ANTIMICROBIAL ACTIVITY OF THE ETHYL ACETATE EXTRACT OF *Chrysophyllum albidum* (African Star Apple) COTYLEDONS

BY

IFIJEN EROMOSE DANIEL

PHA1305030

DEPARTMENT OF PHARMACEUTICAL MICROBIOLOGY AND BIOTECHNOLOGY

FACULTY OF PHARMACY

UNIVERSITY OF BENIN

BENIN CITY

JANUARY, 2020

ANTIMICROBIAL ACTIVITY OF THE ETHYL ACETATE EXTRACT OF *Chrysophyllum albidum* (African Star Apple) COTYLEDONS

BY

IFIJEN EROMOSE DANIEL

PHA1305030

SUPERVISED BY

DR (MRS). UPE BABAIWA

A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICAL MICROBIOLOGY AND BIOTECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DOCTOR OF PHARMACY (PHARM.D) DEGREE OF THE UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

JANUARY, 2020

CERTIFICATION

This is to certify that this project was carried out by Ifijen Eromose Daniel, in the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Benin under my supervision.

_____ _____

IFIJEN DANIEL EROMOSE **Date**
PROJECT STUDENT

_____ _____

DR. (MRS). UPE BABAIWA **Date**
PROJECT SUPERVISOR
Ag. HEAD OF DEPARTMENT

_____ _____

DR (MRS) UPE BABAIWA **Date**
Ag. HEAD OF DEPARTMENT

DEDICATION

I dedicate this book to the Almighty God for the gift of Life and to my Parents for their continuous support and prayers.

ACKNOWLEDGEMENT

I express profound gratitude to God for granting me the grace and to conduct this research.

To my supervisor Dr (Mrs) Upe Babaiwa, I mustn't fail to thank her for her time, knowledge and immense contribution to my work. Your knowledge base is outstanding and I am forever grateful.

I want to thank my parents Mr Ifijen Dennis, Mrs Theresa Ifijen and siblings Miss Sandra Ifijen, Miss Sarah Ifijen, Miss Divine Ifijen, Master Dennis Ifijen and Miss Testimony Ifijen for their support all through Pharmacy school.

I also want to thank Dr. Allen Iboi CEO of Dreamlife/Graceville Pharmacy for your mentorship and immense support.

To the lecturers in the department of Pharmaceutical Microbiology and the entire Faculty of Pharmacy at large, that you for impacting me with the requisite skills required for my practice of the profession.

Also I want to thank Mr. John and Mr. Monday for their assistance in making this project a reality.

To my project colleagues in the Department of Pharmaceutical Microbiology Biotechnology: Ezekiel, Uyiose, Oge, Isaac, Alex. Working with you guys was exciting and educating thanks also for your support.

Table of Contents

ABSTRACT

Multi-Drug Resistance developed by pathogens to most of the antibiotics present in the world today has led to an increase in the interest on plant derived compounds as substitute.

In this study the antimicrobial activity of ethyl acetate extract of *Chrysophyllum albidum* cotyledon was determined, as well as the phytochemical constituents, proximate parameters and chemical constituents of *C. albidum* cotyledon.

The antimicrobial activity of the seed extract was evaluated against clinical isolates (*Stapyhlococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtili*s, *Klebsiella spp, Aspergillus nigans)* using agar-well diffusion method, Proximate analysis and phytochemical studies were carried out using standard method as described by Association of Official Analytical Chemist(AOAC).

A two phase (liquid:oil) brown coloured extract with a yield of 2.530% was obtained. The extract inhibited the growth of all test bacteria with an inhibition zone diameter (IZD) of 25mm – 20.5mm.

The phytochemical result revealed the presence of alkaloids, phenolic, flavonoid, tannins, terpenoids, carbohydrate, proteins, reducing sugars, phytosterol and amino acids.

Proximate analysis have shown that the cotyledons contain appreciable quantity of fat (11.67%), protein (11.09%), crude fibre (4.67%), moisture (6.67%), carbohydrate (62.34%) content and ash value (4.23%).

The ethyl acetate extract of *C. albidum* cotyledons possess antibacterial activity but lacked antifungal activity. The observed antimicrobial activities of this plant may be due to the presence of alkaloids, phenolics, tannins and flavonoids.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.0 INTRODUCTION

Antimicrobials are agent that kills micro-organisms or inhibit their growth. Antimicrobial agents have been used for over 40 years (Dada-Adegbola *et al*; 2014). The studies of medicinal plants used in folklore remedies have attracted the attention of many scientists in finding solutions to the problems of multiple resistances to the existing synthetic antibiotics. Most of the synthetic antibiotics now available in the market have major setback due to the multiple resistance developed by pathogenic micro–organisms against these drugs (Akinpelu *et al*; 2008). It is a well-established fact that some plants possess antimicrobial properties such as the activity of *Cremaspora triflora* and *Masea lanceolata* leaf extract against Gram-positive organisms (Ishaku *et al*; 2017). Many plants in Africa have been found to have antimicrobial properties e.g *Amaranthus spinosus*.

Africa star apple (*Chrysophyllum albidum*) is a medicinal plant that belong to the Sapotaceae family which has up to 800 species and make up almost half of the order Ericales(Ehiagbonare et al 2008). The generic name is derived from the Greek word Chrysos meaning ‘gold' and Phyllos meaning ‘leaf'(Quattrocchi; 2000). The genus is native to tropical regions throughout the world, with the greatest number of species in Northern South America. *Chrysophyllum albidum*

is a dominant canopy tree of lowland mixed rain forest; it is primarily a forest tree species. It is widely distributed from West Africa to the Sudan with an eastern limit in Kakamega forest, Kenya (Quattrocchi; 2000). It is distributed throughout the Southern parts of Nigeria. The plant often grows to a height of 36.5m though it may be smaller and the stems are often branched and buttress at the base. It flowers in January to February. The fruits are sub-spherical in shape, about 3cm in diameter, usually 5 celled and contain an edible sweet fruit pulp. The skin or peel is orange to golden yellow when ripe and the pulp within the peel may be orange, pinkish or light yellow and within the pulp are at least five seeds which are not eaten (Oladipo *et al*; 2015).

Fig 1: C. albidum fruit.

Fig 2: C. albidum seeds.

Table 1:1 Scientific classification

Kingdom	Plantae
Division	Angiosperm
Class	Eudicots
Order	Ericales
Family	Sapotaceae
Genus	Chrysophyllum
Species	*C. albidum*
Local names	*Agbalumo* (Yoruba), *Udara* (Igbo), *Khada* (Hausa)

Extracts from different parts, including the barks, leaves, roots and seeds of *C. albidum* have been used for the treatment of different ailments, such as yellow fever, malaria, certain skin diseases, stomach ache, diarrhoea, vaginal and infertility problems as well as dermatological and urinary related infections.

The extracts have also found use as liniments and in stopping microbial growth in open wounds. The extracts of the leaves and fruits using different solvents of varying polarity have shown antimicrobial and antioxidant properties in-vitro and in-vivo (Oputah *et al;* 2016).

The fruit of *Chrysophyllum albidum* has been found to have a very high content of ascorbic acid with 1000 to 3,300mg of ascorbic acid per 100g of edible fruit or about 100 times that of oranges and 10 times that of guava or cashew (Amusa *et al,* 2003). The plant could be employed as a source of natural antioxidant boosters in the treatment of some oxidative stress disorders in which free radicals have been implicated (Orijajogun *et al,* 2013).

The cotyledons are used as a remedy for malaria and yellow fever, while the leaves are used as palliative for the treatment of dermatological problems, stomach ache and diarrhoea (Idowu *et al*, 2006). Studies suggest the cotyledons within the hard seed coat are useful as unguents for the treatment of vagina infections (Akubugwo and Ugbogu, 2007).

Most of the documented antimicrobial activities *C. albidum* seeds have been based on the extraction with Water or Methanol (George Oladije A *et al* 2018). Others employed the use of partition ethyl acetate and n-hexane or other organic solvent.

1.1 EXTRACTION TECHNIQUES

Extraction is the process of isolation of active ingredients or active substances or active medicaments from raw materials of either plants or animals or directly from the natural sources with the help of solvent is called extraction or the

removal of desired soluble constituents from a substance leaving out those which are unwanted with the aid of solvent.

