EFFECT OF AQUEOUS EXTRACT OF MONKEY SUGARCANE (*Costus afer*) ON SOME CLINICAL ISOLATES

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A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF DEGREE OF B.Sc. (Hons.), MICROBIOLOGY.

NOVEMBER, 2025.

CERTIFICATION

This is to certify that this project work was carried out by **ELIZABETH ADEWUYI** of the Department of Microbiology, Faculty of Life sciences, University of Benin, Benin city, Edo state, in partial fulfilment of the requirement for the award of degree of Bachelor of Science (B.Sc.), Microbiology.

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PROF. E.A. OPHORI **DATE**

(Project Supervisor)

_____ _____

PROF. E.O. IGBINOSA **DATE**

(Head of Department)

DEDICATION

This project work is dedicated to God Almighty for his mercies, love, grace, strength, wisdom, guidance and provision he has shown me.

I also dedicate this research work to my family, The Heart of Warri (HWT), The Love Family (TLF) and my friends for all their unwavering support amidst all stress and expenses.

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ABSTRACT

Ethnomedicine or traditional systems of medicines have suggested means to increase the body's natural resistance to microbial infections. A number of medicinal plants have been reported to possess antimicrobial activities. This study aimed at determining phytochemical constituents, proximate composition, and the antibacterial activity of aqueous extract of *Costus afer* (Monkey Sugarcane) on clinical isolates such as *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa*. The plant samples were collected from a farmyard in Ughelli, Delta State. Samples were air dried for ethanol and aqueous extraction, using standard method. The antibacterial activities of the ethanol and aqueous extract of *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa,* were determined by the disc diffusion method. The result of this study revealed the presence of phytochemical constituents including; flavonoids (2.69 ± 1.53), saponins (4.86 ± 0.23), alkaloids (1.93 ± 0.11), tannins (88.96 ± 5.77) and phenols (59.70 ± 3.29). Proximate analysis revealed the moisture content 22.4%, ash content was 11.2%, crude fibre content was 3.9%, crude protein content was 8.0%, crude fat content was 0.7%, while carbohydrate content accounted for 53.8% of its total composition. The study investigated the determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC), and the identification of bacterial isolates, *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa.* The antibacterial activity of aqueous extract of *Costus afer* on clinical isolates such as *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* showed that the organism was not sensitive to the aqueous extract. The bacterial isolates showed greater resistance to commercially available antibiotics against *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* in the susceptibility test. Antioxidant assays such as the DPPH radical scavenging activity, Ferric Reducing Antioxidant Power (FRAP), and Total Antioxidant Capacity (TAC) were conducted to assess the extract's free radical scavenging ability.

CHAPTER ONE

INTRODUCTION

1.0 Background of Study

Ethnomedicine or traditional system of medicines have suggested means to increase the body's natural resistance to pathogenic diseases. A number of medicinal plants have been claimed to possess immunomodulatory activity (Alanazi *et al.,* 2023). Screening plant extract for their immunostimulant property, it may be possible to get effective, cheaper new molecular entity for the treatment of various infections. It is believed that such extract will not produce microbial resistance and will not have adverse effects since they are from natural plant origin (Swaroop *et al.,* 2021). In the last few years, the use of medical plants for therapeutic purposes has been on the increase globally. It is evaluated that about 80% of the world population depends mainly on medicinal plants for their health care delivery system (Ulriksen *et al.,* 2022). Extracts from medicinal plants which are a combination of diverse Phyto-constituents that interact with several molecular targets in an individual or organism to elicit physiological activities or pharmacological response (Modaresi and Khodaddi, 2014) are currently prepared as tablets, snips, tinctures, sauces, encapsulated powders and lozenges. In developing countries like Nigeria, it is a common practice that plant products or remedies are administered over a long period of time without attention to the likely toxicity or side effects (Antony *et al.,* 2025). Various organic compounds are derived from plants which are important in combating different diseases that we are constantly exposed to. Knowing these phytochemicals and their specific uses will go a long way in treating diseases in the medical as well as pharmaceutical field (Zebeaman *et al.,* 2023). Plants have provided mankind with herbal remedies for many diseases for many centuries till date. They continue to play a major role in primary health care as therapeutic remedies in developing countries. The role of plants in folklore medicine is

attributed to the presence of phytochemicals; which are non-nutritive plant chemicals that have disease preventing or curative properties (Alanazi *et al.,* 2023).

Costus afer, which is commonly called bush sugar cane or monkey sugar cane (Nyanayo, 2006), belongs to the family Zingiberaceae is a monocot and a tropical plant which is relatively tall, herbaceous, unbranched with creeping rhizome. It is commonly found in moist or shady forest of West and Tropical Africa (Ezeabara *et al.,* 2025). *Costus afer* is a useful medicinal plant that is highly valued for its anti-diabetic, anti-inflammatory and anti-anchoritic properties in South-East and South-South Nigeria (Anekwe-Nwekeaku, 2024). Its alleviation of Carbon Tetrachloride-induced Hepatic Oxidative stress and toxicity has also been reported (Ezeabara *et al.,* 2025). C. afer is used traditionally for the treatment of malaria, measles, stomach ache, rheumatoid arthritis, hepatic disease, eye defects and could also serve as an antidote for snake poison (Anekwe-Nwekeaku, 2024).

Costus afer has been reported to contain bioactive components including diosgenin, saponins, aferosides A-C, dioscin, paryphyllin C, flavonoid, glycosides, kaempferol 3-L-rhamnophyranoside (Ezeabara *et al.,* 2025). There is also a report on acute and sub-chronic toxicity studies of aqueous leaf and stem bark extract of *Costus afer* (Udem and Ezeasor, 2010). In the use of plant extract for treatment it is necessary to ensure that the extracts do not have adverse effects on liver, kidney, blood and other organs and tissues of the body. Blood is a specialized body fluid and has four main components, plasma, red blood cells (RBCs), white blood cells (WBCs) and platelets. RBCs are bright-red and contain hemoglobin which helps to carry oxygen from the lungs to the rest of the body and then returns carbon (iv) oxide from the body to the lungs for exhalation. The white blood cell protects the body from infection. Platelets helps in blood clotting process. Any deleterious effect on the blood affects the entire system of the body (Zebeaman *et al.,* 2023). *Costus afer* is used in the treatment of many ailments. It is necessary to ascertain that the use of this plant extract is not deleterious to the blood. It is against this backdrop that I investigated the effects of aqueous extract of monkey sugarcane (*Costus afer*) on some clinical isolates.

1.1 Aim and Objectives of the Study

The aim of this study was to determine the phytochemical constituents, proximate analysis and antimicrobial effect of the ethanol and aqueous extract of *Costus afer* on selected bacterial isolates; *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa.*

The specific objectives of this study were;

1. To determine the phytochemical constituents of the aqueous leaf extract of *Costus afer* using qualitative and quantitative analytical techniques.

2. To determine the proximate constituents of *Costus afer*

3. To determine the antibacterial effects of *Costus afer* on *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* using disc diffusion methods.

4. To determine the antibacterial effects of commercially available antibiotics on *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa.*

5. To evaluate the antioxidant activity of the aqueous extract using DDPH radical scavenging assay, Total Antioxidant Capacity (TAC) and Ferric Reducing Antioxidant Power (FRAP) methods.

6. To determine MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) of aqueous leaf extract of *Costus afer* against bacterial isolates (*Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa*).

CHAPTER TWO

LITERATURE REVIEW

2.1 *Costus Afer* Plant

Medicinal plants are of particular importance because they contain useful secondary products with high potency in the management of human ailments. It is generally assumed that the active constituents contributing to the efficacy of the medicinal plants are the phytochemicals, minerals and vitamins. Plants have provided mankind with herbal remedies to diseases for many centuries till date. They continue to play a major role in primary health care as therapeutic remedies in developing countries. The role of the plants in folklore medicine is attributed to the presence of phytochemicals, which are non-nutritive plant chemical that have disease preventive or curative properties (Eze *et al.,* 2022). Medicinal plants have various effects on living systems, some are sedatives, analgesics, antipyretics, cardio-protective, antibacterial, antivirals and antiprotozoals (Uzochukwu & Okafor, 2023).

Costus afer is a belonging to the family Zingiberaceae. It is a tall, perennial, herbaceous and un-branched tropical plant with a creeping rhizome and it is commonly called bush cane or monkey sugar cane. It was also reported that in Africa *Costus afer* is found in the forest belt of Senegal, South Africa, Guinea, Niger and Nigeria (Ezeabara *et al.,* 2025). In Nigeria, bush cane is known by various local names as Ukhuere-oha in Benin, Okpete or Okpoto among the lkwerre and Igboid speaking tribes of the South-South and South-Eastern region of Nigeria. It is also called Ireke Omoder in Western Nigeria, Kakizawa in Northern Nigeria, Akan Asante in Ghana and in Cameroon, Monkey sugarcane (Iwu, 2009). Parts of plants (leaves, stem and rhizome) harvested from the wild plant are commonly used as medicinal herbs in the treatment of various ailments (Adejumo & Onyema, 2020). The juice from the leaves has a wide reputation in folk medicine for the treatment of diarrhea, vomiting and dysmenorrhea.

The plant is commonly found in moist or shady forest of West and Tropical Africa (lwu, 2023) and can be employed in polyherbal therapies. They have the synergistic, potential, agonistic/antagonistic pharmacological agents within themselves that work together in a

dynamic way to produce therapeutic efficacy with minimum side effects. Conclusively, monkey sugarcane (*Costus afer*) extract could be used in boosting the immune system.

The taxonomical classification of the plant is as follows;

Kingdom: Plantae

Division: Spermatophyta

Order: Zingiberales

Family: Zingiberaceae

Genus: Costus

Species: afer

The plant possesses leaves with parallel venation with mid rib, simple, oblong-lanceolate, deep green adaxially, light green abaxially, globous on both sides, base cuneate to obtuse, margin entire, undulate, apex acute to long acuminate, lateral veins 5-7 pairs mid rib prominent on both sides. The different parts of the plant have been employed in different studies; the leaves, stem and rhizome have been reported. When the leaves are crushed, they produce aromatic odour. Arhoghro *et al.,* (2014) carried out a study on the aqueous extract of the leaves.

Costus afer is an evergreen, perennial rhizomatous herbaceous species, 2.5-4m tall, with thin stems and spiral leaves with closed tubular sheath of green colour with purple spots and 4.8mm long ligula, coriaceous and globous. It is characterized by an incomplete horizontal rim around the nodes of the shoot which is covered with only some erect hairs. It possesses 1 or 2 flowers per bract; the bracts are pale green often with reddish upper margin, coriaceous, ovate-triangular and the inflorescence of *Costus afer* can terminate a leafy or leafless shoot as is known in many species of Costus (Chukwu *et al.,* 2022).

Costus afer can be propagated by seed and also by stem cutting or rhizome cuttings. The stems and rhizomes are cut into pieces 2.5cm long and planted in a mixture of sand and peat moss with high survival rates under controlled storage conditions (Anyanwu *et al.,* 2023). In vitro storage of multiple shoot cultures of *Costus afer* was successful; after I year of storage under liquid paraffin, high survival rates (70-100%) were found and even after 2 years 75% of the

cultures of *Costus afer* remained viable (Anyasor *et al.,* 2013). *Costus afer* is a host of the African root and tuber scale *Stictococcus veyessierei.* The stems and rhizomes of *Costus afer* are harvested from the wild or from plants grown in home gardens whenever the need arises. In Ethiopia, *Costus afer* is harvested on a large scale from the wild for medicinal purposes, resulting in further aggravated by habitat destruction caused by slash-and-burn, commercial logging and firewood collection. In West and Central Africa, *Costus afer* remains a common species in forest undergrowth and is not liable to genetic erosion (Katiyar *et al.,* 2012). The prospect of the plant is that it is tropical African and several have been confirmed by pharmacological tests. *Costus afer* also have some value as an ornamental plant.

