In-vitro Phytochemical and Antimicrobial Evaluation of *Garcinia cola, Aframomum melegueta* and *Zingiber officinale* against Selected Upper Respiratory Tract Pathogens

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Certification

This is to certify that **Grace Temitope OYATUNDE** with matriculation number **LCU/PG/003080** carried out this research work titled '***In-vitro* Phytochemical and Antimicrobial Evaluation of *Garcinia cola, Aframomum melegueta* and *Zingiber officinale* against Selected Upper Respiratory Tract Pathogens**' in the Department of Biology Science, Faculty of Natural and Applied Sciences, Lead city University, Ibadan, Oyo State, for the award of Master Degree in Medical Microbiology and that this has not been previously submitted.

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Dedication

This work is dedicated to God Almighty.

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Abstract

The use of plants for treating health issues has been a tradition since ancient times. With the rise in bacterial resistance to antibiotics and the side effects of antibiotics, there has been a growing emphasis on using plant extracts and their derivatives as a source of drugs for various human diseases. This research focuses on screening the phytochemicals and antimicrobial activities of three medicinal plants: *Garcinia cola, Aframomum melegueta,* and *Zingiber officinale Roscoe*. The plants were tested individually and in different combinations with various concentrations. The effects of the plant extracts were examined *in-vitro* using petroleum ether, ethanol, and aqueous solutions at different concentrations on pathogenic bacteria including *Staphylococcus aureus, Streptococcus pneumonia, Klebsiella pneumonia,* and *Pseudomonas aeruginosa*. The zone of inhibition of *Aframomum melegueta*, *Zingiber officinale Roscoe* and *Garcinia cola* ranged from 19.5 ± 1.12, 14.5 ± 1.12, and 13.5 ± 1.5 and the combined antibacterial activity of *Aframomum melegueta* and *Garcinia cola, Garcinia cola* and *Zingiber officinale Roscoe, Aframomum melegueta and Zingiber officinale Roscoe* ranges from 24.2 ± 1.73, 23.23 ± 0.05, and 14.5 ± 1.18, respectively. While combinations of *Garcinia cola, Aframomum melegueta,* and *Zingiber officinale Roscoe* have the highest concentration of 26.1±0.05.The MIC revealed that the plants extracts inhibited bacterial growth at concentration as low as 3.12 mg/mL, especially in ethanol extracts of *Garcinia cola* and *Zingiber officinale.* The MBC reults showed that petroleum ether extracts of *Garcinia cola* and *Zingiber officinale* effect at lower concentration compared to ethanol with *Staphylococcus aureus* being the most susceptible *Pseudomonas aeruginosa* the most resistant. The result of the standard antibiotics test showed that the different bacterial isolates used were inhibited by some antibiotics provided. Phytochemical analysis of the plants revealed the presence of alkaloids, tannins, saponins, terpenoids, steroids, flavonoids, glycosides, and phenols. The quantitative analysis showed ranges of 0.52 mg/mL, 0.0019 mg/mL, 0.24 mg/mL, and 0.286 mg/mL and ethanol proving to be an excellent solvent for extraction.

Keywords: Medicinal plants, Minimum inhibitory concentration,Plant Extracts,Antibiotics, Bacteria isolates.

Word Count: 300

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List of Acronyms

Abbreviation	Meaning
MDRM	Multidrug-resistant microorganisms
K	Pelumenial
SXT	Septrin
Z	Zinnacef
AM	Amoxicillin
R	Rocephin
APX	Ampiclox
CPX	Ciprofloxacin
S	Streptomycin
CN	Gentamicin
PEF	Perfloxacin
E	Erythromycin
OFXB	Tarivid
AU	Augmentin
SP	Sperfloxacin
URTI	Upper Respiratory Tracts Infection
WHO	World Health Organisation

MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration

Chapter One

Introduction

1.1 Background to the Study

Plants are indispensable for life on Earth, serving a critical function in sustaining ecological balance by supplying essential resources that are vital for human survival[1]. They serve as the primary source of nutrition, bioactive elements, and vital essentials for both survival and environmental conservation. Plants represent a diverse kingdom of living organisms encompassing of trees, herbs, shrubs, grasses, lianas, ferns, and mosses.

