**CHAPTER ONE**

**INTRODUCTION**

* 1. **BACKGROUND OF STUDY**

African flora in general has an important reserve of aromatic food and medicinal plants. It was demonstrated that medicinal plants play an important role in the pharmacopoeia (Badiaga, 2011). Indeed, according to WHO (2002), about 80% of Africans have recourse to traditional medicine that involves the use of plant active principles to treat most diseases. Thus, a medicinal plant is defined as a plant that one or more of its parts including a substance can be used for therapeutic purpose or as a precursor of the synthetic antimicrobials (Sofowora, 1984). Medicinal plants are of great importance in the health of the individuals and communities. It was the advent of antibiotics in the 1950s that led to the decline on the use of plant derivatives as antimicrobials (Marjorie, 1991).

Medicinal plants contain physiologically active components which over the years have been exploited in the traditional medical practices for the treatment of various ailments (Adebanjo, *et al.,* 1983).

Medicinal plants represent a rich source of antimicrobial agents (Manesh and Satish, 2008). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper price than modern medicine (Mann *et al,* 2008). Plant generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs. In Benin, several ethno botanical studies have focused on identifying medicinal plant species (Sopkon and Quinsaui, 2002; Bieke, 2004). The cashew (*Anacardium accidentale)* plant,a member of genus *Anacardium* belonging to the family Anacardiaceae has been an important plant. Its leaves, bark, roots and stem are traditionally used for the treatment of numerous diseases such as allergy, cough, stomach ache, diarrhea and skin infections (Chabi *et al.*, 2013). Besides these medicinal uses, cashew plays several other important roles. Its wood is used mainly in carpentry, firewood or turned into charcoal (Akinwale, 2000) whereas the resins are used in the manufacture of plastics and natural insecticides (Carvalcante *et al,* 2003).

In contrast to conventional medicine which seeks the origin and causes of diseases and infections, traditional medicine goes directly to the illness. The scientific study of the use of plants allows establishing a link between the two medicines and at times could eventually relieve populations (Badiaga, 2011). Nowadays infectious diseases are responsible for 45% of death in low income countries and 50% of premature deaths worldwide (Gangove, 2007). Groundnut (*Arachis hypogea)* is a geocarpic ( ripening its fruit underground) plant. It is native to Brazil and comes widely distributed throughout South America. In the sixteenth century the Portuguese brought them from Brazil to West Africa and they are introduced to China, Japan, and Malaysia republic. Many fungi grow on solid substrate and secret extracellular enzyme that break down various polymers to molecules that are then absorbed by fungal colony. Consequently, enzyme derived from filamentous fungi have diverse roles in nature being involved in bio-degradation of many types of agricultural matters. Many fungi such as *Aspergillus niger, Aspergillus fumigatus, Candida tropicali* are pathogenic fungi. Fungi that produce mycotoxins are microbes responsible for spoilage. The organism is haploid and has no diploid phase except for the sexual sporangium.

**1.2** **AIM**

To determine the antifungal potential of cashew (*Anacardium occidentale)* leaves extract on fungi isolates of groundnut seed.

**1.3** **OBJECTIVES**

* To extract the active components of cashew leaves using ethanol.
* To isolate fungi from groundnut seed
* To determine the anti-microbial potential of the extract.
* To determine the minimum inhibitory concentration of the extract of cashew leaves (*Anacardium occidentale*) on the fungi isolates of groundnut seed.
* To determine the minimum fungicidal concentration

**CHAPTER TWO**

**2.0 History of Cashew (*Anacardium occidentale)***

Cashew (*Anarcardium occidentale*)Linn tree has been used medicinally worldwide. It is a multipurpose tree whose leaves, stem and bark extracts are used extensively for the treatment of different diseases (Arekemase *et al.,* 2011). The Anacardiaceae family has 76 genera divided into five tribes (Anacardiaceae, Dubineae, Rhoceae, Semecarpeae and Spondiadeae) covering about 600 species (Correia, *et al.,* 2006). *Anacardium occidentale* is an abundant tree in the northeast of Brazil and the states of piaui, ceara and rio grande of north. It represents 90% of cashew production in Brazil. This specie is evident for its antioxidant (Melo-caracante *et al.,* 2003) anti-inflammatory (Olajide *et al.,* 2004), antibacterial antifungal and larvicides (Behrauan *et al.,* 2012*)*. It is a tree rich in anthocyanins, carotenoids ascorbic (vitamin c), flavonoids and other polyphenols as well as mineral components. The antimicrobial activity of cashew leaf extract has been documented by several researchers (Omojasola and Awe 2004; Ifesan *et al*., 2013), different parts of the cashew plant have been used in the treatment of allergies/ inflammation, GIT syndromes endocrine defects, cardiovascular problems, respiratory tract dysfunctions and in the treatment of cancerous growth and tumor (Arekemase *et al*., 2011).

**2.1 Taxonomic Hierarchy of *Anacardiun occidentale***

Kingdom Plantae

Subkingdom Tracheobionta

Division Magnoliophyta

Class Magnoliopsida

Subclass Rosodae

Order Sapindales

Family Anarcardiaceae

Genus *Anacardium* L

Specie *Anacardium occidentale* L

**2.2 Morphology and geographical distribution of *Anacardium occidentale*.**

*Anacardium occidentale* has a height of 5-10m, but in clay land can reach up to 20m. It has a crooked trunk at 25-40cm in diameter. The leaves are oval, obovais, leathery, glabrous; rosy when young, it has vinaceas flowers arranged in terminal panicles (Lorenzi, 2008). According to Gomes (2010), cashew tree is spread around the world, between latitudes 270 N in Southern Florida and 280S of southern Africa and also in low latitude regions near the equator between the parallel 150N and 150S in coasted areas, typically tropical South America, Africa and Asia. The family is rich in important secondary metabolites with varieties of interesting biological activities (Abu-Reidah *et al.*, 2015).

**2.3 Chemical compounds present in *Anacardium occidentale* and its biological roles.**

In the phytochemical analysis of cashew leaves, it is reported that it has (E)-B- ocimene, alpha-copaene and 5-cardienol; while the fruit contains palmitic, oleic acids, furfural,4-hydroxydodecanoic acid, lactone, E-hexenal, (Z)-hex-3-enol and hexadecanol (Mala, *et al.,* 2000). Cashew is rich in anacardic acid, cardionol and cardol along with other alkyl phenolic compounds (Trevisan *et al.,* 2006). It is also evident to have monomeric phenols, flavonoids, glucosides such as myricetin and glycosidic hexoside, pentoside, rhamnosides and glycosidic anthocyanins (Michodjehoun-Mestres *et al*., 2009).