The stem bark is used to make table mats and baskets, it is also used to make papers and the stem in house construction in DR Congo. The sap can be used to coagulate latex and also in skin nourishing creams (Ukpabi & Nwachukwu, 2022). In West Africa, *Costus afer* is used as a fodder for small ruminants and poultry and the leaves serve as a feed for snails. It is also widely used for ceremonial and religious purposes.

Fig 2.1: Image of Monkey Sugarcane (*Costus afer*)

2.2 Health Benefits of *Costus Afer*

Costus afer have numerous health benefits. An infusion of the inflorescence is taken to treat tachycardia. The same infusion or a rhizome infusion is taken to stomach complaints. A stem decoction, the mashed or chewed stem or the pounded fruit sometimes mixed with sugar cane juice are taken to treat cough, respiratory problems and a sore throat. The smoke of the dried stem is also inhaled to treat cough (Akande *et al.,* 2023).

Leaf sap is used as eye drops to treat eye troubles and a nose drops to treat headache with vertigo and in frictions to treat oedema and fever. Leaf sap or a rhizome decoction is taken to treat malaria. Stem sap is applied to treat urethral discharges, venereal diseases, jaundice, prevent miscarriage and treat different skin ailments. A stem decoction is widely taken to treat rheumatoid arthritis (Omokhua *et al.,* 2022). An infusion of the dried aerial parts is taken to treat hypertension. The powdered stems are used as an enema to treat worms and hemorrhoids. The pulped stems taken in water are strongly diuretic (Ezeani & Uche, 2021).

In Nigeria, the debarked stem is chewed to treat nausea and to quench thirst. A cold water extract of the stem is taken to treat small epileptic attacks. Rhizome pulp is applied to abscesses and ulcers to mature them, applied to the teeth to cure toothache, and mixed with water it is taken to treat diarrhoea and amoebic dysentery. A rhizome decoction or the raw rhizome is taken to treat leprosy and venereal disease. In Gabon, the stem sap is rubbed on the body to treat colic (Ezejiofor *et al.,* 2021). *Costus afer* leaves are used to treat inflammation, rheumatism, arthritis, cough, hepatic disorders and helminthic infections. Malaria is caused by plasmodium parasites, the parasites are spread to people through the bites of infected female Anopheles mosquitoes called malaria vectors. Resistance to the commonly used anti-malaria are very high and indoor house spraying to prevent and control the vector has not totally solve the problem.

The use of traditional herbs in treating ailments, especially in rural and poor areas is becoming increasing popular due to their efficacy in managing health conditions. *Costus afer* is one of the most common herbs used by people to treat several ailments. It is used in the treatment of

health problems like diabetes, stomach ache, inflammation, gout and arthritis. According to medical research, the stem and leaves of the herb are rich in micronutrients and macronutrients. They contain high number of flavonoids, glycosides, paryphyllin C and aferosides essential in treating many health problems. The plant also contains saponins, alkaloids, anthraquinones, terpenoids, tannins and cardiac glycosides (Ezeabara *et al.,* 2025). For instance, the oil from the herb is used in treating cough, asthma, gas dysentery and cholera. The oil helps to stimulate digestion and can be used as a tonic also. C. afer oil is also used in foods and beverages serving as a flavouring component (Okoro *et al.,* 2024).

A study of C. afer stem extract in alloxan-induced damaged pancreatic cells showed that it can protect the pancreas. The leaf and stem extract of this plant were able to reverse histopathological damage of the pancreatic β-cells in alloxan induced diabetes mellitus. The result of the study indicates that C. afer leaves and stem extract have pancreatic islet cell protective and regenerative effect that could be useful in managing type 1 diabetes mellitus (Ogueke *et al.,* 2022).

Many research has strongly supported the anti-diabetic effect of C. afer. Extracts from the plant help in managing diabetes by increasing the uptake of glucose into muscle cells without using insulin. The herb is powerful and can help those with diabetes manage their high blood sugar levels (Omokhua *et al.,* 2022). It is also very efficient on out digestive system as it helps cure indigestion by erasing stomach cramps and nausea. It also aids in clearing out gas from out bowels and fight against fermentation in our stomach. A study also showed that C. afer extract can help on treating steatosis. It improved the lipid profile as indicated by lowering serum total cholesterol, triglyceride and low-density lipoprotein (LDL) levels to normal (Onwuka *et al.,* 2021).

It has been established that C. afer contains high anti-inflammatory properties and antioxidants, making the herb a very efficient treatment for those with liver problems. The study concluded that the plant possessed pharmacological activity against alcohol liver cirrhosis. The oil from the plant when rub on the face helps to smoothen the face and clear acne. The oil contains

compounds that are antibacterial, antifungal and anti-inflammatory. The leaves and stems of *Costus afer* help to removes toxins from the kidney and liver. The herb releases anti-inflammatory effects which help to reduce swelling and relieve pain in the kidney caused by infections. An animal studies indicated that C. afer leaves have nephroprotective properties on the kidney (Amabe *et al.,* 2021). It also fights oxidative stress due to its antioxidant properties and also serve as an antibacterial and antifungal. However long-term use of the plant extract is not recommended because of its effect on decreasing the haemoglobin and red blood cells which can lead to anemia. Also, the plant extract has been implicated in abortion (Enaibe *et al.,* 2020) however, there is no evidence in literature that substantiates this statement hence further study is required in this regard.

2.3 Phytochemicals Composition of *Costus Afer*

The use of plants in the treatment of different ailments have been attributed to the presence of bioactive compounds in the plant parts extracted with different solvent. Arinze et al*.,* (2021) reported from their study that C. afer contain saponin, alkaloid, flavonoids and cardiac glycosides in the aqueous extract of the leaves. The presence of this phytochemicals which are non-nutritive plant chemicals have disease prevention or curative properties. These pharmacological agents work in dynamic ways to produce therapeutic efficacy with minimum side effects. Hence knowing these phytochemicals and their specific uses will go a long way in treating diseases in the medical as well as pharmaceutical fields (Eze & Okafor, 2022). Hence the boosting of the immune system and the beneficial effect on serum cholesterol concentration can be attributed to the presence of these phytochemicals in the plant extract. C. afer have been reported to contain bioactive component including diosgenin, saponins, aferosides A-C, dioscin, paryphyllin C, flavonoid, cardiac glycosides, kaempferol 3 (Obinna & Eze, 2021; Anyanwu *et al.,* 2023).

Elele and Kingsley (2023) carried out a phytochemical test on the aqueous extract of C. afer and showed the presence of alkaloids, flavonoids, saponins, tannins and phenols. From the

analysis, the aqueous extract of C. afer showed high content of tannins and phenols, and the lowest is alkaloids, it showed the quantity of flavonoids and alkaloids as 2.69 ± 1.53 and 1.93 ± 0.11 while saponins, tannins and phenols have value of 4.86 ± 0.23, 88.96 ± 5.77 and 59.70 ± 3.29 respectively. The presence of these phytochemical constitutes can act as secondary metabolites with certain degree of therapeutic effects, which has been shown by other previous researchers to be responsible for the healing effects of most plant parts (Ogundele et al., 2021). Recent studies have shown that a wide range of medicinal plants used as herbal remedies may have adverse effects in both male and female animals including infertility, increased foetal death, teratogenesis and abortion depending on the phytochemical constituents though in a dose-dependent manner (Adeleke *et al.,* 2020). Obinna *et al.,* (2019) reported that the polar leaf extracts of *Portulaca oleracea limn* indicated the presence of the following phytochemicals; carbohydrate, saponins, cardiac glycosides, triterpenoids, steroids, anthraquinones and alkaloids in trace amounts. There is also the assertion that the type of solvents employed the extract yield, the available biologically active compounds as well as the resulting pharmacological activities of the plant materials (Onyeka *et al.,* 2021).

Preparation in ethnomedicine is usually aqueous based, hence the choice of aqueous extract in many studies. According to Widyawati *et al.,* (2022) methanol and ethanol can dissolve polar compounds such as sugar, amino acid and glycoside, phenolic compounds with low and medium molecular weight and medium weight polarity, aglycones of flavonoid, anthocyanin, terpenoid, saponin and tannins.

Anyanwu et *al.,* (2023) worked on the phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of *Costus afer*. The phytochemical analysis of the extracts revealed the presence of flavonoids, phenols, anthraquinones, cardiac glycoside and terpenoids. In addition, aqueous extract of C. afer tested positive for alkaloids and tannins. However, all extracts tested negative for saponins, phlobatanins and cardenolides. Alkaloids are known to have anti-microbial, antifungal and anti-inflammatory effect (Adekunle *et al.,* 2022) and also acts as an anti-hypertensive agent. The folkloric use of C. afer in the treatment of sore throat,

diarrhoea, haemorrhage and wound healing might be due to the presence of tannins. Cardiac glycosides have been reported to be effective in the treatment of congestive heart failure and regulation of heart beat. Anthraquinones can induce laxative effect and hence the use of C. afer as laxative and nervous system depressant may result from the presence of anthraquinones (Ayoola *et al.,* 2008). Flavonoids and phenols are potent water-soluble antioxidants when prevent oxidative cell damage suggesting antiseptics, anticancer, anti-inflammatory effects and mild anti-hypertensive properties (Okafor et al., 2024).

2.4 Anti-Oxidant Activity of *Costus Afer*

Plant phenolics are major group of compounds acting as primary antioxidants or free scavengers. The therapeutic potential of antioxidants in controlling degenerative diseases with marketed oxidative damage from reactive oxygen species or free radicals have been reported. Aqueous extract C. afer with high phenolic content showed higher antioxidant and inhibition of lipid peroxidative activity than methanol extract. These suggested its potential in the treatment and prevention of various oxidative related diseases (Chukwu *et al.,* 2022). Therefore, stem extract of C. afer could be exploited as sources of free radical scavenges and bioactive metabolites for nutritional, medicinal and commercial purposes (Ezeani *et al.,* 2021).

Reactive oxygen species (ROS) possess a strong oxidizing effect and induce damage to biological molecules, including proteins, lipids and DNA with concomitant changes in their structure and function. The major nutritional antioxidants are vitamin E (tocopherol), vitamin C (ascorbic acid) and β-carotene, maybe beneficial to prevent several chronic disorders (Iwu & Kalu, 2023).

Considerable interest has arisen in the possible reinforcement of antioxidant defenses, both for chemoprevention and treatment purposes. The extract of C. afer showed a powerful scavenging activity of hydroxyl radicals and acted as a chelator of iron. The interaction of the extract with iron (II) was studied and the results justify the high efficiency of the extract as an agent protecting from iron-induced oxidative damage (Uzochukwu & Okafor, 2023).

The assessment of plasma lipid profile is required for the state of wellbeing of every individual as cardiovascular diseases and coronary heart diseases arises from oxidative stress are silent, serial killers of our age. There is also report on acute and sub chronic toxicity studies of aqueous leaf and stem bark extract of C. afer (Udem *et al.,* 2023). C. afer, a medicinal plant plays a role in the alleviation of Carbon Tetra-Chloride-Induced Hepatic Oxidative Stress and Toxicity has also been reported (Okoro *et al.,* 2024). Also in recent years, attention has been

focused on the role of bio-transformation of chemicals of highly reactive metabolites that initiate cellular toxicity. Many compounds including clinically useful drugs can cause damage through metabolic activation of the chemical to highly reactive compound such as free radicals, carbenes and nitrenes.

Moreover, the hepatoxicity of paracetamol, a widely used antipyretic-analgesic drug, produces acute hepatic damage on accidental over dosage. It is established that, a fraction of paracetamol is converted via the cytochrome p450 pathway to a highly toxic metabolite, N-acetyl-p-benzo-quinamine (NAPQ 1) which is normally conjugated with glutathione and excreted in urine. Overdose of paracetamol depletes glutathione stores, leading to accumulation of NAPQ 1, mitochondrial dysfunction and development of acute hepatic necrosis. Studies demonstrated that paracetamol induced hepatoxicity can be modulated by substance that influence p450 activity (Anyasor *et al.,* 2013).