Methods used in extraction process are Infusion, Decoction, Maceration, Percolation and Digestion. Some of the solvents used in extraction process are Water, Alcohol, Ether, Ethyl acetate, Chloroform and Light petroleum

1.1.1 MACERATION

In this process solid materials are placed in a stopper container with the whole of the solvent and allowed to stand for at least 3 days (3-7days) with frequent agitation, until soluble matter is dissolved. The mixture is then strained (through sieves/nets), the marc pressed and combined liquid clarified (cleaned by filtration) or by decantation after standing. Stopper container is usually taken to reduce the loss of solvents by evaporation. If the volume of solvent is reduced by evaporation the extract may become concentrated which may not be desired.

1.2 DRUGS ISOLATED FROM PLANTS

Drug discovery from medicinal plants led to the isolation of early drugs such as cocaine, codeine, digitoxin, and quinine, in addition to morphine, of which some are still in use (Newman et al., 2000; Butler, 2004; Samuelsson, 2004).

Artemisinin Comibination Therapy (ACT) drugs which includes Artesunate, Anteether, Antemether and Dihydroartemisinin are potent antimalarial drugs and is derived from artemisinin, a sesquiterpene lactone isolated from *Artemisia annua* L. (Asteraceae), a plant used in traditional Chinese medicine (TCM) (Graul 2001).

Galantamine is a natural product discovered through an ethno botanical lead and first isolated from *Galanthus woronowii* Losinsk. (Amaryllidaceae) in Russia in the early 1950s, It is approved for the treatment of Alzheimer's disease, slowing the process of neurological degeneration by inhibiting acetyl cholinesterase (AChE) as well as binding to and modulating the nicotinic acetylcholine receptor (nAChR) (Heinrich and Teoh, 2004; Pirttila et al., 2004).

Tiotropium has recently been released to the United States market for treatment of Chronic Obstructive Pulmonary Disease (COPD), It is an inhaled anti-cholinergic bronchodilator, based on ipratropium, a derivative of atropine that has been isolated from *Atropa belladonna* L. (Solanaceae) and other members of the Solanaceae family (Barnes et al., 1995; Dewick, 2002; Mundy and Kirkpatrick, 2004). Tiotropium has shown increased efficacy and longer lasting effects when compared with other available COPD medications (Barnes, 2002; Mundy and Kirkpatrick, 2004).

Nitisinone is a medicinal plant-derived drug that works on the rare inherited disease, tyrosinaemia, demonstrating the usefulness of natural products as lead

structures (Frantz and Smith, 2003). Nitisinone is a modification of mesotrione, an herbicide based on the natural product leptospermone, a constituent of *Callistemon citrinus* Stapf. (Myrtaceae) (Hall et al., 2001b; Mitchell et al., 2001). All three of these triketones inhibit the same enzyme, 4-hydroxyphenylpyruvate dehydrogenase (HPPD), in both humans and maize (Hall et al., 2001b; Mitchell et al., 2001). Inhibition of the HPPD enzyme in maize acts as an herbicide and results in reduction of plastoquinone and tocopherol biosynthesis, while in humans the HPPD enzyme inhibition prevents tyrosine catabolism and the accumulation of toxic bioproducts in the liver and kidneys (Hall et al., 2001b).

Anticancer agents from plants currently in clinical use can be categorized into four main classes of compounds: vinca(or Catharanthus) alkaloids, epipodophyllotoxins, taxanes, and camptothecins. Vinblastine and vincristine were isolated from *Catharanthus roseus* (L.) G. Don (Apocynaceae) (formerly *Vinca rosea* L.) have been used clinically for over 40 years (van Der Heijden et al., 2004). The vinca alkaloids and several of their semi-synthetic derivatives block mitosis with metaphase arrest by binding specifically to tubulin resulting in its depolymerization (Okouneva et al., 2003). Podophyllotoxin was isolated from the resin of *Podophyllum peltatum* L. (Berberidaceae) but was found to be too toxic in mice so derivatives were made with the first clinically approved drug being etoposide (Gordaliza et al., 2004). The epipodophyllotoxins bind

tubulin, causing DNA strand breaks during the G2 phase of the cell cycle by irreversibly inhibiting DNA topoisomerase II (Gordaliza et al., 2004). Paclitaxel was originally isolated from *Taxus brevifolia* Nutt. (Taxaceae) and was clinically introduced to the U.S. market in the early 1990s (Wall and Wani, 1996; Oberlies and Kroll, 2004). The taxanes, including paclitaxel and derivatives, act by binding tubulin without allowing depolymerisation or interfering with tubulin assembly (Schiff et al., 1979; Horwitz, 2004). Camptothecin was isolated from *Camptotheca acuminata* Decne. (Nyssaceae) but originally showed unacceptable myelosuppression (Newman, 2004). Interest in camptothecin was revived when it was found to act by selective inhibition of topoisomerase I, involved in cleavage and reassembly of DNA (Cragg and Newman, 2004). Together, the taxanes and the camptothecins accounted for approximately one-third of the global anticancer market in 2002, over 2.75 billion dollars (Oberlies and Kroll, 2004). Numerous derivatives of all four compound classes have been synthesized, some of which are currently in clinical use. All of these natural products have led to significant biological discoveries related to their unique mechanisms of action.

1.3 MEDICINAL PLANTS USED AS ANTIMICROBIAL AGENT

The Onion (*Allium cepa*) which is also known as the bulb onion, common onion and garden onion is the most widely cultivated species of the genre

Allium. Wide ranging claims have been made for the effectiveness of onions against conditions ranging from the common cold to heart diseases, diabetes, osteoporosis and other diseases. They contain compounds believed to have inflammatory, anticholesterol, anticancer and antioxidant properties such as quercetin. Preliminary studies have shown that increased consumption of onions reduces the risk of head and neck cancers (Parmar and Rawat, 2012). Asian onions have the most eye irritating chemical reaction. Half bake onions is used in Malta and Bulgaria traditional cure for urchin wounds and inflammation in finger and fingernails respectively by tying the onion around the affected part.

Allium sativum (Lilicaceae) which is commonly known as garlic have been found to have antimicrobial, antiviral and antifungal activities. In 1858, Louis Pasteur observed garlics antibacterial activivty and it was used as an antiseptic to prevent gangrene during World War I and World War II. More recently it has been found from a clinical trial that a mouthwash containing 2.5% fresh garlic shows good antimicrobial activity, although the majority of the participants reported an unpleasant taste and halitosis (Parmar and Rawat, 2012). It has been found to enhance thiamine absorption and therefore reduces the likelihood for developing the thiamine deficiency beriberi (Ried *et al*, 2010)

Carica papaya belongs to the family Caricaceae. It has the following names; pawpaw, pawpaw tree, papaya, papayer, tinti, pepol, chich put. The plant is

also described in a documented property forms and it acts as an analgesic, amebicide, antibacterial, cardiotonic, cholagens, digestive, emanagogue, febrifuge, hypotensive, laxative, pectoral, stomachic and vermifuge. *Carica papaya* conatins two important compounds chymopapain and papain, which are supposed to aid in digestion. Papain is used in the treatment of arthritics. The young leaves and to a lesser degree other parts contain carpain an active bitter alkaloid which has a depressing action on heart. The plant is a strong amoebicide(Parmar and Rawat, 2012).

1.4 CHEMICAL CONSTITUENTS OF *C.ALBIDUM*

The sweet fleshy fruit is an excellent source of vitamin C, iron and is used as a thichkener, flour and raw materials for some manufacturing industries. The plant could be employed as a source of natural antioxidant boosters in the treatment of some oxidative stress disorders in which free radicals have been implicated (Orijajogun *et al*, 2013).

1.5 ANTIOXIDANT

The identification of antioxidant from natural products has become a matter of great interest in recent studies for it cell protective role in nullifying the destructive effects of reactive oxygen species (ROS) (Liao et al., 2012).

