EFFECTS OF *ADANSONIA DIGITATA*(L)SEED OIL ON MICROBIAL ISOLATES AND INSECT PEST(*DERMESTES MACULATUS*, DEGEER) OF SMOKE-DRIED*CLARIAS GARIEPINUS* (BURCHELL, 1822)

BY

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DECLARATION

I declare that the work in this dissertation entitled 'Effects of *Adansonia digitata* (L)Seed Oil On Microbial Isolates And Insect Pest (*Dermestes maculatus*, DeGeer) Of Smoke-dried *Clarias gariepinus* (Burchell, 1822)'has been carried out by me in the Department of Biological Sciences. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled ‘Effects of *Adansonia digitata* (L)seed oil on microbial isolates and insect pest (*Dermestes maculatus*, Degeer) of smoke-dried*Clarias gariepinus* (Burchell, 1822)’ by Maymunah Ummjamil MAZAI meets the regulations governing the award of the degree of M.sc Biology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

The study assessed the effects of *Adansonia digitata* (L) seed oil on *Dermestes maculates* (DeGeer) and isolated fungi and bacteria of smoke-dried *Clariasgariepinus* (Burchell, 1822).Samples of smoke-dried catfish were bought randomly from six retailers in Samaru Market, Zaria. Baobab seeds were collected from trees within Zaria and subjected to soxhlet extraction using petroleum ether as the solvent. The extracted oil was subjected to phyto-chemical, physico-chemical and fatty acid examination using methods of International Union of Pure and Applied Chemists and Gas Chromatography Mass Spectrometry respectively . Fungal isolates were identified using morphological and microscopical characteristics. Bacterial isolates were identified by Gram stain reaction as well as microgen identification kits. The oil was diluted to different concentrations of 5%, 10%, 15%, 20% and 25% by mixing with requisite amounts of Dimethyl Sulphoxide solvent. Microbial sensitivity to the different concentrations of oil was done by pouring appropriate concentration of oil into marked wells made with 8mm cork borer on potato Dextrose Agar for fungi and Mueller-Hinton agar for bacteria. The zones of inhibition were measured. Larvicidal effects were determined by soaking 5g of fish in varying volumes of oil and 15 larva introduced with three replications. Mortality readings were taken at 24, 48, 72 and 96 hours for each treatment. *A. digitata* seed oil had the following phytochemicals: Carbohydratesn (70.08%), Phenols (0.55%), Saponins (0.10%), Tannins (15.6%), Flavonoids (3.33%) and Alkaloids (19.10%) with carbohydrates having the highest proportion at a percentage of 70.08 followed by alkaloids at 19.10% and saponins having the lowest proportion at a percentage of 0.10. The relative density of the oil was 0.9029±0.0003, saponification level was 61.71±0.043mg/g, peroxide value 3.17±0.06mg/g. Eight fatty acids were identified including pentadecanoic acid, N-hexadecanoic acid, pentafluoropropionic acid, stearic acid, and oleic acid. The fungal

isolates were found to be *Aspergillus niger*, *Monilia sitophila*, *Stemphylia* sp, *Microcarpum narum*, *Trychophyton tonsurans*. Seven *Bacillus* species and three *Staphylococcus* species were identified. There was no significant ($P \leq 0.05$) fungal sensitivity to the oil as shown by the lack of zones of inhibition. There was significant bactericidal activity of the oil.*Bacillus circulans* was resistant to the extract. *Paenibacillus macerans* had the highest susceptibility to the test oil with *B. subtilis* having the lowest. The least concentration of 0.004ml/g did not result in any larval mortality for the first 48hours but by the 96^{th} hour, only 27% mortality was recorded. The highest concentration of 0.256ml/g gave a near perfect larval mortality by 24 hours and a total mortality by the 72^{nd} hour. The study came to the conclusions that *Adansonia digitata* seed oil contained significant amounts of phyto-chemicals and also had significant effects on isolated bacteria as well as *Dermestes maculatus* but had no effects on isolated fungi.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Fish is one of the cheapest animal protein sources, being used increasingly to correct protein deficiency (Akinwumi, 2011).It is the most important animal protein food available in the tropics, and it represents about 14% of all animal protein source to consumers on a global basis (Abolagba and Igbinevbo, 2010).Fish is a food of excellent nutritional value, providing high quality protein and a wide variety of vitamins and minerals, including vitamins A and D, phosphorus, magnesium, selenium, and iodine in marine fish. Its protein - like that of meat - is easily digestible and favourably complements dietary protein provided by cereals and legumes that are typically consumed in many developing countries. Fish and fish products constitute more than 60% of the total protein intake in adults especially in the rural areas in Nigeria. They are widely accepted by Nigerians across socio-economic, age, religious and educational barriers.Fish plays an important role in the life and wellbeing of most of the vast population of Nigeria. Dried, smoked or roasted, it is sold in the remotest villages as well as the urban cities. Its harvesting, handling, processing and distribution provides livelihood for millions of people as well as foreign exchange earnings to many countries (Al-Jufaili and Opara, 2006).

The catfish, *Clarias gariepinus* is a very important freshwater fish in Nigeria (Idodo-Umeh, 2003) belonging to the family Clariidae.*Clarias gariepinus* is the most popular, widely cultivated and mostly smoked fish in Nigeria (Aderolu and Akpabio, 2009). They have the ability to live out of water for several hours or in damp muddy places for several months and so,usually referred to as mudfish. It enjoys wide acceptability in most part of

the country because of its unique taste, flavor and texture (Daramola *et al.*, 2014). The importance of catfish cannot be over emphasized because it provides food for the populace, allows for improved protein nutrition as it has a high biological value in terms of high protein retention in the body, higher protein assimilation as compared to other protein sources, low cholesterol content and one of the safest sources of animal protein (Adebayo and Daramola, 2013).

It is commonly found in swamps, streams, rivers and lakes and is an omnivorous bottom feeder, feeding on detritus, plankton, insect larvae, insects, worms, gastropods, crustacean and small fishes such as Tilapia and *Alestes* spp. (Idodo-Umeh, 2003).

Fish is highly susceptible to autolysis and microbial spoilage which could result in the loss of thousands of metric tonnes of fish per year (Olonitola *et al.,* 2006). Spoilage is usually accompanied by change in physical characteristics. Change in colour, odour, texture, colour of eyes, color of gills and softness of the muscle are some of the characteristics observed in spoiled fish. The major fish preservation methods are sundrying, salting and smoking (Eyo, 2001). Smoking is a traditional preservation method that is used to prepare fish for long storage. In tropical countries, fish are generally heavily smoked at high temperatures. These high temperatures are usually high enough to eliminate the non-sporulating spoilage bacteria. However the spores of *Bacillus* sp survive even with longer periods of smoking (Sinha, 2011). Prevention of microbial spoilage of fish may be achieved by different methods such as smoking, drying, freezing, salting and use of modified atmospheric storage (Gupta and Gupta, 2006). Smoke dried fish may spoil due to growth of halophilic bacteria or moulds (Sinha, 2011).

Insect infestation of cured fish by blowflies and beetles is an important cause of post harvest losses (Wahedi and Kefas, 2013) and it is also the cause of most prominent losses in quality and quantity of stored, dried fish in Nigeria (Ayuba and Omeji, 2006). It is estimated that between 25% - 75% of the nutritional value of fish is lost during attack by

insect pests (Johnson and Esser, 2005).Bolaji (2005) reported that despite the rudimentary nature of traditional processing methods, the lack of control over the drying rate, sometimes results to under- or over-drying and expose fish to wind, dust, dirt, insect infestation and contaminants such as flies.

Adansonia digitata L belongs to the family Malvaceae and is used for so many purposes ranging from food, medicine to fuel (Orwa *et al.,* 2009).The fruit pulp, seeds, leaves, flowers, roots, and bark of baobab are edible and they have been studied by scientists for their useful properties. The seeds contain appreciable quantities of phosphorus, magnesium, zinc, sodium, iron, manganese, calcium and they have high levels of lysine and thiamine. Baobab is said to have biological properties including antimicrobial, anti-malarial, anti-diarrhoeal, anti-anaemia, anti-asthma, anti-viral, anti-oxidant and anti-inflammatory activities amongst others (Rahul *et al*., 2015).

1.2 Statement of Research Problem

Some of the traditional fish processing methods are associated with contamination which may be injurious to consumers (George *et al*, 2014). Bacteria, fungi and insect pests are major agents of spoilage that causes huge loss of post harvest fishes (Akinwumi, 2011). For long, fungi were regarded as causing only an aesthetic spoilage of food. In 1966 the famous "Turkey X" diseases killed10,000 turkey poultry in Great Britain; Western world became aware that common spoilage molds could produce significant toxigenic fungi and potentially toxic compounds have been discovered. Aflatoxins, a group of toxic metabolites produced by certain *Aspergillus* species have been found to be carcinogenic, tetratogenic and mutagenic to several species of experimental animals (Adebayo-Tayo *et al.*, 2008).Smoking provides very little control against insect pests (Okorie, 2003).The traditional methods of processing are often inefficient and unhygienic involving substantial post harvest losses in terms of mould, fragmentation, infestation by flies and

beetles, loss of quality by charring etc. (Okereke *et* al, 2014). The use of insecticides, fungicides and bactericides pose health hazards to consumers (Udomkusonsri *et* al., 2007).

1.3 Justification

Fish constitute about 40% of animal protein intake of Nigerians (Eyo, 2001) and it is a low acid food that supports the growth of pests and pathogens (Wahedi and Kefas, 2013).In Nigeria, fish drying/smoking is the most adopted technique for fish preservation resulting in 95% of the total artisanal landing being smoked or sundried (Ugwu *et al.*, 2005; George *et al*, 2014)). This could be as a result of the fact that most of the coastal communities have no access to electricity to preserve or process their products, as such, smoke-drying method still remain predominant in Nigeria. Because of the many hazards of chemical control, focus is changing to the use of natural products such as plant oils in the control of pests and diseases of smoke-dried fish.Plant oils have been proven to have potent anti-microbial activity (Upadhyay *et al.*,2010).

1.4 Aim of the Study

The aim of the study is to assess the effect of *Adansonia digitata* seed oil on *Dermestes maculates,* a common insect pest `of smoke-dried *C. gariepinus* and isolated fungi and bacteria of smoke-dried *C. gariepinus*.

1.5 Objectives

1. To determine the phytochemicals present in the seed oil of *A. digitata*.
2. To determine the fungicidal effect of *A. digitata* seed oil on isolated fungi of smoke-dried *C. gariepinus.*

3. To determine the bactericidal effect of *A. digitata* seed oil on isolated bacteria of smoke-dried *C. gariepinus.*
4. To determine the repellant and larvicidal effects of *A. digitata* seed oil on *Dermestes maculatus*, a common pest of smoke-dried *C. gariepinus*.