Plants are very important to human existence, especially as food and medicine for nutrition and treatment of human and animal diseases. They are used in all cultures of the world and have been relied upon for millennia to support, promote, and restore human health[2].

The primary source of many useful chemicals and/or medications is medicinal plants. In Europe, around 1300 medicinal herbs are used, with 90% of them coming from wild species. Medicinal plants are the basis for the treatment of numerous diseases in traditional African medicine, as well as other methods of treatment from diverse civilizations around the world. About 80% of the world's population still relies completely on traditional medicine or herbal medicine to treat diseases, mainly in Africa and other developing countries[3].

A medicinal plant is a plant that contains substances that can be used for medicinal purposes or to synthesize valuable drugs. Medicinal plants are used in traditional and allopathic medicine systems and can be used to treat a variety of conditions. This description enables one to differentiate between medicinal plants that have been scientifically shown to have therapeutic qualities and ingredients and those that are thought to be medicinal but have not yet undergone extensive scientific study[4]. Medicinal plants are considered to be a rich source of ingredients that can be used for the development and combination of better medicines. The phytochemical compounds found in plants such as alkaloids, glycosides, polyphenols, and terpenes[5].

Researchers continue to focus on the use of plant material to cure a variety of ailments, and traditional medicines created from plants are becoming more and more significant as alternative medicine to treat a wide range of conditions. Many impoverished nations think that these plant-based drugs are more affordable and safer. The usage of these plant products has expanded in both industrialized and developing nations due to the emergence of novel diseases and microbial resistance[6].

Antimicrobials are medications used to prevent and cure infectious disease in people, animals, and plants. These medications include antibiotics, antivirals, antifungals, and antiparasitic agents[7]. Medications called antibiotics are used to treat and prevent bacterial infections. When bacteria adapt to the use of antibiotics, antibiotic resistance develops. Antibiotic resistance raises mortality, lengthens hospital stays, and increases healthcare expenses[8]. Everywhere in the world, antibiotic resistance is escalating to alarmingly high levels. Our capacity to cure common infectious diseases is in danger due to the emergence and global spread of new resistance mechanisms. Because antibiotics are less effective, treating a rising number of infections, including gonorrhea, septicemia, pneumonia, tuberculosis, and foodborne illnesses, is become harder and occasionally impossible[9]. One of the main causes of bacterial resistance is the frequent use of synthetic antibiotics; this resistance may be linked to biological phenomena like membrane permeability, mutations, physicochemical changes, and runoff dynamics in the target bacterium. Bacterial strains possess the genetic capacity to rapidly develop and transmit resistance to widely used antibiotics, in contrast to other microorganisms. Scientists are searching for novel compounds with antibacterial properties and potential for use as raw materials in the development of new treatments because drug resistance to antibacterials is becoming a serious worldwide issue[10].

With at least 1.27 million fatalities globally and around 5 million in 2019, antimicrobial resistance poses danger to public health on a global scale[11]. *Garcinia cola* from the *Clusiaceae* family and the order *Malpighiales* has over 180 members from all over the world. *Garcinia cola* is a forest tree from sub-Saharan Africa that has been called the "miracle plant" because virtually every part of it has proven to be of medicinal value. It originally grows from Sierra Leone to southern Nigeria and then to Zaire and Angola, but is widely distributed by humans and often grows near communities. It is a tree that grows in the rainforest on the coast of Central and West Africa[12]. It can be found in tropical African nations as well as Asia. It reaches a height of roughly 30m. With peach skin, yellow meat, and three or four seeds with a brown seed coating, orange fruits are smooth and reddish-yellow in color. The seed is a consumable nut. While the grains are pale and punctured with resinous vesicles, the seed's shell is dark with branching lines. The flesh is golden-orange, occasionally reddish, and the fruits are yellow, reddish, and orange. The indumentum of the greenish-white blooms is reddish[13].