Antioxidants are compounds that inhibit the chemical reaction that can produce free radicals, thereby leading to chain reaction that can damage the cells of humans. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes(e.g., catalase and superoxide dismutase), produced internally, or supplied exogenously through dietary antioxidants such as Vitamin C and Vitamin E (Dabelsteinn, *et al* 2007). Thus antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Falodun et al., 2011).

Extensive studies has been carried out to demonstrate the use of antioxidants in important industrial processes; such as the prevention of mental corrosion, the vulcanization of rubber and the polymerization of fuels in the fuelling of internal combustion engines (*Valko et al*., 2017).

Antioxidant activity could be measured simply by placing the fat in a closed container with oxygen and measuring the rate of oxygen consumption. However, it was the identification of vitamin A, C and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in the biochemistry of living organisms (Vertuani *et al* 2004).

Thus antioxidants are radical scavengers which protect human body against free radicals that can cause pathological condications such as Ischemia, anemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson disease, mongolism, aging process and perhaps dementias. Majority of active

antioxidants of plant origin are flavonoids, flavones, isoflavones, anthiocyannins, cumarins, lignans, catechins, phenols and isocatachins (Makari *et al*., 2008).

1.6 ANTIMICROBIAL SUSCEPTIBILITY TEST

Antimicrobial susceptibility test (AST) is used to determine the efficacy of potential antimicrobials, from biological extracts against a number of different microbial species. AST methods are used to screen plant extracts for antimicrobial activity but are largely used to determine the usefulness of an antimicrobial in combating infections by determining its minimum inhibitory concentration (MIC).

The MIC is the lowest concentration that under defined in Vitro conditions prevents the growth of bacteria within a defined period of time. It is expressed in mg/L or mg/ml. In clinical research in vitro susceptibility are particularly important if an organism is suspected to belong to a species that has shown resistance to frequently used antimicrobial agents. They are also important in epidemiological studies of susceptibility and in comparison of new and existing microbial agents (EUCAST, 2000).

AST standard test can be conveniently divided into diffusion and dilution methods. Common diffusion tests include agar-well diffusion and bio-

autography; while dilution methods are the conventional reference methods for AST (Tenover *et al*., 1995). The agar-disk diffusion technique can only be used for AST of pure substances because when it is applied to mixtures containing constituents, which exhibit different diffusion factors, results may be unreliable (Silva *et al.,* 1996). In this method 6mm paper disk is saturated with filtered, sterilized plant extracts at the desired concentration are placed onto suitable solid agar medium pre inoculated with the test organism. The plates are then incubated at appropriate temperature for 24hours. Zones of inhibitions are measured from the circumference of the disk to the circumference of inhibition zone or recorded as the difference in diameter between the disk and the growth free zones around the disk (Silva *et al.*, 1996).

In agar-well diffusion method, standardized inoculum culture is spread evenly on the surface of gelled agar plate. Wells of between 6 and 10mm are aseptically punched on the agar using sterile cork borer allowing at least 30mm between adjacent wells. The plates are incubated at appropriate temperature for 24 hours. Zones are also measured in millimetres. Extracts with zone of inhibition in the range of 24-26 mm are said to show high antibacterial activity, while zones between 15-18 mm is defined as intermediate in activity (kyung *et al*., 2003)

Another specific parameter often used in AST is minimum bactericidal concentration (MBC). This is the lowest concentration of an antimicrobial agent,

expressed in mg/L, mg/mL or mg/L which under defined in vitro conditions reduces by 99.9% (3 logarithms) the number of organisms in a medium containing a defined inoculum of bacteria within a defined period of time. The reduction is usually expressed as the proportion of inoculum (number of living colony forming units (CFU) introduced) that is rendered incapable of reproduction on subculture within that period. The effect can be presented as a time-kill curve, in which an inoculum is incubated with the antimicrobial agent and samples are tested for numbers of surviving CFUs at defined time interval.

1.6 JUSTIFICATION

The emergence of multidrug resistant micro-organism has rekindled interest on plant derived compounds as substitutes for synthetically produced antimicrobial agents. Although the antimicrobial properties of African star apple have been investigated most studies have been base on the ethanol and aqueous extract but not much have been done on the ethyl acetate extract.

1.7 OBJECTIVES

This study aims to evaluate the antimicrobial property of *C. albidum* cotyledon using ethyl acetate as solvent for extraction.

1.8 AIMS

- To determine the antimicrobial activity of C. *albidum* seed extract against selected organism and fungi.
- To determine the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentrations (MFC) of susceptible isolates.
- Evaluate/Carry out proximate analysis on powdered cotyledon (*C. albidum*).
- Determine chemical constituent responsible of extract using the GC-MS

CHAPTER TWO

MATERIALS AND METHODS

2.0 MICROBIOLOGICAL MEDIA

Nutrient Agar (Titan Biotech Ltd, India), Nutrient broth (Titan Biotech Ltd, India), Sabouraud Agar (Lifesave Biotech, USA)

2.1 EQUIPMENT

Hot air oven size two (2) (Gallenkamp, UK), Autoclave (model YX-2803, Lincoln Mark Medical, England), furnace, Rotary Evaporator (Stuart/Julabo F10, United Kindgom), pH meter, Abbe refractometer, spectrophotometer (Spectrum lab 23A, China) and weighing balance.

2.2 GLASSWARE

Petri dishes, Beakers, conical flask, test tubes, pipettes, measuring cylinders, bottles (MacCartney, Universal and Bijou), glass funnels, maceration jar, glass stirrer and burettes, viscosity bottles.

2.3 REAGENTS AND CHEMICALS

Analytical grade Ciprofloxacin powder (by Sigma Aldrich), Fluconazole manufactured (by Biochemika India), ethyl acetate, Methylated spirit, Dragendorff's reagent, Wagner's reagent, Hager's reagent, Mayer's reagent, 1% alcoholic naphthol, sulphuric acid, Benedict's reagent, 20% sodium hydroxide, acetic anhydride, 1% gelatin solution, 10% Sodium hydroxide, 5% ferric chloride, hydrochloric acid, lead acetate, chloroform, petroleum ether, ammonia solution, nitric acid, ninhydrin solution, aluminium chloride, folin solution.

2.4 OTHER MATERIALS

Micro-pipette, cotton wool, cork borer (10mm in diameter), izal, methylated spirit, bunsen burner, sterile swab sticks and sun dried seeds.

2.5 TEST MICRO-ORGANISMS

Clinical isolates of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella aerogenes, Yeast* and *Aspergillus niger* obtained from the University Of Benin Teaching Hospital, Benin-City, Edo State.

2.6 PREPARATION OF CRUDE EXTRACT

The fresh fruits of *Chrysophyllum albidum* used for this study were collected and identified by Dr. Mrs Babaiwa, Head of Department (H.O.D.), Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, University of Benin, Benin City.

The fruits were cleaned with water, seeds were extracted, rinsed and sun dried for a couple of weeks. The seeds were pulverized using a Phillip kitchen blender, after which 500g was macerated with one and a half litres (1.5L) of ethyl acetate for 72 hours. During this period, the solution was stirred at intervals to allow a proper permeation extraction solvent into the cotyledon. After 72 hours the content in the maceration jar was filtered using what man filter paper No 42 (125mm). The extract was concentrated using a rotary evaporator which was weighed and kept at 4^0C in an airtight container unt il when required for use.

2.7 AUTHENTICATION OF MICRO-ORGANISMS

All test micro-organisms were authenticated by Gram-staining techniques (based on their colour, arrangement, shape and colour of dye retained) and biochemical reactions (based on specific enzymes present in different organisms).

2.8 STANDARDIZATION OF TEST MICRO-ORGANISMS

Test micro-organisms were sub-cultured on freshly prepared Nutrient agar plates for bacteria and Sabouraud Dextrose agar for fungi, and incubated for 24hrs at 37^0C for bacteria and for 48 hours at room temperature for fungi respectively. Portions of the streaked bacterial and fungal colonies were transferred into test tubes containing 8ml of sterile nutrient broth and incubated for 12 hours and 48 hours at 37^0C respectively. The growth of bacterial and fungal suspension obtained was compared to that of freshly prepared Barium sulphate opacity standard (0.5mL of 1% barium chloride to 99.5mL of 1% Sulphuric acid (0.36 Normal). The turbidity was adjusted by adding more sterile nutrient broth to match 0.5 McFarland standards (10^{-6}CFU/mL and 10^{-6}spores/mL).