1.6 Hypotheses

1. *Adansonia digitata* seed oil does not contain any significant phytochemical.
2. *Adansonia digitata* seed oil has no significant fungicidal effect on isolated fungi of smoke-dried *C. gariepinus.*
3. *Adansonia digitata* seed oil has no significant bactericidal effect on isolated bacteria of smoke-dried *C. gariepinus.*
4. *Adansonia digitata* seed oil has no significant repellant and larvicidal effect on *Dermestes maculates*, a common pest of smoke-dried *C. gariepinus*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The African Catfish, *Clarias gariepinus*

African catfish, *Clarias gariepinus* are a diverse group of ray-finned fish, named for their prominent barbels, which resembles a cat's whiskers. The African catfish, genus *Clarias* has very high commercial value in Nigeria owing to its flavour and taste. *Clarias gariepinus*, which is generally considered to be one of the most important tropical catfish species for aquaculture, has an almost Pan-African distribution, ranging from the Nile to West Africa and from Algeria to Southern Africa. They also occur in Asia Minor (Israel, Syria and South of Turkey). (FAO, 2015)

Clarias spp. inhabit calm waters from lakes, streams, rivers, swamps to floodplains, many of which are subject to seasonal drying. The most common habitats frequented are floodplain swamps and pools in which the catfish can survive during the dry seasons due to the presence of their accessory air breathing organs. They are omnivorous feeders feeding on all types of food items (FAO, 2015). The taxonomic hierarchy of the African catfish is as follows:

Kingdom: Animalia

Subkingdom: Bilateria

Infrakingdom: Deuterostomia

Phylum: Chordata

Subphylum: Vertebrata

Infraphylum: Gnathostomata

Superclass: Osteichthyes

Class: Actinopterygii

Subclass: Neopterygii

Infraclass: Teleostei

Superorder: Ostariophysi

Order: Siluriformes

Family: Clariidae

Genus: *Clarias*

Species: *Clarias gariepinus* (Burchell, 1822).

2.2 Fish Smoking in Nigeria

The phenomenon of fish preservation is enunciated by the simple principle of making fish unfavourable for the growth of spoilage organisms. In Nigeria, fish drying is the most adopted technique since it is within the socioeconomic levels of artisanal fishermen contributing over 90% of domestic fish supply. Smoked fish constitute a major source of animal protein for a vast majority of the population in Nigeria, particularly the rural population. These products can be kept for 2 to 4 weeks in market stalls with poor storage facilities. Smoke drying method is carried out in traditional smoking kilns of clay, cement blocks, drums or iron sheets. In Nigeria, over 80% of fish caught from artisanal waters are processed through sun drying or smoked partly due to unavailability of ice for preservation of fresh fish products (Ashamo and Ajayi, 2003). Dried fish products have their oil content and moisture content considerably reduced.Okereke *et al.,* (2014) reported that the protein content of smoked fish was considerably higher and attributed it to the effect of drying which removed moisture thereby concentrating the protein content. Effiong and Fakunle (2012) came to the conclusion that the protein content of smoked dry fish was much higher than that from cow, pork and mutton.

2.2.1 Smoked dried fish pest infestation

Dried fish products may deteriorate very rapidly from infestation by pestiferous insects, mainly beetle species in the genera *Dermestes* and *Necrobia* (Tunaz and Uygun, 2004,). Improperly dried fish or adequately dried fish that has reabsorbed moisture could harbour large population of blowfly belonging to the genus *Chrysomyia* (Ajayi *et al.*, 2006). Eke *et al.* (2008) reported the presence of both resident pests such as *Dermestes maculatus, Necrobia rufipes, Lardoglyphus konoi, Theridion saxatile* and *Triboliumcastaneum*, and visiting pests such as *Periplaneta americana*, *Lucilia spp*, *Calliphora spp* and *Musca domestica* in the dried fish sampled and further asserted that most of the dried fish sampled had more than one type of pest infesting it.

2.2.2 Smoked dried fish microbial spoilage

Fish drying occurs slowly over a fire to remove its moisture content which aids bacterial activities and spoilages. Fish may harbour myriad microorganisms; the most prevalent being bacteria and fungi. As a result of pollution of water bodies, pathogenic organisms may be introduced to these aquatic ecosystems from which fish are harvested. Sources of pollution vary and could include faecal contamination too. As a result, water bodies may contain high numbers of coliform and these organisms would also be present in seafood harvested from such water systems. The nutrients present in fish provide a good medium for microbial growth.

After processing, the products are placed in locally made baskets or jute sacs ready for transportation to various markets in the country. Often, the products are not properly packaged and stored. Consequently, re-absorption of moisture and post processing contamination of fish occur.

2.2.2.1 Fungal spoilage of smoked dried fish

Fungi have a worldwide distribution, and grow in a wide range of habitats, including extreme environments such as deserts or areas with high salt concentrations (Vaupotic *et al.*, 2008) or ionizing radiation (Dadachova *et al.*, 2007). Some can survive the intense UV and cosmic radiation encountered during space travel (Sancho *et al.*, 2007). Among the commonest different fungi found to be associated with smoked fish samples in Nigeria are *Aspergillus flavus, A. terens, A. fumigatus, A. niger, Mucor* sp., *Cladosporium* sp., *Penicillium* sp., *Candida tropicalis* and *Fusarium moniliformis* (Adebayo-Tayo *et al.*, 2008 and Fafioye *et al.*, 2002). Moulds may be present without producing any toxin (Bennett and Klich, 2003), but the presence of toxigenic fungi increases the risk for mycotoxin production.

2.2.2.2 Bacteriological spoilage of smoked dried fish

Spoiling of smoke dried fish as a result of presence of bacteria has been reported. Bacteria commonly associated with smoke dried fishes include those of the following genus: *Staphylococcus, Streptococcus, Bacillus, Salmonella* (Ibrahim *et al.*, 2014;Felix and Adegbehingbe, 2015). Sikoki and Aminigo (2002) reported that changes in the moisture content of smoked fish were most significant during the first week of storage and that bacterial population increased during this period.

2.3 The Baobab Plant, *Adansonia digitata*

Baobab (*Adansonia digitata* L.) is a large iconic tree indigenous to Africa where it is found in many countries. It is an emblematic, culturally important and physically majestic sub-tropical tree. The baobab has been referred to as “arbre a palabre”, meaning the place in the village where the elders meet to resolve problems. In the past decade, it has attracted the interest of several pharmaceutical companies and researchers due to its various traditional uses (medicinal, nutritional and cosmetic). Recently, the European Commission

authorised the import of baobab fruit pulp as a novel food (Buchmann *et al.,* 2010) and it was approved in 2009 by the Food and Drug Administration as a food ingredient in the United States of America (Addy, 2009). Due to the high demand for commercial baobab products in EU and United States, this tree with its edible fruits needs to be conserved and treasured (Sanchez *et al.,* 2010).

Baobab products (e.g. fruits, seeds, leaves, bark) contribute to the livelihood of many populations in Africa as it is a source of food, fibre and medicine (Codjia *et al.,* 2001; Sidibe and Williams, 2002; Chadare *et al.,* 2009; De Caluwé *et al.,* 2009). More than three hundred traditional uses have collectively been documented in Benin, Mali, Zimbabwe, Cameroon, the Central African Republic, Kenya, Malawi, South Africa and Senegal (Buchmann *et al.,* 2010). Various plant parts (e.g. leaves, bark, and fruit pulp), have traditionally been used for immuno-stimulant, anti-inflammatory, analgesic, insect repellent and pesticidal properties, in the treatment of diarrhoea and dysentery in many African countries, and have been evaluated as a substitute for imported western drugs. Some of the traditional medicinal uses of baobab are presented in Appendix I. Baobab products (e.g. seed oil, fruit pulp) are increasingly being commercialised and exported around the world leading to increased pressure on this resource (Sidibe and Williams, 2002).

2.3.1 Origin of the baobab plant

The origin of the vernacular name "baobab" is uncertain. However, most scientists believe it is derived from the Arabic name 'buhibab' meaning fruit with many seeds. The genus name *Adansonia* is used in honour of Michel Adanson (1727–1806) who brought the seeds to Paris in 1754 and who was the first person to provide a comprehensive description accompanied by a drawing of the plant (Esterhuyse *et al.,* 2001) after a trip to West Africa (Senegal). The species name *digitata* (hand-like) was selected in reference to the shape of

the leaves. Several names are used to describe the baobab depending on its geographical location (Vermaak *et al.,* 2011).

2.3.2 Botanical description, habitat and distribution

The baobab is found in many African countries. Eight baobab species have been identified globally and six species found on the island of Madagascar are endemic to that region (Wickens and Lowe, 2008). It is postulated that the centre of evolutionary origin of the genus *Adansonia* is Madagascar (Drake, 2006). The African species *A. digitata* is indigenous to, and widely distributed throughout the savannas and savanna woodlands of sub-Saharan Africa (Wickens and Lowe, 2008). The only species which is not endemic to the African continent is *A. gibbosa* (A. cunn.) Guymer ex D.A. Baum, native to Australia (Drake, 2006; Wickens and Lowe, 2008). In Southern Africa, *A. digitata* is commonly found in Malawi, Zimbabwe, Mozambique and South Africa especially in the warm parts of the Limpopo Province, while in West Africa, it is found in Mali, Benin, Senegal, the Ivory Coast, Cameroon and Burkina Faso. In East Africa, the plant is found in countries such as Kenya, Uganda and Tanzania (UNCTAD, 2005; Lamien-Meda *et al.,* 2008).

The baobab is a massive deciduous tree easily distinguishable by its huge trunk. It is regarded as the largest succulent plant in the world with a diameter of 10–12 m and a height of 23 m or more (Chadare *et al.,* 2009). Some baobab trees bear leaves only for three months per year. During the leafless period physiological processes such as photosynthesis take place in the trunk and branches, utilising water stored in the trunk (Gebauer *et al.,* 2002). The large egg-shaped fruit capsule is covered with velvety hairs, and contains many seeds. It is estimated that it takes between eight and twenty-three years before the baobab produces seeds and the mature plant (over 60 years) can produce more than 160–250 fruits per year (UNCTAD, 2005). The baobab produces large pendulous

white flowers from October to December (Watson, 2007). Assogbadjo *et al.* (2005) showed correlation between soil minerals and/or soil type and fruit production. For instance, the higher the pH– KCl, the percentage of total nitrogen, organic carbon and organic matter, the higher the number of seeds produced by an individual baobab. It was also noted that the higher the clay and crude silt content of the soil, the better the fruit production.

Baobab is restricted to hot, semi-arid regions, dry woodland and stony places with low rainfall (less than 1500 mm annually) (Gebauer *et al.,* 2002) and grows on a wide range of well-drained soils, from clays to sands, but not on deep unconsolidated sands, where it is unable to obtain sufficient moisture or anchorage (Wickens and Lowe, 2008). In Africa, the plant grows at Latitude of 16° N and 26° S in areas not receiving more than one day of frost per year.

2.3.3 Traditional and modern uses of baobab

Throughout Africa the baobab is regarded with awe by most indigenous people; some even consider it bewitched (Wickens and Lowe, 2008). Almost all parts of the tree are used in traditional medicine in Africa although this varies from one country to another. The various parts of the plant (leaves, bark, seeds) are used as a panacea, that is, to treat almost any disease but specific documented uses include the treatment of malaria, tuberculosis, fever, microbial infections, diarrhoea, anaemia, dysentery, toothache, etc. (Van Wyk and Gericke, 2000; Brendler *et al.,* 2003; Tapsoba and Deschamps, 2006; Wickens and Lowe, 2008; De Caluwé *et al.,* 2009; Nguta *et al.,* 2010).

In India, it is reported that baobab pulp is used externally with buttermilk for the relief of diarrhoea and dysentery, while the young leaves are crushed and used to treat painful

swellings (Sidibe and Williams, 2002). Furthermore, it is reported that the leaves of baobab are used to treat various conditions including internal pains, disease oftheurinary tract, otitis, asa tonicand for insect bitesand Guinea worms (Sidibe and Williams, 2002). The dried leaves are used in many West African countries as an insect repellent (Denloye *et al.,* 2006).

