

Phytochemical Constituents, Proximate Composition and Antibacterial Activity of Ethanol Extract of Costus Afer (Monkey Sugar Cane) on Escherichia Coli and Klebsiella Pneumoniae

Masikoro Collins Oghenekparobor

reachcollinsm@gmail.com

University of Benin <https://orcid.org/0009-0006-5906-2654>

Research Article

Keywords: Costus afer, Monkey sugarcane, Phytochemical analysis, Proximate composition, Antimicrobial activity, Escherichia coli, Klebsiella pneumoniae, Ethanol extract, Aqueous extract, Disk diffusion method, Traditional medicine, Medicinal plants, Antibiotic resistance, Ethnomedicine, Soxhlet extraction

Posted Date: October 20th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7843092/v2>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: The authors declare no competing interests.

CHAPTER ONE

1. INTRODUCTION

1.0 Background of the Study

Traditional and ethnomedical medicine both contend that the body's natural defenses against microbial illnesses can be strengthened. Antimicrobial properties of therapeutic herbs have been reported (Omokhua, 2011). It may be possible to identify novel molecular entities that are both inexpensive and efficient in treating various ailments by assessing plant extracts for their immunostimulant properties. These extracts are believed to be safe and won't cause microbial resistance because they are derived from natural plant sources (Ezejiofor et al., 2017). The use of medicinal plants for therapeutic reasons has increased recently on a global scale. It is believed that the majority of the world's population—about 80% of people—relies on medicinal plants for their primary source of healthcare. (2014) Bala et al. These days, supplements, tinctures, sauces, encapsulated powders, lozenges, and tablets are made from plant extracts. Combining a variety of phyto-constituents, these extracts interact with several molecular targets within an organism or individual to produce pharmacological or physiological reactions (Modaresi and Khodaddi, 2014). Giving plant-based foods or medicines for long periods of time without taking into account their possible toxicity or negative consequences is a common practice in underdeveloped countries like Nigeria.

Numerous chemical components found in plants can help combat the range of diseases we are constantly exposed to. It would be helpful to comprehend these phytochemicals and their specific uses for medical and pharmaceutical therapy of disorders (Enwuru et al., 2008). Plants have long provided mankind with natural remedies for a variety of ailments. Nonetheless, they

play a vital role in primary healthcare as therapeutic interventions in developing countries. Phytochemicals are the reason plants are used so extensively in traditional medicine; they are non-nutritive plant compounds with the ability to prevent or treat disease.

Costus afer is a tropical monocot plant that is quite tall, herbaceous, unbranched, and has a creeping rhizome. It is also known as bush sugar cane or monkey sugar cane (Nyanayo, 2006). It belongs to the family *Zingiberaceae*. It is frequently found in the moist or shady woodlands of West and Tropical Africa (Iwu, 2009). *C. afer* is a useful medicinal plant that is well-known for its anti-inflammatory, anti-diabetic, and anti-anchoretic properties in South-East and South-South Nigeria (Soladoye & Oyesika, 2008). Bioactive compounds such kaempferol 3, L-rhamnopyranoside, diosgenin, saponins, aferosides A–C, dioscin, and paryphyllin C have been reported to be present in *Costus afer* (Aweke, 2007). Additionally, it has been shown to lessen the toxicity and oxidative stress on the liver brought on by carbon tetrachloride (Ukpabi *et al.*, 2012). Furthermore, Okoko (2009) demonstrated that *C. afer* stem extracts reduced the tissue damage that paracetamol caused in rats. *C. afer* has historically been used to treat a wide range of ailments, such as hepatic disease, rheumatoid arthritis, stomach problems, snake poisoning, measles, and malaria (Iwu, 2009; Omokhua, 2011).

1.1 Aim and Objectives of the Study

The aim of this study was to determine the phytochemical constituents, proximate composition and antimicrobial activity of the ethanol and aqueous extract of *Costus afer* on selected bacterial isolates; *Escherichia Coli* and *Klebsiella pneumonia*

The specific objectives of this study are;

- I. Determine the phytochemical constituents of the ethanol and aqueous leaf extract of *Costus afer*,
- II. Determine the proximate constituents of *Costus afer*,
- III. Evaluate the antibacterial effects of *Costus afer* on *Escherichia coli* and *Klebsiella pneumoniae*; and
- IV. Assess the antibacterial effects of commercially available antibiotics on *Escherichia coli* and *Klebsiella*.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Costus afer* Plant

Because medicinal plants can provide extremely potent secondary compounds that are used to treat human illnesses, they are particularly significant. It is well acknowledged that vitamins, minerals, and phytochemicals are the active components of medicinal plants that give them their therapeutic properties. Plants have historically been used for millennia to treat illnesses with herbal treatments, and in developing countries, they continue to be an important part of primary health care. Plants have a folk-medical relevance because of phytochemicals, which are non-nutritive plant compounds with medicinal or preventative qualities (Arhoghro *et al.*, 2014). Different medicinal plants have different effects on biological systems; sedatives, analgesics, cardio-protection, antipyretics, antibacterials, antivirals, and antiprotozoals are a few characteristics. One plant whose effects have been demonstrated to differ is *costus afer* (Ukpabi *et al.*, 2012).

Costus afer is a plant that belongs to the Zingiberaceae family. This tall, perennial, herbaceous, unbranched tropical plant is also known as bush cane or monkey sugar cane. It originates from a rhizome that spreads. Furthermore, it was claimed that the woodland belt of Nigeria, Senegal, South Africa, Guinea, and Niger may include *Costus afer* (Omokhua, 2011).

Costus afer is usually found in wet or shady forests of West and Tropical Africa and can be used in polyherbal therapy (Iwu, 2009). They contain synergistic, prospective, agonistic/antagonistic pharmacological substances that interact dynamically to achieve therapeutic effectiveness with minimal adverse effects. Similarly, Arhoghro *et al.* (2014) examined the phytochemical composition and effects on the lipid profile and other haematological indicators in

wistar rats of an ethanolic leaf extract of *Costus afer* and *Cleome rutidosperma* combo. Considering this, they studied the combined extracts of the two widely used plants—*Costus afer* and *Cleome rutidosperma*—which have been used for many years to treat a range of ailments. The investigations showed that when compared to the control, the extract greatly reduced the amount of packed cell volume (PVC), haemoglobin (HB), and lymphocytes but dramatically increased the number of white blood cells (WBC) and neutrophils. In conclusion, the combined leaf extract may have an unofficial impact on blood cholesterol content and be utilized to strengthen the immune system.

The plant is classified according to the following taxonomy;

Kingdom: Plantae

Division: Spermatophyta

Order: Zingiberales

Family: Zingiberaceae

Genus: *Costus*

Species: *afer*

Costus afer is an evergreen, perennial rhizomatous herbaceous species 2.5–4 m tall, with slender stems and spiral leaves with a closed, tubular sheath that is green in colour and speckled with purple. It also has a 4.8 mm long, coriaceous, globose ligula. It is distinguished by a mostly bald horizontal ring surrounding the shoot nodes, which are sparsely coated with upright hairs. It has one or two blooms per bract; the bracts are coriaceous, ovate-triangular, pale green, and frequently have a reddish upper edge. As is recognized in many *Costus* species, the inflorescence of *Costus afer* can terminate a leafy or leafless branch (Omokhua, 2011).

2.2. Health benefits of *Costus afer*

Costus afer has a number of health benefits. An infusion of the inflorescence is used to treat tachycardia. Inhaling the smoke from the dried stem is another method for treating cough (Akbar, 2011). Leaf sap is applied topically to treat heat and edema, as well as to relieve vertigo and migraines. Leaf sap or rhizomes are decocted to treat malaria. Applying *Costus afer* stem sap helps treat urethral discharges, repair jaundice, prevent miscarriages, and cure a variety of skin ailments. A stem decoction is a typical treatment for rheumatoid arthritis (Omokhua, 2011). An infusion of the dried aerial sections is used to treat hypertension. The stems that have been pulverized are utilized topically to treat worms and hemorrhoids. Pulped stem water ingestion has a strong diuretic impact.