Aframomum melegueta is a spice with a similar composition to ginger, belonging to the same family *Zingiberaceae*. *Aframomum melegueta* has various names such as Grain of Paradise, Atare (Yoruba), Chitta (Hausa) or Guinea pepper, it is a seed with many medicinal properties and its benefits to humanity seem endless. *Aframomum melegueta* is a perennial herbaceous plant native to swampy habitats along the West African coast of Nigeria[14]. The purple tubular flowers develop into 5-7 cm long pods with many small, reddish-brown seeds. It is most commonly found in the countries of Ghana, Liberia, Côte d'Ivoire, Togo, and Nigeria. Traditionally, *Aframomum melegueta* can be mixed with other herbs to treat common ailments such as body aches, diarrhoea, sore throat, catarrh, constipation, and arthritis in West Africa, Nigeria. It is a perennial herb cultivated for its valuable medicinal and

pharmacological effects, such as antimicrobial, hepatoprotective, anticancer, and antidiabetic effects[15].

Ginger (*Zingiber officinale* Roscoe), belonging to *the Zingiberaceae* family and the genus *Zingiber*, has long been widely consumed as a spice and herbal remedy. Phenolic compounds are mainly gingerols, shogaols, and parasols, which explain the various biological activities of ginger[16]. Ginger is rich in active ingredients such as phenolic and terpene compounds. In fresh ginger, gingerols are the main polyphenols, such as 6-gingerol, 8-gingerol, and 10-gingerol. Several studies have shown that ginger effectively protects against oxidative stress. The basic mechanisms of antioxidant activity were studied in cell models. Ginger extract exhibited antioxidant effects in human chondrocyte cells with interleukin-1β (IL-1β) mediated oxidative stress.

Plants produce substances known as phytochemicals ("Phyto" means "plant"). Fruits, vegetables, cereal fibers, beans, and other plants contain them. Non-nutritive plant compounds with disease-preventive or disease-protective qualities are called phytochemicals. These are naturally occurring bioactive substances that interact with fiber and minerals to provide protection. They can be found in plant foods, leaves, or other plant components. According to recent studies, they can lower a person's risk of developing a number of inflammatory or chronic illnesses. Among the popular phytochemicals in fruits include flavonoids. Soybeans have isoflavones, and tomatoes contain lycopene. These are naturally occurring substances found in plants that may shield the liver by offering nutrients or therapeutic benefit[17].

1.2 Statement of the Problem

Over the years, several species of bacteria have developed resistance to chemically produced antibiotics. Microorganisms are rapidly acquiring resistance to new drugs, shortening the commercial life cycle of many antibiotics and discouraging large pharmaceutical companies from investing in the discovery and development of synthetic drugs[18].

Antibiotic abuse, including the use of antibiotics for viral illnesses, is associated with the problem of antimicrobial resistance. It also results from doctors administering inappropriate dosages of antibiotics, as well as people asking doctors to prescribe antibiotics for less serious ailments, which leads to an overuse of antibiotics[19].

1.3 Justification of the Study

Infectious diseases seem to be a serious health problem in underdeveloped countries and are a problem for people. Infections involve a complex interaction of parasites and their effects. The emergence and spread of antibiotic resistance, as well as the development of new strains of pathogens, is a major concern for the global health community. In today's world, with multiple resistance to human pathogens, there is a constant need to develop new antimicrobials from other sources including plants. The goal of this research is to find the alternative treatment that is safer, more cost-effective, and readily available to society.

1.4 Aim and Objectives of the Study

This study aims to investigate the antibacterial effect of aqueous, ethanol and petroleum ether extracts of *Zingiber officinale* (Ginger), *Garcinia kola* (Bitter cola), *Aframomum melegueta* (Alligator pepper) on four respiratory pathogens.