In Ghana, the bark is used as substitute for quinine to relieve fever (Shukla *et al.*, 2001). The oil extracted from the seeds is used against diarrhoea and hiccup (De Caluwé *et al.*, 2009). In Africa, people infected with malaria parasites consume a mash containing dried baobab bark as a febrifuge in order to treat the fever associated with this illness (Wickens and Lowe, 2008). In Mali, it is reported that swollen joints are treated by rubbing a paste made with baobab fruit into the affected area (Wickens and Lowe, 2008).

The baobab is extremely important to humans and animals in the dry areas of Africa because it offers shelter, a source of nutrition, clothing as well as raw material for many useful items. In Benin, the bark has been used for making ropes (De Caluwé *et al.*, 2009). Baobab is used for several purposes and these include: fruit for food; oil from the seeds; rope, cordage and cloth from the bark fibre; tannin for curing leather from the tree bark; glue from the pollen grain of the flowers; pulp for making paper from the harvested tree (although of low quality), seasoning and as an appetizer (Sidibe and Williams, 2002; Nhukarume *et al.,* 2008).

In some countries in West Africa, the leaves, fruit pulp and seeds are the main ingredient in sauces, porridges and beverages (Chadare *et al.*, 2009; De Caluwé *et al.,* 2009; Yusha'u *et al.,* 2010). Recently, baobab has been referred to as a "superfruit" based on its nutritional profile (e.g. vitamin, fatty acid, mineral) (Gruenwald, 2009). The nutritional

value of baobab is onlybriefly discussed since acomprehensive reportonthenutritive aspects is already available (Chadare *et al.*, 2009; De Caluwé *et al.*, 2009). The major interest in baobab products is as a result of its ascorbic acid and dietary fibre content. The level of vitamin C contained in fruit pulp is high and can range from 2.8 to 3g/kg (Vertuani *et al.*, 2002). It was noted that baobab fruit pulp has very high vitamin C content (280–300mg/100g), which is seven to ten times more than oranges (51mg/100g) (Manfredini *et al.*, 2002). One study demonstrated that the consumption of 40g of baobab pulp provides 100% of the recommended daily intake of vitamin C in pregnant women (19–30 years) (Chadare *et al.*, 2009). Sidibe and Williams (2002) recommended that baobab leaves should be stored as whole leaves rather than ground leaf powder in order to preserve the high vitamin content.

The calcium content found in the fruit pulp varies according to authors and the origin of the samples tested. Brady (2011) reported a calcium content of 344.2mg/100g sample which differs from the value of 295.0mg/100g reported by Osman (2004). Other authors have found different levels of calcium in the fruit (211 to 2160mg/100g) (Lockett *et al.*, 2002). Similarly, the level of potassium in the fruit pulp was found to be 1578.5mg/100g sample (Brady, 2011) and 1240.0mg/100g (Osman, 2004). The leaves are particularly rich in calcium (307 to 2640mg/100g dw), they are known to contain good quality proteins and it is estimated that it contains three to five times more calcium than milk. The seeds have a relatively high lipid content of 11.6 to 33.3g/100g dry weight (Brady, 2011).

Several studies have revealed that baobab is rich in fibre (approximately 44%) which can balance the intestinal flora. However, the fibre content varied considerably between studies which could be due to several factors such as the origin of the plant material and the extraction procedure used.

2.3.4 Use of baobab seed oil

The oil obtained from seeds is used to treat skin ailments, thus it may have some cosmetic applications (Sidibe and Williams, 2002). Fixed oils are important ingredients in cosmetic products and amongst these is baobab seed oil. It is suitable for use on the skin as it is non-irritating and non-allergenic (Wren and Stucki, 2003). Other properties of pharmaceutical/cosmetic importance include that it is excellent for restoring and re-moisturising the skin due to its high penetrability and nourishing properties. It can also be used to treat eczema and psoriasis (PhytoTrade Africa).

Baobab oil or fruit pulp contains several vitamins (Nkafamiya *et al.*, 2007) that are essential for skin care. Theseincludevitamins A and F (rejuvenation and cell renewal); vitamin E (anti-oxidant and anti-ageing effects) (Nyam *et al.*, 2009) and vitamin D3, which increases calcium absorption and decreases blood pressure in the elderly (Wasserman, 2004). The oil is said to alleviate pain from burns and regenerates the epithelial tissues in a short time, thereby improving skin tone and elasticity (MurungaProducts).

The oil can be used as a protecting, nourishing, moisturising, soothing and regenerating agent. Studies have also shown that the oil contains anti-oxidants (Nkafamiya *et al.*, 2007), which can protect the skin against premature ageing and prevents the appearance of wrinkles (Africajou). Alone or combined with other ingredients, it is also used to aid in skin healing (small cuts, chapping) or as a mask for hair care (dry, brittle hair, split ends) (Africajou). Linoleic acid (found in baobab seed oil) is the most frequently used fatty acid in cosmetic products as it moisturises the skin, aids in the healing process of dermatoses and sunburns and is used for the treatment of *Acne vulgaris* (Lautenschläger, 2003).

2.3.5 Chemicalcomposition of baobab seed oil

Several classes of compounds have been identified from various parts of baobab (fruit pulp, seed oil, leaves, roots) including terpenoids, flavonoids, sterols, vitamins, amino acids, carbohydrates and lipids (Shukla *et al.,* 2001).Compounds such as campesterol, cholesterol, isofucosterol, β-sitosterol, stigmasterol and tocopherol (α, β, γ, and δ) have been detected in the seed oil.The major hydrocarbons in the seed oil were n-alkanes (57.3%) and squalene (39.5%). Fatty acids present in the seed oil include linoleic and oleic acids in high concentration as well as lesser amounts of palmitic, stearic and arachidic acids (Codjia *et al.,* 2001; Sidibe and Williams, 2002; Osman, 2004; Nkafamiya *et al.,* 2007).

2.3.6 Biological activity of baobab

2.3.6.1 Antibacterial activity

Baobab plant parts have been used for centuries to treat microbial infections both in humans and animals and many scientific studies have been carried out in order to validate its traditional antimicrobial uses. Kubmarawa *et al.* (2007) found that *A. digitata* extract was not active against *Candida albicans* (ATCC 10231), *Staphylococcus aureus* (ATCC 13709), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 9637) and *Bacillus subtilis* (NCTC 8236). This was in contrast to a study carried out by Yagoub (2008) who investigated the antibacterial activity of a solvent extract of *A. digitata* against E. coli isolated from urine and water. The results clearly indicated that the solvent extract inhibited bacterial growth with the inhibition zone ranging from 20 to 30mm depending on the concentration at which the sample was tested (Yagoub, 2008). In a recent study, Masola *et al.* (2009) investigated the antimicrobial activity of baobab plant parts (stem and root barks) against Gram-positive bacteria, Gram-negative bacteria and yeast. The results indicated that the aqueous and ethanolic root and stem bark extracts inhibited the growth

of various micro-organisms with the MIC values ranging from 1.5 to 6mg/ml. The antibacterial activity of the plant could be attributed to the presence of tannins, phlobatannins, terpenoids and saponins in the stem bark (Masola *et al.*, 2009).

2.3.6.2 Anti-Inflammatory activity

The anti-inflammatory activity of the fruit extracted with hot water was tested in vivo using the rat paw formalin-induced oedema test. The extract tested at a dose of 400 and 800mg/kg inhibited formalin-induced oedema. After 24h administration of the aqueous extract, the mean swelling of the foot was 1.81 and 1.75 mm for 400mg/kg and 80 mg/kg, respectively, in comparison to the negative control (6.35 mm). The DMSO fruit pulp extract and aqueous leaf extract showed significant inhibition against cytokine IL-8 (Vimalanathan and Hudson, 2009).

***2.3.6.3** Anti-insecticidal activity and repellency*

Smoke from pellets of *A. digitata* leaves was assayed for toxicity and repellency against adult (0–7 days old) *Anopheles gambiae* (African malaria mosquito), *Musca domestica* (housefly) and *Periplaneta americana* (American cockroach) insects. The results indicated that the smoke caused the death of *A. gambiae* and *M. domestica*, but not *P. americana*. The median lethal dose for*A. gambiae*was 0.47, 0.50and 0.20 g for thepellets containing 0, 0.01 and 0.05 g of d-allethrin, respectively, while it was 0.46, 0.52 and 0.46 g for *M. domestica* (Denloye *et al.*, 2006).

Krishnappa *et al.* (2012) carried out Preliminary phytochemical analysis of *A. digitata*which showed the presence of triterpenoids and saponins. The LC 50 and LC 90 values of hexane, benzene, chloroform, and methanol extracts of *A. digitata* against *Anopheles stephensi* larvae in 24 h were 111.32, 97.13, 88.55, 78.18 and 178.63, 176.19, 168.14, 155.42 mg/l, respectively. The repellent activity of methanol extract was found to

be most effective and at higher concentration of 6 mg/cm 2 benzene, chloroform hexane and methanol extracts provided 100% protection up to 150, 180, 120 and 210 min against *Anopheles stephensi*, respectively. The preliminary study indicated that *A. digitata* showed larvicidal and repellent activities against *Anopheles stephensi* and could be used for controlling mosquitoes.

2.3.6.4 Inhibitory effects of trypanosoma by baobab products

The capacity of *A. digitata* to reduce the mobility of *Trypanosoma brucei*, which causes sleeping sickness, was evaluated using four different extracts (petroleum ether, chloroform, water and methanol) obtained from the leaves and the bark. The time at which mobility stops ranged between 10 and 45 min for the root bark, while with the leaves, the mobility seized between 25 and 45 min, when various extracts were tested at 2 mg/ml (Atawodi, 2005). In another study, the in vitro trypanocidal activity of baobab was microscopically investigated against *T. brucei brucei* and *T. congolense*, which causes nagana in animals. The methanol root extract exerted a noticeable effect on motility after 50 and 55 min for *T. brucei brucei* and *T. congolense*, respectively (Atawodi *et al.*, 2003).

2.3.6.5 Antiviral activity of Adansonia digitata

Several in vitro and in vivo studies have been carried out to determine the antiviral activity of various baobab plant parts. Ananil *et al.* (2000) investigated the antiviral activity of several plants against the herpes simplex, sindbis and polio viruses. The baobab extract from leaves was found to have the most potent effect. Vimalanathan and Hudson (2009) investigated the antiviral activity of *A. digitata* leaves, fruit-pulp and seed extracted with water, DMSO and methanol. The study was conducted using the minimum inhibitory concentration method against influenza virus, herpes simplex virus and the respiratory syncytial virus. The influenza virus was very susceptible, while the respiratory syncytial

virus was resistant. The leaf extract exhibited the most promising activity against the influenza virus with the MIC value ranging from 0.12 μg/ml (DMSO) to 2.8 μg/ml (water). The activity of the leaf extract was promising against the herpes simplex virus (MIC value: 1.0 to 11.7 μg/ml), while the pulp and the seed exhibited much lower activity (MIC value N72.5 μg/ml). The study clearly demonstrated variation in biological activity when different plant parts are investigated. Furthermore, the anti-HSV activity was considerably enhanced by light, especially long wavelength UV although they all showed 'dark' antiviral activity as well. Thus, all the extracts contained antiviral photosensitisers (Hudson *et al.,* 2000; Vimalanathan and Hudson, 2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Zaria, a city in Kaduna State, Nigeria with Latitude 11° 4' 0" North, and Longitude 7° 42' 0" East. *A. digitata* seeds were collected from trees within Zaria and environs. Microcidal work was carried out in Microbiology Laboratory of the Department of Microbiology, Ahmadu Bello University (ABU), Samaru, Zaria.Larvicidal work was done in the Entomology Laboratory of the Department of Biological Sciences, ABU, Samaru, Zaria.Extraction of oil and phyto-chemical screening of the oil was carried out at the Pharmacognosy Laboratory of the Faculty of Pharmaceutical Sciences, ABU, Samaru, Zaria.Physico-chemical screening and fatty acid composition of the oil was carried out at the National Research Institute for Chemical Technology(NARICT), Zaria.