The leaves are rich in alkaloids, essential oils, tannins (Ayepola and Ishula, 2009), saponins, cardenolides and others (Onosanwo *et al.,* 2012). In addition, hydrolysable tannins, phenols, flavones, flavonols, xanthones, chalcones catechins (Santos *et al*., 2013), terpenoides and other phenolic compounds (Doss and Thangavel, 2011) have also been reported. Boiled extract from the new leaves of cashew has for wound healing property (Mazzetto *et al.,* 2009) while the adult leaf extract inhibits the action of the enzyme tyrosinase, demonstrating a therapeutic potential for skin pigmentation problem (Abdul *et al.,* 2008). A recent study showed anti-ulcer actions by the hydro-ethanolic extract of cashew (0.1%), leaving increased gastric acid section. This demonstrates its anti-helicobacter pylori effect (Ajibola, *et al.,* 2010). The aqueous extract of leaves of *Anacardium occidentale* showed hypoglycaemic activity in streptomycin induced diabetic rats at a dose of 175mg/kg, where repeated administration of this dose (twice/day) significantly reduced the blood glucose level ( p<0.01) by 43% in diabetic rats (Sokeng *et al.,* 2001). Petroleum ether and ethanolic extracts of cashew leaves shoed antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans* and *Aspergillus niger* (Dahake *et al*., 2009; Doss and Thangavel, 2011; Onasanwo *et al.,* 2012).

**2.4 Anti-microbial properties of cashew**

**Disease**  **Cashew part** **Reference**

Toothache leaf, bark Khare, 2007; Vanghese, *et al.,*2013

Veneral disease leaf, bark Akash, *et al.,* (2009)

Fungal skin disease (dermatomycosis) bark, leaf Franca, 1993, Anke *et al.,* 2003

Thrust protozoal leaf Akah *et al.,* 2009

Malaria bark, leaf Obembe *et al.,* 2012 ; kunle *et al.,*2013

**2.4.1 Antibacterial activity**

**Dental infection**

The oral cavity contains several microenvironments that can be colonized by bacteria and consequently support the formation of biofilms. Biofilms are involved in the etiology of the most common diseases of the oral cavity such as dental carries and periodontal diseases (Martin *et al.*, 2012). *Streptococcus* mutants as commonly found in the human oral cavity and contributes significantly to tooth decay (Biswas *et al.*, 2012). The presence of bioactive ingredients in the cashew leaves like carbohydrates, tannins, saponins, resins, alkaloids and flavonoids add to their antimicrobial activities. A phytochemical screening analysis on *Anacaerium occidentale* leaves has showed the presence of high concentration of tannins in the aqueous extract of the leaf (Abulude *et al.*, 2010). According to Khare (2007) the bark of cashew has been used for the treatment of toothache due to the presence of flavonoids which disrupt the microbial membrane of *streptococcus* mutants. An extract from cashew peduncle bagasse also showed bacteriostatic and bacteriocidal activity on *S*.mutants and eventually inhibited biofilm formation (Furtado *et al*., 2014).

**Veneral disease**

These are infections that are commonly spread by unprotected sex especially in vaginal intercourse, anal sex and oral sex (Patrick *et al*., 2013). Akash *et al.,* (2009) reported that the leaf and bark of cashew are useful in the treatment, since extract of cashew had been reported for their antimicrobial activities.

**2.4.2 Anti-fungal activity**

**Thrush**

Thrush is a disease caused by *Candida albicans* in the mouth or in the vagina. Akash *et al.,* (2009) reported the anti-fungal effect of cashew leaf extract on two fungi where the minimum inhibitory concentrations (MICs) for ethanolic and petroleum ether extracts were found to be 15.62 and 31.25ug/ml respectively for *Candida albicans.* It was observed that the zone of inhibition of the ethanolic extract was higher with 17mm while the petroleum ether extract was 13m in diameter. Anke *et al*., (2003) reported that the triterpenoids are responsible for the antimicrobial activity against *Candida albicans*. Triterpenoids disrupt the cytoplasmic membrane of this organism which is often indicated by the leakage of intracellular constituents.

**Dermatophycosis**

Dermatophycosis is a skin disease caused by fungi; the causative organisms in most cases include species of *Candida, Trichophyton* and *Cryptococcus* (Anke *et al*., 2003). The organisms had to be reported to be killed or inhibited by the extract of cashew bark and leaf is due to the bioactive components such as triterpenoids, phenolic and volatile oils reported by Ifesan *et al.,*(2013) to be active against fungal isolates. The mechanisms thought to be responsible for

phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds possibly through reaction with sulfhydryl groups or through more non-specific interactions with the proteins (Aiswarya *et al*., 2011).

**Malaria**

*Anacardium occidentale* leaves and bark have been reported for their amoebicidal, antioxidant and astringent properties. The extracts are used to treat malaria (Razalia *et al*., 2008; Orwa *et al*., 2009). The tannin that is present in both the leaf and stem extracts of cashew was said to be responsible for the antimicrobial activity against *Plasmodium specie* which is the causative organism of malaria (Obembe *et al*., 2012).

**2.4.3 Anti-inflammatory activity**

**Bronchitis**

This is an inflammation of the mucous membrane of the bronchi. The larger and medium airways that carry airflow from the trachea int the more distal part of the lung parenchyma. The symptoms might include cough and production of sputum (Lee and Denis, 2004). The component of the leaf and bark that might be responsible is tannin. Tannins had been reported to inhibit acetic acid induced writhing responses in mice and were found to antagonize that permeability, increasing effects to the rats of certain mediators of inflammation and inhibit the migration of leucocytes to an inflammatory site (Mota *et al*., 1985).

**Inflammation**

Pawar and Pal (2002) reported the anti-inflammatory effect of cashew leave extract as shown in carrageenan induced rat paw edema. The acetone soluble part of methanolic extract showed better analgeric and anti-inflammatory activity. Another possible mechanism by which the extract exhibits anti-inflammatory effect is through the inhibition of nitric oxide producing inflammatory cells (Olajide *et al*., 2004).

**Dyspepsia**

This is a condition of impaired digestion. It is a medical condition characterized by chronic or recurrent pain in the upper abdominal fullness and feeling full earlier than expected when eating (Talley and Vakel, 2005). The ability of the cashew leaf has also been attributed to presence of flavonoids found in the leaf of cashew (Jellin *et al*., 2002).