The Specific objectives are to:

i.Evaluate phytochemical composition of *Zingiber officinale* (ginger), *Garcinia kola* (bitter cola) and *Aframomum melegueta* (alligator pepper) on selected upper respiratory tract pathogens.

ii. Quantitatively and Qualitatively determine the phytochemical composition of *Zingiber officinale* (Ginger), *Garcinia kola* (Bitter cola), *Aframomum melegueta* (Alligator pepper)

iii. Evaluate the antibacterial activity of *Zingiber officinale* (Ginger), *Garcinia kola* (Bitter cola), *Aframomum melegueta* (Alligator pepper) on selected pathogens of the upper respiratory tract.

iv. Evaluate the combination of the efficacy *of Zingiber officinale* (ginger), *Garcinia kola* (bitter cola) and *Aframomum melegueta* (alligator pepper) in various combinations on selected upper respiratory tract pathogens

v. Compare the antibacterial effectiveness of *Zingiber officinale* (Ginger), *Garcinia kola* (Bitter cola), *Aframomum melegueta* (Alligator pepper).

vi. Determination of the Minimum Inhibitory Concentration And Minimum Bactericidal Concentration of solvent fractions *Zingiber officinale* (Ginger), *Garcinia kola* (Bitter cola) and *Aframomum melegueta* (Alligator pepper) on selected upper respiratory tract pathogens.

1.5 Significance of the Study

The field of biomedical research is actively investigating the use of plants as promising sources for pharmaceuticals that can aid in the prevention and treatment of various human diseases. The World Health Organization (WHO) has acknowledged antibiotic resistance as a pressing global health security concern that demands intervention across all sectors of government and society. This research endeavor focuses on developing novel approaches to antibiotic production from medicinal plants, employing phytochemicals obtained from extracts of three different plant varieties.

1.6 Scope of the Study

This study includes a comparative evaluation of the antibacterial effect of *Garcinia kola, Aframomum melegeta* and *Zingiber officinale Roscoe* on selected upper respiratory tract pathogens. The study also includes the determination of the minimum inhibitory concentration (MIC) *of Garcinia kola, Aframomum melegeta and Zingiber officinale Roscoe* alone and in combination against selected bacterial strains. The effect of the combination of the plants extracts on bacterial growth were also studied. Some phytochemicals were identified for this requirement.

1.7 Limitation of the Study

This study was limited to an *In-vitro* evaluation of the medicinal plants used,few phytochemicals were analysed and these study focus on only on few selected upper respiratory tract pathogen excluding other relevant pathogens.

1.8 Operational Definitions of Terms

Antimicrobial: Antimicrobials refer to substances that either kill or inhibit the growth of microorganisms. These microorganisms encompass a range of entities, including bacteria, viruses, protozoa, and fungi, such as powdery mildew and mold. Antimicrobials, which comprise antibiotics, antivirals, antifungals, and antiparasitics, are utilized in the prevention

and treatment of infections affecting humans, animals, and plants. Microorganisms that exhibit resistance to these antimicrobials are often referred to as "superbugs"[20].

Antioxidant: An antioxidant is defined as any substance that hinders or mitigates oxidative damage to a target molecule. The defining feature of an antioxidant is its ability to neutralize free radicals through mechanisms involving redox hydrogen donors and agents that quench singlet oxygen. Antioxidants can be categorized into natural sources, such as plants, and synthetic compounds, including tetra-butylhydroquinone, butylated hydroxytoluene, and butylated hydroxyanisole, all of which can effectively scavenge free radicals. However, there is a growing trend to phase out synthetic antioxidants in favor of natural alternatives, which are perceived to be safer and devoid of adverse effects[21].

Plant Extracts: The extraction of materials or products from plants often involves the use of chemicals such as ethanol. A plant extract refers to an active ingredient or substance that possesses specific properties and is isolated from plant tissues for designated applications. Plant extracts find a wide range of uses, including in natural food products, pharmaceuticals, cosmetics, technological adjuvants, and as chemical alternatives to additives[22].

Bacteria: Bacteria represent a diverse category of unicellular microorganisms characterized by the presence of cell walls, yet they do not possess organelles or a structured nucleus; some species within this group are pathogenic and can cause disease[23].

Minimum inhibitory concentrations: The minimum inhibitory concentration (MIC) refers to the smallest amount of an antimicrobial agent that prevents the observable growth of a microorganism following a 24-hour incubation period[24].