3.2 Sample Collection

Samples of smoke-dried *C. gariepinus* were bought from different retailers in Samaru market and transported to the laboratory immediately.

3.3 OilExtraction of *Adansonia Digitata* Seeds

The seeds(Plate I) were washed in clean tap waterand dried under the sun for two days until very dry (Plate II). They were then pulverized with a mortar and pestle and then subjected to Soxhlet extraction using petroleum ether as the solvent. The pulverised seeds were placed inside the extraction thimble which was then loaded into the main chamber of the Soxhlet extractor. The petroleum ether was placed in a distillation flask which was then placed on the heating element. The Soxhlet extractor was placed atop the flask and a reflux condenser was placed atop the extractor. The solvent was heated to release the vapors which cools and drips back into the chamber dissolving the oil which was then



Plate I: *Adansonia digitata*pulp containing the seeds



Plate II: Sun-dried*Adansonia digitata* seeds

collected in the distillation flask and the solvent returned back to repeat the cycle. This process was maintained for a period of 24 hours to ensure maximum extraction. The extracted oil was exposed to air for about 24 hours to enable evaporation of the solvent after which it was stored in a tightly covered opaque bottle until usage.

3.3.1Phyto-chemical screening of the extracted oil (according to Evans, 2009)

The extracted oil was subjected to a series of phyto-chemical tests to determine the phytochemical compounds present in the oil.

3.3.1.1 Test for carbohydrates

This was done using the Molisch test. Few drops of the Molisch reagent was added to a small portion of the extract in a test tube and concentrated sulphuric acid was added down the side of the test tube to form a lower layer. A reddish colored ring at the interphase indicated the presence of carbohydrates.

3.3.1.2 Test for free anthracene derivatives.

This was done using the Bontregers test. Chloroform (2mls) was added to a portion of the extract in a dry test tube and was shaken for 5 minutes. It was then filtered and the filterate shaken with equal volume of 10% ammonia solution. A bright pink color in the aqueous (upper) layer indicates the presence of free anthraquinones.

3.3.1.3 Test for unsaturated steroid and triterpenes

This was done using the Liebermann-buchard test. To 2mls of the extract, 2mls of acetic acid anhydride was added and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Color changes were observed

immediately and over the period of one hour. Blue to blue-green color in the upper layer and a reddish, pink or purple color indicate the presence of triterpene.

3.3.1.4 *Testfor cardiac glycoside*

This was carried out using the keller-Kiliani test where 2mls of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred in to a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the testtube to form a lower layer at the bottom. The interphase was then observed carefully for a purple-brown ring. This indicates the presence of deoxy sugars and a pale green color in the upper acetic acid layer indicated the presence of cardiac glycosides.

3.3.1.5 Test for saponin glycoside

This was done using the Frothing test where 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30mins. A honeycomb froth that persists for 13mins indicated the presence of saponins.

3.3.1.6 Test for tannins

This was carried out using the Ferric chloride test. To a portion of the extract, 4 drops of ferric chloride solution was added. A greenish-black precipitate indicates the presence of condensed tannins while hydrolysable tannins give a blue or brownish-blue precipitate.

3.3.1.7 Test for flavonoids

This was done using the sodium hydroxide test where 5 drops of 10% sodium hydroxide was added to the extract. A yellow coloration indicates the presence of flavonoids.

3.3.1.8 Test for alkaloids

This was done using Mayer's test. To a portion of the extract (2ml), 4 drops of Mayer's reagent were added. A cream precipitate indicates the presence of alkaloids (Evans, 2009).

3.3.2 Physico-chemical analysis of the extracted oil

3.3.2.1 Determination of iodine value

The iodine value was determined using wijs method IUPAC (1979). The oil sample (0.20g) was weighed into a round neck conical flask and 10ml of carbon tetrachloride and 25ml wijs reagent was added, shaken and placed in the dark for one hour. After the set time, 20ml of 10% potassium chloride and 15ml of distilled water were added. This was titrated against 0.1M sodium thiosulphate using 1% starch solution as indicator. A blank test was carried out under the same conditions. The result was calculated thus:

$$IV = \frac{12.69 \times T \times (V_1 - V_2)}{\text{Mass of test sample (g)}}$$

Where T = molarity of sodium thiosulphate solution

V_1 = vol of thiosulphate solution used for the blank test

V_2 = vol of thiosulphate solution used for test sample

3.3.2.2 Determination of saponification value (SV)

The method described by IUPAC (1979) was used. Two gram of the sample was weighed into a round neck conical flask and 25ml of ethanolic 0.5M potassium hydroxide was added and boiled for one hour. Thereafter, 0.5ml of phenolphthalein was added and titrated with 0.5M HCL solution. A blank test was carried out simultaneously. The result was calculated thus:

$$S.V = \frac{56.1 \times T (V_2 - V_1)}{\text{Mass of test sample}}$$

Where T = concentration (molarity) of HCL used

V_2 = volume of HCL used for the blank

V_1 = volume of HCL used for the test sample

3.3.2.3 Determination of acid value (AV)

The acid value was determined using IUPAC (1979) method. A solvent mixture of 25ml 95% ethanol and 25ml diethyl ether was neutralized with 0.1M ethanolic KOH using phenolphthalein as indicator. Four grams of oil sample was dissolved in the neutralized solvent mixture and titrated with 0.1M KOH. The result was calculated thus:

$$AV = \frac{56.1 \times T \times V}{M}$$

Where: T = molarity of ethanolic KOH used

V = volume of KOH used

M = mass in grammes of test sample

3.3.2.4 Determination of peroxide value (PV)

The peroxide value was determined using IUPAC (1979) method. Three grams of the extract was weighed in a conical flask and 10ml chloroform was added to dissolve the oil sample. This was followed by addition of 15ml acetic acid and 1ml 5% KI solution. The mixture was shaken and kept in the dark for 5 minutes after which 75ml was added and titrated with 0.002M sodium thiosulphate. The result was calculated thus:

$$PV = \frac{V \times T \times 100}{M}$$

Where V = volume of sodium thiosulphate solution used

T = Molarity of thiosulphate

M = mass in gramme of test sample

3.3.2.5 Determination of unsaponifiable matter

Five grams of the oil sample was weighed into a round neck round bottom flask containing antibumping granules and 50ml of 1M ethanolic KOH solution was added. A reflux condenser was attached and boiled gently for 1 hour. Then 100ml water and 100ml diethyl ether were used to transfer the solution from the flask to the separating funnel. The extraction of the aqueous ethanolic soap solution was carried out three times and the three extracts were washed with 40ml distilled water. The washing continued until the wash water no longer gives a pink colour with phenolphthalein. The ethereal solution was transferred into a weighed conical flask and evaporated to dryness on a water bath. Acetone (5ml) was added and dried. Drying continued in an oven set at 103±2°C for 15mins. After weighing the residue, 4ml diethylether and 20ml ethanol previously neutralized to faint pink colour with phenolphthalein was added and titrated with 0.1M ethanolic KOH to the same final color. The result was calculated thus:

$$\text{Unsaponifiable matter (\%)} = \frac{(m_1 - 0.28VT) \times 100\%}{m}$$

where: m= mass in grammes of test sample

m_1 = mass in grammes of the residue

V = volume of 0.1M KOH used

T = Molarity of KOH used

3.3.3 Determination of fatty acid composition

The fatty acid composition of the oil sample was carried out using gas chromatography mass spectrometry (GC-MS) machine with code GCMS-QP2010 PLUS, SHIMADZU JAPAN

3.4 Determination of Fungicidal Effect

One gramme of the sample was soaked in 9ml of peptone water to form the stock solution and this was serial diluted to 10^5 after which 0.1ml was spread plated on potato dextrose agar (PDA) and inoculated at room temperature for one week. The isolates were then identified under the microscope according to identification keys by Barnett and Hunter (1972). Agar slants were also prepared for the isolates to store them until further use (Cheesebrough, 2004).

3.4.1 Fungicidal sensitivity studies

Stock solution was prepared by pouring T80 solution into the PDA agar slants and gently shaken to release the spores into the solution. The stock was then spread plated onto potato dextrose agar (PDA) plates with sterile swab sticks. Using an 8mm cork borer, 5 holes were cut into each plate to hold the treatments/concentrations. The oil was serially diluted with dimethyl sulphoxide (DMSO) solution to the following concentrations: 25%, 20%, 15%, 10%, and 5%. The control was done using Teconazole disks of 10µg. The treated plates were then incubated at ambient temperature for 5 days.

3.5 Determination of Bactericidal Effect of *Adansonia digitata*

3.5.1 Isolation of bacterial species from *Clarias gariepinus*

The stock solution was prepared by soaking 1g of the smoke-dried *C.* gariepinus in 9ml of peptone water which was serially diluted to 10^5 and 0.1ml was spread-plated on nutrient agar (NA), Mannitol Salt Agar (MSA), Eosine Methylene Blue Agar (EMB) and MacConkey agarplates and then incubated for 24hours. The total bacterial plate count was done from the NA plates and the result was calculated thus:

$$\frac{\text{Total number of colonies counted} \times \text{dilution factor}}{\text{Volume of inocula}}$$

and expressed in colony forming unit per gram (cfu/gm).

The isolates were then stored in slants made of NA until further use.

The isolates were identified by preparing a smear by using a sterile loop to place a drop of sterile water on the slide, res-sterilizing the loop and using it to take a small sample of bacterial isolates which was gently stirred into the water on the slide. The smear was allowed to dry and thereafter heat fixed. The slides were flooded with crystal violet (primary stain) and allowed to stand for a minute after which it was gently rinsed with distilled water. Then flooded again with Gram's iodine (mordant) and allowed to stand for another minute and then rinsed with distilled water. The smear was then decolorised using acetone and immediately rinsed with water. The slides were further counter stained with safranine and allowed to stand for 30seconds then rinsed with distilled water, allowed to dry and then viewed under the microscope at ×100 objective. Gram positive bacteria were those which appeared purple while the Gram negative bacteria were those which appeared pink. The bacterial isolates were further subjected to identification to species level by means of commercially produced identification test kit (Cheesebrough, 2004).

3.5.2Bactericidal activity of *Adansonia digitata* seed oil

This was done according to the methods of Kirby Bauer disc diffusion. The bacterial isolates were standardized using the 0.5 McFarland turbidity standard with a cell population of approximately 1.5×10^8CFU/ml. The standardized isolates were then plated on Mueller-Hinton agar using sterile swab stick to spread it on the plate after which 5mm disc borer was used to cut out 5 places for the 5 concentrations and labelled 1-5 where 1 represents 25% and 5 represents 5%. The oil concentrations of 5%, 10%, 15%, 20% and 25% were then poured into the appropriate holes using syringes and incubated at room temperature for 24hours (Plate III). The control was plated with the standardized isolates

and a commercially produced ciprofloxacin disc was placed at the centre (Plate IV). The zones of inhibition were measured in mm with a metre rule for each of the concentrations.

