enzymes.

**4.2.8.4 Histology of the lungs**

The lungs section from the treated rats and the control rats showed normal histology. This reaserch finding revealed normal histological feature of pulmonary tissue such as normal shape of alveoli, thin septate between alveoli and normal distribution of collagen fibre even at higher concentration of 40 mg/kgbw of the fraction of bitter leaf and neem leaf. This indicate that the fractions from the bitterleaf and neem leaf had no significant effect on the

lungs of the treated groups. However,. This findings disagrees with the findings of Amal and Mona (2014) on the toxicity effect of carbon tetrachloride ($CCl_3$) on the lungs of experimental mice during a 28 days subacute experiment Although, the mechanism behind the histological changes exerted by medicinal plants on tissues of vital organs is not well understood but documented research findings suggest that such toxicity depends on the formation of some free chemical radicals..

# CHAPTER FIVE

## 5.0 CONCLUSION AND RECOMMENDATIONS

### 5.1 Conclusion

The n- hexane and methanol crude extracts of the leaves of *Vernonia amygdalina* and *Azardirachta indica* showed appreciable antifungal inhibitory activity on clinical strains of *Candida albicans*. *Vitellaria paradoxa* and *Moringa oleifera* were inactive . Thus leaves of *Vernonia amygdalina* and *Azardirachta indica* contained sufficient amount of bioactive substance with antifungal potentials . The fractions may be acting in synergy with the crude extract although the positive control (fluconazole) showed higher potentials perhaps due to differences in pharmacokinetics and

dynamics properties of the compounds. The gas chromatograph mass spectrophotometer analysis (GCMS) revealed probable compounds found in the active fraction. The significant increase ($P>0.05$) in Aspertate Amino transferase (AST) and Alanine amino transferase (ALT) level observed at 600 mg/kgbw of *Vernonia amygdalina*(bitter leaf) and *Azardirachta indica* (neem) extract shows hepatotoxicity at a higher dose only.Haematological parameters of the test animals were all within the normal range and as well may serve as an indicator for further pharmacological evaluation.

### 5.2 Recommendations

1. The promising anticandida fractions of *Vernonia amygdalina* and *Azardirachta indica* could be recommended as candidates for the search for new antifungal drugs
2. The Medicinal plants used in these study should be tested on a large number of microoraginsms. Since the phytochemical constitutents in medicinal plants tend to show different mechanisms of action against microorganisms.

3. More medicinal plants should be tested on the various strains of *Candida albicans* used in this study in other to determine their wide spectrum of activity.
4. More sensitive method of purification and analysis like the nuclear magnetic resonance(NMR) should be employed in the analysis of the active fractions of these plants.
5. Since the name of this active components of this medicinal plants were known through GCMS, they could be used as clue to formulate drugs for the treatment and control of infections caused by this organisms.
6. Since there was toxicity test and hispathological findings, it is necessary that toxicology studies should be done on all plants recommended for drug development. In addition, no drug should be consumed without proper findings of the toxic effect.
7. Since there are challenges in antibiotic drug development especially in aspect of microbial resisitance, antifungal lipids and other potent compounds especially those identified in this studies should be used as a way forwad in combating and also to serve as a potential way of drug resistance. Fatty acids could be biotectnologically used owing to their invivo administration.
8. The fractions of *Vernonia amygdalina* and *Azardirachata indica* should also be used to develop effective drugs against the diseases caused by *Candida albicans*.

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# APPENDICES

## APPENDIX A
## Research, ethical and publication approval of Minna General Hospital

# NIGER STATE HOSPITALS MANAGEMENT BOARD
## GENERAL HOSPITAL MINNA

23rd May, 2017
HMB/GHM/136/VOL.III/449

The Dean,
Department of Microbiology,
Federal University of Technology,
Minna.