A stem decoction is a typical treatment for rheumatoid arthritis (Omokhua, 2011). An infusion of the dried aerial sections is used to treat hypertension. The stems that have been pulverized are utilized topically to treat worms and hemorrhoids. Pulped stem water ingestion has a strong diuretic impact. Because there is substantial resistance to the commonly used anti-malaria treatments, interior house spraying to prevent and control the vector has not completely eliminated the issue. Elele and Umukoro (2019) therefore documented the administration of *C. afer* stem aqueous extract on albino mice infected with *Plasmodium*. The ability of *C. afer*'s aqueous stem extract to shield mice from plasmodium infection suggests that it may have anti-malaria properties, according to the study's findings.

Traditional herbs are being used more and more to treat illnesses because of their efficacy in managing health issues, especially in rural and impoverished areas. *Costus afer*, commonly referred to as okpete, is one of the most widely used herbs in medicine. Numerous conditions, such as gout, diabetes, arthritis, stomachaches, and inflammation are treated with it. The stem and leaves

of the herb are a good source of macro- and micronutrients, according to medical study. They include high concentrations of atherosides, paryphyllin C, and flavonoids glycosides, which are essential for the treatment of many diseases. The plant also contains tannins, alkaloids, anthraquinones, terpenoids, cardiac glycosides, and saponins (Adedapo et al., 2007). For instance, the oil from the herb is used to treat asthma, cough, gas dysentery, and cholera. The oil promotes digestion in addition to being a tonic. Food and drink also use *C. afer* oil as a flavoring element (Arhoghro et al., 2009). Studies on pancreatic cells damaged by alloxan demonstrated that *C. afer* stem extract can protect the organ. The histological harm that alloxan induced to the pancreatic β -cells in diabetes mellitus was restored by the plant's leaf and stem extract. The study's conclusions indicate that *C. afer* leaves and stem extract have effects that protect and regenerate pancreatic islet cells, which may make them useful in the management of type 1 diabetes mellitus (Arhoghro et al., 2012).

According to a recent study (Udem and Ezeasor, 2010), the stem extract of *C. afer* can shield the rats' livers from alcohol-induced liver damage. According to the study's findings, the plant has pharmacological efficacy against liver cirrhosis caused by alcohol. When applied to the face, the plant's oil helps to reduce acne and smooth the complexion. Compounds with antibacterial, antifungal, and anti-inflammatory properties are included in the oil.

The stems and leaves of *Costus afer* aid in the liver and kidney's detoxification. The anti-inflammatory properties of the herb aid in decreasing kidney discomfort and edema brought on by infections. According to an animal study, the leaves of *C. afer* exhibit nephroprotective effects on the kidney (Amabe *et al.*, 2016). Because of its antioxidant qualities, it also combats oxidative stress and acts as an antifungal and antibacterial agent. However, because the plant extract lowers haemoglobin and red blood cells, which might result in anaemia, long-term usage is not advised.

2.3. Phytochemicals Composition of *Costus afer*

The use of plants in the treatment of a variety of disorders has been connected to the presence of bioactive compounds in plant components that are extracted using different solvents. Arhoghro et al. (2014) state that flavonoids, cardiac glycosides, alkaloids, and saponins are present in the ethanolic extract of *C. afer* leaves. Phytochemicals are plant compounds that are not food. They can either prevent or treat disease. These pharmaceuticals feature dynamic modes of action that produce therapeutic effectiveness with minimal side effects. It would therefore be advantageous to understand these phytochemicals and their specific uses for the treatment of illnesses by pharmaceutical and medical means (Enwuru, 2008). Therefore, it is possible to attribute the presence of these phytochemicals in the plant extract to both the immune system's strengthening and its favorable effect on blood cholesterol content. Aweke (2007) states that bioactive components found in *C. afer* include paryphyllin C, diosgenin, flavonoids, cardiac glycosides, aferosides A–C, dioscin, and kaempferol 3. Alkaloids, flavonoids, saponins, tannins, and phenols were found in the phytochemical investigation Elele and Kingsley (2019) conducted on the aqueous extract of *C. afer*. The study's findings showed that the aqueous extract of *C. afer* included high concentrations of saponins, tannins, and phenols with values of 2.60.18, 2.540.18, and 0.180.03, respectively, and flavonoids and alkaloids with values of 290.18 and 4.60.13, respectively.

Since aqueous preparation is the norm in ethnomedicine, aqueous extract is the method of choice for many studies. According to Widyawati *et al.* (2014), methanol and ethanol can dissolve phenolic compounds with low and medium molecular weight and medium weight polarity, as well as aglycones of flavonoids, anthocyanins, terpenoid, saponins, and tannins. Polar compounds like sugar, amino acids, and glycosides can also be dissolved (Dehkharghanian *et al.*, 2010).

The phytochemical components and antioxidant properties of *Costus afer* stem extracts in methanol and water were studied by Anyasor *et al.* (2010). Through phytochemical examination of the extracts, the presence of flavonoids, phenols, anthraquinones, cardiac glycosides, and terpenoids was determined. Additionally, studies on the aqueous extract of *C. afer* showed promising outcomes for alkaloids and tannins. Cardenolides, phlobatannins, and saponins, however, tested negative for all extracts. In addition to its antimicrobial, antifungal, and anti-inflammatory qualities, alkaloids are known to have an anti-hypertensive effect (Okwu and Okwu, 2004). Owing to its tannin content, *C. afer* has long been used to heal wounds, bleeding, diarrhea, and sore throats (Okwu and Okwu, 2004). Cardiac glycosides have reportedly been used successfully to treat congestive heart failure and regulate pulse.

2.4. Anti-oxidant Activity of *Costus afer*

Plant phenolics are an important class of compounds that act as a main antioxidant or free scavenger. Antioxidants have been shown to offer therapeutic potential in lowering degenerative disorders linked to oxidative damage caused by reactive oxygen species or free radicals. The high phenolic content water extract of *C. afer* showed higher inhibition of lipid peroxidative and antioxidant activities than the methanol extract. These suggested that it might be helpful in the treatment and prevention of certain illnesses linked to oxidative stress. Therefore, biologically active metabolites and free radical scavengers in the food, pharmaceutical, and other industries may employ *C. afer* stem extract (Anyasor *et al.*, 2010).

Reactive oxygen species (ROS) are potent oxidizers that damage biological components, changing their structure and function in DNA, lipids, and proteins. β -carotene, vitamin C (ascorbic acid), and vitamin E (tocopherol) are the primary antioxidants present in diet. Many chronic disorders may be prevented by these antioxidants. Much attention has been aroused by the

possibility of chemoprevention and therapeutic uses to boost antioxidant defenses. The *C. afer* extract showed high hydroxyl radical scavenging capabilities in addition to chelating iron. In addition, it had a strong inhibitory effect on the peroxidation of phospholipids in rat brain and protected DNA against bleomycin or copper-phenanthroline systems. Anwanni and Atta (2006) found that the extract had great efficacy as a preventive measure against oxidative damage caused by iron. The study also looked at the extract's interaction with iron (III).

2.5. Antimicrobial Activity of *Costus afer*

The antibacterial chemical components found in medicinal plants are helpful in combating a range of ailments to which humans are frequently exposed (Enwuru, 2008). Numerous detrimental microorganisms have been shown to be susceptible to the antibacterial activity of *C. afer*. Among the bacterial species against which the *C. afer* extract showed effectiveness in an in vitro agar diffusion method were *Salmonella typhi*, *Bacillus pumilus*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Shigella* spp., and *Klebsiella pneumonia*. Furthermore, studies have demonstrated the efficacy of *C. afer* against *Saccharomyces cerevisiae*, *Aspergillus fumigatus*, *Trichoderma* spp., and *Aspergillus flavus* (Nyananyo, 2006). The bioactive chemicals present in *C. afer* have the potential to cause this activity as they alter the growth patterns and biochemical activities of different pathogenic bacteria that are associated with different human disorders. *C. afer* thus significantly benefits humankind's ability to prevent and cure a wide range of illnesses.

Malaria is brought on by parasites called Plasmodium. Female Anopheles mosquitoes carrying the parasites that bite people are known as malaria vectors. The popular anti-malarial drugs have a high rate of resistance, and substitute drugs have significant risks. Spraying inside

homes to prevent and manage the vector hasn't completely eliminated the issue, and female *Anopheles* mosquitoes have developed resistance. A number of malaria projects have also been hampered by operational and financial problems. Higher plants are therefore a source for scientists trying to create new antimalarial drugs. There are reports that *C. afer* possesses specific antimalarial properties. Aqueous extract from the stem of a bush can (*Costus afer*) and infected albino mice were used in Elele and Umukoro's (2019) investigation of the effects of these materials on *Plasmodium berghei*. The results of the study show that the aqueous stem extract of *C. afer* has anti-malaria properties against *Plasmodium berghei*-infected mice. Given that the plant extract is also able to influence hematopoietic stem cells' ability to produce red blood cells (RBCs), oral administration of the extract during the study (at doses between 100 and 500 mg/kg body weight) raises the possibility that *C. afer* stem extract may be a safe and effective natural anti-malaria medication.