2.9 PREPARATION OF STOCK SOLUTIONS

Ciprofloxacin stock solution of 0.5mg/ml was prepared in 10% DMSO solution. Fluconazole 2mg/mL was used as stock solution. The extract was used directly.

2.10 ANTIMICROBIAL SUSCEPTIBILITY TESTS

Antimicrobial susceptibility was carried out using the agar well diffusion method. Sterile nutrient agar which was prepared in an autoclave was poured (30mL each) into sterile Petri dishes and allowed to set. The Petri dishes were then dried in a hot air oven for about 10minutes at 40^0C. The dried plates were then streaked with the test microorganism with the aid of a sterile swab stick. With the aid of a sterile cork borer, four wells were bored into the agar plate and the base sealed with 2 drops of molten nutrient agar. Two of the wells were filled with 200µL of the extract while the other two holes were filled with standard Ciprofloxacin.

The procedure was repeated with Sabouraud Agar and standard fluconazole was used. The plates were all incubated in an upright position at 37^o C for 18 - 24hours for bacteria and at room temperature for 48 hours for fungi.

The inhibition zone diameters were measured in millimetres as an index of the killing or inhibitory action of the extract or standard against the test organism.

2.11 DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

The agar dilution method of Afolayan and Meyer (1997) was used for the determination of MIC of the extracts, ciprofloxacin and fluconazole on the susceptibility of microorganisms. The nutrient agar and sabouraud agar were prepared following the manufacturer's instructions and placed in water bath at 50^0C. The stock solution of extract was filtered and incorporated into the molten nutrient agar and sabouraud agar at different volumes to obtain a range of concentrations between 25 mg/mL - 400mg/mL. The agar extract, agar ciprofloxacin/ fluconazole mixtures were poured into separate sterile plates and allowed to set. The micro-organisms were streaked into the solidified agar plates. Negative and positive controls were set up for each of the experiments. All plates were incubated at upright position for 18-24 hours at 37^0C and at 25-28^0C temperatures for 48 hours. All experiments were done in triplicates.

The MIC was defined as the lowest concentration of extract, Ciprofloxacin and fluconazole that inhibited growth of test micro-organisms.

2.12 DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATION (MBC) AND MINIMUM FUNGICIDAL CONCENTRATION (MBC).

Sample were taken from the culture plates with no visible growth in the MIC test and plated out and incubated at 37^0C for 24 hours for bacterial culture plates and 25-28^0C temperatures for 48 hours for fungal culture plates. After the incubation, the plates were observed for growth. MBC/MFC was defined as the lowest concentration of extract and standard antimicrobial agent that showed no growth of test isolates.

2.13 PRELIMINARY PHYTOCHEMICAL ANALYSIS

Approximately 5g of the powdered cotyledon of *C. albidum* was weighed into a conical flask and boiled with 50ml of distilled water for 30 minutes. The solution was filtered What man filter paper while still hot and allowed to cool. The filtrates obtained were screened for the presence of alkaloids, tannins, saponins, anthraquinones, flavonoids and phenolic compounds.

2.13.1 General Tests for Alkaloids

Approximately 2 drops of Dragendorff's reagent was added to 2mL of filtrate and the formation of a reddish brown precipitate was recorded as positive outcome for the presence of alkaloids. Other test for alkaloid carried out are Wagner's, Hager's and Mayer's where the formation of brown, yellow and bulky precipitates respectively were considered as positive result for the test of alkaloids.

2.13.2 Tests for Carbohydrates

2.13.2.1 Molisch Test

Exactly Two (2) mL of filtrate was added to 2 drops of 1% alcoholic napthol followed by 2mL of concentrated sulphuric acid. Observation of a violet ring at the interface of both liquid layers was considered as positive result for the presence of carbohydrate.

2.13.3 Tests for Reducing Sugars

2.13.3.1 Fehling's Test

Exactly Two (2) mL of filtrate was added two (2) drops of Benedict's reagent (A mixture of equal volumes of Fehling's solutions A and B). The resulting solution was heated over a boiling water bath for three (3) minutes.

The formation of a brick red precipitate was a positive result for the test of reducing sugars.

2.13.3.2 Tollen's Test

Dilute ammonia hydroxide was added drop wise manner to silver nitrate solution containing a few drops of 10% sodium hydroxide until the precipitate of silver oxide almost completely dissolved. A few drops of the extract were added to the mixture above. Observation of silver mirror precipitate along the side of the test tube was taken as a positive result.

2.13.4 Keller Kiliani's Test for Deoxysugars

Few drops of dilute acetic acid containing a trace of 5% ferric chloride was added to 2mL of filtrate. The resulting mixture was transferred to the surface of concentrated sulphuric acid.

Formation of a violet ring at the interface of the two liquid layers is indicative positive test for deoxysugars.

2.13.5 Test for Saponins

2.13.5.1 Frothing Test

Exactly One (1) mL of filtrate was diluted with 10ml distilled water and shaken vigorously for one minute. Observation of persistent frothing was recorded as positive result for presence of saponins.

2.13.6 Test for Tanins

2.13.6.1 Gelatin Test

Exactly two (2) Ml of filtrate sample was added 2mL of 1% gelatine solution in 10% NaCl and formation a cloudy precipitate was recorded as positive result for tannins.

2.13.7 Test for Phenolic compounds

2.13.7.1 Ferric chloride Test

Exactly two (2) mL of filtrate was added 5mL of distilled water followed by 2 drops of 5% ferric chloride solution. A blank test was done by adding 2 drops of 5% ferric chloride solution to 5mL of distilled water. Presence of the formation of intense colouration in the test tube was recorded as positive for the presence of phenolic compounds.

2.13.7.2 Folinciocalteu's Test

Approximately 5mL of filtrate was added 0.5mL 10% folinciocaulteu's phenol reagent followed by 5mL of 7% Na_2CO_3 and observation of intense purple colour as recorded as positive result for the presence of phenolics.

2.13.8 Test for Flavonoids

2.13.8.1 Shinoda test

A little amount of magnesium powder and a few drops of concentrated Hydrochloric acid were added to 1mL of the filtrate. Observation of a red colour is recorded as a positive result for the presence of Flavonoids.

2.13.9 Test for Terpenoids

2.13.9.1 Salkowski Test

Exactly Five (5) Ml of the filtrate was mixed with 2mL of chloroform and a few drops of concentrated sulphuric acid were carefully added to form a layer.

Observation of reddish brown colouration was recorded as positive result for the presence of Terpenoids.

2.13.10 Test for Anthraquinone Derivatives

2.13.10.1 Bontreger's Test

Exactly Two (2) mL of filtrate was shaken with 2mL of petroleum ether. The ether layer was washed with 2mL distilled water and shaken with dilute ammonia solution. Observation of a pink colour was observed on addition of ammonia solution was recorded as a positive result for test for anthraquinones.

2.14 PROXIMATE ANALYSIS

2.14.1 Ash content determination

The total ash content was determined according to the standard method, (AOAC, 2000). Three clean dried crucibles were labelled 1–3 and 1g of powdered seeds was weighed into each crucible. The crucibles with their content were heated in the Gallenkamp muffle furnace 2 at 600^0C for 4 hours or until light grey residue

was obtained. The Gallenkamp muffle furnace was switched off, allowed to cool and all crucibles were removed, cooled in a desiccator and reweighed. The percentage ash was calculated.

2.14.2 Moisture content

Moisture content was determined using weight difference method (AOAC, 2000; Anklam *et al* 2001). The weight of the dry sterile crucible was taken after cooling in a desiccator. Two grams (2g) of powdered sample were weighed into three (3) pre-weighed evaporating crucibles. The crucibles containing the samples were reweighed before placing them in a hot air oven at 105^0C temperature for 3 hours. After drying, the crucibles and the content were allowed to cool in a desiccator containing silica gel. The crucible and dried samples were weighed. The weight of the sample, the moisture content loss and the percentage moisture content were then determined and recorded.