3.6 Determination Of Larvicidal Effect

The *Dermestes maculates*larva were collected from smoke-dried fishes from market sellers and transferred into containers with perforated covers. Some pieces of smoke dried fish were added to the containers to serve as food for the larvae until usage. The larvae were starved for a period of 24hrs prior to usage. Fresh*C. gariepinus* were collected, smoke-dried and stored in foil paper until usage for treatment for larvicidal bioassay.

3.6.1Larvicidal bioassay

The smoke dried fish was heat sterilized in the oven at 60°C for one hour and then cooled to room temperature. The fish were then weighed and treated with the oil sample in volumes as described by Adebote *et al*.(2006). The treated fishes were then introduced into separate kilner jars and a control was setup without oil treatment. Fifteen (15) late instar larvae of *D. maculates* were introduced into each jar containing treated fish as well as the control and each jar was labeled for oil volume and time of larval inoculation. The experiment was replicated thrice for each oil volume. The setup was maintained for 96 hours and mortality of larvae in treatment and control were assessed at 24, 48, 72 and 96 hours exposure period (Plate V).

3.7 Data analyses

Probit analysis was used to determine the median lethal volume (LC_{50}) of the oil against the larvae.One way Analysis of variance (ANOVA) was used to determine the significant differences between mean mortalities of the oil against the larvae at 5% level of

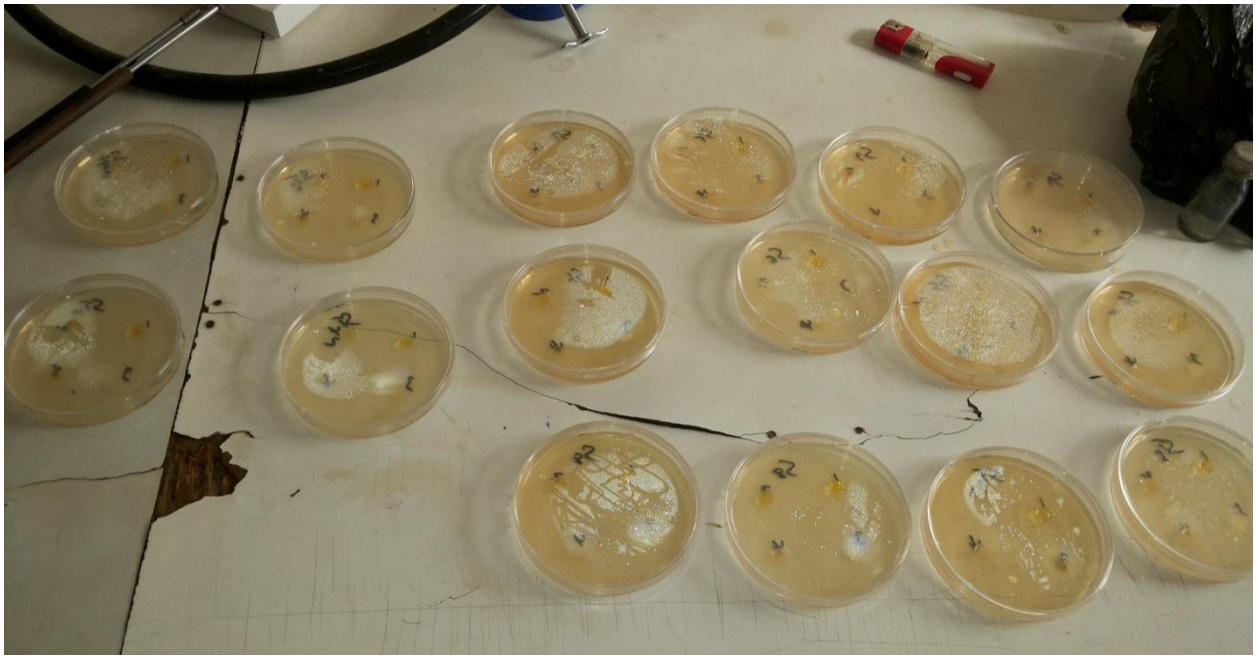
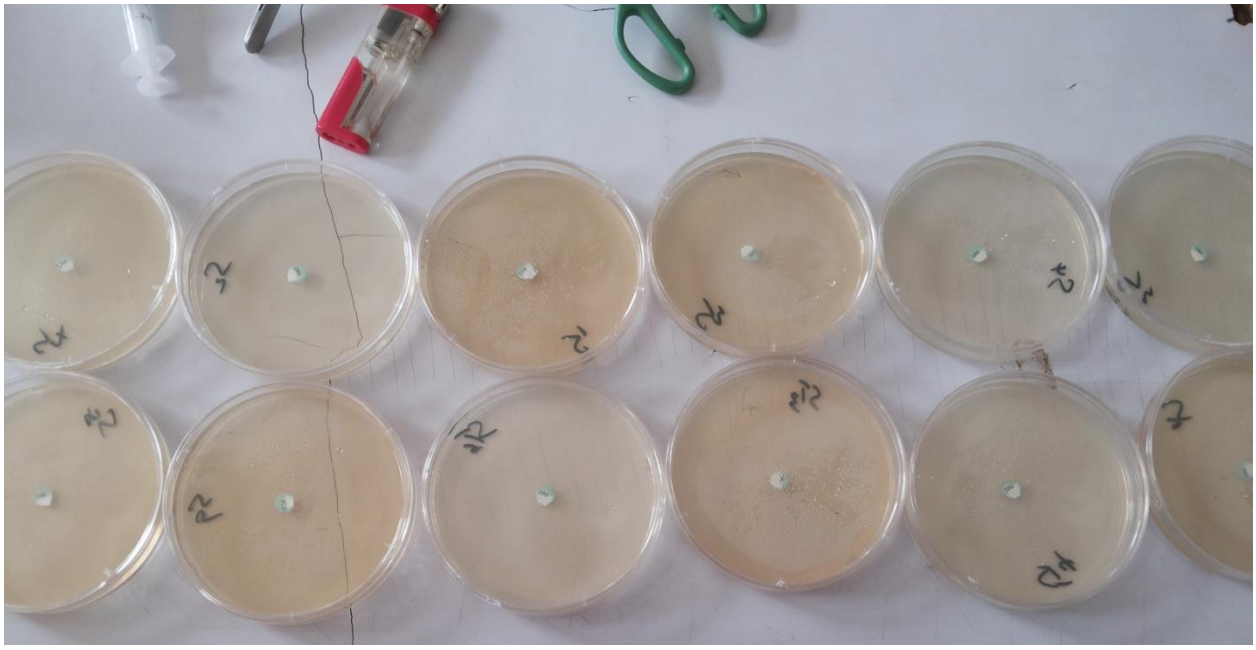


Plate III: Experimental setup for bacterial sensitivity studies



PlateIV: Control setup for the bacterial sensitivity study



Plate V: Experimental setup for larvicidal work

significance and also used to determine the significant differences in the effects of the oil against the isolated bacteria. Duncan's multiple range test (DMRT) was used to rank the means where significant differences occurred.

CHAPTER FOUR

4.0 RESULTS

4.1Chemical Screening Of The Extracted Oil

The result of the phyto-chemical screening of *A. digitata* seed oil indicated the presence of carbohydrates, phenols, saponins, tannins, flavonoids and alkaloids (Table 4.1). The quantitative phyto-chemical analysis showed carbohydrates to be highest in proportionat a percentage of 70.08followed by alkaloids at 19.10% and saponins having the lowest proportionat a percentage of 0.10 in the extracted oil (Table 4.2).

The physico-chemical screening of the oil indicated the relative density to be 0.9029±0.0003, saponification level at 61.71±0.043mg/g, peroxide value at 3.17±0.06mg/g. Other physico-chemical parameters tested for and their values are as shown in Table 4.3.

The fatty acid composition of the oil showed the presence of eight fatty acids with pentadecanoic acid having the lowest retention time of 15.25with an area over height of 1.84 followed by N-hexadecanoic acid with a retention time of 15.940. The fatty acid with the highest retention time was pentafluoropropionic acid with a time of 20.557. Other fatty acids found in the oil were stearic acid, oleic acid, and others as shown in Table 4.4.

4.2 Identification of Fungal Isolates

Fungal growth after incubation of the sample on potato dextrose agar, are shown in Plates V and VI. After identification, the fungi isolates were found to be *Aspergillus niger, Monilia sitophila, A. niger, Stemphylia* sp*, Microcarpum narum, Trychophyton tonsurans* (Table 4.5)

Table 4.1:Phyto-chemical constituentsof *Adansonia digitata* seed oil

Phytochemical	Remarks
Carbohydrates	Present
Anthraquinones	Absent
Phenols	Present
Saponins	Present
Triterpenes	Absent
Tannins	Present
Flavonids	Present
Alkaloids	Present

Extraction of oil was done through Soxhlet extraction method using petroleum ether as solvent.

Table 4.2: Quantitative phyto-chemical components of the *Adansonia digitata* oil

Phytochemical	Proportion (%)
Flavonoids	3.33
Saponins	0.10
Alkaloid	19.10
Tannins	15.6
Phenols	0.55
Carbohydrates	70.08

Table 4.3:Physico-chemical values of *Adansonia* digitata seed oil

Parameter	Value
Relative density	0.9029±0.003
Refractive index	1.4668±0.0002
Saponification value	61.71mg/g±0.043
Peroxide value	3.17mg/g±0.046
Acid value	1.70mg/g±0.029
Iodine value	5.08mg/g±0.018

Note:

All results are presented as Means±SE

Table 4.4: Fatty acid composition of *Adansonia digitata* seed oil extract

Peak	Name	Retention time	Area/height
1	Pentadecanoic acid	15.425	1.84
2	n-hexadecanoic acid	15.940	5.71
3	Elaidic acid	17.140	1.81
4	Oleic acid	17.697	13.12
5	Stearic acid	17.849	4.24
6	Palmitic acid	18.888	2.06
7	13-docosenoic acid	19.977	1.98
8	Pentafluoropropionic acid	20.557	2.20