It is believed that these polyphenols are responsible for *C. afer* extract's antibacterial activity against *Vibrio cholerae*, *Staphylococcus aureus*, and *Bacillus subtilis* (Ezejiofor et al., 2017). The plant extract also possesses anti-diarrheal qualities since it acts against *E. coli*, a common pathogen associated with diarrhea. One possible explanation for its anti-diarrheal qualities could be its effect on the digestive system.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Collection, Identification and Authentication of Materials

Plant Collection; The plant used in this study was collected from a farmyard in Ughelli, 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* and *Klebsiella* 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. (Mahasneh *et al.*, 1999).

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°C. The extract was stored at 40°C for further use.

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: The extract was mixed with a few drops of 0.1% ferric chloride. A positive test result is indicated by a brownish green or blue-black colouring.
2. Test for terpenoids: The extract was treated with a mixture of chloroform and concentrated tetraoxosulphate (iv) acid. A good indicator of terpenoids' existence is the presence of reddish-brown colouring.
3. Test for flavonoids: Tetraoxosulphate (vi) acid in concentrated form was added to the extract after 10 millilitres of diluted ammonia solution was added. Flavonoids are present when a yellow tint arises and vanishes while a person stands still.
4. Test for alkaloids: A test tube full of extract was filled with chloroform. If an alkaloids look pink, they are not present, however if they appear brown, they are present.
5. Test for steroids: Concentrated tetraoxosulphate (vi) acid was added to the extract after acetic anhydride was added. Steroids are indicated by a colour that ranges from violet to blue or green.
6. Test for saponins: After the extract was added to a test tube and shaken briskly, the presence of phenols was determined by the absence of persistent froth.

7. 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 Proximate Analysis

3.4.1. Moisture content determination

Equipment: oven, weighing balance

Apparatus: beaker, Spatula crucible/beaker, desiccator

Procedure:

- To ensure the crucible is dry, bake it at 105°C for an hour (or 5 minutes if using a beaker).
- Place the crucible or beaker in the desiccator and let it cool for about half an hour.
- Use an electronic balance to weigh the crucible and note the result as W1.
- One gramme of the sample is weighed into the beaker or crucible beforehand. W2
- The beaker/crucible and its contents are oven dried for three hours at 105°C. After that, they are weighed, allowed to cool for ten minutes in a desiccator, and then they are placed back in the oven until a constant weight (W3) is achieved.

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

$$W2 - W1$$

3.4.2 Crude Protein Determination

Equipment: weighing balance, spectrophotometer

Apparatus: conical flask, heating mantle, volumetric flask,

Reagents: 0.1N HCl, conc. H₂SO₄, 40% NaOH, Boric acid indicator, mixed catalyst (copper sulphate, and sodium sulphate)/Digestion mixture.

Procedure

- A conical or volumetric flask should be filled with 0.5–1 g of the sample.
- • Include 1 gram of mixed catalyst or a mixed catalyst tablet with 10 millilitres of concentrated H₂SO₄.
- • After spinning the flask to fully combine the contents, it is heated for around two hours to initiate the digestion process and turn the liquid a pale green colour.
- Following cooling, the digest was transferred to a 100 ml volumetric flask and its volume was increased with distilled water (keep note of the volume of water used to reach the 100 ml threshold).
- • To capture any ammonium created during the distillation process, standardized boric acid and roughly three drops of methyl red indicator are added to the volumetric flask of the apparatus.
- • In the distillation tube, 25 millilitres of diluted digest and 25 millilitres of gradually added 40% NaOH were added.
- • After at least ten minutes of distillation, the NH₃ that is produced is collected as NH₄OH in a conical flask with 25 millilitres of 4% boric acid and methyl red indicator.
- • NH₄OH causes a yellowish colour to develop during distillation.
- • After that, 20 millilitres of distillate are titrated against a standard 0.1N HCl solution until a pink hue appears.
- % 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 = milliequivalent weight of Nitrogen

3.4.3 Ash Content Determination

Equipment: oven, weighing balance

Apparatus: crucible, desiccator, muffled furnace

Procedure

- Pre-dry the crucibles
- Following these steps:
 - Weigh the crucibles and record the results (2 for each sample) as W1;
 - Place the crucible on the weigh and zero it;
 - Add 1g of sample to each crucible as W2;
 - Record the weight of the crucible and sample;
 - Place the crucible and sample in a muffle furnace and leave for 3 hours;
 - Weigh it again and place it back in the oven until it reaches a constant weight W3.
- Percentage ash is then calculated as follows and average taken.

Calculation:

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

Where

W1 = weight of crucible

W2 = weight of crucible and sample before ashing

W3 = weight of crucible and sample after ashing

3.4.4 Lipids (Fat Extraction) Determination

Equipment: weighing balance, oven

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

Reagent: petroleum ether

Procedure:

- Crush the previously dried sample to make it easier for the organic solvent to enter.
- Weigh a filter paper and note W1, then zero the reading and weigh one gram of sample on the paper (note the combined weight of the filter paper and sample as W2).
- Pour petroleum ether halfway into the conical flask of the Soxhlet extractor.
- Insert the sample with the filter paper into the Soxhlet extractor's sample holder; the extractor has a reflux condenser.
- Modify the heat source and let the mixture boil.
- It is left to siphon around the barrel for more than five hours at a pace of five to six drips per second of condensation.

- The filter paper holding the sample is taken out, and the condenser is disconnected.
- For one to two hours, dry the filter paper with the sample inside it at 105°C in the oven.
- Dry till it attains a constant weight W3.

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

W1

3.4.5 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 2g of sample into 250ml volumetric flask and record W0
- Pour in 100ml of 1.25% sulfuric acid and simmer for 30 minutes. Pass the mixture through muslin cloth or filter paper, and then thoroughly rinse with hot distilled water to guarantee that all of the acid has been removed from the sample.
- Using a spatula, separate the residue and return it to the flask
- For thirty minutes, slowly boil 100 millilitres of 1.25% NaOH.
- To guarantee the total removal of base from the sample, filter through muslin cloth or filter paper and thoroughly rinse with hot distilled water.

- Remaining residue can be separated using a spatula and then placed back into a crucible. To rinse the residue off the muslin cloth and help balance the acid and base in the residue, add a few drops of acetone.
- Then, move the crucible into a muffle furnace at 3000C for one hour.
- Cool in a desiccator and weigh W2.
- Move the residue into an oven at 1050C for two to three hours.

Calculation:

$$\% \text{ crude fibre} = \frac{W1 - W2}{W0} \times 100$$

Where

W1 = weight of sample after oven drying

W2 = weight of sample after ashing

W0 = weight of sample

Nitrogen free extract (carbohydrate)

$$\text{N.F.E} = 100 - (\% \text{ moisture} + \% \text{ ash} + \% \text{ fat} + \% \text{ crude fibre} + \% \text{ crude protein})$$

3.5 Histochemical Study

The chemical constituents in the tissues were localized by treating freehand sections of leaves, stem, rhizome, and root materials with the appropriate reagent. The freshly unstained and stained sections were contrasted. The sections were put on a slide so that a compound microscope could view them. Alkaloids, phenols, tannins, proteins, and other phytochemicals were examined in the mounted sections using a compound microscope (Matias et al., 2016; Sussma et al., 2019).