2.14.3 Determination of crude fibre content

Percentage of crude fibre was determined by the method, (Udo, Ogunwele, 1988), in which 2g of coarse sample was weighed into a $1dm^3$ conical flask. Water ($100cm^3$) and $100cm^3$ of 1.25% of H_2SO_4 were added and boiled gently for 30 minutes. The content was filtered through what men no. 1 filter paper. The residue was scrapped back into the flask with a spatula and $100cm^3$ of

1.25% NaOH were added and allowed to boil gently for 30 minutes. The content was filtered and residue was washed thoroughly with hot distilled water, and then rinsed once with 1; 1 Ethanol and acetone to neutrality. It was allowed to dry and scrapped into the crucible and dried to consent weight at 104 water, then rinsed once with 1; 1 Ethanol and acetone to neutrality. It was allowed to dry and scrapped into the crucible and dried to consent weight at 105^0C in an air oven. It was then removed and cooled in a dessicator. The sample was weighed (W_1) and ashed at 300^0C for 90 minutes in a Gallen kamp muffle furnace. It was finally cooled in a dessicator and weighed again (W_2). The percentage crude fibre was calculated using;

% crude fibre

Where;

W_0 = Weight of sample, g

W_1 = Weight of dried sample, g

W_2 = Weight of ash sample,

2.14.4 Determination of crude protein content

The crude protein of the sample was determined using the micro- Kjeldahl method described by AOAC (1990). The principle of this method is based on the transformation of protein and that of the other nitrogen containing organic compounds, other than nitrites and nitrates into ammonium sulphate by acid digestion.

Sample nitrogen + H_2SO_4 (aq) ---- catalyst $(NH_4)_2SO_4$ (aq)

$(NH_4)_2SO_4$ (aq) + 2NaOH (aq) ---- $2NH_3$ (aq) + H_2O + Na_2SO_4 (aq)

NH_3 (aq) + $2H_3BO_3$ (aq) ---- NH^{+4} (aq) + H_2BO^{-3} (aq)

H^+ (aq) + H_2BO^{-3} (aq) --- H_3BO_3 (aq)

About 1.0g of the homogenized peel was weighed into Kjedahl flask. To the sample was added a 1: 2 mixture of perchloric acid and H2SO4. The sample was left to settle for 20 minutes and then transferred to a digester for one hour till sample turned colourless. The sample was then left to cool under fume-cupboard. The digest was distilled in a 500 ml flask using 100 mL distilled H2O, 50 mL NaOH and selenium tablet. The flask was immediately covered and placed in a condenser. Attached to the condenser was a conical flask containing 50 ml boric acid which changed from red to green and volume was made up to 100 mL. The solution was then titrated using phenolphthalein indicator against standardized sulphuric acid till the colour turned to red. From the entire procedure, the % Nitrogen was calculated.

2.14.5 Carbohydrate content

The nitrogen free extract which represent the digestible carbohydrate was determined by difference. The value was obtained by subtracting the sum of the

percentage of moisture, crude fibre, crude fat and ash from 100. The energy values (Kcal/100g) were determined by multiplying the values of carbohydrate, lipid and protein by factors 4, 9 and 4 respectively and the total sum expressed in kilocalories (Imran et al 2007). All proximate values were carried out in triplicates and expressed in percentage (Hussain et al 2009a, b and 2010 a, b and c).

2.15 GC-MS analysis

GC-MS analysis was carried out on GC-MS QP2010SE Shimadzu, Japan System. The carrier gas was helium with a constant flow rate of 3.33mL/min. The inlet temperature was maintained at 250^0CThe oven temperature was programmed initially at 60^0C for 3-4 minutes, and then ramped at 12^0C/min for to increase to 240^0C. The temperature was gradually kept at 60^0C for at a rate of 10^0C ending with 25 minutes. Total run time was 45 minutes. The MS transfer line was maintained at a temperature of 300^0C. The source temperature was maintained at 230^0C and the MS Quad at 150^0C. The ionization mode was used when electron ionization model at 70eV. Total Ion Count (TIC) was used to evaluate for compound identification and quantization. The spectrum of the separated compound was compared with the database of the spectrum of known compound saved in the NIST02 Reference Spectra Library. Data analysis and

peak area measurement was carried out using Agilent chemstation and Pherobase Software in comparison with data reported in literature.

2.16 STATISTICAL ANALYSIS OF VARIANCE

Data generated from antimicrobial sensitivity test (inhibition zone diameter, IZD) would be presented in mm +/- standard error of mean, while MIC and MBC values will be presented as mg/ml for extract and ug/ml for standard drug. IZD and MIC values for extract and standard drug against each isolate were subjected to one - way Analysis of Variance (ANOVA) at $p = 0.05$ level of significance

CHAPTER THREE

RESULTS

3.1 Percentage yield of extract

The percentage yield of the ethyl acetate extract of *C. albidum* cotyledon and physical properties are showed in Table 3.1

3.2 Antimicrobial susceptibility test

A positive result for this test is determined by the presence of an inhibitory zone (inhibition zone diameter) on seeded agar plate. The inhibition zone diameter (IZD) is measured in millimetres (mm) as an index of the killing or inhibitory action of the test agent against a given organism.

In this study all of the micro-organisms used were susceptibility to the killing or inhibitory effect of ethyl acetate extracts and all the test micro-organisms were susceptible to the standard drug as showed in Table 3.2.

3.3 Minimum inhibitory concentration

The minimum inhibitory concentration for all the organisms used was 25mg/ml as shown in Table 3.3.1

The MBC results of the test extract as shown in Table 3.3.1 shows that at a concentration of 50mg/mL *Staphylococcus aureus, Klebsiella aerogenes, Bacillus subtilis Escherichia coli and Aspergillus niger s*howed no growth on sub-culturing the MIC plates on fresh agar plates.

3.4 Gas Chromatography – Mass Spectrometry (GC-MS) results

The GC-MS results show the presence of a total of 17 components with 5-7 major components

Table 3.1: Physical properties and percentage yield of ethyl acetate extract of *C. albidum* cotyledon extract.

PARAMETERS	VALUES
Solvent	Ethyl acetate
Extract texture	Oily
Colour	Brown
Percentage yield (w/w)	2.530%

Table 3.2: Susceptibility test of ethyl acetate extract of *C. albidum* cotyledon extract

Test Micro-organisms	Ethyl acetate extract (mm)	Ciprofloxacin (mm)	Fluconazole (mm)
K. spp	23.22±0.13	22±0.90	NA
S. aureus	25.10±2.28	25±0.94	NA
B. subtilis	20.50±1.87	32±0.50	NA
E. coli	24.50±0.76	34±0.50	NA
P. aeruginosa	24.75 ±1.89	32±0.50	NA
Candida albican	NZ	NA	23±0.6
Aspergillus nigan	NZ	NA	23±0.6

KEY

NA No Activity

NZ No Zone

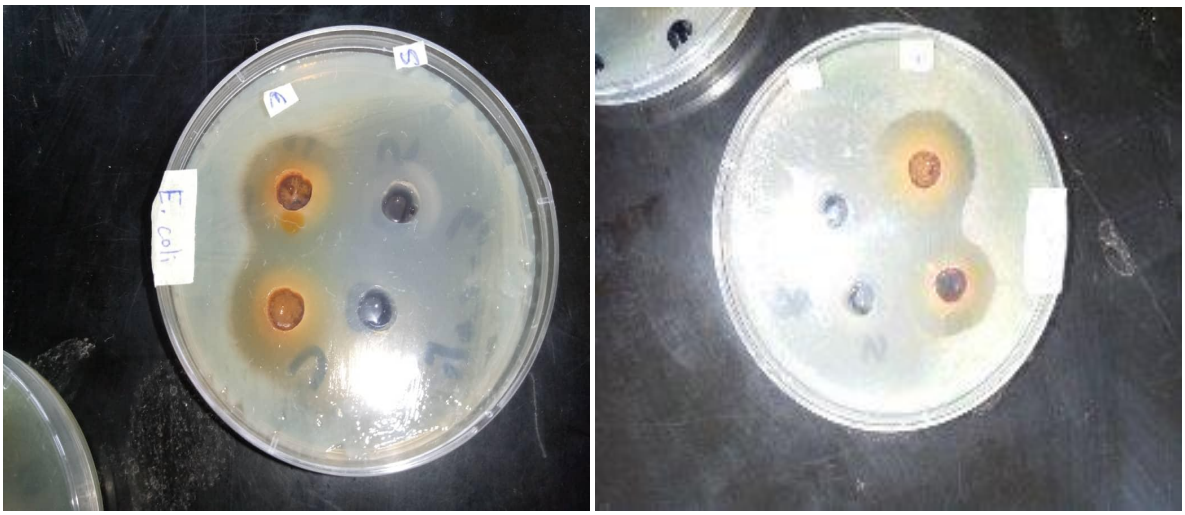

Fig 3: Susceptibility result for E. coli and B. subtilis respectively.