**Through:**
**Adeoye Daniel Owoyele,**
Department of Microbiology,
Federal University of Technology,
Minna.

**RE: ADEOYE DANIEL OWOYELE (Ph.D/SLS/2015/776)**
**RESEARCH, ETHICS AND PUBLICATION COMMITTEE'S APPROVAL TO CONDUCT RESEARCH.**

The bearer, a student of your department sought for permission to conduct research on **"EVALUATION OF ANTIFUNGAL ACTIVITIES OF SOME MEDICINAL PLANT EXTRACTS AND FRACTIONS AGAINST CLINICAL ISOLATES OF CANDIDA ALBICANS".**

The committee after going through his proposal has given him the approval to conduct the Research.

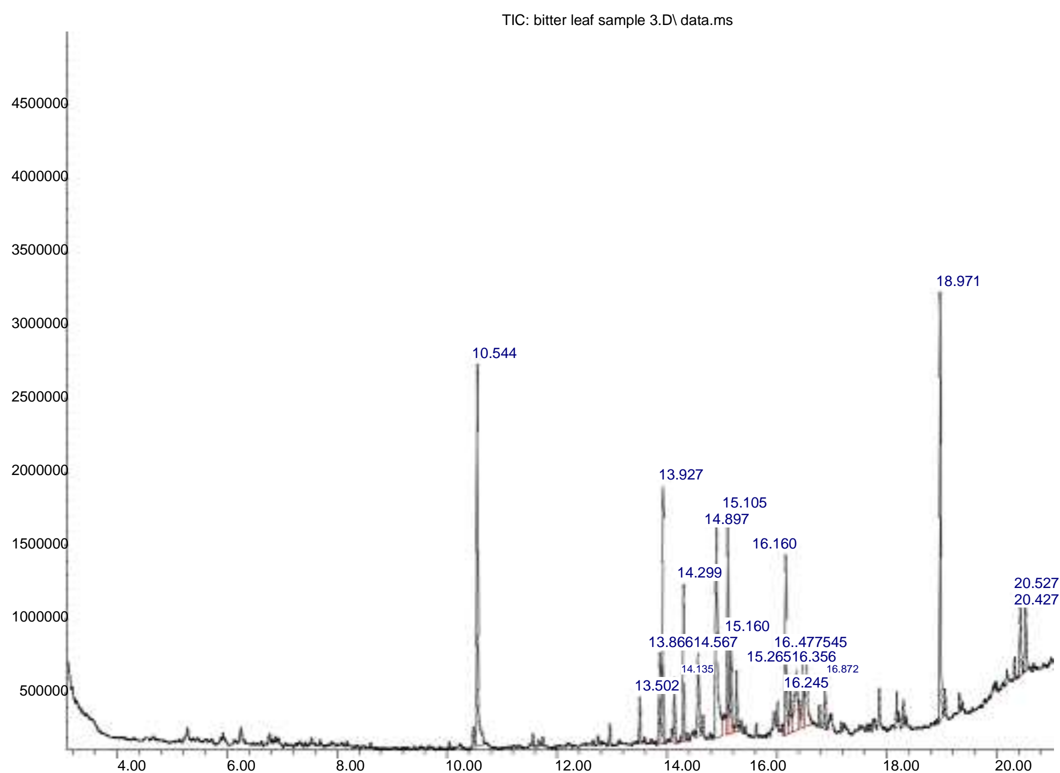
A copy of his final findings must be submitted to the committee as a pre-requisite to this approval.

Thank you for your cooperation.