3.6 Qualitative Phytochemical Screening

- a. Test for tannins: Add 2-3 drops of 5% FeCl solution to approximately 2-3 mL of extract. The development of a green or bluish-black colour indicates the presence of tannins.
- b. Test for saponins (Foam formation test): 5 mL of de-ionized water was added to approximately 2 to 3 mL of extract. Strong shaking caused the foam to continue to form. After 15 minutes of standing, the honeycomb froth—which displays saponins—was collected.
- c. Test for flavonoids (Shinoda test): Add 4-5 drops of concentrated hydrochloric acid and a few pieces of magnesium ribbon to 1 millilitre of extract. The development of the pink or red colour indicates the presence of flavonoids.
- d. Test for terpenoids (Salkowski test): Add 2 mL of chloroform and conc. H₂SO₄ to 0.5 mL of extract. Terpenoids are indicated by the reddish-brown colour at the interface.
- e. Test for carbohydrates (Molisch's test): The oil-water layers' interphase can be seen to exhibit a reddish-violet zone when 1 millilitre of extract is combined with 1 millilitre of concentrated H₂SO₄. It is indicated that glycosides and carbohydrates are present.
- f. The test for anthraquinone (Bontrager's test): A volume of 1 mL was combined with 5 mL of benzene. It was also shaken and filtered. After adding 5 mL of 10% ammonium hydroxide, the contents were shaken. The presence of anthraquinones is indicated by a red, pink, or violet colour in the lower ammoniacal phase.
- g. Test for cardiac glycosides (Keller-Kiliani test): One millilitre of the extract was combined with two millilitres of acetic acid and one or two drops of 2% ferric chloride solution. Next, this mixture was added to a second test tube containing two millilitres of concentrated H₂SO₄. The sample shows the presence of cardiac glycosides and a brownish-coloured ring at the interphase.

- h. Test for steroids/phenol (Liebermann-Burchard test): 1 mL of extract was mixed with about 2 mL of acetic acid. Conc. H₂SO₄ was cautiously added after the solution had cooled on an ice bath. The test for steroids is confirmed when the colour changes from violet to blue or bluish-green.
- i. Test for alkaloids: 2 mL of 1% HCl were added to 1 mL of extract, and the mixture was heated. Additionally, 4–5 drops of Mayer's reagent were added. The test for alkaloids is validated by the formation of a white or cream-coloured precipitate.
- j. Test for coumarins: One millilitre of the sample extract was taken, and filter paper moistened with diluted NaOH was placed in a test tube. It took three to five minutes to heat the sample. In addition, the filter paper was inspected for yellow-coloured fluorescence under ultraviolet light (365 nm), which validates the coumarin test. (Saraf et al., 2010) (Yadav et al., 2011).

3.7 Quantitative Phytochemical Screening

3.7.1 Determination of Total Tannins

The modified Folin-Denis method was used to determine the content of tannins (Polshettiwar et al., 2007). The technique measures the blue colour that results from tannin-like compounds reducing phosphotungstic-molybdic acid in an alkaline medium. 0.5ml of the Folin-Denis reagent and 1ml of the 7.5% Na₂CO₃ solution were combined with 0.5ml of the extract (1 mg/ml) and a standard solution of tannic acid (10-150 µg/ml). After adding 3.4 millilitres of distilled water, the absorbance at 700 nm was determined. In milligrams of tannic acid equivalent per gram of extract, the total tannin content was reported. (Polshettiwar and Ganjiwale, 2007)

3.7.2 Determination of Total Flavonoids

Flavonoids possess antioxidative properties through multiple mechanisms, including scavenging of free radicals, chelation of metal ions like copper and iron, and inhibition of enzymes that

generate free radicals (Benavente-Garcia, 1997). Flavonoids have the ability to scavenge almost all known ROS, depending on their specific structure.

How to do it: In methanol, prepare the quercetin standard (10–100 µg/ml) and extract (1 mg/ml). Add 2.5 ml of water, 0.1 ml of potassium acetate (1M), and 0.5 ml of aluminum chloride (10%) in ethanol to 0.5 ml of extract and standard. Measure the absorbance at 415 nm after 30 minutes of room temperature incubation.

3.7.3 Determination of Alkaloids

Equipment: weigh balance

Apparatus: Beaker, pipette, measuring cylinder

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

Preparation of Reagent

To prepare 40ml of 10% acetic acid, dilute 4ml acetic acid in 36ml ethanol

To prepare 100ml 0.1M ammonium hydroxide, dilute 0.68ml ammonium hydroxide in 99.32ml of water

Procedure

- Weigh 1 gram of the sample into a beaker, add 40 millilitres of 10% acetic acid in ethanol, cover, and let stand for 4 hours before filtering.
- After concentrating the filtrate to a quarter of its initial value in a water bath, add three drops of concentrated or conc. ammonium hydroxide to the extract drop by drop until the precipitation is finished.
- Following three hours of mixture sedimentation, the precipitate is filtered and washed with four millilitres of 0.1M ammonium hydroxide. The supernatant is disposed of.
- After drying, the residue is weighed.
- The following formula is used to calculate the sample's alkaloid content:

Calculation

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

Where

W= weight of sample

W₁= weight of empty filter paper

W₂= weight of filter paper and dry residue

3.7.4 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 32ml water

To prepare 5ml 5% NaCl, dissolve 0.25g NaCl in 5ml of water

Procedure

- One gram of the sample is heated in a water bath for 2.5 hours while being constantly stirred, and the mixture is filtered after 20 millilitres of 20% aqueous ethanol is added.
- After extracting the residue again from the filter paper using 20 millilitres of 20% ethanol and heating it for 2.5 hours while stirring continuously, the residue is filtered.
- The filtrates are mixed collectively.
- 4 ml of diethyl ether is added to the concentrate in a SEPARATOR FUNNEL and vigorously agitated, from which the aqueous layer was recovered and the diethyl ether layer was discarded. • The combined extract is evaporated to 8 ml over a water bath at 90°C.
- Carry out step six again.
- Add 12 millilitres of n-butanol, then extract twice with 2 millilitres of 5% NaCl.
- The NaCl is thrown away.
- Weigh a dry crucible and heat the residual solution in a water bath until all of the evaporation has occurred.
- After being put into a crucible, the solution was dried in an oven to a consistent weight.

Calculation:

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

Were

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

3.7.5 Determination of Cyanogenic Glycoside

In a conical flask, 1g of sample was mixed with 200ml of water and left to stand for 2 hours to allow autolysis to take place. After adding an antifoaming agent (tannic acid), 20 millilitres of 2.5% sodium hydroxide (NaOH) was added to the sample before full distillation was completed in a 250-millilitre conical flask. In order to observe continuous turbidity, which indicates the end point, 100 millilitres of cyanogenic glycoside, 8 millilitres of 6 millilitres of ammonium hydroxide, and 2 millilitres of 5% potassium iodide were added to the distillate(s), mixed, and titrated with 0.02 millilitres of AgNO₃ (silver nitrate) against a black background.

Content of cyanogenic glycoside in the sample was calculated as:

$$\text{Cyanogenic glycoside (mg/100g)} = \frac{\text{titre value (ml)} \times 1.08 \times \text{volume}}{\text{aliquot volume (ml)} \times \text{sample weight (g)}} \times 100$$

3.8 Preparation of Plant Extract

After phytochemicals, *Costus afer* was extracted into an aqueous and an ethanolic extract, which were then refrigerated at 40C until isolates were obtained and prepared for antimicrobial testing. Concentrations of 80 mg/ml, 40 mg/ml, 20 mg/ml, and 10 mg/ml were used to prepare the extracts.

3.8.1 Preparation of Ethanol Plant Extract

Following plant collection, the leaves were shade-dried for seven (7) days at room temperature (32–35 oC) to constant weight. Cowan's (1999) cold maceration extraction technique was applied. In a two-litre conical flask, fifty grams of dried *Costus afer* leaves were weighed, ground into a fine powder, and then dissolved in one thousand millilitres of 70% ethanol. For an effective extraction, the flask was shaken vigorously every 30 minutes and allowed to stand at room

temperature for 72 hours. To get rid of the plant sample particles, the mixture was filtered using cotton wool and Watman's No. 1 filter paper. The resulting clear solution was transferred to an evaporating dish over a steam bath after being concentrated with a rotary evaporator at 45°C under low pressure. The resulting solid dried powder was labelled and stored in sterile screw-capped bottles that had been previously weighed. At this point, the extract was kept at room temperature.

3.8.2 Preparation of Plant Aqueous Extract

We used an impact mill (Model 474/54, Christy and Morris Ltd. Process Engineers, Chelmsford, England) to grind the dried stems into a fine powder. After mixing 3.0 L of distilled water with 150 g of the ground material, it was left for 72 hours. A sterile glass rod was used to stir the mixture every six hours while it was being run through filter paper. With a yield of 5.53%, the filtrate was concentrated in vacuo in a rotary evaporator at 40°C. Prior to use, the concentrated extract was placed in universal bottles, labelled, and chilled at -4 °C. (Owolabi and Nworgu, 2009).