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Chapter Two

Literature Review

2.1 Overview of Plants

In biological classification, a plant is identified as a eukaryote that is part of the kingdom Plantae. These organisms are autotrophs, utilizing photosynthesis to generate their own food[1]. They are able to capture energy through the green pigment (chlorophyll) in the chloroplast and use carbon dioxide and water to produce sugar as food and oxygen as a by-product. As autotrophs, plants are often located at the beginning of the food chain. Since time immemorial, human civilization has used them as food, medicine, clothing, and shelter. Plants are a source of bioactive molecules with antibacterial, antiparasitic, healing, analgesic, anti-inflammatory, and tissue-repairing properties, providing a potential alternative to treatment[2]. About 80% of the world's population in developing countries uses plants to treat various diseases[3].

Approximately 80% of individuals residing in developing nations derive advantages from the use of traditional herbal remedies [4]. Humans consume 28,187 medicinal species, compared to an estimated total of 374,000 plant species. In addition, the WHO has listed and identified more than 20,000 species of medicinal plants as possible sources of new drugs[5]. Recipes for medicinal plants have been developed in more than 100 countries. More than 30,000 antimicrobial molecules have been extracted from plants, and antimicrobial activity has been detected in more than 1340 species. In addition, between 14% and 28% of higher plant species are thought to have medicinal properties, and 74% of bioactive chemicals generated from plants have been shown to have ethnomedicinal effects[6].

Plants are an important source of medicine and have a major impact on global health. Herbs or medicinal plants are recognized as an important potential source of therapeutic or

therapeutic assistance[7]. Around the world, the use of medicinal herbs has taken over the healthcare system. This includes the use of medicinal plants as a potential resource for maintaining health and conditions, in addition to using them to treat diseases. Two-thirds of the world's population, or many countries, rely on herbal medicine for their primary medical needs. They are less likely to cause negative side effects and are more culturally acceptable, compatible and adaptable to the human body[8].

2.1.1 Medicinal Plants

Medicinal plants are an extraordinary endowment from the natural world, contributing significantly to human health endeavors. From ancient times, individuals have identified and utilized these natural products and their derivatives as key sources of medicinal therapies. They persist in offering valuable bioactive ingredients that are appropriate for pharmacological applications. According to contemporary estimates, roughly 10% of the 250,000 species that constitute the plant kingdom have been investigated or recognized for their therapeutic potential in treating various diseases[9]. Medicinal plants are considered to be the major biological source of medicines used in modern medicine, traditional medicine and as chemical elements or models of manufactured medicines[10]. Identification of widely available and medically important metabolites in plants would reduce the overuse of rare and well-known medicinal plants. The use of plants for medicinal purposes continues to this day, mainly in the form of traditional medicine, which is now recognized by the World Health Organization as a building block of primary health care. A medicinal plant is a plant containing substances in one or more of its organs which can be used for therapeutic purposes or which are precursors to the synthesis of useful medicines. This description distinguishes between medicinal plants, whose medicinal properties and ingredients have been

scientifically proven, and those that are considered medicinal but have not yet been the subject of in-depth scientific research.

Compared to conventional medicine, many herbal and herbal therapies have a long history of use in the prevention and treatment of various conditions[11].Medicinal herbs are widely distributed among plant sources and have a wide range of therapeutic uses[12]. The value of these plants or herbs lies in their secondary metabolites, which are not nutritious but can exert certain physiological effects in humans against various types of infectious diseases and metabolic disorders[13]. Different plant species around the world have been studied for their therapeutic properties, and biological activity varies from plant to plant because they have different physiological effects on the human body. Medicinal plants play an important role in public health, especially in developing countries because of their better cost and low toxicity. Intensive use of herbs with therapeutic properties does not lead to poisoning, while excessive use of allopathic drugs is associated with side effects. Drug residues can lead to the growth of resistant microorganisms that are difficult to treat; As a result, the world is looking for safer alternatives[14].

The natural antioxidants in fruits and vegetables are inversely proportional to the risk of chronic diseases such as atherosclerosis, coronary artery disease, cancer, diabetes mellitus, neurodegenerative diseases and aging. Therefore, natural antioxidants are an alternative strategy for preventing and treating these diseases. Due to their oxidative activity, phenolic compounds are potential substances for the prevention and treatment of many diseases related to oxidative stress[15].