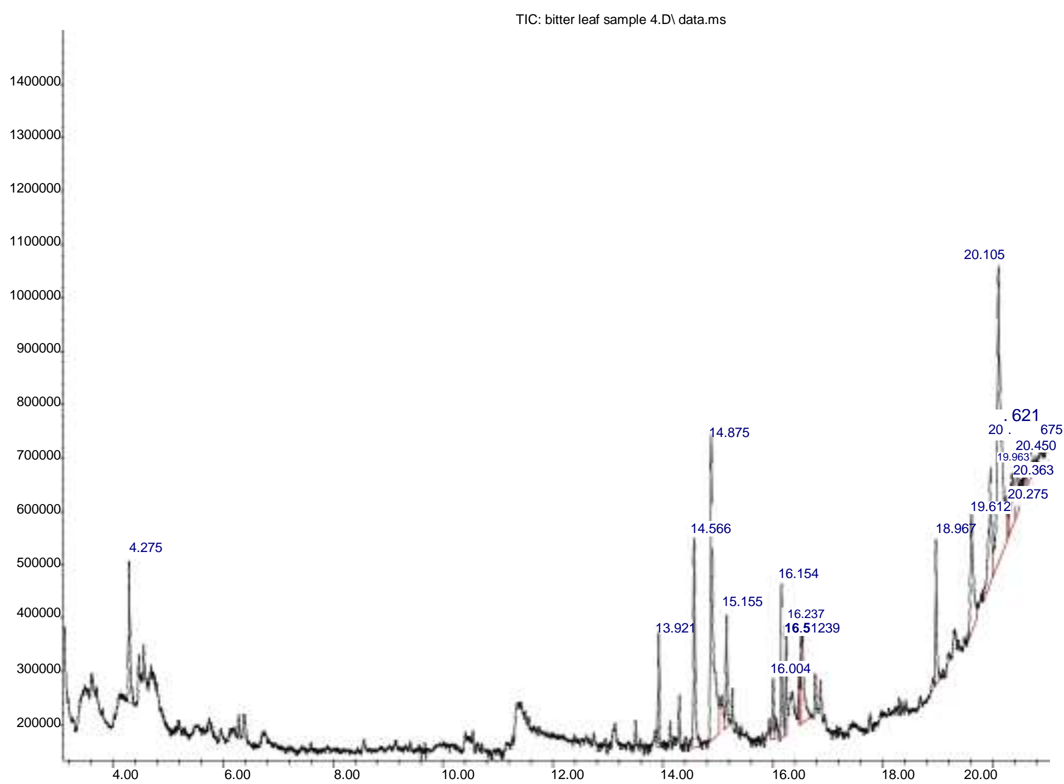
**Pharm. Ibrahim Shehu Madara**
B.Pharm. MPharm.
Secretary REPC
For: **Medical Director**