**Diarrhoea**

The bark and leaves of cashew are a rich source of tannins, a group of plant chemicals with documented medicinal activity. Banso and Adeyemo (2007) had earlier reported that tannins isolated from the medicinal plant possessed remarkable toxic activity against bacteria and fungi. The relative antimicrobial action of tannins can be related to the ability to inactivate microbial adhesins, enzymes and cell envelope transport protein. Tannins cause contraction of the surface with which they come in contact and reduce permeability. When consumed they reduce the permeability of the intestinal lining of the intestine which reduces the amount of toxins from the microorganisms assimilated into the blood. This reduces the harmful effect the toxins may have as well as the body’s reaction to the diarrhoea and dehydration (Ojesanmi and Ajao, 2011)

**2.5 GROUNDNUT**

Groundnut (*Arachis hypogaea*) also called peanut is a geocarpic (ripening its fruit underground ) plant. It is a native of Brazil; it comes widely distributed throughout South America at early date. In the sixteenth century in Portugese brought them from Brazil to West Africa and the Spaniard to this crop the pacific to Philippine from where they are introduced to China, Japan, Malaysia, and Indonesia. The plant is extensively cultivated in tropical, subtropical regions all over the world including the temperate region, International Crop Research Institute for Semi-Arid and Tropical (ICRISAT 2005). Groundnut is grown for its oil and protein content .It is both a rainforest and savanna crop which have it major producing countries in West African including Gambia, Nigeria, Togo, Benin, Ghana, Ivory Coast. Groundnut seeds are rich in mono unsaturated fats, the type of fat that is emphasized in the healthy heart diets. Seeds generally associate with certain saprophytic or parasitic microorganisms which perpetuate in the seed lots on the advent of favourable conditions. Groundnut seeds are highly susceptible to fungi such *as Rhizopus, Aspergillus flavus, Penicillium, Fusarium.* Infection of groundnut by species of *Aspergillus* occurs both at pre-harvest and post-harvest stage (Dharmaputra *et al.,* 2003, Craufurd *et al.,* 2006 and Goncales *et al.*, 2008). Groundnut plants are highly susceptible to fungal contamination including toxin producing fungi. However, toxigenic fungal pathogens are important constraints to the production of the crop affecting the quality of the seeds during storage. Generally there several toxigenic fungi types, but predominant fungi *Aspergillus, Fusarium* and *Alternaria*.

**2.5.1 TAXONOMY OF GROUNDNUT SEED**

Kingdom Plantae

Subkingdom Tracheobionta

Superdivision Spermatophyta

Division Magnoliophyta

Class Magnoliopsida

Subclass Rosidae

Order Fabales

Family Fabaceae

Genus *Arachis* L.

Species *Arachis hypogaea* L.

**2.5.2 ECONOMIC IMPORTANCE OF GROUNDNUT**

Groundnut also called peanut, goober pinder, earthnut, was grown on average of sixty-one million acres (twenty-five million ha) in 2000-2003.The leading countries growing groundnut are China, India, Nigeria, United States and Indonesia. Production in 2000-2003 averaged about thirty-eight point five million tons (35million MT) or about 1,300 pounds per acre (1,400kg/ha).about i.9 million tons (1.7 million MT) were produced in the united states in 2000-2003 on about 1.3 million acres (540,000 ha). Yield averaged about 2800 pounds per acre (3100 kg/ha).The leading states in peanut production are Georgia, Texas, Alabama, North Carolina and Florida. Peanut grown from oil in much of the world but all but about 15% of the US crop is used for food. About 6 pounds per capita are consumed as peanuts and peanut products in the United States (John *et al*., 2006).

**2.5.3 HISTORY OF GROUNDNUT**

The groundnut plant is a native of South America where closely related wild species are found. It may have originated on the mountainous part of Northern Argentina in the state of Matto Grosso, Brazil. Peanuts were grown in Mexico by the beginning of the Christian era. Early slave ships carried groundnut to Africa and years later introduced them to the colonial United States from Africa. Commercial development of peanut industry began about 1876. A rapid increase in production occurred after 1900 when the weevil caused serious damage to the cotton crop (John *et al*., 2006).

**2.5.4 BOTANICAL DESCRIPTION**

Cultivated peanut (*Arachis hypogea*) is a member of the legume family. It is a pea rather than a nut. It is unknown in the wild state but about fifteen species that bear some resemblance to the cultivated types are found in South America.

Peanut has been considered a short day plant but it is mostly photoperiod insensitive (day neutral) because floral initials may be present in un-germinated seeds. The peanut plant is a low-growing annual with a central upright stem. The numerous branches vary from prostrate to nearly erect. The innately compound leaf consists of two pairs of leaflets but occasionally a fifth leaflet is borne on a slender petiole. Peanut varieties are readily separated in the bunch and runner types. The nuts are closely clustered about the base of the plant of the erect or bunch type. The runner varieties nuts scattered along the prostrate branches from base to tip. The peanut has a well-developed taproot with numerous lateral roots that extend several inches into the ground. Most roots have nodules but bear very few root hairs.

Peanut is an indeterminate plant that flowers while still growing vegetatively. The flowers are borne in the leaf axils, above or below ground, singly or in clusters of about three. Under filed conditions it is not uncommon to find the blossoms with their yellow petals 3 inches below the soil surface. The calyx consists of a long, slender tube crowned with foe calyx tips, the corolla is borne at the end of the calyx tube. After pollination takes place, the section immediately behind the ovary (the gymnophore or peg) elongates and pushes the ovary with the soil where the pod develops (George, 2005).

**2.5.5 USES OF GROUNDNUT**

Groundnuts are not harvested are used for livestock feed (hogged off). The equivalent of more than 100 million pounds (45 million kg) of unshelled peanuts or 6% of the threshed crop, is used for planting. An average of more than 500 million pounds (225 million kg) of shelled peanuts is crushed, producing more than 200 million pounds (90 million kg) of oil. About 6.5 pounds (3 kg) per capital are consumed as peanuts and peanut products excluding crushing. Annual world production of peanut oil is about 5.5 million tons (5 million MT). It is important edible vegetable oil in Europe, Asia and Africa where peanut butter is almost unknown.

Green leafy peanut hay is as nutritious as good quality alfalfa hay. Nutrient content of field cured hay nay be reduces as present or more through loss of leaves, weathering, excessive stems, mustiness and dirt content (John *et al*., 2006).

**2.5.6 CHEMICAL COMPOSITON**

Peanuts are high in both protein and oil. In general, the vines with the nuts contain 10 to 20% protein or without the nuts, about 7% protein. Unshelled peanuts contain about 25% protein and 33% fat. The nuts contain 40-48% oil and from 25-30% protein. The nuts contain amino acids including cysteine that are essential for animal growth. They are rich in phosphorus like other large legume seeds, peanuts are an excellent source of the vitamins; thiamine and riboflavin as well as good source of niacin.

**2.6 THE MICROORGANISM: FUNGI**

Fungi are any members of the group of eukaryotic organisms that includes microorganisms such as yeasts and molds as well as the more familiar mushrooms. These organisms are classified as a kingdom, which is separated from the other eukaryotic life kingdoms of plants and animals.

A characteristic that places fungi in a different kingdom from plants, bacteria and some protists is chitin in their cell walls. Fungi are heterotrops; they acquire their food by absorbing dissolved molecules, typically by secreting digestive enzymes into their environment. Fungi are the principle decomposers in ecological systems. Growth is their means of mobility, except for spores (a few of which are flagellated) which may travel through air or water.

Fungi include symbionts of plants, animals or other fungi and also parasites. They have long been used as a direct source of human food, in the form of mushrooms and truffles; as a leavening agent for bread; and in the fermentation of various food products such as wine, beer and soysauce.