2.1.2 Natural Medicinal Product as Repair Products

Natural products have received particular attention in recent years as a valuable resource for drug discovery due to their abundant resources and long history of clinical use[16]. A return to

nature has become a trend believed to address the relationship between humans and the environment. Natural active ingredients have always played an important role in the discovery of drugs for the treatment of various diseases due to their natural properties, less toxicity and reduced amount of drug residues in the body[17]. They can also be used as lead compounds to lay the foundation and give direction to the development of biopharmaceuticals. Natural products are obtained mainly from plants, animals and minerals, the main source of which are plants. Most plant materials, especially Chinese medicinal plants, have remarkable therapeutic effects, mainly due to their active ingredients, but only 1-5% of plants have been scientifically studied and have therapeutic value. Therefore, the discovery, separation and identification of new lead compounds from natural products remains an important task[18].

Natural products are reported to have been used for treatment since ancient times and have so far been the basis for half of new drugs serving as parent form or as optimized derivatives[19] .In recent decades, the accumulation of evidence has shown that the therapeutic effects of Natural product in a number of diseases, including cancer, bacterial infections, metabolism and autoimmune diseases, can be meditated on their anti-inflammatory, antioxidant, anti-apoptotic and immunoregulatory properties. Compared to conventional therapies, Natural Products have several advantages, including different mechanisms of action, multiple chemical structures, and a natural ability to repair[20]. The exploration of natural products as bioactive compounds has led to the development of a plethora of best-selling drugs in the last century through isolation, characterization, chemical synthesis, or study of biosynthesis in modern society. However, many natural products are only available in very small quantities from natural sources, especially from higher plants and marine organisms[21].

2.1.3 Antimicrobial Activity of Plants

Microorganisms are inherently present in the environment, allowing them to easily contaminate food during various stages such as harvesting, slaughtering, processing, and packaging[22].These microorganisms possess the ability to endure harsh conditions typically employed in food preservation, including low temperatures, modified atmosphere packaging, and vacuum packaging, and they can also withstand conventional pasteurization methods. Plants exhibit a remarkable capacity to produce aromatic compounds, predominantly phenols and their oxygen-substituted derivatives. A significant number of these compounds are classified as secondary metabolites, with over 12,000 having been identified, which is estimated to represent less than 10% of the total diversity. These compounds often function as defensive agents for plants, protecting them from microbial pathogens, insects, and herbivores[23]. For instance, terpenoids are responsible for the characteristic scents of plants, while compounds such as quinones and tannins contribute to pigmentation. Furthermore, numerous compounds are integral to the flavor of plants, such as the terpenoid capsaicin found in chili peppers, and many culinary herbs and spices also yield beneficial medicinal compounds[24].

Antimicrobials derived from plant sources are often regarded as safer alternatives to synthetic agents due to their natural origins. It is estimated that approximately 25% of contemporary pharmaceuticals are based on plant-derived compounds. These plant substances may interact with different targets compared to conventional antimicrobials, leading to distinct mechanisms of action against microbial pathogens. The antimicrobial properties of plant compounds can be attributed to several mechanisms. For instance, certain phytochemicals, such as carvacrol, thymol, and eugenol, can disrupt microbial cell membranes, while others, like cinnamaldehyde, can modify cellular metabolic processes. Additionally, some plant-derived compounds are effective in inhibiting biofilm formation, as seen with trans-

cinnamaldehyde, carvacrol, thymol, and geraniol. Furthermore, plant antimicrobials may impede the synthesis of bacterial capsules, exemplified by salicylic acid and its derivatives. Certain plant metabolites can also diminish bacterial virulence by modulating quorum sensing. Another significant mechanism involves the reduction of microbial toxin production. Moreover, plant metabolites can serve as resistance-modifying agents, which are increasingly recognized as promising strategies to address bacterial resistance. Research has indicated that these plant compounds can enhance the efficacy of antibiotics by acting as resistance modifiers[25].