3.9. Biochemical Tests

- 1. Indole Test:** The purpose of this test was to identify the isolate that is capable of separating indole from tryptophan in buffered peptone water. Typically, the test is employed to help distinguish between gram-negative bacteria, particularly those belonging to the Enterobacteriaceae family. Using a sterile pipette, approximately 3 millilitres of the prepared peptone water were transferred into test tubes. Next, a well-isolated colony of bacteria was selected using new, sterile loops, and inoculated into the test tubes. The tubes were then incubated for 48 hours at 37°C. The inoculated tubes were then filled with 0.5 ml of Kovac's indole reagent after incubation. After gently shaking the tubes for ten minutes, the surface layer's red colour was checked. An indole positive reaction is indicated by a red ring on top of the tube.

- 2. Citrate Test:** This particular medium is used to test an organism's ability to use citrate as its only source of carbon. It is frequently employed to distinguish between Enterobacteriaceae members. The enzyme citrase hydrolyses citrate into oxaloacetic acid and acetic acid in organisms that can use it as a carbon source. After that, the oxaloacetic acid hydrolyses to produce CO₂ and pyruvic acid. In the event that CO₂ is created, it reacts with the medium's constituents to form an alkaline compound, such as Na₂CO₃. The pH indicator (bromothymol blue) changes from green to blue due to an alkaline pH.
- 3. KOH test:** The gram-negative bacteria test, also known as the KOH test, is used to determine their identity. Gram-negative bacteria's cell walls' thin coating of peptidoglycan dissolves in KOH, but gram-positive bacteria's cell walls are unaffected. Within the first thirty seconds, the organisms should become thick, stringy, and form long strands for a positive outcome. Gram negative bacteria exhibit this.
- 4. Triple Sugar Iron Agar (TSIA) Test:** The Triple Sugar Iron Agar (TSIA) test is intended to separate Enterobacteriaceae—a family of gram-negative intestinal bacilli—from other Enterobacteriaceae groups or genera. All Enterobacteriaceae species are capable of fermenting glucose and producing acid. Based on the fermentation of glucose and lactose or sucrose and the generation of hydrogen sulphide (H₂S), the differentiation is made. Touch a well-isolated colony with the tip of a straight inoculation needle. To inoculate TSI, stab through the middle of the medium to the tube's bottom first, and then streak the agar slant's surface. After leaving the cap loose, incubate the tube for 18 to 24 hours at 35°–37°C in room temperature. A red slant/yellow butt reaction, indicating an alkaline/acid balance, is specific to the fermentation of dextrose. A yellow slant or yellow butt reaction, also known as an acid/acid reaction, denotes the fermentation of lactose, sucrose, or

dextrose. A red slant, red butt, or alkaline/alkaline reaction There is no fermentation of carbohydrates as a result. Discolouration of the media: occurs when H₂ is present. Gas generation: Cracks or bubbles in the agar suggest that gas is being produced (CO₂ and H₂).

- 5. Oxidase test:** Two drops of freshly made 1% oxidase reagent (phenylenediamine) were applied to filter paper for the oxidase test. After the test organisms were smeared on the paper, a deep purple colouration within five to thirty seconds indicated a positive result.

3.10. Antimicrobial Activity Studies.

Two methods (the Agar well method and the disc diffusion method) were used in the investigational study for antibacterial activity against different pathogenic bacterial strains, and the outcomes from each were compared. The disk diffusion method was limited to assessing the antibacterial activity of the corresponding crude extracts qualitatively. The agar well diffusion test was utilized to optimize the antibacterial activity.

3.10.1 McFarland Standard Solution

A 0.5 McFarland standard solution was previously prepared by combining 0.5 mL of a 1.175% barium chloride dihydrate (BaCl₂·2H₂O) solution with 99.5 mL of 1% sulphuric acid (H₂SO₄). These viable cells were used to create a solution of cells of 1.5x10⁸cfu/ml by continuously inoculating cells from the nutrient agar plate with a sterile loop (flamed at intervals to ensure sterility) until a certain turbidity was reached. (Aryal Sagar et al., 2021)

3.10.2 Agar Disk Diffusion Method

After 0.2 millilitres of cell suspension were inoculated onto the agar surface, each bacterial sample was distributed across sterile agar plates using a sterile wire loop. Whatman filter paper disks with a 2.5 µL infused capacity and a diameter of approximately 5 mm were prepared and sterilized

before being used. After each disk was infused with a different crude extract at a concentration of 40 mg/disk, the bacterial pre-spread agar plates were used as the substrate. The samples were given 30 minutes to diffuse at room temperature. For twenty-four hours, the diffused petri plates were incubated at $35 \pm 0.5^{\circ}\text{C}$. The diameter (mm) of the inhibition zones was used to calculate the microbial growth following incubation. (Pochapski *et al.*, 2011)

3.10.3 Agar Well Diffusion Test

Using a sterile wire loop, each bacterial inoculum was distributed across the sterile agar plate. The agar plates were prepared with 20 μL capacity wells that had a diameter of approximately 4 mm. The initial concentration of crude extract used for the agar well diffusion method was 80 mg/mL, subsequent concentrations. The zones of inhibition were observed to merge with one another as a result of the crude extract's increased concentration. The crude extracts' concentration was subsequently lowered to 20 mg/mL. The sample solution (all crude extracts), negative control (different solvent extracts), and positive control (PC) (streptomycin, 30 $\mu\text{g}/\mu\text{L}$) were added to each well and allowed to diffuse for 30 minutes at room temperature. The antibacterial activity of the plates was measured by measuring the inhibition zones (mm) after they were incubated for 24 hours at $35 \pm 0.5^{\circ}\text{C}$. (2017) Khayyat et al. In this study, no statistical analysis was done.

3.10.4 Antibiotic Susceptibility Test

The modified Kirby-Bauer disc diffusion technique was utilized to ascertain the isolates' susceptibility to antibiotics. Mueller Hinton agar was used to culture the isolates for a brief 24-hour period at 37°C . Each isolate was inoculated onto sterile agar plates as a suspension. The antibiotic sensitivity disc and the plates were left to settle. After letting the plates settle, the antibiotic sensitivity disc was put on top of them. Following a 24-hour incubation period at 37°C ,

the zones of inhibition on the plates were measured and noted. The Clinical Laboratory Standard Institute's guidelines were used to interpret the results that were obtained.

CHAPTER FOUR

RESULTS

Table 1 shows the Proximate composition of *Costus afer*. The obtained moisture content of *Costus afer* was 23.11%, 23.25%, and 22.90%, while the crude protein content was 8.12%, 11.04% of the total included ash (7.98%, 7.83%). 3.76 percent crude protein, 10.86 percent, and 10.80% 3.85%, 3.81%, fat (9.71, 0.78, and 0.69%), and carbohydrates (53.26%, 53.28%, and 53.97%).

Table 2 shows the photochemical constituents of *Costus afer*. As can be seen from the table, the phytochemicals found in monkey sugar cane include flavonoids, saponins, alkaloids, tannins, phenols, and glycosides, with saponins having the lowest proportion content.

Table 3 shows the biochemical test and their results for the clinical isolates *Escherichia coli* and *Klebsiella sp.*

Table 4 shows the antibacterial activity of aqueous and ethanol extract of *Costus afer* at different concentrations of 80mg/ml, 40mg/ml, 20mg/ml, 10mg/ml on *Escherichia coli* and *Klebsiella sp.* using the disc diffusion method. showing no zone irrespective of concentrations. Therefore, the aqueous and ethanol extract of monkey sugar cane cannot be utilized to treat diseases caused by these bacteria (*Klebsiella sp.* and *Escherichia coli*) because it has no antibacterial activity on these clinical isolates.

Table 5 shows the antibacterial activity of aqueous and ethanol extract of Monkey sugar cane at different concentrations of 80mg/ml, 40mg/ml, 20mg/ml, 10mg/ml on *Escherichia coli* and *Klebsiella sp.* using the Agar well method. showing no zone irrespective of concentrations. Hence, the aqueous and ethanol extract of Monkey sugar cane has no antibacterial effects on these clinical isolates and cannot be used for the treatment of diseases caused by these bacteria (*Escherichia coli* and *Klebsiella sp.*).