Since the 1940s, fungi have been used for the production of antibiotics and more recently, various enzymes produced by fungi are used industrially and in detergents. Many species produce bioactive compounds called mycotoxins such as alkaloids and polyketids, that are toxic to animals including humans. The fruiting structures of a few species contain psychotropic compounds and are consumed recreationally.

**2.6.1 FUNGI ASSOCIATED WITH GROUNDNUT SEEDS.**

It is reported that 25% of the world’s crops are affected by mold or fungal growth. In india around 82% of groundnut produced is used for edible oil production, 12% as seed and 5% as feed. The seeds are found to be responsible for disease transmission because they carry a number of pathogens which get associated either in the field or in the poor harvest storage condition. Fungi like *Aspergillus niger, Aspergillus flavus, Alternaria dianthicola, Culvularia lunata, Fusarium solan, Fusarium oxysporum, Fusarium equiseti, Macrophomina phaseotina, Rhizopus stolonifer, Penicillium digitatum and Penicillum chysogenum causes* decoularation, rotting, shrinking seed necrosis, loss in germination capacity and toxification to oil seeds (Chavan and Kakde, 2008).

According to Bhattacharya and Raha, (2002) among different species, *Aspergillus niger* and *Rhizotonia bataticola* were serious in reducing germination of seed by about 30% after 6 months of storage. Peanut/groundnut with their subterranean growth habit was invaded by both *Aspergillus flavus* and *Aspergillus parasitcus* (Raju and Krishnamurty, 2003).

Chavan found that species of *Aspergillus, Penicillum, Fusarium, Rhizopus and mucor* were commonly occurring post-harvest molds in storage conditions. Ibiam and Egwu (2011) reported that among different species of fungal infection. *Aspergillus* was the most predominant one in groundnut.

**2.6.1.0 ASPERGILLUS**

*Aspergillus* is a genus consisting of a few hundred mold species found in various climates worldwide. It was first catalogued in 1729 by the Italian priest and biologist Pier Antonio Micheli. While viewing the fungi under the microscope, Micheli was reminded of the shape of an aspergillus ( holy water sprinkler, from latin spargere (to sparkle). *Aspergillus* is an asexual spore forming structure common to all *Aspergillus* species. Around one third of the species are also known to have a sexual stage (Geiser, 2009).

**2.6.2 AFLATOXINS CONTAMINATION IN GROUNDNUT**

**2.6.2.1 Overview of Aflatoxins**

Aflatoxins are polypeptide derived secondary metabolites produced from *Aspergillus flavus* and other closely related subspecies *Aspregillus parasiticus.* They are highly toxic, mutagenic and carcinogenic to animals (Gilbert *et al*., 2002).They are a group of structurally related polyketide mycotoxins that contaminate many agricultural commodities, such as almond, coffee, corn, cottonseed *(Gossypium* spp.), groundnut, rice (*Oryza sativa* L.), soybean, sunflower, and wheat (Soliman 2002., Yazdanpanah *et al*., 2005, Bhat *et al*.,2010). In addition, milk and milk products can be contaminated as a result of cows being fed on aflatoxin-contaminated feed. Aflatoxin B1 (AFB1) is the most common of the six forms of aflatoxins, AFB1, AFB2, AFG1, AFG2, AFM1 and AFM2.

**2.6.2.2 History of Aflatoxins**

Aflatoxins were discovered in 1960 when more than 100,000 young Turkeys died in England over the course of a few months from an apparently new disease that was termed “Turkey-X disease”. It was soon found that the mortality was not limited to turkeys. Ducklings and young pheasants were also affected. After a careful survey of the outbreaks, the disease was found to be associated with the Brazilian groundnut meal. An intensive study of that groundnut meal revealed its toxic nature as it produced typical symptoms of Turkey-X disease when consumed by poultry and ducklings. A study on the nature of the toxin suggested its origin from the fungus Aspergillus flavus. Thus, the toxin was named “aflatoxin” by virtue of its origin from A. flavus (Guo *et al*., 2008). This was the event which stimulated scientific interest and gave rise to modern mycotoxicology. Research on aflatoxins led to a “golden age” of mycotoxin research during which several new mycotoxins were discovered (Bennette, 2010). Other important mycotoxins produced by *Aspergillus, Fusarium and Penicillium* include ochratoxin, patulin and fumonisins. Among all mycotoxins and polyketide compounds synthesized by fungal species, aflatoxins (the most potent hepatotoxic and carcinogenic metabolites) continue to receive major attention and are most intensely studied.

**2.6.2.3 Types of Aflatoxins**

There are four major types of aflatoxins (namely AFB1, AFB2, AFG1and AFG2) among at least 18 structurally related mycotoxins (Wogan, 1966). Aflatoxins designated by B1and B2show strong blue fluorescence under UV light, whereas the G1and G2 forms show greenish yellow fluorescence. *Aspergillus flavus* produces aflatoxin B1and B2. Other toxic compounds produced by A. flavus are cyclopionic acid, kojic acid, nitropropionic acid, aspenoxin, aflam and aspergillic acid. A. parasiticus produces aflatoxin G1and G2in addition to B1and B2, but not cyclopionic acid (Yu, 2004). Aflatoxin B1 is considered to be the most important of the four because it is the most toxic and has been classified by the International Agency for Research on Cancer a probable human carcinogen (International Agency for Research on Cancer IARC). Level of carcinogenicity is B1>G1>B>G2in that order. AflatoxinB1is predominant, the most toxic and most potent hepatocarcinogenic natural compound ever characterized. Aflatoxin M1and M2 are other significant members of the aflatoxin family and are oxidative forms of aflatoxin B1, which are modified in the digestive tract of some animals and humans and can be isolated from milk. Aflatoxins like any other mycotoxins are a subclass of substances which originated as a result of secondary metabolism of fungi. Unlike primary metabolites, these secondary metabolites are not essential for the growth of the fungi but have survival functions in nature (Luchese *et al*., 1993). Genes required for aflatoxin production have persisted in fungi for more than 100 million years (Cary *et al*., 2006).Expression of secondary metabolite biosynthesis genes does not occur at high growth rates, which indicates that the synthesis of these metabolites occurs during growth repression.