The estimated number of plant species ranges from 250,000 to 500,000; however, only a limited number have been investigated for their antimicrobial properties. Historically, humans have utilized plant materials to combat infectious diseases, often without an understanding of the underlying pathogens. In contemporary times, various herbs continue to play a significant role in traditional medicine for treating infectious diseases across numerous nations, including Armenia. This reliance on herbal remedies has notably intensified in recent decades. There are three primary methodologies for selecting plants for antimicrobial evaluation. The first involves identifying antimicrobial activity in plants traditionally employed in herbal medicine. The second method focuses on exploring the antimicrobial characteristics of flora endemic to specific regions or countries. The third strategy entails assessing the antimicrobial effectiveness of various plants against specific microbial agents[26].

2.2 Review of Plants Used

2.2.1 *Garcinia cola*

Scientific Classification of *Garcinia cola*[27]

Kingdom: Plantae

Phylum: Spermatophyta

Class: Dicotyledonae

Order: Malpighiales

Family: Clusiaceae

Genus: *Garcinia*

Species: *Garcinia cola*

Garcinia cola is a member of the *Clusiaceae* family, and it is referred to by various names in different languages, including Mijingoro in Hausa, Agbilu in Igbo, and Orogbo in Yoruba. This species is characterized by its notably bitter flavor, which has led to its common designation as Bitter kola, as well as the name male kola, attributed to its purported aphrodisiac properties[28]. It is native to several countries in West and Central Africa, including Benin, Cameroon, Gambia, Congo, Ivory Coast, Mali, Gabon, Ghana, Liberia, Nigeria, Senegal, and Sierra Leone. *Garcinia cola* typically flourishes in subtropical and tropical lowland rainforests, with a notable presence in the southwestern states of Nigeria and Edo state. This species can grow into a medium to large tree, reaching heights of up to 30 meters and a trunk diameter of 100 centimeters[29].The flowering period occurs from December to January, while the fruiting season spans from July to October, with harvesting taking place intermittently as the ripe fruits naturally fall. This species exhibits a slow growth rate, characterized by extended periods of flowering and fruiting. The fruit itself is orange, smooth, and reddish-yellow, resembling peach skin, and contains three to four seeds encased in a brown layer. The seeds, which are nut-like and marked with dark branched lines, are commonly consumed[30].They are traditionally chewed during cultural and social ceremonies, valued for their aphrodisiac effects, and are often offered to guests as a gesture of hospitality and respect[31].

2.2.2 Importance of *Garcinia cola*

Garcinia cola serves as an alternative to hops in the beer brewing process and plays a role in preventing spoilage. Organoleptic assessments indicate that beer produced with *Garcinia cola* exhibits a slightly increased bitterness compared to that brewed with traditional hops[32].

This plant is also prominent in traditional medicine practices. The bark possesses detoxifying properties, and its powdered form is utilized in tumor treatment. The juice is effective for managing parasitic skin diseases, while the latex is administered internally for gonorrhea and applied externally for various conditions. The seeds are often chewed for their aphrodisiac effects and are used to treat dysentery, relieve colic, and alleviate symptoms of coughs and colds. Moreover, the seeds are employed in the management of diabetes, bronchitis, liver ailments, and throat infections[33].

Garcinia cola is currently classified as "vulnerable" on the IUCN Red List of Threatened Species, likely as a result of deforestation and significant wild harvesting. This species is extensively utilized for its medicinal properties, which include antiviral, anti-inflammatory, antidiabetic, bronchodilator, and antigenotoxic effects. The seeds of Garcinia cola are traditionally employed as a remedy for ailments such as cough, laryngitis, and liver disorders[34]. The seeds are also known to contain various phytochemicals, including saponins, tannins, flavonoids, proteins, glycosides, reducing sugars, starch, sterols, and triterpenoids. Although the presence of antinutrients such as oxalates and phytates has been noted, the seeds are generally regarded as safe for consumption, with few reports of adverse effects from excessive intake. Flavonoids, which are low molecular weight compounds, serve as natural antioxidants that can eliminate free radicals and convert them into harmless substances. Additionally, they influence immune cell activation and provide protective effects

against oxidative and excitotoxic stress in the central nervous system, while also demonstrating antitumor activity.