2.5 Antimicrobial Activity of *Costus Afer*

Medicinal plants possess different organic compounds which possesses antimicrobial activity that are important in combating different diseases that we are constantly exposed to (Enwuru, 2008). C. afer have been shown to possess antimicrobial activity against some pathogenic microorganisms (Eze *et al.,* 2022).

In an in vitro agar diffusion technique, C. afer extract showed activity against bacterial species such as *Bacillus pumilus, Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigella spp. and Klebsiella pneumonia.* It also showed activity against *Saccharomyces cerevisiae, Trichoderma spp, Aspergillus flavus and Aspergillus fumigatus* (Okoro & Ezeani, 2021). This activity might be due to different bioactive compounds present in C. afer which alters the biochemical activities and growth patterns of different pathogenic microorganisms implicated in different diseases of man. Thus C. afer is of great benefit to mankind in preventing and treating different ailment.

Malaria is caused by plasmodium parasites. The parasites are spread to people through the bites of infected female Anopheles mosquitoes called malaria vectors. Resistance to the commonly used anti-malaria drugs are very high and toxicity to alternative. Indoor house spraying to prevent and control the vector has not totally solved the problem and the female anopheles' mosquitoes have developed resistance. Also, several malaria programs have been hampered by financial and operational problems. Hence researchers have source for new antimalarial drugs from higher plants. C. afer have been reported to have some antimalarial properties. Elele and Umukoro (2023) worked on the effect of the aqueous extract of bush C. afer stem on *Plasmodium berghei*. The result of their study shows that the aqueous stem extract of C. afer possesses anti-malaria activity as seen in the ability to suppress *Plasmodium berghei*. The oral administration of the plant extract indicates to a far-reaching end that C. afer stem extract would be a promising natural ant-malaria product devoid of side effects upon use as it also influences hematopoietic stem cells to produce red blood cells (RBC) especially when administered within the dose range of 100-500mg/kg body weight investigated.

These polyphenols found in the extract of C. afer account for its activity against *Bacillus subtilis, Staphylococcus aureus and Vibrio cholerae* (Amabe *et al.,* 2021). The plant extract also possesses anti-diarrhoeal activity since it has effect against *E. coli* which is a common organism implicated in diarrhoea. Its anti-diarrhoeal activity might also be attributed to its effect on intestinal tract. C. afer is a useful medical plant that is highly valued for its ant-diabetic properties in South-East, South-West and South-South, Nigeria (Onwuka *et al.,* 2021). The stem of the plant was also used to examine antidiabetic, analgesic and anti- inflammatory properties. The different chemical constituents of the plant, especially the polyphenolics, flavonoids, triterpenoids and other chemical compounds present in the plant maybe involved in the observed anti-inflammatory, analgesic and hypoglycemic effect of the plant extract. This led to the assertion that the plant extract can be used in the management of adult-onset type 2 diabetes mellitus in some rural African communities (Atawodi, 2005). The antiviral activity of

the plant extract was also studied against Herpes simplex virus type 2 and it inhibits the late event in HSV-2 replication and also antagonizes the cytopathic effect of Human Immunodeficiency Virus (HIV) which is of great importance in medicine and pharmacological studies (Okafor *et al.,* 2024).

2.6 Immunomodulatory Activity of *Costus Afer*

Immunomodulatory is a procedure which can alter the immune system of an organism by interfering with its function. If it results in an enhancement of immune reaction it is named as an immunostimulant drug which primarily implies stimulation of non-specific system. Immunosuppression implies mainly to reduce resistance against infections, stress and may occur on account of environmental or chemotherapeutic factors. Immunostimulant and immunosuppression both need to be tackled in order to regulate normal immunological functioning. They both have their own standing and search for better agents exerting these activities is becoming the field of major interest all over the world (Adebayo & Kretti, 2023). Natural adjuvants, synthetic agents, antibody reagents are used as immunosuppressive and immunostimulant agents. But there are major limitation to the general use of these agents such as increased risk of infection and generalized effect throughout the immune system (Adebayo and Kretti, 2023).

Ethno medicine or traditional system of medicine have suggested means to increase the body's natural resistance to disease. A number of medicinal plants have been claimed to possess immunomodulatory activity of which C. afer is one of them (Omokhua, 2011). Screening plant extract for their immunostimulant property it may be possible to get effective, cheaper new molecular entity for the treatment of various infections. It may be hoped that such extract will not produce microbial resistance and will not have adverse effects since they are from natural plant origin.

C. afer leaf extract may contain substances that usually accompanies anemia. This suggests that C. afer may have the potential to induce anemia in the body of users. This serves as a caution to those who use it in the treatment of ailments. In a sub chronic toxicity study, there was a significant reduction of haemoglobin and total red blood cell count, indicating that the long-term use of aqueous extract of C. afer could result in anemia (Udem and Ezeasor, 2010). There was significant increase in WBC count and neutrophils and a decrease in PVC, HB and lymphocyte compared to the control. The increase in WBC in combined ethanolic leaf extract suggest that they might have a good potential to boost the immune system. However, a significant reduction of PVC and HB of the combined leaf extract treated animals is suggestive of anemia.

According to Oyedeji and Bolarinwa (2012), animals with elevated leucocyte count are capable of producing antibodies in the process of phagocytosis and as such are conferred with high degree of immunity.

In summary, medicinal plants of various types and degree possesses immunomodulatory activities as shown in experiment with different rat model. However long-term use is not recommended due to their ability to induce anaemia since they reduce haemoglobin and packed cell volume (PVC). Hence, ethnomedicine that is the use of monkey sugarcane plant aqueous extract for therapeutic purpose have been on the increase globally. It is evaluated that about 8% of the world population depend mainly on medicinal plants for their health care delivery system (Bala *et al.,* 2022).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection, Identification and Authentication of Materials

Plant Collection: The plant used in this study was collected from a bush Ughelli Town, Delta State, Nigeria. The plant was identified and authenticated in the herbarium unit of the Department of Plant Biology and Biotechnology, University of Benin.

Microbial sample: Clinical isolates of *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* were collected from University of Benin Teaching Hospital (UBTH) Medical Microbiology Laboratory. The bacterial isolates were kept at 4°C on an agar slant. The sample was sub-cultured for 24 hrs. in nutrient agar at 37°C before any susceptibility test.

Material (Reagents and Apparatus) used in antimicrobial testing of *Costus afer*:

Petri dishes, MacConkey agar, Biochemical reagents, Inoculating loop, Filter papers, commercially available antibiotic discs, Flavour bottles, Mueller Hinton Agar, Tryptone soy broth, Monkey sugar cane stems, Bunsen burner, Alcohol and Sterilizer, Autoclave, Test tubes, measuring pipette, Analytical-grade chemicals such as hexane, methanol, ethyl acetate, and nutrient agar were procured from a Microbiology and Medical store (Pyrex) in Benin.

3.2 Preparation of the Extract

The leaves of *Costus afer* plant were dried at room temperature for two weeks. The dried leaves were then milled into fine powder. The powdered leaves were soaked in water and kept at room temperature for 72 hours with intermittent mixing. The mixture was filtered using Whatman Qualitative Grade-1 filter paper and the resultant filtrate was concentrated to dryness over a hot bath at 40 ℃. The extract was stored at 40℃ for further use.

For the aqueous extraction, another 100 g of the powdered sample was soaked in 500 ml of distilled water for 72 hours, filtered, and concentrated using a water bath at 60 °C. The extracts

were stored in well-labeled airtight containers and kept in a refrigerator at 4 °C for subsequent use in analyses (Mbah *et al.,* 2023; Ezeabara *et al.,* 2025).

3.3 Phytochemical Analysis

Phytochemical analysis was carried out for *Costus afer* as described by Gboeloh et al., (2014). The following procedures were used:

1. **Test for Tannins:** A few drops of 0.1 % Ferric Chloride was added to the extract. A brownish green or a blue-black coloration shows a positive test.
2. **Test for Flavonoids:** 10ml dilute ammonia solution was added to the extract followed by the addition of concentrated tetraoxosulphate (VI) acid. Appearance of a yellow coloration which disappears on standing shows the presence of flavonoids.
3. **Test for Alkaloids:** Chloroform was added to the extract in a test tube. Appearance of pink colour indicates the absence of alkaloids while appearance of brown colour indicates the presence of alkaloids.
4. **Test for Phenols:** Ethanol was added to the extract in a test tube, followed by a few drops of 1 % ferric chloride. The formation of green or blue coloration indicated a positive test.
5. **Test for Saponins:** The extract was placed in a test tube and shake vigorously, if no persistent foam it indicates the absence of saponins.

3.4 Preparation and Sterilization of Culture Media

All culture media were prepared according to the manufacturer's instructions. Sterilization will be at 121 ºC at 15 psi for 15 min unless otherwise stated by manufacturer.

3.4.1 Nutrient Agar

Twenty-eight grams (28 g) of nutrient agar were dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of

distilled water in a conical flask. The medium will be placed in an autoclave to sterilize it for 15 minutes at 121 °C. After sterilization, the flask was allowed to cool.

3.4.2 Mueller Hinton Agar

38 g of Mueller Hinton agar were dissolved in 1000ml of distilled water and boil to completely dissolve agar. The autoclave was sterilized for 15 minutes at 15 psi (121^{o} C). It was then cool to 60^{o} C and before pouring into sterile Petri dishes.

3.5 Confirmatory Test for Isolates

MORPHOLOGICAL ANALYSIS

3.5.1 Gram Staining:

This test was done to confirm the cell type of the bacteria to be used. Gram staining techniques was used for differentiation between Gram-positive and Gram-negative bacteria. Organisms that retain the primary stain are called Gram positive while those that do not retain the primary stain when decolorized are called Gram negative. The non-retention of the stain is due to the cell composition. The Gram stain procedure is as follows:

A smear of the bacteria isolate was made on grease free slide and heat fix by passing over flame. The smear was flooded with crystal violet which is the primary stain for 1 min then washed with distilled water. Subsequently the slides were flooded with Lugol's iodine solution for 30 sec and then washed off with distilled water. 95 % alcohol was used for decolorization for 10sec and immediately washed off with distilled water. Finally, the smear was counter stained with saffranin for 1 min and washed off. The slides were allowed to air dry before observing under the microscope using an oil immersion objective lens of ×100 magnifications to view the slides.

3.6 Biochemical Identification

Biochemical test was carried out so as to help in the identification of the bacteria isolates as phenotypic (cultural) characteristics is not sufficient. The various biochemical test carried out are shown below;

3.6.1 Oxidase Test

This is mainly used to differentiate between *Pseudomonas* from other Gram-negative rods. Oxidase test was carried out to identify bacteria species that will produce cytochrome oxidase enzyme. *Staphylococcus aureus* and *Escherichia coli* which are Gram-positive and Gram-negative respectively were employed as control. A piece of filter paper using sterilized wire loop 2-3 drops of freshly prepared oxidase reagent (1% aqueous tetramethyl-3-phenyl nediamine dichloride) was added. A positive oxidase test is indicated by purple colouration within 10 seconds.

3.6.2 Urease Test

This is used to test organisms that have the ability to produce the enzyme urease which catalyzes the breakdown of urea to produce ammonia. The test is usually used to differentiate organisms like *Proteus mirabilis* from other non-urease positive organism. A sterilized medium was dispensed into test tubes aseptically and the test bacteria isolated were inoculated into the medium and incubated at 37 ºC for 24 hours. A change in colour from yellow to red-pink confirmed the presence of urease.