Table 3.4. Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Test Micro-organisms	Ethyl acetate extract (mg/ml)		Ciprofloxacin (µg/ml)	
	MIC	**MBC**	**MIC**	**MBC**
Eschericia coli	25	50	0.02	0.02
Klebseilla spp	25	50	0.02	0.02
Staphylococcus aureus	25	50	0.02	0.02
Bacillus subtilis	25	50	0.02	0.02
Pseudomonas aeruginosa	25	50	0.02	0.02

Table 3.5: Proximate analysis of powdered *C. albidum* cotyledon

PROXIMATE PARAMETERS	VALUES IN PERCENTAGE
Moisture content	6.67%±0.021
Total Ash	4.23%± 0.003
Crude Fibre Content	4.67%± 0.67
Crude Protein	11.09%±0.33
Ether Extract/Crude fat content	11.67%±0.008
Carbohydrate Content	62.34%± 0.0001

Table 3.6: Preliminary phytochemical constituents of powdered *C. albidum* cotyledon

PHYTOCONSTITUENTS	OCCURRENCE
Alkaloids	+
Carbohydrate	+
Flavonoids	+
Phenolics	+
Proteins	+
Reducing sugars	+
Tannins	+
Saponins	-
Amino acids	+
Phytosterol	+

KEY

+ Present

- Absent

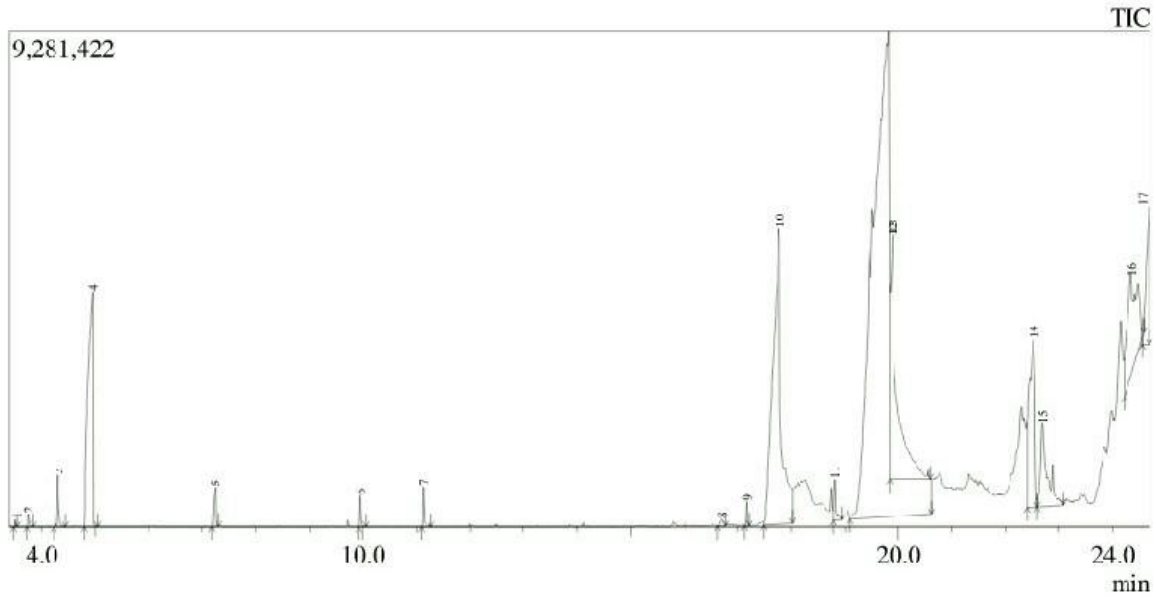

Fig. 4 **Spectra of Gas Chromatography – Mass Spectrometry (GC-MS)**

Analyzed by: $Ronald Ibia$
Analyzed: 11/9/2019 5:48:14am
Sample Type: Unknown
Level #: 1
Sample Name: BABA 4.
Sample ID: BABA 4.
IS Amount: [1]=1
Sample Amount: 1
Dilution Factor: 1
Vial #: 5
Injection Volume: 1.00
Data File: C:\GCMSsolution\Nov19\BABA 4.qgd
Org Data File: C:\GCMSsolution\Extract\BABA 4.qgd
Method File: C:\GCMSsolution\Extract\Extract SAVED 2.qgm
Org Method File: C:\GCMSsolution\Extract\Extract SAVED 2.qgm
Report File:
Tuning File: C:\GCMSsolution\System\Tune1\A111019.qgt
[Comment] BABA 4.
Modified by: Admin
Modified: 11/17/2019 11:09:26 am

Table 3.7: Chemical constituents of ethyl acetate extract of *C. albidum* cotyledon

Peak number	Compound	Retention time (minutes)	Peak area (%)
1	Propanoic acid	3.511	0.03
2	Propanoic acid	3.744	0.08
3	3-Penten-2-one	4.302	0.34
4	2-Pentanone	4.954	6.00
5	Ethanol	7.232	0.39
6	Acetamide	9.954	0.20
7	Formic acid	11.124	0.27
8	Eicosanoic acid	16.695	0.13
9	Hexadecanoic acid	17.162	0.15
10	n-Hexadecanoic acid	17.765	13.27
11	9-Octadecenoic acid (Z)	18.812	0.43
12	Oleic Acid	19.894	54.65
13	cis-9-Hexadecenal	19.896	8.68
14	4, 4, 6a, 6b, 8a, 11, 11, 14b-Octamethyl-	22.520	5.12
1,4,4a,5			. 15
2-Propen-1-amine	22.691	2.86	
16	Betulin	24.350	4.99
17	Betulin	24.692	2.42

CHAPTER FOUR

DISCUSSION

4.1 Percentage yield of extracts

The colour of oil extracted was deep brown; it has a pleasant and sweet smelling odour. The oil content of the seed was 2.530% which was lesser than the literature value of ethanol, alcohol and water extractive value of 7.7 %, 7.09% and 11.67% respectively (Sam *et al*., 2008; Egharevba *et al*.,2015d), suggests that water would be a better solvent of extraction than alcohol. The difference in oil yield could be attributed to variation in genes; climate, plant species, soil condition and improper processing techniques such as prolong exposure of harvested seeds to sunlight which is capable of impairing the oil yield considerably. It can also be attributed to factors such as the extraction process employed (Mahale and Goswami-Giri, 2012).

4.2 Antimicrobial Susceptibility test

In this screening test, ethyl acetate extracts of the powdered cotyledon of *C. albidum* were found to be effective against *Stapyhlococcus aureus,*

Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, Klebsiella aeroginese but not against *Aspergillus nigans* and *Candida albicans* as showed in Table 3.2. Extract was considered to be active at a zone of inhibition diameter of > 10mm (Usman and Haruna 2005). The ethyl acetate extract showed antimicrobial activity (IZD; 20.5 – 25). The result that was obtained is similar to the work of Orijajogun *et al*, (2014), in which the ethyl acetate extract of the seed was found to be active against Escherichia *coli, Stapyhlococcus spp* and *Klebsiella spp* .

The observed antimicrobial activity of this extract may be due to the presence of secondary metabolites the cotyledon of this palnt possesses. Alkaloids, phenolics, tannins, terpenoids and flavoniods have been found to have high antimicrobial activity against *Staphylococcus aurues, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* (Compean and Ynalvez ; 2014).