**2.6.2.4 Implications of Aflatoxins for Human and Animal Health**

Numerous epidemiological studies have established the connection between aflatoxin consumption and incidence of liver cancer in humans (Wild and Gong, 2010). Acute aflatoxin poisoning is rare, 25% of these cases are fatal, and chronic exposure rates are high, especially in developing counties. As such, many countries established legal limits for aflatoxin concentrations allowed in foods, specifically groundnuts have intended for human consumption (Reddy *et al.,* 2010). Developing countries have especially high incidences of aflatoxicosis because regulation and testing is prohibitively expensive, and food is scarce because uncontaminated alternatives are not available. Aflatoxins suppress the immune system of susceptible populations in humans, such as young children and HIV/AIDS patients. In Ghana, changes in the percentages of immune cell subsets following aflatoxin B1exposure reduced cell immunity, decreased human resistance to infections and reduced immune responses to vaccinations. Aflatoxin is a carcinogen and acute aflatoxicosis results in human deaths. Aflatoxins also cause oxidative stress, liver necrosis, haemorrhage and death in broiler chickens, pigs and cattle (Eraslan, *et al.,* 2005; Osweiler 2005).The carcinogenic effects of aflatoxin in animals are well established and highly species dependent. Since aflatoxin, specifically aflatoxin B1(AFB1), is one of the most carcinogenic chemicals studied, the FDA, World Health Organization (WHO), and European Union (EU) limit permissible concentrations in foods (groundnut feeds) intended for animal feed as follows: for dairy animals (20 g/kg), immature animals (20 g/kg), breeding beef cattle, breeding swine, or mature poultry (100 g/kg) and finishing swine of 100 lbs or greater (200 g/kg) (Food and Drug Administration FDA 2008).

**2.6.2.5 AFLATOXIN PRODUCING FUNGI**

**Ecology and Biology of Aflatoxigenic Fungi sAflatoxins are produced by four Aspergillus species.** These include

*Aspergillus flavus* Link ex Fr, *Aspergillus nomius Kurtzman*, Horn and Hesseltine, *Aspergillus parasiticus* Speare, and Aspergillus tamari (Goto and Ito, 1996). The agronomical and economically most important aflatoxin producers are the closely related *Aspergillus flavus*, hence the name aflatoxin, and *Aspergillus parasiticus*. Both species are soil-borne fungi that grow on living and decaying plant matter. These fungi produce aflatoxins on various commodities, but they are aconcern on corn, groundnut and cottonseed. A. flavus can be distinguished from A. parasiticus by its smoothspores and yellow-green colonies on potato dextrose agar (PDA) medium.

A. parasiticus produces dark yellow-green conidia with nearly spherical vesicles that produce roughened conidia. It can be readily distinguished from *Aspergillus flavus* by its rough-walled conidia (Klich and Pitt, 2002). Dominant aflatoxins produced by *Aspergillus flavus* are B1and B2, whereas *Aspergillus parasiticus* produces two additional aflatoxins G1and G2 (Payne, 1998). *Aspergillus flavus* of the section Flavi is the most common species involved in pre-harvest aflatoxin contamination of crops and causes a flat root or yellow mold. *Aspergillus flavus* is the most common mycotoxin-producing fungus found in groundnuts; this is true across various climates and geographic regions. Aflatoxigenic fungi are soil-borne imperfect filamentous fungi, which are saprophytic during most of their life cycle, and grow on wide variety of substrates, including decaying plant and animal debris. Two major factors that influence soil populations of these fungi are soil moisture and soil temperature. These storage fungi can grow at temperatures ranging from 12 to 48 oC with optimum of 25 to 42 ºC, and at water potentials as low as -35 MPa. Under high soil temperatures and low moisture, which are associated with drought stress, these fungi become highly competitive and dominant, produce abundant inocula, and outcompete other microflora on corn, cotton, and groundnut. Neither A. flavus nor A. parasiticus has a known sexual stage; they reproduce only by asexual means, but undergo genetic recombination through a parasexual cycle.

Morphology of the conidiophore, which bears asexual spores, is the most important taxonomic character in the identification of Aspergillus. Other important morphological structures used in identification are cleistothecia, hulle cells, and sclerotia. These fungi can survive either as mycelium or as resistant structures known as sclerotia (Hedayaati, *et al*., 2007).

**2.6.2.6 Occurrence of Aflatoxin Contamination in Groundnut**

Aflatoxin contamination can occur in pods and seed in the soil near harvest, during harvest, and post-harvest in storage. Pre-harvest infection by *Aspergillus spp.* and the environmental factors that lead to colonization, infection of the seeds, plants, and aflatoxin accumulation have been reviewed in detail (Payne, 1998). Initial inoculum in groundnuts most likely originates in the soil. Inoculation and colonization are dependent on soil pathogen population, temperature and moisture content of the soil. The two most important conditions that favor pre-harvest invasion and aflatoxin contamination of groundnut seeds are the simultaneous occurrence of excessively high soil temperature and late season drought stress. Neither heat nor drought alone can induce high levels of pre-harvest contamination. Insect and mechanical damage to the pod increase the opportunity for invasion by *Aspergillus* and the consequent accumulation of aflatoxins. Similarly, post-harvest aflatoxin contamination is most attributable to improper storage of the pods and seed. Conditions important for aflatoxin formation during storage are high humidity and high temperature.

**2.6.2.7 Management of Aflatoxin Contamination of Groundnut**

Management of aflatoxin contamination in groundnuts is complex. Both preventative and curative procedures may be necessary. Aflatoxin occurrence and severity in field crops is largely a matter of uncontrollable natural events. The complete elimination of aflatoxin from human food, while desirable, is almost impossible to achieve, as aflatoxin is distributed in a wide range of agricultural products where it is an unavoidable natural contaminant. However, certain practices such as the “farm to fork” policy of the European Union (EU) can be put in place to reduce aflatoxin levels. Pre-harvest and post-harvest management strategies employed to reduce aflatoxin in food result in lower productivity, but better quality. This is confirmed by Hameeda *et al.,* (2006) who stated that commodities contaminated with aflatoxin have a lower market value and cannot be exported. The authors also reported that animals feed with groundnut meal contaminated with aflatoxin have lower productivity and slower growth. Dohlman, (2003) proposed a strategy to reduce both health risks and the economic costs associated with mycotoxins. Food producers must be more aware of mycotoxin effect, and therefore, handling practices that would minimize mycotoxin contamination. Moreover, another solution would be to encourage the adoption of process-based guidelines (good agricultural practices (GAPs) or good manufacturing practices (GMPs).

Hazard analysis and critical control points (HACCP) are employed to reduce unacceptable aflatoxin levels from insect damage to the developing crop in the field or when the mature crop is exposed to moisture prior to harvest or during handling, transportation and storage. However, small-scale industries, subsistence production and food insecurity make the economics and enforcement of aflatoxin regulations impractical. Current management of aflatoxin contamination starts in the farmer’s field and continues through harvest, drying, storage and processing (Vincelli and Parker, 2002).

**2.6.2.7.1 Cultural Practices Used in the Management of Aflatoxins**

**Habitat Management:** Certain cultural practices, such as summer ploughing, selection of appropriate planting date to take advantage of periods of rainfall to avoid end-season drought effects, seed dressing with systemic fungicides or biocontrol agents, maintaining good plant density in the fields, removal of premature dead plants, managing insect pests and diseases, timely harvesting, exclusion of damaged and immature pods, quick pod drying, controlling storage insect pests and storing the pod and seed with less than 10% moisture content can prevent fungal infection and proliferation. The use of safe and efficient mechanical threshers, and seed storage bins are other cultural for reducing aflatoxin in groundnuts. Although most of the options are cost-effective and practical under subsistence farming conditions, they remain largely un-adopted by farmers. Researcher efforts resulted in devising appropriate combination of practices (timely harvesting, windrow drying and threshing) that are more compatible with socio-economic profiles and farming practices which contribute to good adoption of the farmers of a particular region. Late season irrigation to alleviate drought stress of plants is effective in reducing aflatoxin contamination in the field. (Waliyar *et al*., 2003; Desai *et al*., 2005; and Waliyer *et al*., 2007).