3.6.3 Indole Production Test

This test was used to determine which of the isolates has the ability to split indole from tryptophan present in peptone water. The test is usually used in differentiating Gram-negative Bacilli especially those of enterobacteriaceae. Five grams of commercially available peptone broth was dissolved in 1litre of distilled water. The medium was then sterilized by autoclaving at 121 ℃ for 15 minutes. The 4 ml of the medium was dispensed into sterile test tube and each of the bacterial isolates was inoculated into the peptone broth. The inoculated media was

incubated at 37 ºC for 24 hours after which few drops of KOVAC reagent was added. KOVAC reagents consist of 150 ml of amylalcohol, 10 g dimethyl amino Benz aldehyde and 150 ml of concentrated hydrochloric acid. Positive test was indicated by the red colouration that occurs immediately at the upper part of the test tube.

3.6.4 Citrate Utilization Test

This test is used to identify which of the isolate can utilize citrate as the sole source of carbon for metabolism. The medium used for this test is Simon`s citrate agar. In the preparation, 22 g of commercially available Simon's citrate agar was dissolved in a litre of distilled water and sterilized by autoclaving at 121º C for 15 minutes. The medium is dispensed into test tubes and the test organism was inoculated by stabling the medium on the tubes using sterile straight inoculation wire containing culture. The tubes were incubated at 37 ºC for about 24 hours. Positive result is indicated by a change in colour from green to bright blue colouration.

3.6.5 Catalase Test

This is a test to detect the presence or absence of catalase enzyme. The catalase enzyme catalyses the breakdowns of hydrogen peroxide to release free oxygen gas and the formation of water. A few drops of freshly prepared 3 % hydrogen peroxide were added onto the bacterial isolates smeared on a slide. The production of gas bubble indicated catalase enzyme positive.

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

3.6.6 Sugar Fermentation and Production of Gases Using Triple Sugar Iron Agar (TSIA)

TSI was prepared following manufacturer's instruction and the prepared media was placed in a test tube and kept in a slant position for it to solidify. The slant and butt of the medium was inoculated with the test bacterium using a sterile loop and it was incubated for 18- 24 hours. The results were read on the basis of acid or alkaline production in the slant or butt region of the tube and gas production was confirmed by the presence of crack or air bubbles in the slant or but region. More so, production of hydrogen sulphide was confirmed by the blackening of

the medium. A prepared laboratory chat was used for result interpretation in line with microbiological standard protocol as well as other biochemical tests carried out on the isolates to confirm or ascertain their identity.

3.7 Antimicrobial Sensitivity of Extract

3.7.1 Inoculation of Plates

This was done by the modified method of Acar and Goldstein using flood-inoculation technique. Bacterial suspension having turbidity equivalent to 0.5 McFarland was freshly prepared and 1 ml of this was transferred onto the Mueller Hinton Agar plate and distributed gently over surface of medium with gentle rocking. The excess fluid was removed from the plate and the plate were kept in incubator at 37 ºC for 30 minutes for drying before application of discs.

3.7.2 Paper Disc Diffusion

This was carried out using the modified method of Bauer *et al*., (2012). Mueller Hinton Agar was prepared and after sterilization, cooled and poured into the petri dishes and allow to solidify. Upon solidification of the agar, sterile paper disc 6mm was soaked in the different concentration of the extract The petri dishes were incubated at 37 ℃ for 24 hours after which the radius of the zone of growth of the isolates were measured using graduated millimeter (mm).

3.7.3 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Using Broth Dilution Method

The Broth dilution method (Nagalakshmi *et al*., 2019) was used for the determination of Minimum Inhibitory Concentration (MIC) of the extract against bacteria. The extracts were diluted into various concentrations 30 mg/ml, 20 mg/ml and 10 mg/ml in a sterile Nutrient broth in test tubes. Using standard wire loop (Hi-media), a loopful of the bacterial culture was

inoculated into test tubes containing various concentrations of extract in Nutrient broth. The tubes were incubated at 37 °C for 24 hours and thereafter observed for growth or turbidity.

The MBC of the extract was determined using the broth dilution method with sub-culturing from MIC tubes showing no visible growth. An aliquot of 0.1 mL from each clear tube (ranging from 12.5 to 100 mg/mL) in the MIC series was spread-plated onto sterile Mueller-Hinton agar (MHA) plates in triplicate. The plates were incubated at 37 ℃ for 24 hrs. The lowest concentration that yielded no colony growth was recorded as the MBC (Omokhua-Uyi *et al.,* 2023).

3.8 Antimicrobial Susceptibility Test

The broth culture of each isolate was standardized using 0.5 McFarland standard. Then, a set plate of sterile Mueller Hinton agar was inoculated with the standardized test organism and the antibiotic disc will be placed on the inoculated medium. The medium was incubated at 37 ^{o}C for 24 hours. After incubation, the plate was observed for zones of inhibition around each of the antibiotics. The diameter of the zone of inhibition was then measured in millimeter (mm) (CLSI, 2005; Brown and MacGowan, 2010). Gram positive antibiotic disc was used for Gram positive organism while Gram negative antibiotic disc was used for Gram negative organism.

3.9 Proximate Analysis

3.9.1 Moisture Content Determination

Equipment: Oven, weighing balance

Apparatus: Beaker, Spatula, crucible/beaker, desiccator

Procedure:

- Oven dry crucible at 105 °C for an hour to ensure dryness, (when using beaker, oven dry for 5 minutes).
- Transfer beaker/crucible into desiccator to cool for about 30 minutes.

- Weigh the crucible in an electronic balance and record as W1
- 1g of sample is weighed into the pre-weighed crucible/beaker W2
- The crucible/beaker and content are oven dried at 105°C for 3 hours, place in a desiccator to cool for about 10 minutes and weigh then put back in the oven till a constant weight is obtained W3.

$$\% \text{ Moisture Content} = \frac{W2 - W3}{W2 - W1}$$

3.9.2 Crude Protein Determination

Equipment: Weighing balance, spectrophotometer

Apparatus: Conical flask, heating mantle, volumetric flask,

Reagents: 0.1 NHCL, conc. H_2SO_4, 40 % NaOH, Boric acid indicator, mixed catalyst (copper sulphate, and sodium sulphate) /Digestion mixture.

Procedure

- Weigh 0.5 g-l g of the sample into a conical or volumetric flask
- Add 10 ml of Conc. H_2SO_4 and 1 g of mixed catalyst or a mixed catalyst tablet.
- The flask is swirled in order to mix the contents thoroughly and then placed on a heat source to start the digestion for about 2 hr till the mixture becomes whitish or a clear light green colour.
- The digest is cooled and transferred to a 100 ml volumetric flask and volume was made up to mark with distilled water (record the amount of water used to make up to the 100 ml mark).
- The standardized boric acid and about 3 drops of methyl red indicator is put in the volumetric flask of the set up to trap any ammonium produced in the process of distillation.
- 25 ml of diluted digest was introduced in the distillation tube as well as 25 ml of 40 % NaOH was gradually added.

- Distillation is continued for at least 10 minutes and NH_3 produced is collected as NH_4OH in a conical flask containing 25 ml of 4 % boric acid and methyl red indicator.
- During distillation, yellowish colour appears due to NH_4OH.
- 20 ml of distillate is then titrated against standard 0.1 NHCL solution till the appearance of pink colour.

% crude protein content is calculated.

Calculation;

$$\% \text{ Crude Protein} = \frac{S \times N \times 0.014 \times D \times 100 \times 6.25}{\text{Weight of sample} \times V}$$

Where,

S = sample titration reading

N = Normality of HCL

D = dilution of sample after digestion

V = volume taken for distillation

0.014 = mill equivalent weight of Nitrogen

3.9.3 Ash Content Determination

Equipment: Oven, weighing balance

Apparatus: Crucible, desiccator, muffled furnace

Procedure

- Pre dry the crucibles
- Weigh the crucibles and record (2 for each sample) as W1
- Put the crucible on the weigh and zero it, then add 1 g of sample into each crucible as W2
- Record weight of crucible and sample.
- Put in a muffle furnace and leave for 3 hours

- It is weighed and put back in the oven and then reweighed till it attains a constant weight W3.
- Percentage ash is then calculated as follows and average taken.

Calculation:

$$\% \text{ ash content} = \frac{W3 - W1 \times 100}{W2 - W1}$$

Where,

W1 = weight of crucible

W2 = weight of crucible and sample before ashing

W3 = weight of crucible and sample after ashing

3.9.4 Lipids (Fat Extraction) Determination

Equipment: Weighing balance, oven

Apparatus: Soxhlet extractor, filter paper, petroleum ether, glass rod, desiccator

Reagent: Petroleum ether

Procedure

- Crush the already dried sample to powder form to facilitate entry of organic solvent
- Weigh a filter paper and record W1, zero the reading and weigh 1 g of sample on filter paper (record weight of filter paper and sample as W2).
- The conical flask of the Soxhlet extractor should be half filled with petroleum ether.
- Transfer the sample with the filter paper into the sample holder of the Soxhlet extractor, the extractor is fitted with reflux condenser.
- Adjust the heat source and allow to boil.
- It is allowed to siphon round the barrel for over 5 hours (condensation rate of 5-6 drops per second).
- The condenser is detached and the filter paper containing the sample is removed.
- Dry the filter paper containing the sample in an oven at 105 °C for 1 -2 hrs.

- Dry till it attains a constant weight W3.

Calculation: % Crude fat = $\frac{W2 - W3 \times 100}{W1}$

3.9.5 Crude Fibre Determination

Equipment: Oven, weighing balance, steam bath, muffle furnace

Apparatus: Beaker, volumetric flask, funnel, filter paper/muslin cloth.

Reagent: 1.25 % Sulphuric, 1.25 % NaOH, Acetone

Procedure:

- Weigh 2 g of sample into 250 ml volumetric flask and record W0
- Add 100 ml of 1.25 % Sulphuric and boil gently for 30 minutes
- Filter through muslin cloth or filter paper and rinse well with hot distilled water to ensure complete removal of acid from sample.
- Separate residue back into the flask with spatula
- Add 100 ml of 1.25 % NaOH and boil gently for 30 minutes
- Filter through muslin cloth or filter paper and rinse well with hot distilled water to ensure complete removal of base from sample.
- Separate residue back into a crucible with spatula and add drops of acetone to rinse the remaining residue from the muslin cloth and help to neutralize the acid and base from the residue.
- Transfer the residue into an oven at 105 °C for 2-3 hours
- Cool in a desiccator and weigh W1
- Then transfer the crucible into a muffle furnace at 300 °C for 1 hour
- Cool in a desiccator and weigh W2

Calculation:

% Crude fibre = $\frac{W1-W2}{} \times 100$

W0

Where,

W1 = weight of sample after oven drying

W2 = weight of sample after ashing

W0 = weight of sample

Nitrogen free extract (carbohydrate)

N.F.E = 100 - (% moisture + % ash + % fat + % crude fibre + % crude protein)

3.10 Histochemical Study

Freehand sections of leaves, stem, rhizome and root materials were taken and treated with the respective reagent to localize the chemical constituents in the tissues. The stained sections were compared with the fresh unstained sections. The sections were mounted on a slide to be observed under a compound microscope. The mounted sections were observed under the compound microscope and were studied for various phytochemicals such as alkaloids, phenols, tannins, proteins etc. (Okafor *et al.,* 2024; Onwudiwe *et al.,* 2023).

3.11 Qualitative Phytochemical Screening

a. **Test for Tannins:** To about 2-3 mL extract, 2-3 drops of 5% FeCl solution. With the formation of green or bluish-black colour, the presence of tannins is indicated.

b. **Test for Saponins (Foam formation test):** To about 2 to 3 mL extract, 5 mL de-ionized water was added. Vigorous shaking resulted in persistent foam formation. It was allowed to stand for 15 min and kept for honeycomb froth, which shows saponins.

c. **Test for Flavonoids (Shinoda test):** To 1 mL extract, a few magnesium ribbon fragments and 4-5 drops of conc. HCL was added. The presence of flavonoids is confirmed by the formation of the pink or red.

d. **Test for Steroids/Phenol (Liebermann-Burchard test):** About 2 mL of acetic acid was added to 1 mL extract. After cooling the solution on an ice bath, conc. H_2SO_4 was added carefully. The development of violet to blue or bluish-green colour confirms the test for steroids.

e. **Test for Alkaloids:** In I mL extract, 2 mL of 1% HCL was added, and the solution was heated. Further, 4 to 5 drops of Mayer's reagent was added. A precipitate white or cream in colour formation confirms the test for alkaloids.