It was suggested that polyphenols act possibly by binding to bacterial dihydrofolate reductase (DHFR) enzymes, inhibition of supercoiling activity of *E.coli* bacterial gyrase by binding to the ATP binding site of gyrase B and binds to bacterial DNA thereby inducing topoisomerase IV enzyme-mediated DNA cleavage and bacterial growth stasis(Sakharkar *et al* and Enwa *et al*).

Tannins are polymeric phenolic substances capable of precipitating gelatinous compounds from solution. Many human physiological activities, such as

stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions, have been assigned to tannins. Thus, their mode of antimicrobial action, as described in the section on quinones may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. The antimicrobial significance of this particular activity has not been explored. The antimicrobial properties of tannins have been reviewed. From these studies, tannins were shown to be toxic to filamentous fungi, yeasts, and bacteria. Condensed tannins have been demonstrated to bind cell walls of ruminal bacteria, thereby inducing bacterial stasis and protease activity (Omojate. *et al* 2014).

Flavoniods are synthesized by plants in response to microbial infection and these compounds have been reported to be effective against many micro-organisms (Kumar and Pandey, 2013b). The observed antimicrobial activity may be due to complexation with soluble proteins and cell wall components. Lipophillic flavonoids may also disrupt microbial cell membrane.

4.3 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The minimum inhibitory concentration (MIC) and the minimum bacteriocidal concentration (MBC) are quantitative indices used to measure the effectiveness

of crude extract against microorganisms. These indices are expressed in microgram or milligram per millilitre. In this study the MIC values obtained varied from 12.5mg/mL – 400mg/mL for the ethyl acetate extract. All the test micro-organism had a MIC of 25mg /mL. At the lowest concentration of 0.2µg of Ciprofloxacin there was no bacteria growth in any of the plates seeded with micro-organism, this is due to the pure nature of these standard antimicrobials compared to the extracts which are still in their crude form with extraneous constituents that tend to dilute the active principle and reduce its activity.

The MBC was 50mg/mL for all the test micro-organisms. The MBC is complementary to the MIC, whereas the MIC test demonstrates the lowest level of antimicrobial agent that inhibits growth, the MBC demonstrates the lowest level of antimicrobial agent that results in microbial death. Compounds with antibacterial activities are generally grouped into bacteriostatic and bactericidal agents.

Bacteristatic agents are biological or chemical agent that stops bacteria from reproducing, while not necessarily killing the organism. They limit the growth of bacteria by interfering with bacterial protein production, DNA replication or other aspect of bacteria cellular metabolism (Pankey and Sabath, 2004). Bactericidal agents kill bacteria's via one of the following mechanisms; inhibition of bacteria cell wall synthesis, irreversible inhibition of protein synthesis, Inhibition of DNA synthesis. There is not always a precise distinction

between them as seen in the MIC and MBC result obtained, as high concentrations of some bacteristatic agents are also bactericidal, whereas low concentrations of some bacterial agents are bacteristatic.

4.4 Proximate and physicochemical parameters of seed and extracts

The results of the proximate analysis of the sun dried powdered cotyledon of *Chrysophyllum albidum* is shown in Table 3.5.

The percentage moisture content of the powdered sample is 6.67% ± 0.021. The value is lower than the values reported by Damilola *et al* (2016) and Florence *et al* which were 9.39% and 28.62% respectively. The moisture content of any food is an index of its water activity which is used to measure or predict the stability and susceptibility to microbial contamination. The high moisture content of the powdered cotyledon would not enhance its storage stability by allowing mould growth and increasing moisture dependent biochemical reactions (Onimawo and Akubor 2012).

The total ash content of the sample is 4.23%± 0.003. The ash value is a measure of the mineral content in the sample. This value is higher than 1.40%breported by Florence *et al* (2015) and also higher than 2.62% and 3.80% reported by Damilola *et al* (2016) and Akubor *et al* (2013) respectively.

The percentage crude fibre content of the sample was 4.67%± 0.67. The crude fibre content of the sample was higher than value reported in the literature by Florence *et al* (2015) which was 1.36% and 2.80% and 2.96% reported by Akubor *et al* (2013) and Damilola *et al* (2016) respectively. The amount of crude fibre may influence the digestibility of the seed and may also help to maintain the normal internal distension of the intestinal tract and thus aid peristaltic movements. Fibre has been reported to prevent heart diseases, diabetes and colon cancer.

The percentage protein observed from the analysis of the sample was 11.09%±0.33. This value is lower than 13.14% reported by Damilola et *al* (2016). Protein is an essential nutrient for repair of worn out tissues and provide support for cells.

The percentage crude fat discovered from the proximate analysis was 11.67%±0.008. This is significantly higher than 0.82%, 7.80%, 9.30% reported by Damilola *et al* (2016), Florence *et al* (2015) and Akubor *et al* (2013) respectively. Fat is an important source of energy protect internal tissue and play a role in essential cell process. It also increases transport of fat soluble vitamins.

The percentage carbohydrate content in the fruit seed was 62.34%± 0.0001.

This high percentage of carbohydrate makes the fruit to be a good source of energy. The energy value obtained from the sample was moderately higher

compared to the values reported by Egharevba *et al*, 2015. The presence of carbohydrate can also suggest of its usage in dietary supplement since carbohydrate plays a significant role as the preferred energy or fuel for muscle contraction and biologic work. Carbohydrates decrease the breakdown of protein and lipids in the body and increase energy level. Thus, carbohydrate is an essential component in dietary fibre and in formulation of animal feeds (Ajayi and Ifedi, 2015).

4.5 GC-MS analysis

Using Gas Chromatography Mass Spectrometer (GC-MS), Seventeen (17) components were detected in *Chrysophyllum albidum* seed essential oil. *Chrysophyllum albidum* ethyl acetate cotyledon extract showed the presence of large 6 to 7 peaks including other minor peaks. The major peaks includes 2-Pentanone, n-Hexadecanoic acid, Oleic Acid, cis-9-Hexadecenal, 4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a, 2-Propen-1-amine and Betulin.

Oleic Acid and n-Hexadecanoic acid (Palmitic acid) belong to the Fatty acid methyl ester group. Most of the fatty acid methyl esters are known to exhibit antifungal, antibacterial or both the properties (Chandrasekran *et al*., 2011). The mechanism of antibacterial activity have been explored using diverse experimental methods to identify that they mainly target bacteria cell

membranes and interrupt crucial process involved in cellular protection and function. The membrane-lytic behaviour of fatty acids stem from their amphipathic properties, leading to overlapping sets of biophysical phenomena including membrane destabilization and pore formation. In particular, membrane –destabilizing activity causes increased cell permeability and cell lysis, leading to inhibition of bacteria cell growth (bacteiostatic action) or cell death (bactericidal action).

Betulin can easily be obtained from more than two hundred plants species, although the richest source of Betulin is the Betulaceae family, especially *Betula alba*, *B. pubescens, B. platyphylla* and *B. pendula* (Dehelean et al. 2012a, b; Lin et al. 2010). The activity of betulin acid has been linked to the induction of the intrinsic pathway of apoptosis also known as programmed cell death, is a programme of damaged or stressed cells, resulting in a sequence of organised reactions leading to the death of the cell (Green and Kroemer, 2004). It may be observed in both physiological and pathological situations.

CHAPTER FIVE

CONCLUSION

This study revealed that *Chyrsophyllum albidum* ethyl acetate cotyledon extracts have inhibitory and bactericidal activity against *Stapyhlococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis* and *Klebsiella pyrogenes* showing its potential as a possible source of new drug. This could be attributed to the presence of the wide range of phytochemicals such as alkaloids, flavonoids, tannins, phenols, steroids and saponins.

Proximate composition of the seeds also indicates that they could be alternative sources of human food and could find immediate application in mixed animal feed.

REFERENCES

Adewoye, EO, Elsie O. Adewoye, Salami, AT, Lawal, TO and Adeniyi, BA (2011).The Antimicrobial and Kill Kinetics of Chrysophyllum Albidum Stem Bark Extracts. In: European Journal of Scientific Research **Vol.56 No.3 pp.434-444**

Ajetunmobi Asibiallau Oladipupo, Towolawi Gafar Abiodun *2014:* Phytochemical Analysis and Antimicrobial Effect of Chrysophillum Albidum Leave Extract On Gastrointestinal Tract Pathogenic Bacteria and Fungi in Human: *IOSR Journal of Applied Chemistry (IOSR-JAC)* ***Volume 7, Issue 1 Ver. II. Page 3-4***

Akubor P.I, Eze J.I (2013). Quality evaluation and cake making potential of sun and oven dried carrot fruit. IntJ.Biosici **2(10): 19 – 27**.