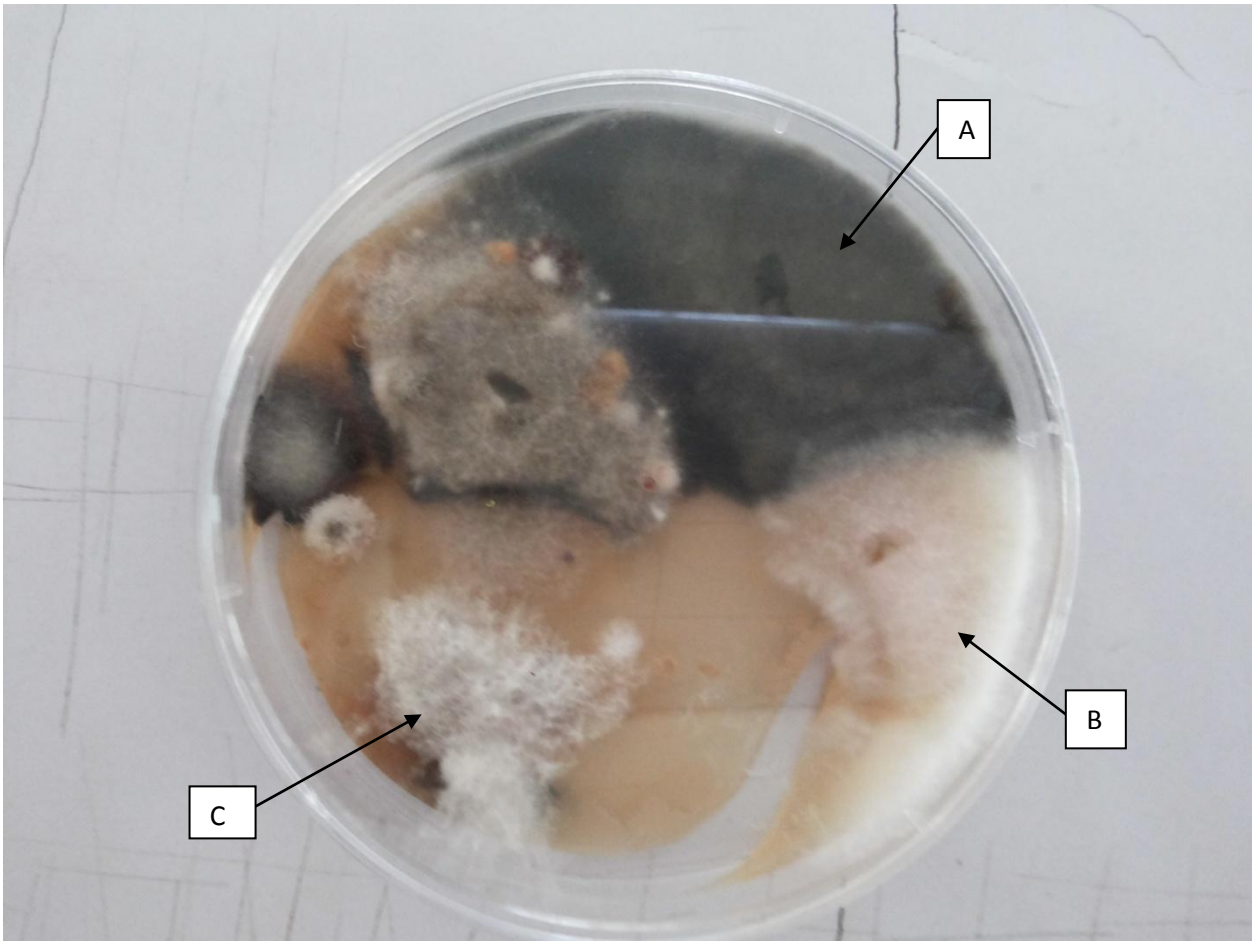


Plate VI: Fungal growth showing colonies of *Aspergillus niger*, *Stemphylium* sp. and *Microsporum narum*

Note:

A: *Aspergillus niger*

B:*Stemphylium* sp.

C:*Microsporum narum*

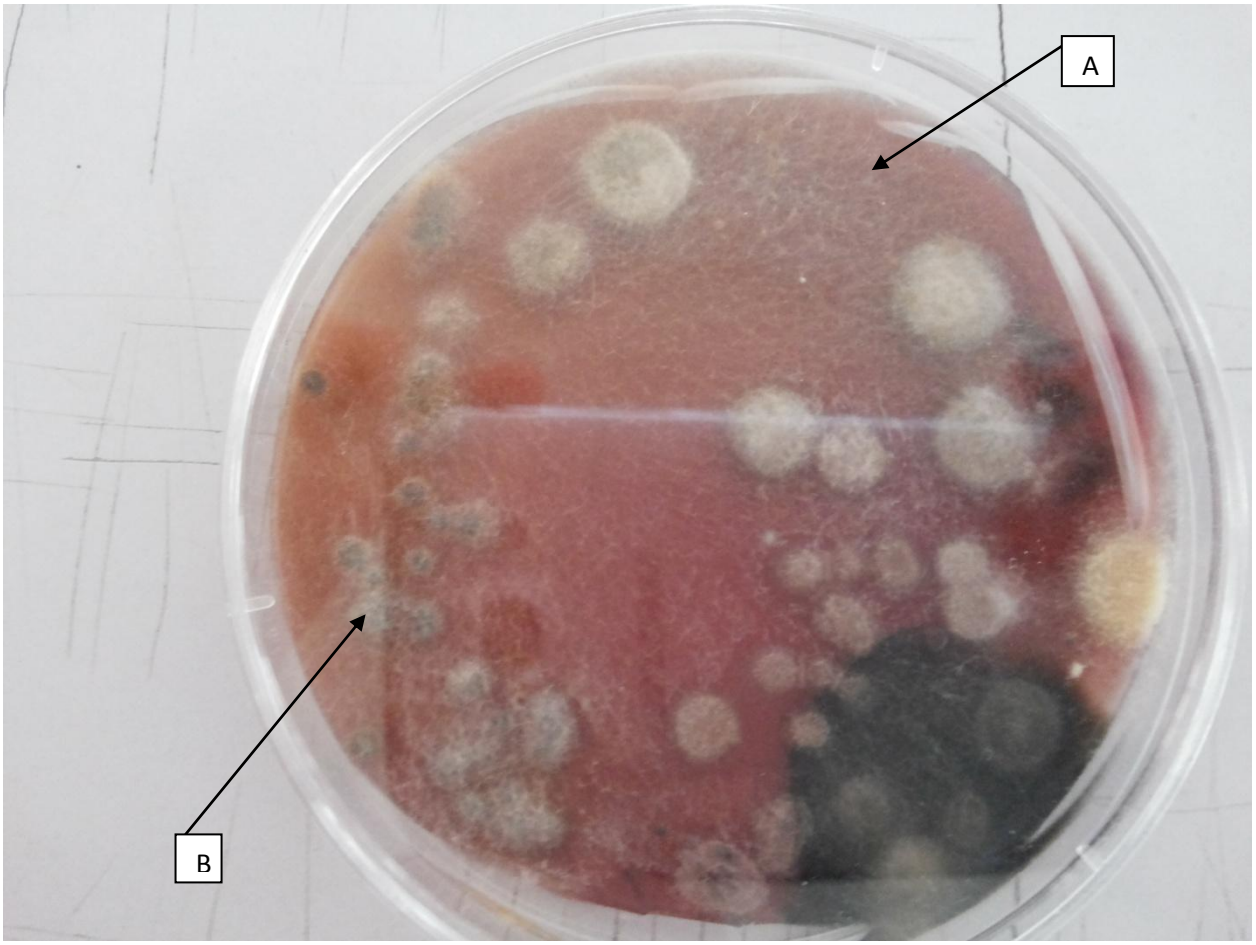


Plate VI: Fungal growth showing colonies of *Monilia sitophilia* and *Trichophyton tonsurans*.

A:*Monilia sitophilia*

B:*Trichophyton tonsurans*

Table 4.5: Fungal Isolates from smoke dried *Clarias gariepinus*

Isolate number	Fungal species
1	*Aspergillus niger*
2	*Monilia sitophilia*
3	*Stemphylium* sp.
4	*Microsporum narum*
5	*Trychophyton tonsurans*

Note:

Identification was done with the aid of microscope 100× magnification and the identification keys of Bannet and Hunter (1972).

4.3 Total Bacterial Plate Count

Sample 6 had the highest bacterial plate count at 3.84×10^{8} followed by sample 2 at 2.12×10^{8} while sample 4 had the lowest bacterial count at 8×10^{7}. The different samples and their bacterial count are shown in Table 4.6.

4.4 Identification of Bacterial Isolates

Upon gram staining reaction, five*Bacillus* species and two*Staphylococcus* species were identified. Table 4.7 shows the bacterial species identified. All bacterial isolates identified were gram positive bacteria. The *Bacillus* species were both long and short rods while the *Staphylococcus* species were cocci.

Upon further subjection to microgen test, the bacterial isolates were further identified to the species level. The species identified includes *B. Coagulans,B. Subtilis, Staphylococcus xylosus* etc. There was also a sub *Bacillus* species *P. macerans* (Table 4.8).

Table 4.6: Total bacterial plate of dried *Clarias gariepinus*

Sample number	Total plate count (cfu/gm)
1	1.6×10^{8}
2	2.1×10^{8}
3	1.3×10^{8}
4	8.0×10^{7}
5	9.9×10^{7}
6	3.8×10^{8}
Average of counts	1.77×10^{8}

Note:

cfu/gm – colony forming unit/gram of bacteria cultured from smoke dried *Clarias gariepinus*.

Table 4.7: Gram staining reaction of bacterial isolates

Sample number	Gram's reaction	Cell morphology	Inference
S1	Gram positive	Short rods	*Bacillus* sp.
	Gram positive	Long rods	*Bacillus* sp.
S2	Gram positive	Short rods	*Bacillus* sp.
	Gram positive	Long rods	*Bacillus* sp.
S3	Gram positive	Short rods	*Bacillus* sp.
	Gram positive	Cocci	*Staphylococcus* sp.
S4	Gram positive	Long rods	*Bacillus* sp.
	Gram positive	Cocci	*Staphylococcus* sp.
S5	Gram positive	Short rods	*Bacillus* sp.
	Gram positive	Long rods	*Bacillus* sp.
S6	Gram positive	Cocci	*Staphylococcus* sp.
	Gram positive	Long rods	*Bacillus* sp.

Note:

S1, S2,.....S6 represents six different retailers from whom the samples of fish were purchased.

Table 4.8: Microgen Bacterial identification to species level

Isolate	Octal code	% Probability	Final identification
1	61376522	81.47	*Bacillus coagulans*
2	77772223	45.00	*Bacillus circulans*
3	67272723	87.08	*Paenibacillus macerans*
4	77172123	76.11	*Bacillus megaterium*
5	77772603	51.28	*Bacillus subtilis*
6	16570	95.00	*Staphylococcus haemolyticus*
7	13770	88.16	*Staphylococcus xylosus*

Note:

The percentage level of confidence as indicated by the biochemical test kit for each species is also indicated.

4.5 Bacterial Sensitivity Tests

The sensitivity tests shows that the higher the concentration, the more the zone of inhibition. *Bacillus circulans* was resistant to the oil. *Paenibacillusmacerans* had the highest susceptibility to the test oil. *B.circulans*has the lowest as indicated by the zones of inhibition (Table 4.9).

The bacterial sensitivity tests after subjection to statistical analysis revealed that at 5% concentration, there is no significant difference (P>0.05) between *B. circulans* and *S. xylosus*. It also shows that there are significant differences (P≤0.05) between the above isolates and the remaining isolates. At 10% concentration level, *P. Macerans* and *B. megaterium* had significantly higher (P≤0.05) zones of inhibition than *B. circulans*. At 15%, *P. Macerans* and *B. megaterium* had significantly higher zones of inhibition as compared to*B. circulans*. At 20%,*P. macerans* was significantly higher (P≤0.05) zones of inhibition than*B. circulans*. Finally, at 25%,*P. macerans* had significantly higher (P≤0.05) zone of inhibition than*B. circulans*. *Paenibacillus macerans* consistently had significantly higher zone of inhibition across all the concentrations (Table 4.10).

Table 4.9: Mean sensitivity of Bacterial isolates using the seed oil of *Adansonia digitata*

Isolate	Diameter zones of inhibition (mm) at varying concentrations of seed oil					
	5%	10%	15%	20%	25%	Control
Bacillus coagulans	9±0	10±0	10±0	13±0.33	15±0.5	11
B. circulans	0	0	0	0	0	15
Paenibacillus macerans	11±0.33	12±1.15	13±0.58	15±0	18±0.33	32
B. megaterium	10±0	12±0.58	13±0	14±0.58	16±0.33	38
B. subtilis	10±0	10±0.33	10±0	12±0	14±0.58	12
Staphylococcus haemolyticus	10±0.33	10±0	12±0	14±0	16±0.58	26
Staphylococcus xylosus	0	10±0	10±0	12±0	14±0.58	22

Note:

- Oil concentrations are a mixture of *A. digitata* seed oil and the solvent DMSO. The percentages indicate the amount of oil (X) to solvent (Y)in the mixture.