Tables 6.1 and 6.2 indicate the bactericidal activity of commercially available antibiotics against clinical isolates of *Klebsiella sp.* and *Escherichia coli*. Despite being resistant to Zithromax and Amoxicillin/Clavulanic acid, two commercially available antibiotics, *Escherichia coli* was susceptible to Sulfamethoxazole-Trimethoprim (Co-trimoxazole), Erythromycin, Pefloxacin, Cephalexin, Rocephin, and Ciprofloxacin. However, *Klebsiella sp.* shown resistance to each of the drugs previously listed.

4.1 Proximate Analysis

Table 1: Proximate composition of *Costus afer*.

	Moisture content (%)	Crude fibre (%)	Ash Content (%)	Crude Protein (%)	Fat (%)	Carbohydrate (%)
<i>Costus afer</i>	23.11	8.12	11.04	3.76	0.71	53.26
	23.25	7.98	10.86	3.85	0.78	53.28
	22.90	7.83	10.80	3.81	0.69	53.97

Table 2: Phytochemical Constituents of *Costus afer*.

ANALYSIS	Flavonoids	Saponins	Alkaloids	Tannins	Phenols	Glycosides
	(µg/ml)	(%)	(%)	(µg/ml)	(µg/ml)	(mg/100g)
	10.50	0.95	1.45	76.78	5.06	34.89
	10.13	1.02	1.20	78.34	5.15	35.60
	10.21	1.02	1.20	80.12	5.09	37.81

Table 3: Characterization and Identification of Bacteria Isolates

Suspected Organism	KOH	TSI	Citrate	Indole	Oxidase	MacConkey agar
<i>Escherichia coli</i>	+	A/A	-	+	-	Pink
<i>Klebsiella pneumonia</i>	+	A/A	+	+	-	Pink

Table 4: Disc diffusion method results for different concentrations of both ethanol and aqueous extract showing zones of inhibition (in mm)

Isolates	Concentration	Extracts (Zone of inhibition)	
		Ethanol (in mm)	Aqueous (in mm)
<i>E. coli</i>	80mg/ml	0.00	0.00
	40mg/ml	0.00	0.00
	20mg/ml	0.00	0.00
	10mg/ml	0.00	0.00
<i>Klebsiella</i>	80mg/ml	0.00	0.00
<i>Pseudomonas</i>			
	40mg/ml	0.00	0.00
	20mg/ml	0.00	0.00
	10mg/ml	0.00	0.00

Table 5 Agar well method results for different concentrations of both ethanol and aqueous extract showing zones of inhibition (in mm)

Isolates	Concentration	Extracts (Zone of inhibition)	
		Ethanol (in mm)	Aqueous (in mm)
<i>E. coli</i>	80mg/ml	0.00	0.00
	40mg/ml	0.00	0.00
	20mg/ml	0.00	0.00
	10mg/ml	0.00	0.00
<i>Klebsiella</i>	80mg/ml	0.00	0.00
<i>Pseudomonas</i>	40mg/ml	0.00	0.00
	20mg/ml	0.00	0.00
	10mg/ml	0.00	0.00

Table 6.1: Antibacterial activity of some commercially available antibiotics on *Klebsiella pneumoniae* and *Escherichia coli*

Isolate	R	CPX	S	SXT	E	CN	PEF	APX
Kleb.	-(R)	-(R)	-(R)	-(R)	-(R)	-(R)	-(R)	-(R)
E. coli	2mm(S)	8mm(S)	4mm(S)	5mm(S)	3mm(S)	3mm(S)	5mm(S)	-(R)

Table 6.2: Antibacterial activity of other commercially available antibiotics on *Klebsiella pneumoniae* and *Escherichia coli*

Isolate	SP	CH	OFX	AM	AU	CN	PEF	Z
Kleb.	-(R)	-(R)	-(R)	-(R)	-(R)	-(R)	-(R)	-(R)
E. coli	6mm(S)	4mm(S)	5mm(S)	4mm(S)	-(R)	3mm(S)	5mm(S)	-(R)

KEY

> R - Resistant

> S - Susceptible

- S - Streptomycin
- SXT - Sulfamethoxazole-Trimethoprim (Co-trimoxazole)
- E - Erythromycin
- PEF - Pefloxacin
- CN - Cephalexin
- APX - Amoxicillin/Clavulanic acid
- Z - Zithromax
- AM - Amoxicillin
- R – Rocephin
- CPX - Ciprofloxacin

4.2 Agar Disk Diffusion Method

The agar disk diffusion method showed no effect at all from the various solvent extracts for *Costus afer*. The agar well diffusion procedure was conducted using a different solvent crude extract of *Costus afer*, according to the overall results. The agar disk diffusion technique's zone of inhibition is displayed in Table 4.

4.2.1 Agar Well Diffusion Test

Similar to the aqueous extracts, the ethanolic extract (EE) exhibited little action against *Klebsiella* and *E. Coli*. (See Table 5). Regarding the studied bacterial pathogens, the aqueous extract showed no evidence of action.

CHAPTER FIVE

DISCUSSION

The result of proximate composition of *Costus afer* presented in Table 1, shows a high carbohydrate content at a mean of 53.77% accounting for the majority of the content of composition 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 a mean moisture content of 23.087%, *Costus afer* shows a fairly high amount of moisture content, which can be attributed to the environment as *Costus afer* commonly grows in areas with high moisture or shady areas where moisture is not so easily lost (Iwu, 2009)

The findings also showed that the aqueous phytochemical composition of *Costus afer* extract contained flavonoids, saponins, phenols, tannins, alkaloids as well as glycosides. Phytochemicals are non-nutritive plant chemicals that, have the ability to prevent or treat diseases, these pharmacological agents have minimal adverse effects while producing therapeutic results in a dynamic way (Enwuru, 2008). It is well known that alkaloids have antifungal, antibacterial, and anti-inflammatory qualities. Strong antioxidants like flavonoids and phenolic compounds also have anti-inflammatory, anti-allergic, and anti-thrombotic properties and they protect against oxidative cell damage as well as degenerative diseases (Anyasor *et al.*, 2010).

While the antibacterial effects of *Costus afer* have been shown in some studies, to have some activity against a number of bacteria like; *Bacillus pumilus*, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Shigella* spp. and *Klebsiella pneumonia* (Nyananyo, 2006), using the disc diffusion method and the agar method, the antibacterial activity of *Costus afer's* aqueous and ethanol extract at concentrations of 80 mg/ml, 40 mg/ml, 20 mg/ml, and 10 mg/ml on *Escherichia coli* and *Klebsiella sp.* in this study showed no zones of inhibition regardless

of concentrations. Hence, the aqueous and ethanol extract of Monkey sugar cane has no antibacterial effects on these clinical isolates and cannot be used for the treatment of diseases caused by these bacteria; *Escherichia coli* and *Klebsiella sp.* The results for the antibiotic susceptibility test of commercially available antibiotics showed that the *Klebsiella* was resistant against both the broad spectrum and narrow spectrum Gram negative antibiotics, and it maintained its resistance against the extract and so many easily available antibiotics. In contrast, the control experiment's *Escherichia coli* was resistant to the extracts of monkey sugar cane but susceptible to tested commercially available antibiotics.

CONCLUSION

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

A more sensible medical application of the plant can be encouraged by the compilation of data on *Costus afer* plant, and the various parts that are used to treat a variety of ailments. Additionally, it can provide data based on evidence for the medicinal plant's clinical development. The plant can be regarded as a nutraceutical because of its high proportions of macro- and micronutrients 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* and *Klebsiella pneumonia* are yet to be seen, as the result of this study therefore shows that *Costus afer* does not

have antimicrobial effect on *Escherichia coli* and *Klebsiella pneumoniae* at the experimented concentration levels of 80 mg/ml, 40 mg/ml, 20 mg/ml, 10 mg/ml.

RECOMENDATION

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 infections.

CONTRIBUTION OF THE STUDY TO KNOWLEDGE

The research identified the use of *Costus afer* aqueous solution at concentration levels of 80 mg/ml, 40 mg/ml, 20 mg/ml, 10 mg/ml, does not have antimicrobial effect on *Escherichia coli* and *Klebsiella*. This contribution advances the field of microbiology by providing data that is significant in the use of phytochemicals and other plant properties in the development of antibiotics and the fight against pathogenic microorganisms.