# APPENDIX B

## Chromatogram of *Vernonia amygdalina* fraction F4 of n- hexane extract

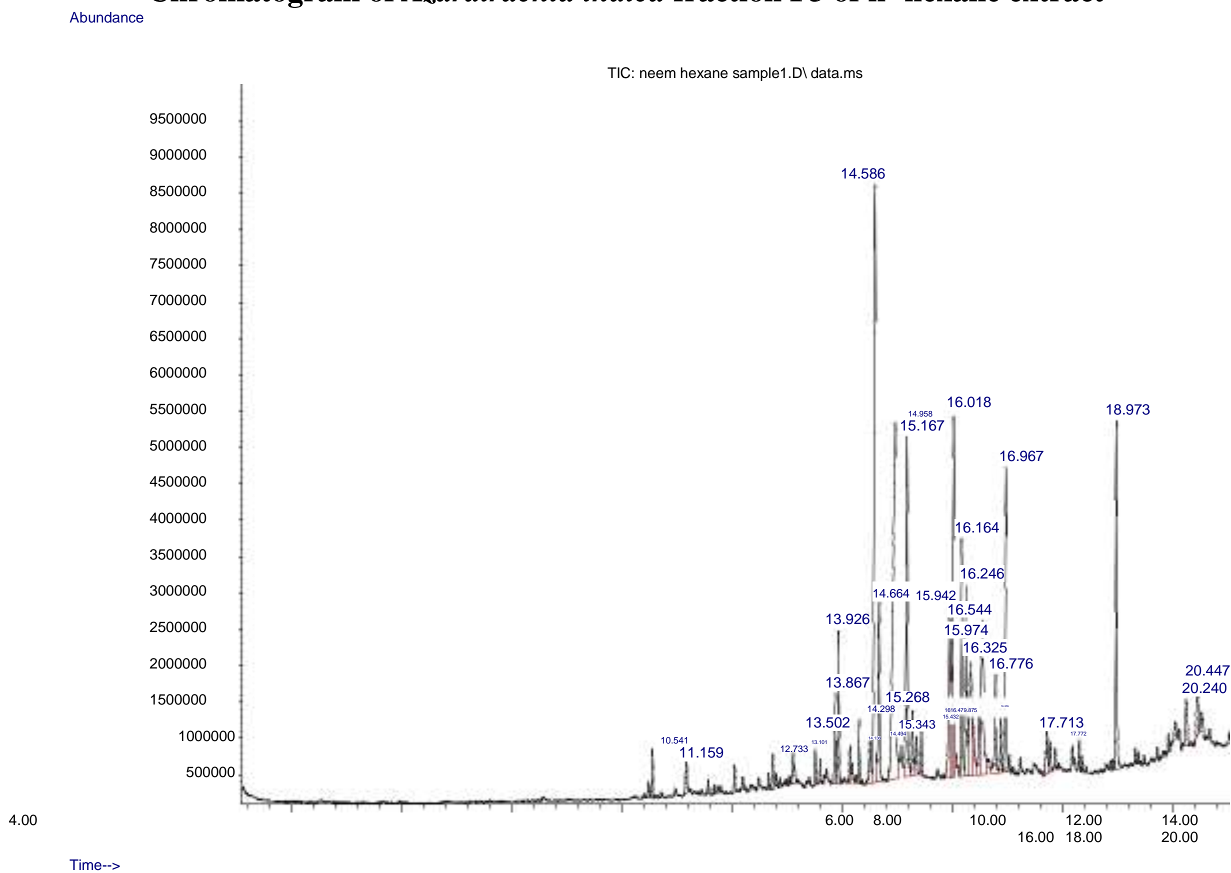


# APPENDIX C
## Chromatogram of *Vernonia amygdalina* fraction F1 of methanol extract

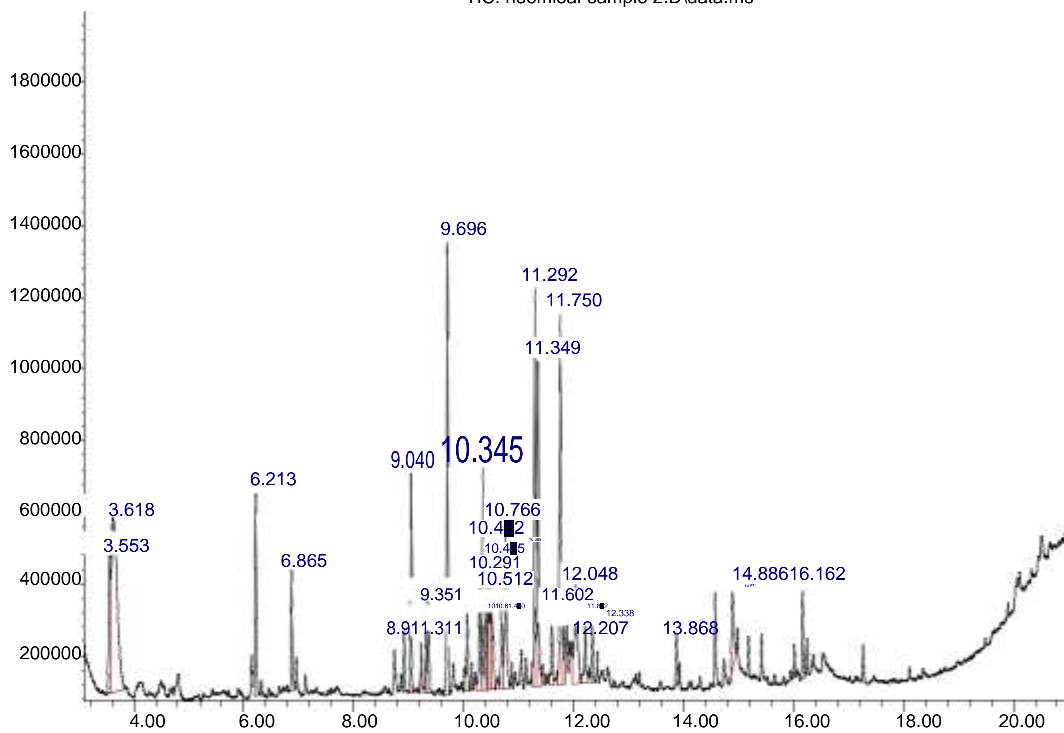


# APPENDIX D

## Chromatogram of *Azardirachta indica* fraction F3 of n- hexane extract

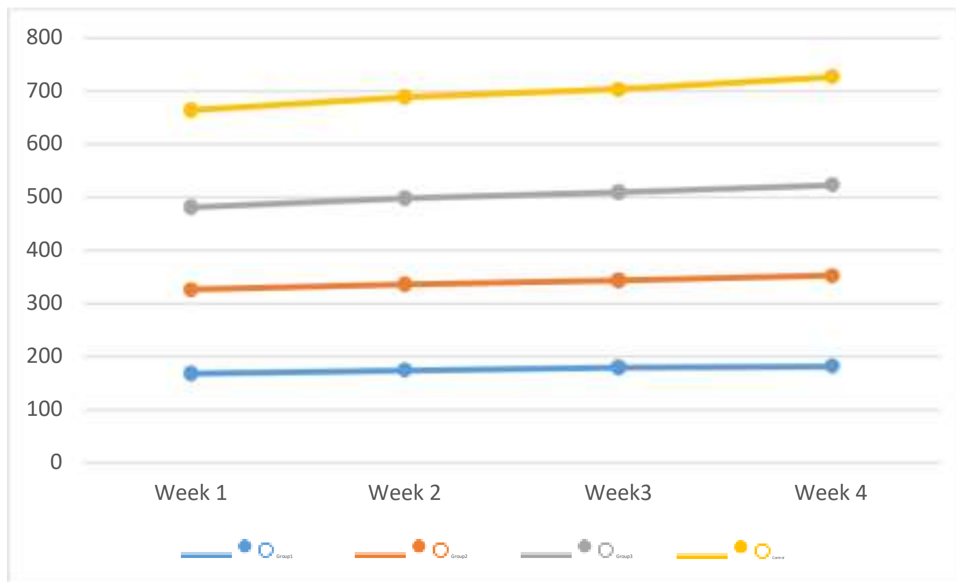