  X : Y

  5 : 95

  10:90

  15:85

  20:80

  25:75

  Control = ciprofloxacin discs

- Results are presented as Means±SE.

Table 4.10:Mean sensitivity of bacterial isolates to *Adansonia digitata* seed oil upon subjection to Duncan's multiple range test

Isolates	5%	10%	15%	20%	25%	Control
Bacillus coagulans	9.00±0.00^{c}	10.00±0.00^{ab}	10.00±0.00^{c}	12.50±0.50^{b}	15.00±1.00^{b}	11.00±0.00
B. circulans	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{c}	15.00±0.00
Paenibacillus macerans	10.50±0.50^{a}	12.00±2.00^{a}	13.00±1.00^{a}	15.00±0.00^{a}	18.00±1.00^{a}	32.00±0.00
B. megaterium	10.00±0.00^{ab}	12.00±1.00^{a}	13.00±0.00^{a}	14.00±1.00^{ab}	15.50±0.50^{ab}	38.00±0.00
B. subtilis	10.00±0.00^{ab}	9.50±0.50^{b}	10.00±0.00^{c}	12.00±0.00^{b}	14.00±1.00^{b}	12.00±0.00
Staphylococcus haemolyticus	9.50±0.50^{bc}	10.00±0.00^{ab}	12.00±0.00^{ab}	14.00±0.00^{ab}	16.00±1.00^{ab}	26.00±0.00
Staphylococcus xylosus	0.00±0.00^{d}	10.00±0.00^{ab}	10.00±0.00^{c}	12.00±0.00^{b}	14.00±1.00^{b}	22.00±0.00

Note: All results are presented as means±SE

Results with the same superscriptalong the column are not significantly different (P>0.05)

4.6*Dermestes maculatus* larval mortality on exposure to *Adansonia digitata* seed oil

The larval mortality results show that the higher the concentration of *A. digitata* seed oilper gram of fish, the more the larval mortality. Table 4.11 shows the percentage larval mortality while using the oil on the larvae of *Dermestes maculates*. The least concentration of 0.004ml/g did not result in any larval mortality for the first 48hours and only in 27% mortality by the 96^{th} hour. The highest concentration of 0.256ml/ggave a near perfect larval mortality by 24 hours and a total mortality by the 72^{nd} hour. The control yielded no larval mortality during the duration of the experiment.

Upon separation of means of the concentrations based on a particular hour, the oil concentration of 0.256ml/g was significantly higher ($P \leq 0.05$) across the hours as compared to the oil concentration of 0.004ml/g(Table 4.12). The most significant was the 96^{th} hour across all the concentrations and there were no difference between the exposure hours across 0.256ml/g concentration. The least significant was the 24^{th} hour across all the concentrations

Table 4.11: Percentage larval mortality using the seed oil of *Adansonia digitata*

Oil concentrations (ml/g)	%mortality at specific exposure period (hours)			
	24hrs	48hrs	72hrs	96hrs
0.004	0	0	13	27
0.016	7	27	33	53
0.032	20	27	40	60
0.256	87	93	100	100
LC_{50}	0.078	0.048	0.028	0.014
Control	0	0	0	0

Table 4.12: Larvicidal Effects of *Adansonia digitata* using Duncan's multiple range test

Concentration (ml/g)	Exposure Period (hours)			
	24	48	72	96
0.004	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	1.33 ± 0.88^{c}	3.33 ± 1.67^{c}
0.016	0.67 ± 0.67^{bc}	3.67 ± 0.67^{b}	5.00 ± 0.58^{b}	7.67 ± 0.88^{b}
0.032	2.33 ± 0.67^{b}	4.33 ± 0.67^{b}	6.00 ± 1.15^{b}	9.00 ± 1.53^{b}
0.256	13.00 ± 1.15^{a}	14.00 ± 0.58^{a}	14.33 ± 0.67^{a}	15.00 ± 0.00^{a}
Control	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}

All results are presented as mean±SE

Means with the same superscript along the column are not significantly different (P>0.05)

CHAPTER FIVE

5.0 DISCUSSION

5.1 Chemical Evaluation of the Extracted *Adansonia digitata* Seed Oil

Compounds such as carbohydrates, saponins, phenols, alkaloids, flavonoids and tannins were found in the baobab seed oil; the presence of carbohydrates and alkaloids are higher than other compunds. The relative density(0.90) of the oil indicate that the oil has the capacity to spread easily over surfaces on application. The refractive index of 1.668 indicates that the *A. digitata* seed oil is lighter than drying oils. A saponification value of 61.71mg/g shows that the oil has fatty acids of average molecular weight. The oil may have a low oxidative rancidity on exposure to air as indicated by the peroxide value of 3.17mg/g.This means that fish treated with the oil may not develop a foul odour or off taste easily. The low acid value of 1.70 is a pointer to the suggestion that the oil has few free fatty acids and has no foul odour or aroma. This may confer an advantage to the seed oil of *A. digitata* and possibly possess acceptability from a wider range of consumers. Shukla*et al.*(2001) reported the presence of terpenoids, flavonoids, sterols, vitamins, carbohydrate and lipids in various parts of the baobab tree including the seed oil. The presence of these compounds has been reported in oils which had antibacterial and larvicidal properties. Kumar *et al.* (2014) reported similar compounds in celery seed oil and confirmed larvicidal action of the oil against *Aedes aegypti* L. Similar properties were recorded from hydroalcoholic extract from *Curtisia dentata* (Burm.f) stem bark and further experimentation revealed broad spectrum activity of the extract against some bacteria (Oyedemi *et al.*, 2012). This study supports the findings ofKamatou *et al*(2011) who reported similar compounds.

Balekar *et al.*(2014) reported that extract of *Wedelia trilobata* L has similar phyto-chemical properties to the ones found in the extract of *A. digitata* and has anti bacterial activity against Gram positive bacteria such as *B. Subtilis* and*S. Aureus*; furthermore that the extract had no effect on yeast and other fungi such as *A. niger*. Tian *et al.*(2010) discovered that the extracted oil from apple seed exhibited an iodine value of 94.14-101.15 g /100 g oil; refractive index (at 40°C) was 1.465-1.466; density (25 degrees C) was 0.902-0.903 mg/ml; saponification value was 179.01-197.25 mg KOH/g oil; and the acid value was 4.036-4.323 mg KOH/g oil. The apple seed oils mainly consisted of linoleic acid (50.7-51.4 g/100 g) and oleic acid (37.49-38.55 g/100 g). Other prominent fatty acids were palmitic acid (6.51-6.60 g/100 g), stearic acid (1.75-1.96 g/100 g) and arachidic acid (1.49-1.54 g/100 g). These results are similar to the propertiesfound in *A. digitata* seed oil. The apple seed oil was also found to be almost completely active against bacteria (Tien *et al*., 2010). This is similar to the result obtained from the present study on *A. digitata* seed oil where the oil has activity against most of the bacteria isolated from the smoked dried *C. gariepinus*.

Steroids, saponins, glycosides, alkaloids and flavonoids are present in *A. digitata* seed oil, perhaps these compounds confers antibacterial and larvicidal properties on the seed oil of *A. digitata*. Castor oil also contains similar fatty acids as baobab seed oil used in the present study and has been implicated as anti-inflammatory, ant-bacterial, anti-ulcer, larvicidal and wound healing among many other functions (Jena and Gupta 2012). Ngele *et al*.(2014) also reported the presence of saponins, steroids, flavonoids, tannins, alkaloids as well as amino acids in unripe epicarp of *Citrus sinensis*(orange) and went further to confirm anti bacterial activity of its extract on *E.coli* and *S. aureus*.

5.2 Isolated Fungi Sensitivity to *Adansonia digitata* Seed Oil

The fungal species associated with the smoke dried *C. gariepinus* in this study belongs to the genus *Aspergillus, Monilia, Stemphylium, Microsporum* and *Trychophyton.Aspergillus* species have been implicated in allergic reactions especially in asthmatic patients when the spores are inhaled. They have also been implicated in respiratory infections when they enter the lungs and continue growing there. *Aspergillus niger* is also implicated in hearing problems and may lead to hear loss. Edema and Agbon (2010) reported that most of the fungi associated with *C. gariepinus* were of the genus *Aspergillus* and *Penicillium*. Fafioye *et al.* (2002), Fafioye and Fafioye (2013)reported that *C. gariepinus*Ikpebivie and Amakoromo (2013) also identified *Aspergillus* spp. as the most occurring fungi of fresh and smoke dried fish in Yenagoa, Bayelsa state.

In this study, the fungal isolates are not sensitive to *A. digitata* seed oilas indicated by the lack of zone of inhibition on the agar.Fungi has been reported to thrive in acidic environments which the oil may have provided as a result of the fatty acids present in the oil. The lack of fungal sensitivity may also be as a result of the presence of flavonoids which has been classified as a fungal secondary metabolite and so may confer on the oil a synergistic effect with the fungal isolates. Dass *et al*.(2013) reported the growth and subsequent biodegradation of *A. digitata* seed oil by the fungus *Aspergillus niger*. This means that the presence of the oil did not prevent the growth and degrading action of the fungi which may also be linked to the acidic nature of the oil.

5.3 Isolated Bacteria Sensitivity to *Adansonia Digitata* Seed Oil

The bacterial isolates in the present study consisted of both *Bacillus* and *Staphylococcus* species,*Bacillus* species being the most predominant. They were all Gram positive bacteria. The presence of bacteria on the smoked fish may be associated with poor handling and inadequate environmental hygiene in places where they are sold. Ikpebivie and Amakoromo (2013)isolated bacteria of the genus *Bacillus*, *Pseudomonas*, *Staphylococcus* among others, and also established that *Bacillus* species were the most predominant of all the isolated species from smoke-dried fishes, which were mostly made up of rods and few cocci and catalase positive except for one. Ibrahim *et al.* (2014) also isolated and identified 6 bacterial isolates including *B. subtilis*from fresh and smoked *C. gariepinus*.

In the present study, *Adansonia digitata* seed oil has bactericidal activity and lack fungicidal activity. Although the control experiment in this study shows a wider zone of inhibition, the seed oil could be used to reduce or prevent bacterial activities in smoke-dried fishes. Yushau *et al*.(2010) reported antibacterial activity of extracts of *A. digitata* stem bark on some clinical bacterial isolates such as *E. coli* and *Staphylococcus* spp.

5.4 Larvicidal Effects of *Adansonia Digitata* Seed Oil

The LV_{50} of the oil for a period of 24 hours was 0.078ml/g and 0.014ml/g for a period of 96 hours. There is a decrease in the volume of oil needed to kill fifty percent of the larva as the period increases. This indicates that if the fish is to be stored for long periods, then lesser volumes of oil will be required. The baobab seed oil exhibits larvicidal activity against the larvae of smoke dried fish, *Dermestes maculatus*in the present study. Larvicidal activity is most

effective in the highest oil volume of 0.256ml/g and least in the lowest volume. Baobab seed oil has been reported to have larvicidal activity against the larvae of different species of mosquito (Krishnappa *et al.*, 2012; Kamba *et al.*, 2014; Kamba *et al.*, 2015).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1Conclusions

Adansonia digitata seed oil contains significant amounts of phyto-chemicals such as carbohydrates, saponins, tannins and other compounds as well as fatty acids such stearic acid, oleic acids, palmitic acid amongst others.