REFERENCES

- Akpan, M. M., Odeomen, C. S., and Nwachukwu, C. N. (2011). Antimicrobial assessment of ethanolic extract of *Costus afer* leaves. *Asian Journal of Plant Science and Research*, **2**(3) 335–341.
- Anaga, A. O., Njoku, C. J., Ekejiuba, E. S., Esiaka, M. N., and Asuzu, I. U. (2004). Investigations of the methanolic leaf extract of *Costus afer*. Ker for pharmacological activities in vitro and in vivo. *Phytomedicine*, **11**(2-3), 242-248.
- Anyanwu, B. O., Ezejiofor, A. N., Nwaogazie, I. L., Akaranta, O., and Orisakwe, O. E. (2020). Low-dose heavy metal mixture (lead, cadmium and mercury)-induced testicular injury and protective effect of zinc and *Costus afer* in wistar albino rats. *Andrologia*, **52**(9), 13-69.
- Anyasor, G. N., Funmilayo, O., Odutola, O., Olugbenga, A., and Oboutor, E. M. (2014). Chemical constituents in n-butanol fractions of *Costus afer* ker Gawl leaf and stem. *Journal of Intercultural Ethnopharmacology*, **3**(2), 78-80.
- Anyasor, G. N., Idowu, D. P., and Nabofa, W. (2020). Evaluation of the hepatoprotective effect of oral administration of aqueous fraction of methanolic extract of *Costus afer* leaves during induction of hepatocellular carcinoma with diethylnitrosamine in rats. *Comparative Clinical Pathology*, **29**, 733-744.
- Anyasor, G. N., Ogunwenmo, O., Oyelana, O. A., and Akpofunure, B. E. (2010). Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of *Costus afer* Ker Gawl.(Costaceae). *African Journal of Biotechnology*, **9**(31), 4880-4884.
- Anyasor, G. N., Onajobi, F. D., Osilesi, O., and Adebawo, O. (2014). Proximate composition, mineral content and in vitro antioxidant activity of leaf and stem of *Costus afer* (Ginger lily). *Journal of Intercultural Ethnopharmacology*, **3**(3), 128.
- Anyasor, G. N., Onajobi, F. D., Osilesi, O., and Adebawo, O. (2015). Hematological and lipid profile evaluation of a hexane fraction of *Costus afer* leaves in arthritic rats. *Pharmaceutical biology*, **53**(11), 1671-1676.
- Anyasor, G. N., Onajobi, F., Osilesi, O., Adebawo, O., and Oboutor, E. M. (2014). Anti-inflammatory and antioxidant activities of *Costus afer* Ker Gawl. hexane leaf fraction in arthritic rat models. *Journal of ethnopharmacology*, **155**(1), 543-551.

- Anyasor, G., Onajobi, F., Osilesi, O., and Adebawo, O. (2014). Hexane fraction of *Costus afer* Ker Gawl leaf inhibited mitochondrial permeability transition, F1F0 ATPase and scavenged nitric oxide and hydrogen peroxide (957.1). *The FASEB Journal*, **28**:957-961.
- Aweke, G. (2007). *Costus afer* (Ker Gawl). Medicinal Plants. *Plant Resources of Tropical Africa*, **11**(1).
- Benavente-García, O., Castillo, J., Marin, F. R., Ortuño, A., and Del Río, J. A. (1997). Uses and properties of citrus flavonoids. *Journal of agricultural and food chemistry*, **45**(12), 4505-4515.
- Boison, D., Adinortey, C. A., Babanyinah, G. K., Quasie, O., Agbeko, R., Wiabo-Asabil, G. K., and Adinortey, M. B. (2019). *Costus afer*: a systematic review of evidence-based data in support of its medicinal relevance. *Scientifica*, **20**:19-20.
- Burkhill, H. M. (1985). Useful Plants of West Africa. *Royal Botanic Gardens*, **1**:130-132.
- BURKILL, I. (1985). Families AD. *The Useful Plants of West Tropical Africa*, 1:4-8.
- Canillac, N., and Mourey, A. (2001). Antibacterial activity of the essential oil of *Picea excelsa* on *Listeria*, *Staphylococcus aureus* and coliform bacteria. *Food Microbiology*, **18**(3), 261-268.
- Davidson, P. M. (1989). Methods for testing the efficacy of food antimicrobials. *Food technology*, **43**:148-155.
- Dhifi, W., Bellili, S., Jazi, S., Bahloul, N., and Mnif, W. (2016). Essential oils' chemical characterization and investigation of some biological activities: A critical review. *Medicines*, **3**(4), 25-30.
- Edeoga, H. O., and Okoli, B. E. (2000). Chromosome numbers of *Costus lucanusianus* (Costaceae) in Nigeria. *Folia Geobotanica*, **35**:315-318.
- Ekpe, L., Inaku, K., and Ekpe, V. (2018). Antioxidant effects of astaxanthin in various diseases—A review. *Journal of Molecular Pathophysiology* **7**(1), 1-6.
- Ekpo, B. A., Bala, D. N., Essien, E. E., and Adesanya, S. A. (2008). Ethnobotanical survey of Akwa Ibom state of Nigeria. *Journal of Ethnopharmacology*, **115**(3), 387-408.

- Eshete, M. A., and Molla, E. L. (2021). Cultural significance of medicinal plants in healing human ailments among Guji semi-pastoralist people, Suro Barguda District, Ethiopia. *Journal of ethnobiology and ethnomedicine*, **17**(1), 1-18.
- Ezejiolor, A. N., Igweze, Z. N., Udowelle, N. A., and Orisakwe, O. E. (2017). Histopathological and biochemical assessments of *Costus afer* stem on alloxan-induced diabetic rats. *Journal of Basic and Clinical Physiology and Pharmacology*, **28**(4), 383-391.
- Ezejiolor, A. N., Igweze, Z., and Amadi, C. N. (2017). Evaluations of some biological properties of ethanolic leave extract of *Costus afer* (ker gawl). *IOSR Journal of Pharmacy and Biological Sciences*, **12**(1), 62-68.
- Ezejiolor, A. N., Orish, C. N., and Orisakwe, O. E. (2013). Effect of aqueous leaves extract of *Costus afer* Ker Gawl (Zingiberaceae) on the liver and kidney of male albino Wistar rat. *Ancient Science of Life*, **33**(1), 4-9.
- Foungbe, S., Sawadogo, D., and Declume, C. (1987). Experimental study of the uterine-relaxant activity of *Alstonia boonei* (Apocynaceae) and *Costus lucanusianus* (Zingiberaceae) traditionally used as anti-abortion agent in the Ivory Coast. In *Annales Pharmaceutiques Françaises* **45**(5), 373-377.
- Gill, L. S. (1992). *Ethnomedical uses of plants in Nigeria*. Uniben press.
- Otuokere, I.E., Okorie, D.O., Orieke, B.E. and Igwe, K.K. (2016), Characterization Of *Costus afer* Stem Ethanol Extract By Gas Chromatography-Mass Spectrometry Analysis. *International Journal of Basic and Applied Chemical Sciences* **6**(2) 79-84.
- Jaganath, I. B., and Crozier, A. (2010). Plant phenolics and human health: biochemistry, nutrition, and pharmacology. *Dietary flavonoids and phenolic compounds*, 1-39.
- Jesus, M., Martins, A. P., Gallardo, E., and Silvestre, S. (2016). Diosgenin: recent highlights on pharmacology and analytical methodology. *Journal of analytical methods in chemistry*, **20**:16-20.
- K Sushma, B., S Ashalatha, K., Ray, P., and R Raveesha, H. (2020). Histochemical and phytochemical analysis of medicinally important plants. *European Journal of Medicinal Plants*, **30**(4), 1-13.
- Kagbo, H. D., and Gospel, L. A. (2020). Phytochemistry and Toxicity of Methanol Root Extract of *Costus lucanusianus*. *Journal of Advances in Medical and Pharmaceutical Sciences*, **22**(3), 12-19.