3.12 Quantitative Phytochemical Screening

3.12.1 Determination of Total Tannins

Tannins content was determined by modified Folin-Denis method (Polshettiwar *et al.,* 2007). The method is based on the measurement of a blue colour formed by the reduction of phosphotungstic-molybdic acid by tannin-like compounds in alkaline medium. 0.5 ml of extract (l mg/ml) and standard solution of Tannic acid (10-150 pg/ml) was added to 0.5 ml Folin-Denis reagent and l ml of 7.5 % Na_2CO_3 solution. Thereafter, 3.4ml of distilled water was added and absorbance was measured at 700nm. The total tannin content was expressed as mg of Tannic acid equivalent/g of extract (Polshettiwar and Ganjiwale, 2007).

3.12.2 Determination of Total Flavonoids

The antioxidatve properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper, and inhibition of enzymes responsible for free radical generation (Benavente-Garcia, 1997). Depending on their structure, flavonoids are able to scavenge practically all known ROS.

Procedure: Prepare quercetin standard in methanol (10 - 100 µg/ml) and extract (1 mg/ml). To 0.5 ml of extract and standard, add 0.5 ml of aluminum chloride (10 %) in ethanol, 0.1 ml of potassium acetate (1 M) and 2.5 ml of water. Incubate at room temperature for 30 mins and measure absorbance at 415 nm (Chukwu et al., 2022).

3.12.3 Determination of Alkaloids

Equipment: Weigh balance

Apparatus: Beaker, pipette, measuring cylinder

Reagent: 10 % acetic acid in ethanol, conc. ammonium hydroxide, 0.1 M ammonium hydroxide

Preparation of Reagent

- To prepare 40 ml of 10 % acetic acid, dilute 4 ml acetic acid in 36 ml ethanol
- To prepare 100 ml 0.1 M ammonium hydroxide, dilute 0.68 ml ammonium hydroxide in 99.32 ml of water

Procedure

- 1 g of sample is weighed into a beaker, 40 ml of 10 % acetic acid in ethanol is added, covered and allowed to stand for 4 hours, then filtered.
- The filtrate is concentrated on a water bath to one quarter of the original value followed by addition of 3 drops of concentrated or conc. ammonium hydroxide drop wise to the extract until the precipitation is complete
- After 3 hours of mixture sedimentation, the supernatant is discarded and the precipitate washed with 4 ml of 0.1 M ammonium hydroxide, then filtered.
- The residue is dried and weighed.
- The alkaloid content of the sample is determined using the following equation:

Calculation:

$$\% \text{ alkaloid} = \frac{W_2 = W_1}{W} \times 100$$

Where,

W = weight of sample

W_1 = weight of empty filter paper

W_2 = weight of filter paper and dry residue

3.12.4 Estimation of Total Phenolic Content

Total phenolic content was determined according to the Folin and Ciocalteau's method (1972). Concentrations (10-20 µg/mL) of Gallic acid were prepared in methanol. Then, 0.5 mL of the sample (1 mg/mL) was mixed with 2.5 mL of a ten-fold diluted Folin-Ciocalteau reagent and 2 mL of 7 % sodium carbonate. The mixture was allowed to stand for 30 min at room temperature then absorbance read at 760 nm. All determinations were performed in triplicates with Gallic acid utilized as the reference control. All determinations were performed in triplicates with Gallic acid utilized as the positive control. The phenolic content expressed as Gallic Acid Equivalent (GAE).

3.12.5 Determination of Saponins Content

Quantitative Test for Saponin

Equipment: Weigh balance, oven, water bath

Apparatus: Beaker, measuring cylinder, conical flask

Reagent: 20 % aqueous ethanol, 5 % NaCl, diethyl ether, N-Butanol

Preparation of Reagent

- To prepare 40ml 20 % aqueous ethanol, dilute 8ml ethanol in 32 ml water
- To prepare 5ml 5% NaCl, dissolve 0.25 g NaCl in 5 ml of water

Procedure

- 20 ml of 20 % aqueous ethanol is added to l g of sample
- The mixture is heated in a water bath for 2.5 hours with continuous stirring, then filtered.

- The residue on the filter paper is re-extracted with another 20 ml of 20 % ethanol and heated for 2.5 hours with continuous stirring, then filtered.
- The filtrates are combined together
- The combined extract is evaporated to 8ml over water bath at 90℃
- 4 ml of diethyl ether is added to the concentrate in a SEPARATOR FUNNEL and vigorously agitated from which the aqueous layer was recovered while the diethyl ether layer was discarded.
- Repeat step six.
- 12 ml of n-butanol is added and extracted twice with 2 ml of 5 % NaCl· The NaCl is discarded.
- The remaining solution is heated in a water bath till evaporation is complete· Weigh a dry crucible
- The solution is transferred into a crucible and was dried in an oven to constant weight.

Calculation:

$$\% \text{ saponin} = \frac{\text{Weight of saponin}}{\text{Weight of sample}} \times 100$$

Where,

Weight of saponin = weight of crucible and residue after oven drying - weight of crucible

3.13 Preparation of Plant Extract

Aqueous extract and Ethanol extract of *Costus afer* were obtained after phytochemicals and stored in the refrigerator at temperatures of 4 °C until isolates were obtained and ready for antimicrobial testing. Extracts were prepared by making into concentrations of 30 mg/ml, 20 mg/ml and 10 mg/ml.

3.13.1 Preparation of Ethanol Plant Extract

After collection of the plant, the leaves were shade-dried at room temperature (32-35 ℃) to constant weight over a period of seven (7) days. The cold maceration extraction method of Cowan (1999) was used. Fifty grams of dried *Costus afer* leaves was weighed and grinded to fine powder and dissolved in 1000 ml of seventy percent ethanol inside a 2-liter conical flask. The flask was shaken vigorously at 30-minute intervals and left to stand for 72 hours at room temperature for effective extraction. The resultant mixture then was filtered with Watman's No.1 filter paper and cotton wool to remove particles of plant sample. The clear solution obtained was concentrated with rotary evaporator at 45 °C under low pressure and later transferred to evaporating dish over a steam bath. The solid dried powder obtained was stored in sterile pre-weighed screw capped bottles and labelled accordingly. The extract was now stored at room temperature.

3.13.2 Preparation of Plant Aqueous Extract

The dried stems were pulverized into a smooth powder using an impact mill (Makers: Christy and Morris Ltd. Process Engineers, Chelmsford. England. Model 474/54). The pulverized material (150 g) was mixed with distilled water (3.0 L) and left for 72 h. The mixture was stirred at 6 h intervals using a sterile glass rod, and passed through a filter paper. The filtrate was concentrated in vacuo in a rotary evaporator at 40 ℃, giving a yield of 5.53 %. The concentrated extract was stored in universal bottles, labelled and refrigerated at -4 ℃ prior to use. (Chandra *et al.,* 2022).

3.14 Antimicrobial Activity Studies

The investigational study for antibacterial activity against various pathogenic bacterial strains was conducted using one technique (Disc diffusion method). The disc diffusion method was used for qualitative evaluation of antibacterial activity for respective crude extracts.

3.14.1 McFarland Standard Solution

These viable cells were used to produce a solution of cells of $1.5x10^3$ cfu/ml by constantly inoculating cells from the nutrient agar plate with a sterile loop (flamed at intervals to ensure sterility) until a certain turbidity was reached that could be compared to a 0.5 McFarland standard solution already prepared by mixing 0.5 mL of a 1.175 % barium chloride dihydrate ($BaCl_2.2H_2O$) solution to 99.5 mL of 1 % sulphuric acid (H_2SO4) (Sagar Aryal *et al.,* 2021).

3.14.2 Agar Disc Diffusion Method

Each bacterial sample was spread on sterile agar plates using a sterile wire loop after 0.2 ml of cell suspension was inoculated on the agar surface. Pre-calibrated Whatman filter paper discs of about 5 mm in diameter and 2.5 µL infused capacity was prepared and sterilized. Each disc was infused with different crude extracts of concentration 40 mg/disc and then sited on bacterial pre-spread agar plates. The samples were allowed to diffuse at RT for 30 min. The diffused petri plates were kept for incubation at 35±0.5 °C for 24 hrs. After incubation, the microbial growth was determined by measuring the diameter (mm) for inhibition zones. (Pochapski et *al.,* 2011).

3.15 Antioxidant Analysis

3.15.1 Determination of Total Antioxidant Capacity

Total antioxidant activity was estimated by phosphomolybdenum assay. The method is based on the reduction of molybdenum (IV) to molybdenum (V) by the extract and the subsequent formation of a green phosphate/molybdenum (V) complex at acid pH.

Preparation of Molybdate Reagent Solution

1 ml each of 0.6 M sulfuric acid, 28 mm sodium phosphate and 4 mm ammonium Molybdate were mixed together. That is 1:1:1.

Procedure

One milliliter of the extract (1 mg/ml) were added to 3 ml of Molybdate reagent solution. These tubes were kept incubated at 95 ℃ for 90 min. After incubation, these tubes were

normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. Ascorbic acid was used as the standard.

3.15.2 Estimation of Diphenyl-2-Picryl-Hydrazyl (DDPH) Radical Scavenging Activity

The free radical scavenging capacity of the leaf extracts against 1,1-diphenyl-2-picrylhydrazyl (DDPH) radical was determined by a slightly modified method of Brand-Williams *et al.,* (1995). The assay is based on the ability of the antioxidant compounds to reduce DDPH by donation of hydrogen resulting in colour change from deep violet to golden yellow. The change in colour from deep violet to light yellow was measured spectrophotometricallly at 517 nm. Briefly, 0.5 mL of 0.3 mm DDPH solution in methanol was added to 2 mL of various concentrations (0.2 - 1.0 mg/ml) of the extracts. The reaction tubes were shaken and incubated for 15 min room temperature in the dark; absorbance read at 517 nm. All tests were performed in triplicate. Ascorbic acid was used as standard control, with similar concentrations as the test samples prepared. A blank containing 0.5 mL of 0.3 mm DDPH and 2 mL methanol was prepared and treated as the test samples. The radical scavenging activity was calculated using the following formula: DDPH radical scavenging activity (%) = $[(A_0-A_1) / (A_0)] \times 100$

Where, A_0 was the absorbance of DDPH radical + methanol.

A_1 was the absorbance of DDPH radical + sample extract or standard. The 50% inhibitory concentration value (IC_{50}) was calculated as the effective concentration of the extract that is required to scavenge 50% of the DDPH free radicals.

3.15.3 Ferric Reducing Antioxidant Power (FRAP) Assay

The Ferric Reducing Antioxidant Power (FRAP) assay was carried out using a modified method of Benzie and Strain (1996). The assay is based on the ability of antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of 2, 4, 6-tri (2-pyridyl)-s-thiazine (TPTZ), forming an intense blue Fe^{2+}- TPTZ complex with an absorption maximum at 593 nm. To 1.5 mL of freshly prepared FRAP solution (25 mL of 300 mm acetate buffer pH 3.6, 2.5 mL of 10 mm 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mm HCL and 2.5 mL of 20 mm ferric chloride ($FeCl_3.6H_2O$) solution was added to 1 mL of the extracts (1 mg/ml) and standard at concentrations of 100-600µM. The reaction mixtures were incubated at 37 °C for 30 min and the increase in absorbance at 593 nm was measured. $FeSO_4$ was used for the calibration curve and ascorbic acid served as the positive control. FRAP values (expressed as mg Fe (II)/ g of the extract) for the extracts were then extrapolated from the standard curve.