The seed oil does not havesignificant fungicidal effect on the fungi isolated from the fish and so the null hypothesis that there is no significant fungicidal effect of the seed oil of *A. digitata* seed oil on isolated fungi of *C. gariepinus* is accepted.

Adansonia digitata seed oil has significant bactericidal effects on the bacteria isolated from the fish.Replacement of chemical based treatments can be done with 25% oil concentration as the majority of the bacterial isolates showed the highest sensitivity at this concentration. As such, the null hypothesiswhich states that there is no significant bactericidal effect of the seed oil of *A. digitata* seed oil on isolated bacteria of *C. gariepinus*is unaccepted.

Adansonia digitata seed oil has significant larvicidal effect on the larvae of *Dermestes maculates,* a pest of smoke dried *C. gariepinus*.The oil concentration of 0.256ml/g of fish proved to be the most effective in killing 87% of the pest after 24 hour exposure periodand 100% at the 96^{th} hour.Therefore, the null hypothesis which states that there is no significant larvicidal effect on the larvae of *D. maculates* by the seed oil of *A. digitata*is hereby rejected.

6.2 Recommendations

1. Fish sellers can use the oil at a concentration of 0.256ml/g to treat their fishes as this kills both the bacteria and the larvae thereby helping to prevent economic and nutritive losses.
2. It is recommended that the seed oil be incorporated into the production of probiotics by pharmaceutical companies.
3. Further research on active constituents resulting in bactericidal and larvicidal effects should be carried out to assist in isolating such constituents for faster action.

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Appendix I: Selected Traditional Medicinal Uses of the *Adansonia digitata* tree in Africa

THERAPEUTIC USES	PLANT PART USED	COUNTRY	PREPARATION	REFERENCE
Fever, diarrhoea	Seeds	South Africa	Mixed with water	Watt and Breyer-Brandwijk (1962)
Dysentery, fever	Seeds, fruits	Cameroon, Central African Republic	Decoction	Watt and Breyer-Brandwijk (1962)
Malaria, fever	Leaves	Sierra Leone	–	Watt and Breyer-Brandwijk (1962)
Coughs	Powdered seeds	South Africa	–	Watt and Breyer-Brandwijk (1962)
Diarrhoea, fever, inflammation, kidney and bladder diseases, blood clearing, asthma	Leaves	South Africa	Infusion	Van Wyk and Gericke (2000)
Fever, dysentery	Leaves, roots	Sudan	–	Gebauer *et al.* (2002)
Anaemia	Bark	Nigeria	Aqueous extract	Adesanya *et al.* (1988)
Malaria	Bark, leaves	Nigeria	Powdered bark mixed with porridge	Ajaiyeoba *et al.* (2004)
Diarrhoea, fever, inflammation, kidney and bladder diseases, blood clearing, asthma	Leaves	Tanzania	Decoction, infusion	Brendler *et al.* (2003)
Dysentery, fever, haemoptysis, diarrhoea	Fruits, seeds	Tanzania	Decoction	FAO (1993)
Refreshing, tonic, diuretic, cystitis, dysentery, hepatic disorders, hypogalactia	Flesh with peel	Burkina Faso	Decoction	Kerharo and Adam (1974) ; Nacoulma (1999)

Toothache, gingivitis	Leaves	Burkina Faso	–	Tapsoba and Deschamps (2006)
Diarrhoea, worms	Leaves, seeds, fruit pulp	Côte d'Ivoire	–	Aké Assi (1992)
Wound healing	Stem bark	Mali	Decoction	Inngjerdingen *et al.* (2004)
Diaphoretic, fever remedy	Leaves	Kenya	Decoction	Abbiw (1990)
Diaphoretic, kidney and bladder diseases, asthma, insect bites	Leaves	–	–	Wickens (1982)
Microbial diseases	Fruits	Nigeria and Senegal	–	Hostettmann *et al.* (2000)

Appendix II: Commercially produced Teconazole disks (10µg)



Appendix III: Commercially produced microgen identification test kit

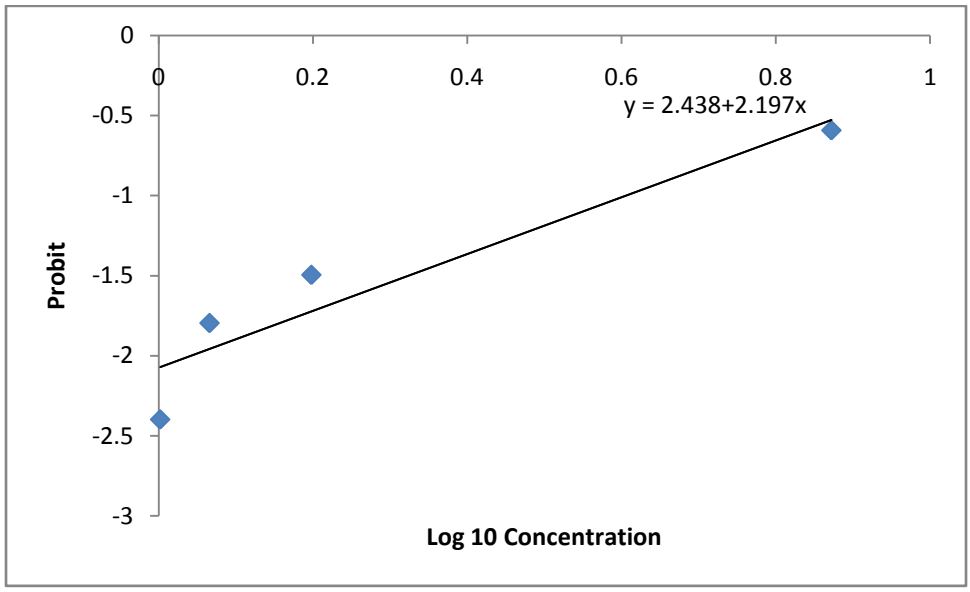


Appendix IV



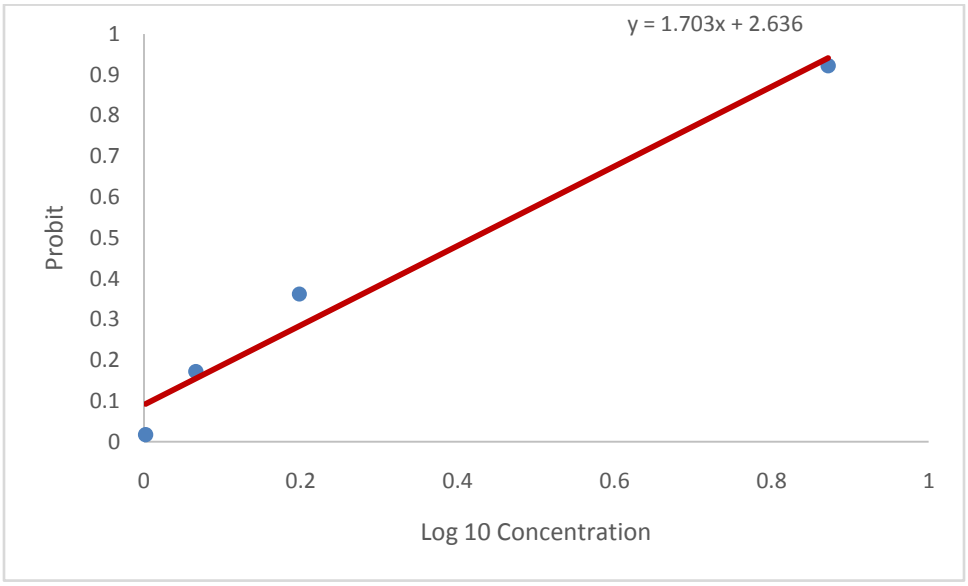
Microgen *Staphylococcus* identification kit

Appendix V



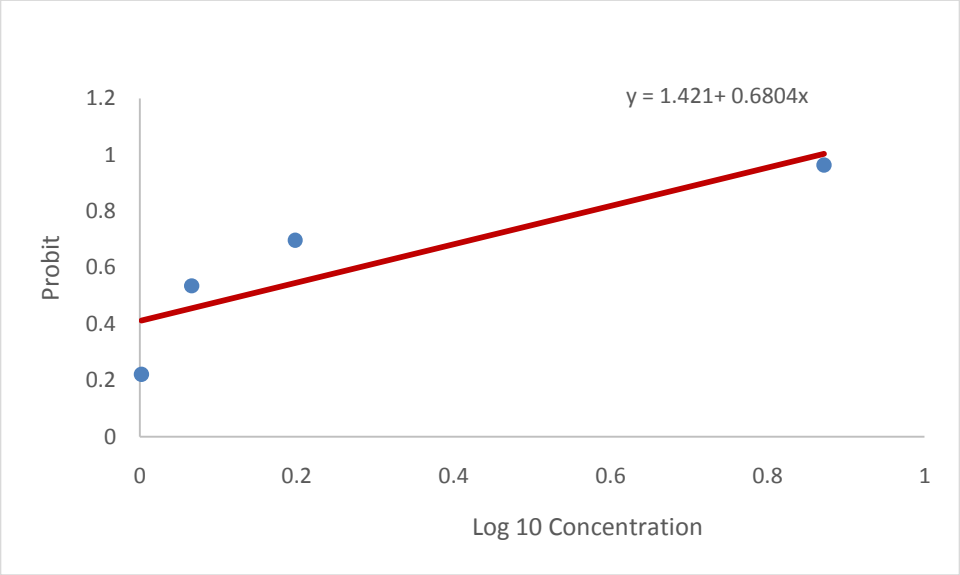
Probit analysis of the larval mortality at 24 hours.

Appendix VI



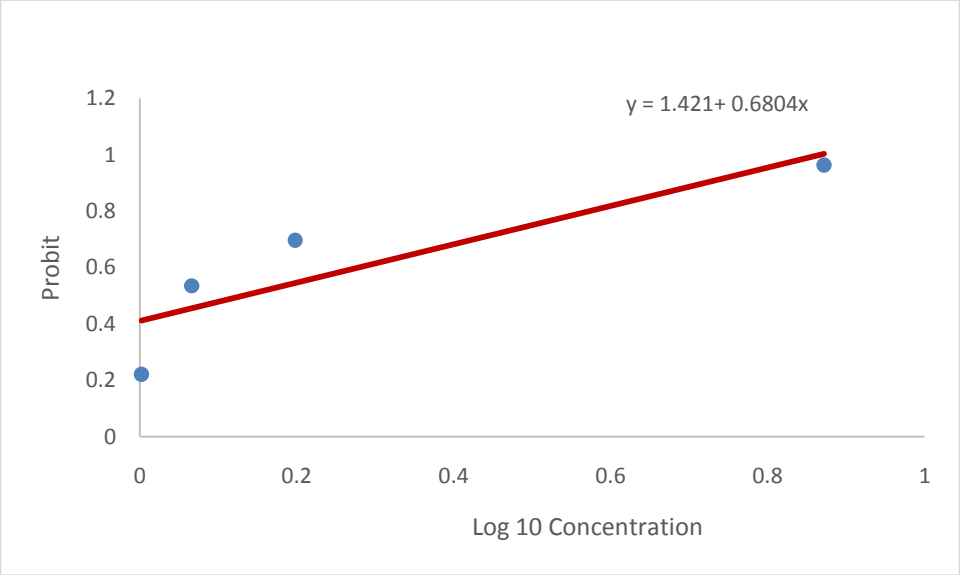
Probit analysis of larval mortality at 48 hours.

Appendix VII



Probit analysis of larval mortality at 72 hours.

Appendix VIII



Probit analysis of larval mortality at 96 hours.