# APPENDIX E
# Chromatogram of *Azardirachta indica* fraction F6 of methanol extract



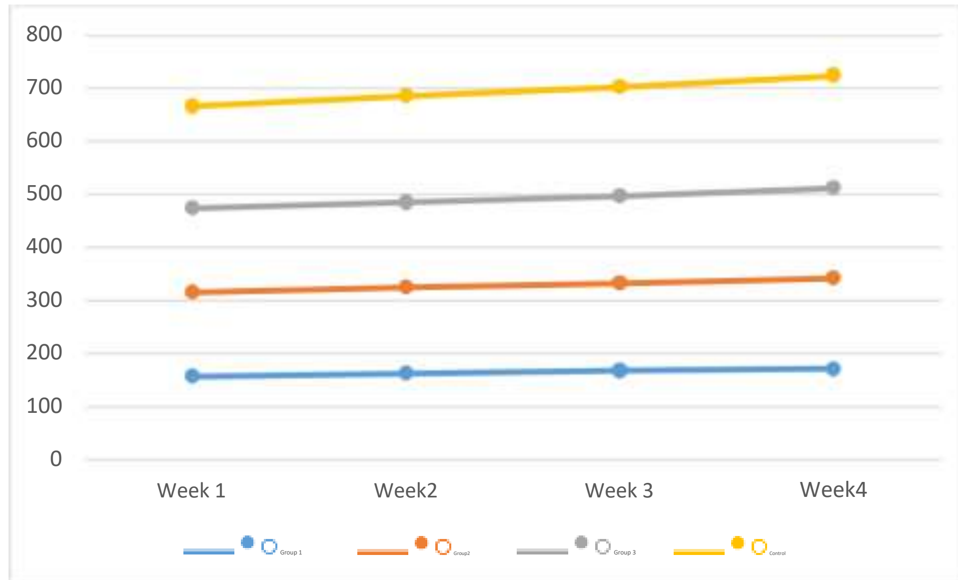
**APPENDIX F**

**Pharmacological effect of *Vernonia amygdalina*(bitterleaf) crude extract on the body weight(g) of Experimental Rats after 28days of Administration**