CHAPTER FOUR

RESULTS

4.1 Sample Identification

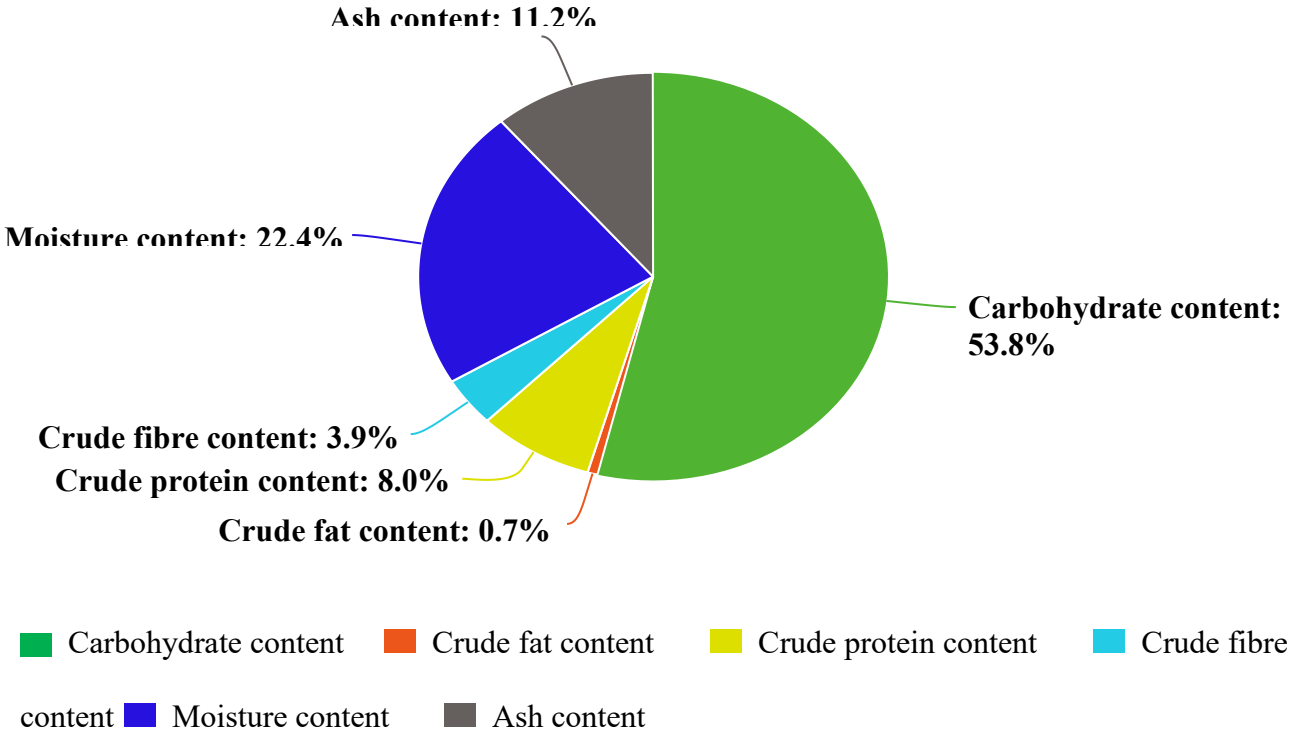

Figure 4.1: Proximate composition of *Costus afer* (Monkey sugarcane)

Table 4.1: Qualitative and Quantitative Estimation of Phytochemical Constituents of *Costus Afer*

Qualitative Phytochemicals

Parameters	Aqueous	Ethanol
Tannin	+	+
Phenol	+	+
Flavonoids	+	+
Alkaloids	+	+
Saponin	+	+

NB:

+ = slightly present

+ + = moderately present

- = absent

Quantitative Phytochemicals

Parameters	Aqueous	Ethanol
Tannin	92.29	85.14
(µg/ml)	82.29	76.57
	92.29	88.00
Phenol	62.95	40.75
(µg/ml)	59.79	38.84
	56.37	40.34
Flavonoids	3.96	2.30
(µg/ml)	0.99	3.25
	3.13	2.65
Alkaloids	2.0	1.4
(%)	2.0	1.4
	1.8	1.2
Saponin	5.0	4.0
(%)	5.0	4.0
	4.6	3.8

Table 4.1 shows the qualitative and quantitative photochemical constituents of Monkey sugarcane. From the table, Flavonoids, Saponins, Alkaloids, Tannins and Phenols are the phytochemicals present in Monkey sugarcane with alkaloids having the least percentage composition and tannin having the highest concentration.

Table 4.2: Identification of Bacterial Isolates

Isolates	Test organism 1 *Escherichia coli*	Test organism 2 *Klebsiella pneumonia*	Test organism 3 *Pseudomonas aeruginosa*
CULTURAL			
Color	Pink	Red	Blue-green
Elevation	Flat	Raised	Flat
Size	1-3 mm	2-4 mm	1-3 mm
Shape	Circular	Mucoid	Irregular

MORPHOLOGICAL			
Gram test	Gram negative	Gram negative	Gram negative
Arrangement	Rods	Rods	Rods
BIOCHEMICAL TEST			
Catalase	+	+	+
Indole	+	-	-
Citrate	-	+	+
Urease	-	+	-
Oxidase	-	-	+
TSIA	A/A	A/A	K/A
Isolates	*Escherichia coli*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*

Table 4.2 shows the cultural, morphological and biochemical test and their results for the clinical isolates; *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa*

Table 4.3: Determination of MIC (Minimum Inhibitory Concentration)

	Aqueous (extract) mg/ml
Escherichia coli	0 mg/ml
Klebsiella pneumonia	30 mg/ml
Pseudomonas aeruginosa	30 mg/ml

Table 4.3 shows the minimum inhibitory concentration of aqueous extract of *Costus afer* at different concentrations of the readings 0 mg/ml, 30 mg/ml and 30 mg/ml on *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* using the disc diffusion method with zones of inhibition on *Klebsiella pneumonia* and *Pseudomonas aeruginosa* but no zone of inhibition on *Escherichia coli.*

Table 4.4: Determination of MBC (Minimum Bactericidal Concentration)

	Aqueous (extract) mg/ml
Escherichia coli	30 mg/ml
Klebsiella pneumonia	0 mg/ml
Pseudomonas aeruginosa	30 mg/ml

Table 4.4 shows the minimum bactericidal concentration of aqueous extract of *Costus afer* at different concentrations of 30 mg/ml, 0 mg/ml and 30 mg/ml on *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* using the disc diffusion method with zone of inhibition on *Pseudomonas aeruginosa* and *Escherichia coli* and no zone of inhibition on *Klebsiella pneumonia.*

Table 4.5: Antibacterial Activity of *Costus afer* on Bacterial Isolates

	Zone of Inhibitions (mg/ml)		
Isolates	**30 mg/ml**	**20 mg/ml**	**10 mg/ml**
Escherichia coli	0	0	0
Klebsiella pneumonia	8.0	0	0
Pseudomonas aeruginosa	8.0	0	0

Table 4.5 shows the antibacterial activity of *Costus afer* aqueous extract at different concentrations of 30 mg/ml, 20 mg/ml and 10 mg/ml on *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa,* this study showed zones of inhibition on *Klebsiella pneumonia* and *Pseudomonas aeruginosa* at 30 mg/ml, having the same concentration of 8.0, but no zone of inhibition on *Escherichia coli.*

Table 4.6: Antibacterial Activity of Commercially Available Antibiotics

Isolates	S	CN	TRX	PEF	CTZ	AU	CPX	CEF	CEP	OFX
***E. coli* (mm)**	9 (R)	8 (R)	9 (R)	-(R)	-(R)	-(R)	-(R)	-(R)	-(R)	-(R)
***Klebsiella* (mm)**	10 (R)	9 (R)	9 (R)	-(R)	-(R)	-(R)	-(R)	-(R)	-(R)	-(R)
***Pseudomonas* (mm)**	10 (R)	-(R)	-(R)	8 (R)	8 (R)	-(R)	-(R)	-(R)	-(R)	-(R)

KEY

- R – Resistant
- S – Susceptible
- S – Streptomycin
- CN – Gentamicin
- TRX – Trimethoprim-Sulfamethoxazole
- PEF – Pefloxacin
- CTZ – Ceftazidime
- AU – Amoxicillin-Clavulanate
- CPX – Ciprofloxacin
- CEF – Cefotaxime
- CEP – Cefepime
- OFX – Ofloxacin

Table 4.6 shows the antibacterial activity of commercially available antibiotics on the clinical isolates of *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa. Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* were all resistant to all the commercially available antibiotics; Streptomycin (S), Gentamicin (CN), Trimethoprim-Sulfamethoxazole (TRX), Pefloxacin (PEF), Ceftazidime (CTZ), Amoxicillin-Clavulanate (AU), Ciprofloxacin (CPX), Cefotaxime (CEF), Cefepime (CEP) and Ofloxacin (OFX).

Table 4.7: Antioxidant Activity of the Aqueous and Ethanol Extracts on TAC and FRAP

Parameters	Aqueous	Ethanol
Total Antioxidant	114.36	139.89
Capacity(µg/ml)	103.09	105.85
	107.98	107.77
Ferric Reducing	3354.6	3094.6
Antioxidant	3470.6	2996.6
Potential (µM)	3216.6	3148.6

Table 4.7 shows the antioxidant activity of the aqueous and ethanol extracts on TAC and FRAP of *Costus afer* giving that the ethanol extract exhibited a higher total antioxidant capacity, while the aqueous extract had greater ferric reducing potential.

Table 4.8: Antioxidant Activity of the Ethanol, Aqueous Extracts and Ascorbic acid control on DPPH (% Inhibition)

(µg/ml)	Ethanol	Aqueous	Ascorbic acid (control)
10	59.38	59.52	91.05
	59.94	58.10	91.19
	61.08	58.52	91.34
20	64.06	60.80	93.75
	64.77	60.94	94.32
	64.35	60.37	94.18
40	67.61	67.33	95.60
	67.19	66.19	95.45
	67.19	66.76	95.74
60	68.89	75.00	96.88
	69.32	75.85	96.59
	68.75	75.71	97.16
80	71.45	78.41	97.73
	72.02	77.84	97.73
	71.02	78.98	97.59
100	75.43	80.68	98.72
	75.00	82.53	98.86
	75.57	81.68	99.01

Table 4.8 shows the antioxidant activity of the ethanol, aqueous extracts and ascorbic acid control on DDPH of *Costus afer* showing different concentrations of 10 µg/ml, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml of DDPH.

CHAPTER FIVE

DISCUSSION

The results of proximate composition of *Costus afer* presented in Fig 4.1, shows a high carbohydrate content at a mean of 53.8%, constituting the bulk of the dry matter of *Costus afer,* which means it can potentially be used by the pharmaceutical and food industries in making of carbohydrate supplements as well as carbohydrate rich foods.

With moisture content of 22.4%, reflects the plant's adaptation to humid, shaded rainforest ecologies in Niger Delta, where water retention supports turgor and secondary metabolites synthesis (Iwu, 2022).

Crude protein content (8.0%), crude fat content (0.7%) and ash content (11.2%) indicate moderate nutritional density. This support its ethno botanical use as a dietary adjunct in anemia and growth disorders among local populations (Anyanwu *et al.,* 2021).

The findings also showed that the aqueous phytochemical composition of *Costus afer* extract contained flavonoids, saponins, phenols, tannins and alkaloids. Phytochemicals are non-nutritive plant chemicals that, confer antioxidant, anti-inflammatory and cytoprotective effects with minimal toxicity (Enwuru, 2023). It is well known that alkaloids have antifungal, antibacterial and anti-inflammatory qualities. Flavonoids and phenolic compounds also have anti-allergic and anti-thrombotic properties reducing and protecting against oxidative cell damage (Anyasor *et al.,* 2021). While tannins form protective complexes with mucosal proteins, supporting gastrointestinal healing.