- Kagbo, H. D., and Obinna, V. C. (2017). Antifertility effect of *Costus lucanuscianus* stem extract in male albino rats. *International Journal of Science and Technology*, **5**(12), 119-123.
- Kagbo, H. D., and Obinna, V. C. (2018). Investigations of methanolic leaf extract of *Costus lucanuscianus* on gestation in albino rats. *International Journal of Pharmacy Chemistry and Biological Science* **89**:14-17.
- Kalio, I. S., Effiong, O., and Iyama, M. J. (2022). Cardiovascular Risk Indices in Apparently Healthy Individuals after 14 Days of Consumption of *Costus afer* Stem Extract. *Journal of Advances in Medical and Pharmaceutical Sciences*, **24**(5), 33-40.
- Khayyat, S. A., and Al-Kattan, M. O. (2017). Phytochemical screening and antimicrobial activities of *Costus speciosus* and *Sea Quist*. *Biomedical Research (India)*, **28**(1), 389-393.
- Knobloch, K., Weigand, H., Weis, N., Schwarm, H. M., and Vigenschow, H. (1986). Progress in essential oil research: 16th international symposium on essential oils; Brunke, E.J., (Ed.) De Walter de Gruyter: Berlin, Germany, pp 429–445,
- Kumar, A., Shukla, R., Singh, P., Prasad, C. S., and Dubey, N. K. (2008). Assessment of *Thymus vulgaris* L. essential oil as a safe botanical preservative against post harvest fungal infestation of food commodities. *Innovative food science and emerging technologies*, **9**(4), 575-580.
- Mahasneh, A. M., and El-Oqlah, A. A. (1999). Antimicrobial activity of extracts of herbal plants used in the traditional medicine of Jordan. *Journal of Ethnopharmacology*, **64**(3), 271-276.
- Matias, L. J., Mercadante-Simões, M. O., Royo, V. A., Ribeiro, L. M., Santos, A. C., and Fonseca, J. M. (2016). Structure and histochemistry of medicinal species of *Solanum*. *Revista Brasileira de Farmacognosia*, **26**:147-160.
- Monago, C., Anacletus, F. C., and Nwauche, T. K. (2016). Hypoglycaemic activity of the aqueous extract of *Costus afer* stems alone and in combination with metformin. *The FASEB Journal*, **30**:1101-1103.
- Moody, J. O., and Okwagbe, K. E. (2003). Topical anti-inflammatory activity of *Costus afer*. *Nigerian Journal of Natural Products and Medicine*, **7**:46-48.
- Moundipa, P. F., Flore, K. G. M., Bilong, C. F., and Bruchhaus, I. (2005). In vitro amoebicidal activity of some medicinal plants of the Bamun region (Cameroon). *African Journal of Traditional, Complementary and Alternative Medicines*, **2**(2), 113-121.

- ThankGod, N. K., Monago, C. C. and Anacletus, F. C. (2014). Antihyperglycemic activity of the aqueous extract of *Costus afer* stem alone and in combination with metformin. *European Journal of Biotechnology and Bioscience*. **1**(5), 19–25.
- Nyananyo, B., Okeke, C. U., and Mensah, S. I. (2006). Physico-chemical conditions and distribution of phytoplankton in the Brass River, Nigeria. *Journal of Applied Sciences and Environmental Management*, **10**(1), 43-45.
- Obinna, V. C., and Kagbo, H. D. (2018). Evaluation of *Costus lucanuscianus* leaf extract for anti-fertility effect in female albino rats. *International Journal of Advanced Research in Biological Science* **5**(1), 153-158.
- Okugbo, T., and Oriakhi, K. (2015). A comparative study of in vitro antioxidant activity and phytochemical constituents of methanol extract of *afmomum melegueta* and *Costus afer* leaves. *Jordan Journal of Biological Sciences*, **147**(3388), 1-7.
- Oladeji, O. (2016). The characteristics and roles of medicinal plants: Some important medicinal plants in Nigeria. *Natural Products: An Indian Journal*, **12**(3), 10-20.
- Omokhua, G. E. (2011). Medicinal and socio-cultural importance of *Costus afer* (Ker Grawl) in Nigeria. *African research review*, **5**(5), 282-287.
- Onwuliri, F. C. (2004). Antimicrobial studies of the extracts of *Acalypha wllkesiana* L. on microorganisms associated with wound and skin infections. *West African Journal of Biological Science*, **15**:15-19.
- Owolabi, O. J., and Nworgu, Z. A. M. (2009). Anti-inflammatory and anti-nociceptive activities of *Costus lucanuscianus* (Costaceae). *Pharmacologyonline*, **1**:1230-1238.
- Owoyale, J. A., Olatunji, G. A., and Oguntoye, S. O. (2005). Antifungal and antibacterial activities of an alcoholic extract of *Senna alata* leaves. *Journal of Applied Sciences and Environmental Management*, **9**(3), 105-107.
- Peters, D. E., and Chibueze, I. (2022). Nutrient's composition of *costus lucanuscianus* stem. *GSC Biological and Pharmaceutical Sciences*, **18**(2), 070-081.
- Pochapski, M. T., Fosquiera, E. C., Esmerino, L. A., Dos Santos, E. B., Farago, P. V., Santos, F. A., and Groppo, F. C. (2011). Phytochemical screening, antioxidant, and antimicrobial activities of the crude leaves' extract from *Ipomoea batatas* (L.) Lam. *Pharmacognosy magazine*, **7**(26), 165-170.

- Polshettiwar, S. A., Ganjiwale, R. O., Wadher, S. J., and Yeole, P. G. (2007). Spectrophotometric Estimation of Total Tannins in Some Ayurvedic Eye Drops. *Indian Journal of Pharmaceutical Sciences*, **69**(4)5-10.
- Rajesh, M. S., Harish, M. S., Sathyaprakash, R. J., Shetty, A. R., and Shivananda, T. N. (2009). Antihyperglycemic activity of the various extracts of *Costus speciosus* rhizomes. *Journal of natural remedies*, **9**(2), 235-241.
- Ratlidge, C., and Wilkinson, S. G. (1988). An overview of microbial lipids. *Microbial lipids*, **1**:3-22.
- Salmerón-Manzano, E., Garrido-Cardenas, J. A., and Manzano-Agugliaro, F. (2020). Worldwide research trends on medicinal plants. *International Journal of Environmental Research and Public Health*, **17**(10), 33-76.
- Sapunyo, W. L., Mbaria, J. M., Kanja, L. W., Omolo, M. J., and Onyancha, J. M. (2023). Phytochemical Screening, Toxic Effects, and Antimicrobial Activity Studies of *Digitaria abyssinica* (Hochst. ex A. Rich.) Stapf (Poaceae) Rhizome Extracts against Selected Uropathogenic Microorganisms. *Evidence-Based Complementary and Alternative Medicine*, **20**:19-23.
- Saraf, A. (2010). Phytochemical and antimicrobial studies of medicinal plant *Costus speciosus* (Koen.). *Journal of Chemistry*, **7**:405-413.
- SHAIKH (2022). Phytochemical, Histochemical, Antibacterial Investigation of Plants. *Turkish Journal of Pharmacy and Science* **19**(2):145-152.
- Sule, W. F., Okonko, I. O., Joseph, T. A., Ojezele, M. O., Nwanze, J. C., Alli, J. A., and Adewale, O. G. (2010). In vitro antifungal activity of *Senna alata* Linn. crude leaf extract. *Research Journal of Biological Sciences*, **5**(3), 275-284.
- Taiwo, A. O., and Bolanle, A. A. (2003). The leaf essential oil of *Costus afer* Ker–Gawl from Nigeria. *Flavour and Fragrance Journal*, **18**(4), 309-311.
- Udem, S. C., and Ezeasor, C. K. (2010). The acute and subchronic toxicity studies of aqueous leaf and stem bark extract of *Costus afer* ker (Zingiberaceae) in mice. *Comparative clinical pathology*, **19**(1), 75-80.
- Ukpabi Chibueze, F., Agbafor Kingsley, N., Ndukwe Okorie, K., and Agwu Akuagwu, N. S. N. (2012). Phytochemical composition of *Costus afer* extract and its alleviation of carbon

- tetrachloride–Induced hepatic oxidative stress and toxicity. *International Journal of Modern Botany*, **2**(5), 120-126.
- Uwah, A. F., Ewere, E. G., and Ndem, J. I. (2015). Hypoglycemic and haematologic effects of crude stem juice of *Costus afer* on alloxaninduced diabetic wistar rats. *American Journal of Ethnomedicine*, **2**(4), 2348-9502.
- Yadav, R. N. S., and Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *Journal of phytology*, **3**(12)4-11.