Akaneme, FI. (2008). Identification and preminary phytochemicalanalysis of herbs that can arrest threatened miscarriage in Orba andNsukka towns of Enugu State. African Journal of.Biotechnology.**7:6-11**.

Amusa N.A., Ashaye O.A., Oladapo M.O., 2003: Biodeterioration of the African Star apple (Chrysophyllum albidum) in storage and the effect on its food value: Afr. J. Biotech. **2: 56 – 59.**

Dada-Adegbola Hannah O. , Olajide Olalekan and Ajayi Bamidele (2014): Comparative Study of Antibacterial Activity of Juice, Acetone, Methanol and Ethanol Leaf Extract of Andrographis paniculata (King of Bitters): E3 Journal of Medical Research **Vol.3(1).006-012**

Dehelean CA, Şoica C, Ledeţi I et al (2012b): Study of the betulin enriched birch bark extracts effects on human carcinoma cells and ear inflammation: Chem Cent J **6:137**.

Egharevba H. O, Ibrahim, J. A, Nduaguba G. A, Kunle O. F(2015).: Phytochemical proximate pharmacognostic analyses and thin layer

chromatography of chrysophyllum albidum seed: *Ewemen Journal of Herbal Chemistry & Pharmacology Research* **Vol.1 (1) Page 6-12**

Egharevba HO, Carew O and Kunle OF (2015d). Phytochemical and Pharmacognostic Analysis of *Ficus thonningii* (Blume Leaves) for Monograph Development. *Int J Basic & Appl Sci* **4(2): 94-100**

Ehiagbonare, JE,Onyibe, HI and Okoegwale, EE.(2008):Studies on the isolation of normal and abnormal seedlings of Chrysophyllum albidum: A step towards sustainable management of the taxon in the 21st Century. Scientific Research and Essay **3(12): 567-570.**

Enwa F. O., C.G. Omojate and C Adonu(2013). A Review on the phytochemical profile and the antibacterial susceptibility pattern of some clinical isolates to the ethanolic leaves extract of *Moringaoleifera* lam (moringaceae). International Journal of Advanced Research. **1(5): 226-238**.

Ettench L. Shoabay M. Smaowi S (2010). Bioactive secondary metobolitis from a new terrestrial Streptomyces sp. TN262. App Biochem Biotechnol. **162: 379 – 393.**

Florence Abolaji Bello and AdiahaAbijail Henry (2015). Storage effects and the postharvest quality of African star apple fruits (Chrysophyllumafricanum) under ambient conditions. African Journal of Food Science and Technology**. 6,35**.

Green DR, Kroemer G (2004) The pathophysiology of mitochondrial cell death. Science **305:626–629**.

Idowu T. O, Iwalewa E. O, Aderogba M. A, Akinpelu B. A and Ogundani A. O.(2006). Biochemical and behavioural effects of eleaginine from Chrysophyllum albidum. Journal of Biological Sciences, **6: 1029-1034**.

I.I.Nkafamiya,U.U.Modibbo, A.J.Manji and D.Haggai(2007). Nutrient content of seeds of some wild plants. African Journal of Biotechnology **6(14): 1665 – 1669**.

Jirenez – Equilin A.E, Roaye T. M.(2005) Antifugal acivities of actinomycetes etc. strains associated with high attitude Sage brush Rhizosphere. Ind. Microbial. Biotechnol. **32: 378 – 381**.

Jones GA (1994). Effects of sainfoin (Onobrychis viciifolia scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. Applied Environmental Microbiology; **60:1374–1375.**

K. Radha Krishnan, F. James and A. Mohan(2016): Isolation and characterization of n-hexadecanoic acid from *Canthium parviflorum* leaves: Journal of Chemical and Pharmaceutical Research **8(8):614-617**

K.L. Compean and R.A Ynalvez(2014). Antimicrobial Activity of Plant Secondary Metabolites: Research Journal of Medicinal Plant 8**(5): 204-213**

Lectcana wanichelcul M., Sawangnop S.A(2008) comparison of two methods used for measuring the antagonistic activity of Bacillus speeres. Walailak J. Sci. Tech **3:161 – 171**.

LJ McGaw, AK Jäger and J van Staden(2002): Antibacterial effects of fatty acids and related compounds from plants: South African Journal of Botany **68: 417–423**

Lin WY, Lin FH, Sadhasivam S, Savitha S (2010): Antioxidant effects of betulin on porcine chondrocyte behavior in gelatin/C6S/C4S/HA modified tricopolymer scaffold: Mater Sci Eng C **30:597–604**.

Madubuike, FN and Ogbonnaya, O (2003): The potential use of White Star Apple seed (Chrysophyllum albidum) and physic Nut(Jatropha curcas) as feed ingredients forrats. Journal of Agricultural Science and Veterinary Medicine **1: 97-105.**

Magaldi, Mata-essayag, Harting de Capriles C. (2004) Well Diffusion for anti-fungal susceptibility testing. Int. J. infection Dis **8:39 – 48**

Mahale M.S and Goswami-Giri A.S.(2011). Composition and Characterization of Refined Oil Compared with Its'Crude Oil from Waste Obtained from *Mangifera indica*. *Asian J. Research Chem*.**Volume 4(9): 1411-1419**

Maunyr Balawiri, Monday Sadiki, Bood Koraich Ibusounda. (Method for in vitro evaluating antimicrobial acitivity; Jainrual of Pharmaceutical Analysis Xi'an Jiaotong University).

Omojate Godstime C, Enwa Felix O, Jewo Augustina O, Eze Christopher O,(2014). Mechanisms of Antimicrobial Actions of Phytochemicals against Enteric Pathogens – A Review. Journal of Pharmaceutical, Chemical and Biological Sciences **2(2):77-85**

Onimawa and Akubor (2012). Proximate composition and selected physicochemical properties of the seed, pulp and oil of sour sop(*Annona muricata*). Plant Foods for human Nutrition 57:165 – 171.

Oputah S. I, Mordi R. C, Ajanaku K. O, Olugbuyiro J. A. O, Olurunshola S. J, Azuh D. E: Phytochemical and antibacterial Properties of Ethanolic Seed Extracts of Chrysophyllum albidum(African Star Apple). Orient J Phys Science;**1(1)**

Orijagun, O. J., Olajide, O . O., Fatokun, A. O., Orishadipe, A. Y and Batari, M. L. (2013). The preliminary chemical constituent of free radical scavenging activity of the exocarp of the fruit extract of African star apple (Chrysophyllum albidum) . International Journal of Pharma Science and Research, **3(3): 72-80**

Orijajogun O. Joyce*, Olajide O. Olutayo and Useh U. Mercy (2014): Biological Analysis and Phytochemical Studies of The Exocarp Fruits Extracts of African Star Apple (*Chrysophyllum albidum G. Don*): Scholars Academic Journal of Pharmacy (SAJP) (Online) Sch. Acad. J. Pharm. **3(5): 379-382**.

Pankey GA, Sabath LD(2004). Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. *Clin. Infect. Dis.* **38(6): 864-70**

Parmar Namita, Rawat Mukesk (2012). Medicinal plants used as antimicrobial agents: A review. International Research Journal of Pharmacy **3(1) Page 31-40**

Quattrocchi, U (2000): CRC World Dictionary of plant Names.IA-C. CRC Press.**P.534**

Reid K., Frank O.R, Stocks N.P(2010). Aged garlic extracts lowers blood pressure in patients with uncontrolled hypertension: A randomized controlled trial. Maturitas **67(2) 144-50**.

Sakharkar M K(2010). Activity and interactions of antibiotic and phytochemical combinations against *P.aeruginosa* in vitro: International Journal of Biological Science; **6(6):556-568.**

Valgas C; De Sauza S.M, Smania E.F.A(2007) Screening methods yb determine contibacterial activity of natural products. Braz J Microbial **38: 369 – 380**.