Antibacterial effects of *Costus afer* have been showed in some studies, to have activity against a number of bacteria like; *Bacillius pumilus, Staphylococcus aureus* (Gram positive), *Salmonella typhi, Shigella spp.* (Gram negative) (Nyananyo, 2006; Prabhu *et al.,* 2017), using the agar disc diffusion method, the antibacterial activity of *Costus afer* aqueous and ethanol extract at concentrations of 30 mg/ml, 20 mg/ml and 10 mg/ml on *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa,* this study showed no zones of

inhibition. Hence, the aqueous and ethanol extract of Monkey sugarcane has no antibacterial effects on these clinical isolates and cannot be used for the treatment of diseases caused by these bacteria; *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa.*

The results for the antibiotic susceptibility test of commercially available antibiotics showed that the isolates; *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* were all resistant to the commercially available Gram negative antibiotics.

The antioxidant, Ferric Reducing Antioxidant Power (FRAP) and Diphenyl-2-Picryl-Hydrazyl (DPPH) is used to reduce molybdenum (IV) to molybdenum (V) also Fe^{3+} to Fe^{2+} and to reduce DPPH respectively of the antioxidant compounds of the extract for both aqueous and ethanol extract forming colors after reduction like; antioxidant forms a green color, FRAP forms an intense blue color and DPPH forms a golden yellow color.

Overall, the results of this study demonstrate that *Costus afer* is a rich source of biologically active compounds with strong antibacterial activity supporting its traditional use as a natural remedy for fatigue, anemia and other oxidative stress-related disorders.

CONCLUSION

The pharmacological characteristics of the *Costus afer* aqueous and ethanol extract presented in this work offer some support for its application as an antifungal, analgesic with antibacterial properties, antioxidant and anti-inflammatory agent in wound healing, diabetes and inflammatory disorders. These results all point to its broad clinical relevance and support its use in the treatment of different diseases.

Monkey sugarcane (*Costus afer*) is a safe, nutrient-dense botanical ideal for functional foods and dietary supplements. It can also be regarded as nutraceutical because of its high proportions of macro and micro-nutrients like proteins and carbohydrates. This, along with the phytochemical abundance evidence, points to why administration of this plant and its extracts has yielded many positive results against many ailments among the local population

and makes it a potential source of many pharmaceutical breakthroughs. However, the antibacterial properties of *Costus afer* aqueous and ethanol extract as a potential pharmaceutical solution against *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* are yet to be seen, as the result of this study therefore shows that *Costus afer* does not have antimicrobial effect on *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa*, as to why these clinical isolates proved resistant to the *Costus afer* extract, more-in-depth research will need to be carried out to get specific answers as, a deeper understanding of this *Costus afer* plant, and its antibacterial components and their particular applications may prove to be potentially beneficial for both pharmaceutical and medical treatment of other microbial infection.

Monkey sugarcane (*Costus afer*) thus emerges not as an antibiotic alternative, but as multifunctional phytomedicine bridging nutrition, oxidative resilience and integrative infection management.

RECOMMENDATIONS

Based on the findings of this study, the following recommendations are made:

1. **Further Research:** Future studies should isolate, purify, and characterize the specific phytochemicals responsible for the observed antioxidant of *Costus afer*.

2. **Toxicological Assessment:** Long-term and chronic toxicity studies should be conducted to evaluate the plant's safety margin, especially at higher doses.

3. **Comparative Solvent Studies:** Additional extraction methods using solvents of varying polarities (methanol, ethyl acetate, etc.) should be explored to determine the optimal solvent for maximum bioactive yield.

4. **Clinical Evaluation:** Human clinical trials are recommended to confirm the therapeutic efficacy and safety of *Costus afer* extracts in managing oxidative stress and blood-related disorders.

5. **Pharmaceutical Development:** Given its rich antioxidant profile, *C. afer* could be formulated into herbal tonics, capsules, or teas for use as a natural hematinic and antioxidant supplement.

6. **Conservation Efforts:** Sustainable harvesting and cultivation programs should be encouraged to preserve *Costus afer* and promote its economic and medicinal utilization.

CONTRIBUTION TO KNOWLEDGE

This study provides new insights into the scientific basis for the traditional use of *Costus afer* as a blood purifier and antioxidant source.

Specifically, it:

1) Established that both aqueous and ethanol extracts of *C. afer* contain bioactive phytochemicals responsible for antioxidant activities.

2) Demonstrated that the plant exhibits significant total antioxidant and ferric reducing capacities comparable to standard antioxidants.

3) Confirmed that *C. afer* extracts can positively influence hematological parameters without inducing toxicity.

These findings contribute valuable data to the field of phytomedicine and support the integration of *Costus afer* into evidence-based herbal therapy and drug development.

REFERENCES

Adebayo, T. S., and Kretti, L. O. (2023). Immunomodulatory agents of natural origin: Mechanisms and applications. *Journal of Phytomedicine and Immunology*, **18**(2): 45–57.

Adedeji, O. A., Okafor, I. E., and Aremu, B. O. (2020). Phytochemical screening and medicinal properties of *Costus afer* in Nigeria: A review. *Nigerian Journal of Natural Product Research*, **4**(3): 89–97.

Adejumo, K., and Onyema, L. (2020). Ethno botanical survey and pharmacological relevance of *Costus afer* in Nigeria. *Journal of Medicinal Plant Studies*, **8**(4): 45–53.

Adekunle, M. C., Eze, V. J., and Nnaji, K. O. (2022). Phytochemical diversity and antimicrobial activity of *Costus afer* leaf extracts. *Journal of Herbal Pharmacology*, **11**(2): 81–92.

Adekunle, M., Eze, V., and Nnaji, K. (2021). Distribution and ecological relevance of *Costus afer* in tropical Africa. *African Journal of Botany*, **13**(2): 101–110.

Adeleke, A. B., Ogunleye, C. E., and Ikechukwu, N. T. (2020). Toxicological implications of bioactive plant constituents: Dose-response perspectives on *Costus afer*. *African Journal of Pharmacognosy*, **15**(3): 67–76.

Ajao, L. O., Eze, N. P., and Nwosu, K. C. (2022). Hepatoprotective and phytochemical analysis of *Costus afer* in paracetamol-induced liver toxicity. *West African Journal of Natural Products*, **10**(4): 145–155.

Akande, T. M., Olatunji, F. T., and Ezeh, N. A. (2023). Traditional therapeutic applications of *Costus afer* and its pharmacological potential. *African Journal of Complementary Medicine*, **19**(1): 25–35.

Amabe, J. E., Ukoha, A. I., and Ezenwa, O. E. (2021). Antimicrobial and antifungal screening of *Costus afer* extracts against selected clinical isolates. *African Journal of Microbiology Research*, **15**(2): 60–71.

Amabe, J. E., Ukoha, A. I., and Ezenwa, O. E. (2021). Antioxidant and nephroprotective potential of *Costus afer* in experimental models. *West African Journal of Pharmacognosy*, **10**(2): 66–77.

Amabe, J. E., Ukoha, A. I., and Ezenwa, O. E. (2021). Hematological and immunological responses to prolonged *Costus afer* extract administration in Wister rats. *West African Journal of Pharmacognosy,* **10**(2): 66–77.

Anaga, A. O., Eze, A. E., and Ofor, S. C. (2020). Reproductive and immunotoxic evaluation of methanolic *Costus afer* extract in pregnant rats. *African Journal of Toxicology Research*, **12**(4): 89–98.

Anyanwu, C. N., Okorie, F. I., and Ene, J. E. (2023). Antioxidant and free radical scavenging potential of aqueous and methanolic extracts of *Costus afer*. *Journal of Medicinal Botany*, **29**(1): 59–69.

Anyanwu, C. N., Okorie, F. I., and Ene, J. E. (2023). Antioxidant and free radical scavenging activity of aqueous and methanolic extracts of *Costus afer*. *Journal of Medicinal Botany*, **19**(1): 59–69.

Anyanwu, C. N., Okorie, F. I., and Ene, J. E. (2023). Comparative phytochemical profiling and antioxidant activity of aqueous and methanolic extracts of *Costus afer*. *Nigerian Journal of Botany*, **29**(1): 58–68.

Anyanwu, C. N., Okorie, F. I., and Ene, J. E. (2023). Phytochemical characterization and antioxidant screening of *Costus afer* leaf and stem extracts. *African Journal of Medicinal Botany*, **29**(1): 58–68.

Anyanwu, C. N., Okorie, F. I., and Ene, J. E. (2023). Propagation techniques and growth performance of *Costus afer* under controlled conditions. *Nigerian Journal of Plant Science*, **29**(1): 23–32.

Anyanwu, C. N., Okorie, F. I., and Ene, J. E. (2023). Quantitative determination of bioactive compounds in *Costus afer* leaf and stem extracts. *African Journal of Medicinal Botany*, **29**(2): 90–99.

Anyanwu, G, O., Njan, A. A., Okorie, C. C., Oguazu, C. E., and Ezejiofor, A. N. (2024). Hematoprotective role of Costuts afer aqueous extract in lipopolysaccharide-induced sepsis in wistar albino rats. Focus on erythrocyte stability and cytokine modulation. *Toxicology reports*, **12**, 100-109.

Arinze, P. E., Chukwu, M. N., and Ikechukwu, E. O. (2021). Phytochemical and hematological effects of combined leaf extracts of *Costus afer* and Cleome rutidosperma. *International Journal of Herbal Pharmacology*, **17**(3): 119–127.

B. A. Amadi, E. N. Agomuo, and C. O. Ibegbulem. (2004). *Research Methods in Biochemistry,* Supreme Publishers, Owerri, Nigeria.

Bala, K. E., Onyekachi, V. O., and Abah, P. C. (2022). Global reliance on medicinal plants for immunological health: An updated assessment. *Journal of Ethno biology and Health*, **8**(1): 55–64.

Benzie, I., F. and Strain, J., J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry* **239**: 70–76.

C. M. Ejikeme, C. S. Ezeonu, and A. N. Eboatu. (2014). "Determination of physical and phytochemical constituents of some tropical timbers indigenous to Niger Delta Area of Nigeria," *European Scientific Journal, vol. 10, no. 18, pp. 247-270.*

Chukwu, C. O., Umeh, E. A., and Iloh, A. B. (2022). Morphological characterization and pharmacognostic evaluation of *Costus afer*. *West African Journal of Natural Medicine*, **11**(1): 87–95.

Chukwu, M. N., Arinze, P. E., and Odo, T. C. (2022). Optimization of plant sample preservation techniques for phytochemical and antimicrobial studies. *African Journal of Laboratory Science*, **19**(3): 44–53.

Chukwu, M. N., Arinze, P. E., and Odo, T. C. (2022). Phenolic composition and DPPH antioxidant properties of *Costus afer* extracts. *African Journal of Natural Products*, **14**(2): 112–122.

Chukwu, M. N., Arinze, P. E., and Odo, T. C. (2022). Phenolic composition and antioxidant properties of *Costus afer* extracts. *African Journal of Natural Products*, **14**(2): 112–122.

Chukwu, M. N., Arinze, P. E., and Odo, T. C. (2022). Spectrophotometric evaluation of flavonoid content in medicinal plant extracts. *Journal of Phytochemical Analysis*, **15**(1): 33–42.

Elele, N. C., and Kingsley, P. O. (2023). Immunostimulatory potential of *Costus afer* leaf extract in laboratory animals. *African Journal of Herbal Research*, **15**(3): 93–102.

Elele, N. C., and Umukoro, B. E. (2023). Antimicrobial efficacy of *Costus afer* extracts using agar well diffusion and MIC assays. *International Journal of Phytomedicine*, **22**(1): 17–26.

Enaibe, B. U., Okorie, J. E., and Ofoegbu, S. C. (2020). Toxicological implications of prolonged herbal extract use: A case of *Costus afer*. *African Journal of Pharmacology and Toxicology*, **14**(2): 74–81.

Enejiofor, M. O., Onyekachi, V. O., and Abah, P. C. (2021). Ethno botanical significance of *Costus afer* in tropical West Africa. *Journal of Herbal Medicine and Research*, **8**(4): 41–50.