**Aflatoxin Management Through Soil Amendments:** Soil amendments with gypsum (as source of calcium), farmyard manure and cereal crop residues applied either singly or in various combinations at different cropping stages would contribute to reduction in the pre-harvest A. flavus infection and aflatoxin contamination in groundnut (Waliyer, *et al*., 2006). Application of gypsum and farmyard manure at the time of sowing was found to be most effective in reducing seed infection and aflatoxin contamination (mean reduction of 80% compared to controls). This management options is feasible because lime and farmyard manure are cheap and easily available in most developing countries.

**Post-harvest Aflatoxin Management Practices:** Several studies in West Africa have shown that aflatoxin contamination increased with the delay in pod removal after lifting the plants and during storage. In addition, traditional heap drying enhanced rapid fungal proliferation and toxin production. Small and immature seeds (beans) contain the highest toxin and segregating such seeds would reduce the contamination in the final product. Rapid post-harvest drying can prevent the further invasion of groundnut seeds by *Aspergillus spp*. Early harvesting and the rapid drying of groundnut kernels to moisture levels below 15% effectively stop aflatoxin accumulation. Storage conditions at low temperatures and reduced humidity are important components for reduced *A. flavus* growth and aflatoxin production. Storage facilities must be regularly monitored to ensure early detection and management of insect infestations. Old grain residue must not be mixed with new grain and storage areas must be sanitized before new grain is stored.

According to Smith, (1989) good warehousing practices largely prevent further increase of post-harvest aflatoxin contamination. The most important factor in preventing aflatoxin contamination in groundnut storage is moisture control. A good warehouse should have a double roof and sidewalls and be adequately cooled and properly ventilated to prevent wetting of the groundnuts. Pods should be dried to less than 9% moisture prior to storage. Controlled atmosphere (CA) in storage with high CO2 and low O2 appears to inhibit *Aspergillus* growth.. In conclusion, the recommended cultural practices include optimizing irrigation; avoiding mechanical and insect pest damage during cultivation, harvesting, storage and processing; optimizing harvest time; rapid post-harvest drying; and storage at low temperature, and managing humidity and seed moisture.

**2.6.2.7.2 Physical Control of Aflatoxin Production**

Sanitation practices, such as mechanical or hand sorting, can reduce aflatoxin levels by removing low-density mould-infected kernels. Ogunsanwo *et al*., (2004) found positive correlations between loss of aflatoxin and dry roasting conditions. Roasting at 140oC for 40 minutes reduced aflatoxin B1and G1by 58.8 and 64.5%, respectively, while roasting at 150oC for 30 minutes resulted in 70 and79.8% reduction in aflatoxin B1and G1, respectively. Cooking and steaming for 1 hour under pressure reduces aflatoxin by up to 60% (Fandohan *et al*., 2005). This is because high temperature breaks the ring of chemical structure of aflatoxin.

**2.6.2.7.3 Biological Control of Aflatoxin Production**

Dorner *et al*., (2000) reported that field application of the non-toxigenic strains not only reduced aflatoxin contamination in the field but also reduced aflatoxin contamination that occurred in storage. Biocontrol agents were used as seed dressing and soil application to determine their effects on population dynamics of A. flavus in the geocarposphere and subsequently on pre-harvest kernel infection of groundnut. Application of 11 kg/ha of an aftoxigenic *A. flavus* strain with a food source such as wheat (Triticum aestivum L.) or sorghum (Sorghum bicolor L.) once a year resulted in the displacement of over 80% of aflatoxin-producing *Aspergillus spp*. in Arizona and Texas (Philips *et al*., 2005).Similarly, significant reduction (20-90%) in A. flavus seed infection was recorded with several treatments over the control (Anjaial *et al*., 2006). These practices have been applied with success in developed countries but have been of limited use in developing countries. In spite of precautions, damage and contamination can still occur, even on undamaged pod and seed, which led to application of various curative methods to eliminate or lower the contamination. Aflatoxin production is also inhibited by lactic acid bacteria (*Bacillus subtilis*) and many other molds. This inhibition may result from many factors, including competition for space and nutrients in general, competition for nutrients required for aflatoxin production but not for growth, and production of anti aflatoxigenic metabolites by co-existing microorganisms. For instance, *Bacillus subtilis*, a bacterium isolated from groundnuts, was found to inhibit the growth of *Aspergillus flavus* in groundnuts.

**2.6.2.7.4 Chemical Control of Aflatoxin Contamination**

Ring opening of the aflatoxin chemical structure occurs at 100°C, followed by decarboxylation, leading to the loss of the methoxy group from the aromatic ring of the aflatoxin molecule. Aflatoxin G1 and G2 are more susceptible to chemical hydrolysis than aflatoxin B1 and B2 because of the presence of two ether linkages in the G group compared to the B group which possess a single ether linkage (Ogunsanwo *et al.,* 2004). Ammonia at 0.5-7% coupled with long exposure time, ambient temperature and pressure has been successfully used to inactivate aflatoxin in contaminated commodities, such as groundnut meal, cotton seed and maize. This process has been approved by safety and regulatory agencies, such as Food and Agriculture Organization (FAO), Food and Drug Administration (FDA), and United States Department of Agriculture (USDA) (Moustafa *et al.,* 2001). Aflatoxin bioavailability was reduced in the gastrointestinal tract of animals by chemisorbents, such as activated carbon, bentonite, clays, and aluminosilicates. At this inclusion rate, NS sequesters aflatoxin resulting in reduced aflatoxin in the gastrointestinal tract and neutralization of its toxic effects. It does, however, not interfere with vitamin and micronutrient uptake (Philips *et al*., 2005).

**CHAPTER THREE**

**MATERIALS AND METHODS**

**3.1 SAMPLE COLLECTION**

The leaves of *Anacardium* *occidentale* were collected from cashew plant tree located at Faculty of Management and Social Science in Godfrey Okoye University, Enugu State, Nigeria on April 2018 and its botanical identity was confirmed by Prof. Amadi J.E. Department of Botany Nnamdi Azikiwe University, Awka. The cashew leaves were collected, rinsed with sterile distilled water and dried at room temperature for 3-4days. The dried leaves were grinded into coarse particles and stored in an enclosed container.

**3.2 COLLECTION OF GROUNDNUT SEEDS**

The groundnut seeds used for this work were bought from Ogbete main market, Enugu state.