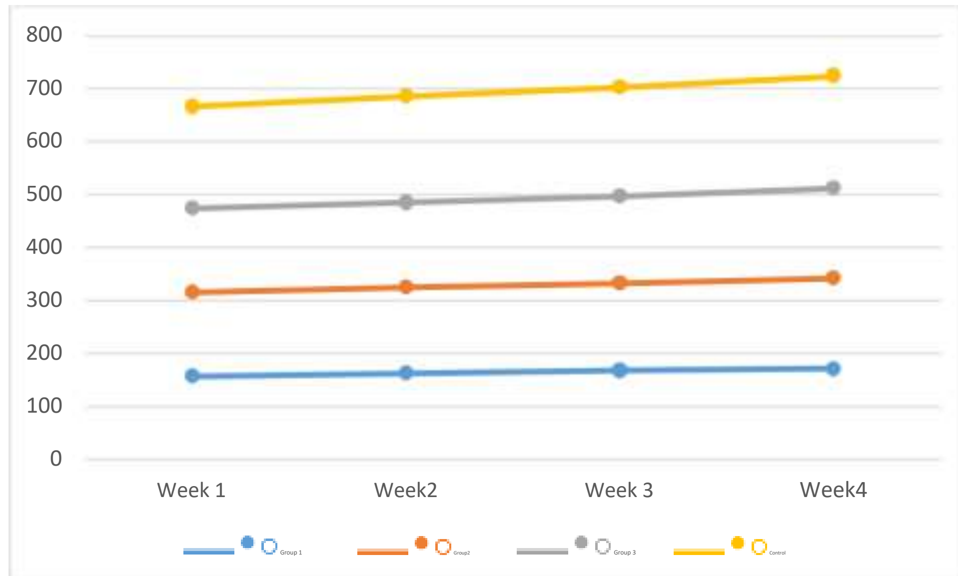
**APPENDIX G**

**Pharmacological effect of *Azardirachta indica*(neem) crude extract on the body weight (g) of Experimental Rats after 28days of Administration**



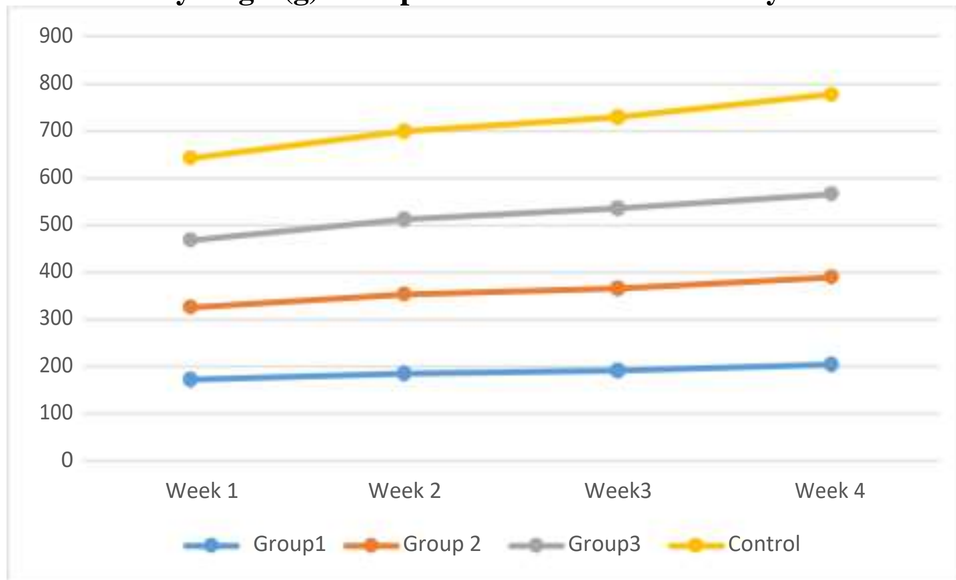
## APPENDIX H

**Pharmacological effect of *Vernonia amygdalina*(bitter leaf) fraction F4 of n – hexane leaf extract on the bodyweight(g) of Experimental Rats after 28days of Administration**