Eze, J. O., and Ogochukwu, T. P. (2022). Phytochemical quantification techniques and pharmacological relevance of Nigerian medicinal plants. *Nigerian Journal of Pharmacognosy*, **18**(3): 101–110.

Eze, J. O., and Ogochukwu, T. P. (2022). Plant-derived immunomodulators and their therapeutic significance in African traditional medicine. *African Journal of Integrative Medicine*, **19**(2): 33–43.

Eze, J. O., and Okafor, F. A. (2022). Phytochemicals as therapeutic agents in traditional medicine: Evidence from *Costus afer*. *African Journal of Phytomedicine*, **18**(2): 33–44.

Eze, J. O., Ike, C. N., and Odu, T. C. (2022). Application of statistical tools in analyzing phytochemical and antimicrobial datasets. *African Journal of Biostatistics*, **13**(2): 65–74.

Eze, J. O., Ike, C. N., and Odu, T. C. (2022). Microbiological techniques for evaluating plant extract activity. *African Journal of Microbial Studies*, **14**(2): 41–50.

Eze, J. O., Ike, C. N., and Odu, T. C. (2022). Morphological identification and authentication of medicinal plants used in ethno medicine in Southern Nigeria. *African Journal of Botany*, **13**(2): 75–84.

Eze, J. O., Ike, C. N., and Odu, T. C. (2022). Phytochemical basis of antimicrobial and pharmacological activities of *Costus afer*. *Journal of Herbal Science*, **19**(2): 54–63.

Eze, J. O., Ike, C. N., and Odu, T. C. (2022). The significance of phytochemicals in ethno medicine and public health. *African Journal of Traditional and Integrative Medicine*, **19**(2): 54–63.

Ezeani, O. C., and Uche, C. E. (2021). Phytotherapy and ethnomedicinal uses of *Costus afer*: A pharmacognostic review. *Nigerian Journal of Medicinal Botany*, **15**(2): 98–108.

Ezeani, O. C., Uche, C. E., and Nwosu, A. F. (2021). Evaluation of antioxidant activity and phytochemical composition of *Costus afer* stem and leaf extracts. *Nigerian Journal of Biochemistry*, **17**(3): 80–90.

Ezeani, O. C., Uche, C. E., and Nwosu, A. F. (2022). Comparative analysis of extraction methods on phytochemical yield and antioxidant potential of medicinal plants. *African Journal of Natural Product Research*, **14**(3): 75–84.

Ezeani, O. C., Uche, C. E., and Nwosu, A. F. (2022). Comparative analysis of extraction and quantification methods for plant phytochemicals. *African Journal of Natural Product Research*, **14**(3): 75–84.

Ezeani, O. C., Uche, C. E., and Nwosu, A. F. (2022). Comparative analysis of antioxidant capacity of medicinal plant extracts using DPPH assay. *Nigerian Journal of Biochemistry,* **17**(3): 80–90.

Ezejiofor, M. O., Onyekachi, V. O., and Abah, P. C. (2021). Antibacterial and antifungal properties of *Costus afer* leaf and stem extracts. *Journal of Herbal Medicine and Research*, **8**(4): 41–50.

Iwu, M. M. (2023). Herbal medicine and ethno pharmacology in sub-Saharan Africa: A review of *Costus afer* and related species. *Phytomedicine Research*, **25**(1): 33–41.

Iwu, M. M., and Kalu, A. N. (2023). Antioxidant profiling and total phenolic quantification in *Costus afer* extracts. *Journal of Phytopharmacology*, **28**(1): 35–45.

Kagbo, D. N., and Obinna, E. F. (2023). Reproductive and gestational effects of *Costus afer* leaf extracts in animal models. *Nigerian Journal of Reproductive Health Research*, **13**(1): 71–80.

Kalu, A. N., Nwachukwu, I. J., and Ezeani, O. L. (2020). Sustainability assessment of medicinal plant harvesting in sub-Saharan Africa: The case of *Costus afer*. *Journal of Environmental Conservation*, **14**(4): 221–230.

Nwachukwu, E. C., and Eze, O. J. (2022). Phytochemical and pharmacological significance of *Costus afer* in traditional medicine. *Journal of Ethnopharmacology Research*, **18**(3): 91–101.

Obi, C. I., Nduka, U. M., and Onyeukwu, C. A. (2023). Reproductive safety evaluation of *Costus afer* in animal models. *African Journal of Reproductive Toxicology*, **8**(1): 45–52.

Obinna, G. C., and Eze, L. N. (2021). Bioactive compounds of *Costus afer* and their pharmacological relevance. *Journal of Medicinal Plant Research*, **15**(6): 230–239.

Obinna, G. C., Eze, L. N., and Okwu, A. P. (2024). Immuno-haematological evaluation of *Costus afer* in laboratory animals. *West African Journal of Medical Botany*, **14**(1): 40–50.

Ogueke, C. C., Ude, S. N., and Obi, J. E. (2022). Alkaloid quantification and antimicrobial correlation in Nigerian medicinal plants. *Nigerian Journal of Experimental Biology*, **9**(2): 89–97.

Ogueke, C. C., Ude, S. N., and Obi, J. E. (2022). Antimicrobial correlation of phytochemical constituents in *Costus afer* and related species. *Nigerian Journal of Experimental Biology*, **9**(2): 89–97.

Ogundele, K. S., Emenike, A. E., and Ezeani, O. C. (2021). Quantitative determination of alkaloids and flavonoids in *Costus afer* and related medicinal plants. *Nigerian Journal of Pharmaceutical Sciences*, **20**(2): 112–121.

Okafor, J. E., Eboh, P. C., and Chinonso, E. N. (2024). Antioxidant and therapeutic potentials of *Costus afer*: Current perspectives. *African Journal of Pharmacology and Therapeutics*, **19**(1): 53–63.

Okafor, J. E., Eboh, P. C., and Chinonso, E. N. (2024). Immunopharmacological prospects of *Costus afer*: A comprehensive review. *Journal of Ethnopharmacology and Therapeutics*, **20**(1): 63–75.

Okafor, J. E., Eboh, P. C., and Chinonso, E. N. (2024). SPSS and Graph Pad-based approaches for analyzing herbal pharmacological data. *Nigerian Journal of Experimental Research*, **20**(1): 57–66.

Okafor, J. E., Eboh, P. C., and Chinonso, E. N. (2024). Standardized methods for preparing medicinal plant materials for phytochemical and pharmacological research. *Journal of Herbal Pharmacology*, **21**(1): 33–42.

Okafor, J. E., Eboh, P. C., and Chinonso, E. N. (2024). Standardized protocols for quantitative phytochemical analysis of herbal extracts. *Journal of Herbal Pharmacology*, **21**(1): 33–42.

Okafor, J. E., Eboh, P. C., and Chinonso, E. N. (2024). Updated protocols for phytochemical screening of medicinal plants: A case study of *Costus afer*. *Nigerian Journal of Natural Products*, **21**(2): 44–53.

Okiemute, R. O., Ewhre, O. L., and Okhimamhe, G. F. (2025). Phytochemical, nutritional and antimicrobial evaluation of *Costus afer* Ker Gawl from Ughelli, Delta State. Implications for ethnomedicinal safety and efficacy. *Journal of Ethnopharmacology,* **312.**

Okoro, N. L., Adeyemi, F. K and Ifeanyi, C. B. (2024). Protective effects of *Costus afer* extract against hepatic oxidative stress. *West African Journal of Toxicology*, **13**(2): 101–111.

Okoro, N. L., Adeyemi, F. K., and Ifeanyi, C. B. (2024). Nutritional and medicinal potentials of *Costus afer* essential oils in tropical diets. *African Journal of Food Science and Nutrition*, **31**(1): 55–64.

Okoro, N. L., Adeyemi, F. K., and Ifeanyi, C. B. (2024). Standardization of inoculum density for reproducible antimicrobial testing of herbal extracts. *West African Journal of Microbiology*, **19**(1): 52–61.

Okoro, N. L., and Ezeani, O. C. (2021). In vitro evaluation of the antibacterial efficacy of *Costus afer* extracts against pathogenic microorganisms. *Nigerian Journal of Biological Sciences*, **11**(3): 112–121.

Omokhua, G. E., Ogu, V. C., and Chinweuba, M. O. (2022). Pharmacological validation and antimicrobial activity of *Costus afer* extracts. *Journal of Ethnopharmacology and Herbal Science*, **28**(3): 110–122.

Onwudiwe, K. C., Ogbuehi, V. C., and Adebisi, T. P. (2023). Comparative phytochemical screening methods for herbal drug standardization. *Journal of Phytochemical Analysis*, **15**(4): 77–88.

Onwuka, A. K., Duru, I. A., and Ene, L. J. (2021). Evaluation of the antibacterial potential of *Costus afer* extracts using standard diffusion methods. *West African Journal of Pharmacology*, **12**(4): 140–149.

Onyeka, F. E., Akintola, D. A., and Ofor, S. O. (2021). Solvent influence on phytochemical yield and bioactivity of *Costus afer* extracts. *International Journal of Plant Biochemistry*, **14**(3): 99–108.

Onyeka, F. E., Akintola, D. A., and Ofor, S. O. (2023). Influence of drying methods on phytochemical integrity and extract yield of tropical medicinal plants. *Nigerian Journal of Medicinal Botany*, **18**(2): 90–99.

Onyekachi, A. F., and Adekunle, M. O. (2023). Comparative herbarium-based authentication of Costus species in West Africa. *Journal of Plant Taxonomy and Biodiversity*, **17**(1): 49–59.

Onyekachi, A. F., and Adekunle, M. O. (2023). Modernized gravimetric and spectrophotometric assays for phytochemical quantification. *African Journal of Pharmaceutical Research*, **17**(2): 61–72.

Onyekachi, A. F., and Okoro, N. L. (2023). Optimization of ethanol extraction for bioactive compound recovery from *Costus afer* leaves. *Nigerian Journal of Herbal Science*, **20**(1): 47–56.

Osuchukwu, C. F., and Onwusonye, K. E. (2021). Haematological and immunological responses to aqueous *Costus afer* extract in rats. *Nigerian Journal of Biochemistry and Molecular Biology,* **35**(2): 112–118.

Owolabi, A. S., Nwokocha, E. O., and Abiola, F. B. (2021). Gravimetric estimation of saponins in tropical herbal species. *African Journal of Plant Biochemistry*, **10**(3): 88–95.

Owolabi, A. S., Nwokocha, E. O., and Abiola, F. B. (2021). Oxytocic and uterine-contractile effects of *Costus afer* in experimental rat uterus models. *African Journal of Physiology and Pharmacology*, **9**(3): 88–96.

Udem, S. C., Ezeasor, C. J., and Amadi, I. A. (2023). Chronic hematological effects of *Costus afer* stem extract in experimental models. *African Journal of Toxicology Studies*, **19**(2): 53–61.

Udem, S. C., Ezeasor, C. J., and Amadi, I. A. (2023). Evaluation of solvent efficiency in phytochemical extraction of medicinal plant species. *Journal of Phytochemical Techniques*, **19**(2): 88–97.

Ukpabi, U. J., and Nwachukwu, E. S. (2022). Ethno pharmacological uses and socio-economic importance of *Costus afer* in Nigeria. *Journal of African Ethno botany*, **16**(2): 75–83.

Uzochukwu, G. M., and Okafor, I. C. (2023). Ethical harvesting practices and conservation strategies for medicinal plants in Nigeria. *Journal of Environmental Conservation*, **14**(4): 120–131.

Uzochukwu, G. M., and Okafor, I. C. (2023). Phytotherapeutic and antimicrobial properties of *Costus afer* in clinical application. *International Journal of Herbal Research*, **21**(1): 42–51.

Widyawati, P. S., Nordin, M. M., and Aziz, R. A. (2022). Extraction solvent polarity and phytochemical recovery from medicinal plants: A systematic review. *Journal of Herbal Science and Technology,* **25**(2): 75–88.