**MATERIALS :** Sabouraud dextrose Agar (SDA), Ethanol, *Anacardium occidentale* leaves, Petri dishes, test tubes, Measuring cylinder, Distilled water, Whatman No.1 filter paper, Slides and cover-slip, syringes, Aluminum foil, Cotton wool, Conical flasks, Microscope, Groundnut seeds, wire loop, autoclave, bijou bottle, cork borer.

**3.3 PREPARATION OF THE PLANT EXTRACT**

The powdered *Anacardium occcidentale* leaves were subjected to extraction by maceration using ethanol extraction technique in which 100g of the powdered plant material was soaked in a conical flask containing 500ml ethanol. This mixture was allowed to stand for 72hours at room temperature with periodic stirring. At the end of extraction, the mixture was filtered using a muslin cloth and later with whatman No.1 filter paper. The filtrate was then concentrated in water bath at 78oC and the extract was stored in a refrigerator at 4oC for subsequent.

**TABLE 1: Parameters used for extraction**

|  |  |
| --- | --- |
| Parameters: | *Anacardium occidentale* |
| Dry powdered leave | 100g |
| Ethanol | 500ml |
| Temperature of water bath | 75oC |
| Time taken for extraction | 72 hours |
| Weight of extract | 90.86grams |
| Alcoholic extract color | Deep green |

**3.4 QUALITATIVE PHYTOCHEMICAL SCREENING**

Phytochemical examination of the extract was carried out for alkaloids, saponins, flavonoids and tannins, cardiac glycoside and oxalates using the standard methods as described by Akinpelu *et al*., 2011, Bala and Edelt, 2011, and Prashant *et al*., 2011.

**Test for Alkaloids**

Zero point five (0.5g) of the plant extract was dissolved in 5ml of 1% HCL on steam bath. A millimeter of the filtrate was treated with drops of Dragendorff reagents. Turbidity or precipitation was taken as indicative of the presence of alkaloids.

**Test for Saponin ‘using foam test’’**

Zero point five (0.5g) of the extract was shaken with 2ml of water. If foam produce persists for ten minutes, it indicates the presence of saponins.

**Test for Tannins**

About 1g of the extract was dissolved in 20ml of distilled water and filtered. Two to three (2-3) drops of 10% FeCl3was added to 2ml of the filtrate. The production of a blackish-blue or blackish-green colouration indicated presence of tannins.

**Test for Flavonoids**

Zero point two (0.2g) of the extract was dissolved in 2ml of methanol and heated. A chip of magnesium metal was added to the mixture followed by the addition of a few drops of concentrated HCL. The occurrence of a red or orange colouration was indicative of the presence of flavonoids.

**3.5 ISOLATION OF THE MICROORGANISMS**

Groundnut seeds collected for the study were crushed into semi-powdery form with aid of sterile laboratory mortar and pestle. Five (5) test tubes were arranged each containing 9ml of distilled water. One gram (1g) of the sample was measured and dissolved in a test tube-1 containing 9ml of distilled water and shaken properly. One (1ml) of the solution was removed from the test tube and transferred into test tube -2, it was shaken properly and same processes were repeated for test tube -3 up to test tube-5. From each of the test tubes 0.1ml was drawn and transferred into Petri dish each containing prepared Sabouraud Dextrose agar (SDA). The inoculated plates were covered and incubated at room temperature (28oC-30oC) for 2 to 7days. The serial dilution was done to disperse the fungal inoculum load in the solution to ease isolation.

**Subculture**

Subculturing was carried out from the inoculated plates. A small portion of different colonies was teased out and placed on the centre of the Sabouraud dextrose agar plates and allowed to grow at room temperate 28oC.

**3.6 IDENTIFICATION OF FUNGAL ISOLATES (using slide culture technique)s**

**Procedure**

Sterilized petri dish and the U- shaped glass rod. Aseptically, with a pair of sterile forceps, the sterile U-shaped glass rod was placed in the petri dish. With sterile forceps, a sterile slide was placed on the U-shaped glass rod. Gently flamed a scalpel to sterilize and cut a 5mm square block of the medium from the plate of prepared Sabouraud dextrose agar. A cube of the agar was picked by inserting the scalpel and carefully transferred the cube agar aseptically to the centre of the slide. With a sterile wire loop, the four sides of the agar were inoculated with the colony of the fungus to be examined. Aseptically a sterile cover-slip was placed on the upper surface of the inoculated agar. One (1ml) of sterile distilled water was added in the petri dish to moisten it. The petri dish was covered and incubated at room temperate for 4-5day maximum of 7days.

**Application of stain** (Using lactophenol cotton blue)

A drop of lactophenol cotton blue stain was placed on a clean microscopic slide. The cover-slip from the slide culture was removed and the cube agar was discarded. Added a drop of 95% ethanol to the growth on the cover-slip. As soon as most of the alcohol has evaporated, the cover-slip mold side was placed down on the drop of lactophenol cotton blue stain on the slide. The slide was examined under the microscope using lower power(x10 objective), then confirmed with higher objective (x40 objective).

**3.7 ANTIFUNGAL ACTIVITY ASSAY**

Zero point five (0.5g) of the plant extract was dissolved in 2ml of Dimethyl sulfoxide (DMSO). Zero point one (0.1) ml of the broth organism was inoculated unto poured plates using spread method. In each plate, well of 5-6mm diameter were made using a sterile cork borer. Each of the wells was filled with 100ul of concentration of the plant extract and allowed to diffuse. The plates were incubated at 28oC for 48hours. The diameter of zones of inhibition was measured and recorded after incubation.

A control to test the activity of the solvent DMSO used to dissolve the extract was carried out to ensure that the activity is not due to the action of the solvent on the test organisms.

**3.8 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)**

The minimum concentrations of the plant extract were determined using the the tube dilution method test with visual assessment of growth of microorganisms (Delarras, 1998). Sterile seven test tubes containing 1ml of DMSO were arranged on test tube rack. To achieve the range of the concentrations of plant extract 0.512g was added to 1ml of DMSO in the tube T1 and allowed to dissolve. The mixture (extract + DMSO) was transferred into test tube 2. For the remaining range, 1ml of the mixture taken from tube T2 was transferred into tube T3.This procedure was repeated until tube T6. The microbial standard to match McFarland (0.5%) standard solution was prepared. One (1) ml of the respective fungal broth culture was added to the experiment (T1 to T6) and incubated for 18hours at 28oc. After incubation period, the fungal growth which leads to development of turbidity was examined in each tube. The MIC was interpreted as the lowest concentration of the extract which showed no growth in the isolates.

**3.9 DETERMINATION OF MINIMUM FUNGICIDAL CONCENTRATION (MFC)**

The minimum fungicidal concentration were determined by first selecting tubes that showed no growth during MIC determination; zero point one (0.1)ml of the broth from each tube was sub cultured onto Sabouraud dextrose agar plates, incubated for 48hours at room temperature. The minimum fungicidal concentration was considered as the lowest concentration that could not produce a single fungal colony (Kambal and Hassan, 2010).