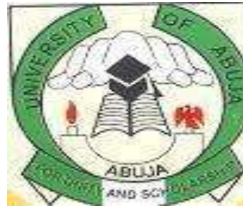


# APPENDIX I

**Pharmacological effect of *Azardirachta indica* on fraction F6 of methanol leaf extract on the bodyweight(g) of Experimental Rats after 28days of Administration**



**APPENDIX J**




**DEPARTMENT OF PATHOLOGY/FORENSIC MEDICINE, FACULTY OF BASIC CLINICAL SCIENCE COLLEGE OF HEALTH SCIENCES UNIVERSITY OF ABUJA**

**HISTOPATHOLOGY REPORT SECTION A (CRUDE EXTRACTS)**

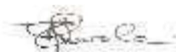
1. Sections of the control kidney and liver showed normal architectural preservation

2. Section from the kidney treated with bitter leaf extract showed mild infiltration of the tubule- interstitial space with lymphocytes. All other achitectures like the glomeruli and collecting duct are free of inflammatory cells.

3. Section from the kidney treated with neem leaf extract showed mild infiltration of the tubule interstitial space with lymphocytes, No other specific features seen.

4. Section from the liver treated with bitter leaf extracts showed mild infiltration of the portal tract with lumphocytes. There was focal infiltration of the liver parenchyma with lymphocytes

5. Section from the liver treated with neem leaf extract shows mild infiltration of the portal tract with lymphocytes .There was focal infiltration of the liver parenchyma with sprineles of lymphocytes.



**DR. OLABODE PETER OLUWOLE MBBS (ABU), FMCPath**
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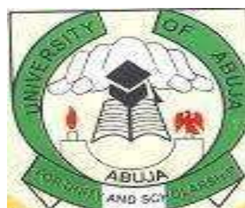
**HISTOPATHOLOGIST** Peter.olabode@uniabuja.edu.ng.
**+2348133129879**

**APPENDIX K**




**DEPARTMENT OF PATHOLOGY/FORENSIC MEDICINE, FACULTY OF BASIC CLINICAL SCIENCE COLLEGE OF HEALTH SCIENCES UNIVERSITY OF ABUJA**

## HISTOPATHOLOGY REPORT SECTION B (FRACTIONS)

1. Sections of the control kidney and liver showed normal architectural preservation

2. Section from the kidney treated with bitter leaf fractions showed mild infiltration of the tubulo- interstitial space with lymphocytes. All other achitectures like the glomeruli and collecting duct are free of inflammatory cells.

3. Section from the kidney treated with neem fractions extract showed mild infiltration of the tubule interstitial space with lymphocytes, No other specific features seen.

4. Section from the liver treated with bitter leaf fractions showed mild infiltration of the portal tract with lumphocytes. There was focal infiltration of the liver parenchyma with lymphocytes

5. Section from the liver treated with neem fractions shows mild infiltration of the portal tract with lymphocytes .

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## APPENDIX L
## CONTRIBUTION TO KNOWLEDGE

This research study:

1. Have been able to identify the specific medicinal plants parts that showed inhibitory activity against *Candida albicans*
2. Demonstrated certain fractions of *Vernonia amygdalina* and *Azardirachta indica* that can confer inhibitory activity against *Candida albicans*
3. Showed compounds through gas chromatography mass spectrophotometer analysis that can aid antifungal activity of the selected test strains
4. Have shown that the extract of *Vernonia amygdalina(*bitter leaf) and *Azardiracta indica*(neem) is hepatotoxic at higher doses but with no adverse effect on the liver at lower doses .
5. Have also shown the fraction can be used for drug development since it toxicity profile were mild compared to the crude extracts.