**CHAPTER FOUR**

**RESULTS**

A variety of rich secondary metabolites such as tannins, alkaloids, saponins, phenols, steroids, glycosides and volatile oil are present in plant general. The ethanol extract of *Anacardiun occidentale* L. leaves result the presence of various phytochemical compounds such as alkaloids, tannins, saponins and flavonoids.

**TABLE 2: Phytochemical screening of extract of *Anacardium occidentale***

|  |  |
| --- | --- |
| **Chemical constituents** | **Status** |
| Alkaloids | + |
| Tannins | + |
| Saponins | + |
| Flavonoids | + |

KEY: + = present, - = absent

**Table 3: Fungal isolates from groundnut seeds**

|  |  |  |
| --- | --- | --- |
| ISOLATES | IDENTIFICATION | CHARACTERISTICS |
| Isolate A | *Aspergillus niger* | Colonies consist of compact white yellow basal felt covered by a dense layer of dark brown to black conidial heads; conidiophores are smooth-walled, turning dark toward the vesicle. |
| Isolate | *Aspergillus fumigatus* | Blue-green, powdery and pale yellow on reverse. Conidial heads are typically columnar and uniseriate with the phialides limited to the upper two thirds of the vesicle and curving to be roughly parallel to each other. |
| Isolate C | *Rhizopus* sp | They are characterized by the presences of stolons and pigmentation rhizoid, the formation of sporangiosphore singly or in group from nodes directly above the rhizoid and pophysate, columellate, multi-spore generally globose sporangia. |

Fungal Isolates

Zones of inhibition

**Figure 1:** Zones of Inhibition of cashew leaves extract on three fungal isolates

**Minimum Inhibitory concentrations**

The table 4 presents the minimum inhibitory concentration of the extract of *Anacardiun occidentale* L leaves on the isolated fungi species. The results showed that the MIC varied according to the isolates. Among the tested isolates, *Rhizopus* and *Aspergillus fumigatus* were more sensitive to the extract with MIC (64mg/ml) while the least sensitive to the extract was *Aspergillus niger* with MIC (128mg/ml). MIC was interpreted as the lowest concentration of the extract (*Anacardium occidentale* L.) which showed clear fluid without development of turbidity (no growth).

**Minimum Fungicidal Concentration**

It was observed that the extract had no fungicidal effect on the isolate *Aspergillus niger*. On the contrary, the extract exerted fungicidal effects on *Aspergillus fumigatus* and *Rhizopus* sp at 128mg/ml respectively.

**Table 4**: **Minimum Inhibitory Concentration (MIC) and minimum fungicidal concentration (MFC)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **The effects of different concentrations of the extract on the isolates** | | | | | | | |
| **Isolates** | 256mg/ml | 128mg/ml | 64mg/ml | 32mg/ml | 16mg/ml | 8mg/ml | MFC mg/ml |
| *Aspergilus niger* | - | **-** | **+** | **+** | **+** | **+** | Nil |
| *Aspergillus fumigatus* | **-** | **-** | **-** | **+** | **+** | **+** | 128 |
| Rhizopus *sp* | **-** | **-** | **-** | **+** | **+** | **+** | 128 |

**KEY: ­** + = growth, - = no growth

**CHAPTER FIVE**

**DISCUSSION**

The phytochemical analysis of the ethanol plant extract was shown in table 2. From the phytochemical analysis, tannins, Saponins, Flavonoids and alkaloids were present in *Anacardium occidentale* (leaves) in the solvent ethanol. Some secondary metabolites such as tannins, alkaloids, flavonoids, phenols and volatile oil are present in *Anacardium occidentale.* This agrees with the work of Kamath and Shabaraya (2016). The phytochemicals are the prime and bioactive compounds of plants that are responsible for the extended biological properties (Kannan *et al.,* 2009).

Flavonoids have been recognized as having a protective effect in plants against microbial invasion by plant pathogens. Flavonoids have been shown to possess important biological activities including antifungal and anti-bacterial activities (Inamdar *et al.,* 2014).

The fungal species were identified in this study are *Aspergillus niger, aspergillus fumigatus* and *Rhizopus* sp. The isolated fungal species were identified using Slide Culture Technique method in which the characteristic features of the particular fungal species were checked on a slide and Atlas for Fungi. The result from the study agrees with work carried out by Ibrahim Abuga, (2014) who found *Mucor, Aspergillus, Rhizopus, Fusarium, Penicillum* and *Curvularia*. It was observed that the ethanol extract of *Anacardium occidentale* gave zones of inhibition 19mm, 22mm 23mm respectively for isolates A, B and C at concentration of 250mg/ml (greater or equal to 15mm shows susceptibility of the isolates to the extract).

The result of this work indicates that the extract of *Anacardium occidentale* have antifungal properties when tested on *Aspergillus niger*, *Aspergillus fumigatus* and *Rhizopus* as shown on figure 1. The highest zone of inhibition was obtained with *Rhizopus* sp and *Aspergillus fumigatus* while *Aspergillus niger* showed lower zones of inhibition.

The difference in the zone of inhibition may be directly related to the susceptibility of each test organism to the cashew leaves extract. The minimum inhibitory concentration was variable depending on the fungal isolate as shown in table 4. However, the highest MIC was (64mg/ml) on *Rhizopus* spand *Aspergillus fumigatus* and (128gm/ml) on *Aspergillus niger.* The extract showed no MFC on isolate A *Aspergillus niger*. On the contrary, the extract exerted fungicidal effects on *Aspergillus fumigatus* and *Rhizopus* sp at 128mg/ml respectively.

The factors responsible for this high susceptibility of *Aspergillus* to the extract may be attributed to the solvent and secondary plant metabolites present in the plant.

The ethanolic extract showed antifungal activity in all the pathogenic isolates (*Aspergillus niger, Aspergillus fumigatus and Rhizopus* sp*.).* The antifungal activities observed may be explained by the presence of large chemical such as tannins, flavonoids, alkaloids and saponins (Omojasola and Awe, 2004). The antifungal activity is due to the bioactive component that may be active against fungal isolates individually or in combination. Fungistatic effect by ethanol extract of *Anacardium occidentale* L. against *A.niger, A. fumigatus* and *Rhizopus* are due to phenol, flavonoids, tannins and alkaloids.

The strong antifungal properties of plant extracts are demonstrated due to the presence of secondary metabolites. Therefore it can be said that the extract of *Anacardium occidentale* (cashew) leaves possess antifungal properties.

**CONCLUSION**

It can be concluded from this study that the ethanol extract of *Anacardium occidentale* L. (cashew) leavescontains bioactive components which individually or in combination confer medicinal properties on the plant and could be responsible for its antifungal properties. Therefore it can be said that the extract of *Anacardium occidentale* (cashew) leaves possess antifungal properties.

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