The astringent taste commonly associated with *Garcinia cola* seeds is largely due to the presence of tannins, secondary metabolites that are well-known for their natural therapeutic applications in treating intestinal disorders such as diarrhea and dysentery. Tannins not only possess antimicrobial properties but also show considerable promise in cancer prevention, and when paired with floral tannins, they reveal a range of medicinal uses. Additionally, *Garcinia cola* extracts have been shown to contain cardiac glycosides and steroid compounds, which correlates with the plant's historical use in addressing chest pain and heart infections[35].

Garcinia cola seeds, while frequently marketed alongside kola nuts, exhibit a distinct chemical profile. In contrast to kola nuts, Bitter cola seeds are characterized by elevated concentrations of phenolic compounds, with no detectable levels of caffeine, theobromine, or catechin. These seeds present a viable opportunity for international trade, as they are commonly exported among neighboring nations, where they can rival locally sourced almonds from regional agroecosystems. Consequently, the seeds available in markets across Benin may originate from Nigeria, Togo, or Ghana. Additionally, the trade of seeds from Nigeria to Cameroon holds significant commercial value[36].

2.2.3 Medicinal Activity of *Garcinia cola*

Garcinia cola is recognized for its therapeutic applications in the treatment of malaria. The fruit is rich in kolaviron, a phytochemical characterized by its antioxidant and anti-inflammatory properties, which contribute to its efficacy against malaria. Additionally, the hypoglycaemic and lipidemic effects of kolaviron derived from kola seeds have been demonstrated to possess antidiabetic, antilipidemic, and antiatherogenic characteristics, indicating significant potential for safeguarding against coronary artery disease. The

medicinal value of bitter kola is substantial, as all its components have been shown to hold medical significance. The seeds are commonly chewed for their aphrodisiac properties or utilized in the management of various ailments, including coughs, dysentery, colds, liver disorders, diarrhoea, laryngitis, bronchitis, and gonorrhoea. Furthermore, bitter kola contains saponins, which enhance cellular function and exhibit detoxifying and cleansing effects. It also promotes lung capacity by facilitating the development of alveolar canals and vesicles, thereby reinforcing lung tissue fibers and contributing to overall bodily strength[37].

2.2.4 Phytochemical Properties of *Garcinia cola*

Phytochemicals are plant-derived compounds that, while not classified as essential nutrients, possess significant protective and preventive effects against various diseases. These naturally occurring bioactive substances are present in different parts of plants, including fruits and leaves, and they interact synergistically with nutrients and dietary fiber to enhance health. Recent studies indicate that phytochemicals can play a crucial role in disease prevention and may lower the risk of numerous chronic and inflammatory conditions. Notable examples of phytochemicals include lycopene found in tomatoes, isoflavones present in soybeans, and flavonoids abundant in various fruits. Flavonoids, a diverse group of polyphenolic compounds, exhibit a range of protective effects, such as anti-inflammatory, antioxidant, antiviral, and anticarcinogenic activities. These compounds are commonly found in foods like oranges, tangerines, berries, apples, and onions[38].

Garcinia cola's phytochemical composition demonstrates that it contains flavonoids (2.041 g/100 g), alkaloids (0.647 g/100 g), glycosides (3.421 g/100 g), phenols (0.147 g/100 g), and saponins (2.471 g/100 g) in relatively low quantities. These phytochemicals are biologically

active compounds present in plants in small amounts, which, while not recognized as essential nutrients, significantly contribute to the defense against degenerative diseases[39].

2.2.5 Economic Importance of *Garcinia cola*

The economic significance of *Garcinia cola* is known throughout West Africa, where its seeds are integral to the sociocultural fabric of tropical communities. Bitter kola nuts are highly regarded for their supposed health benefits, and their consumption in large quantities is not linked to digestive disturbances. Various parts of the *Garcinia cola* plant, including its seeds, nuts, and bark, are commonly employed in traditional African medicine to address a range of health issues. Traditional medicinal practices utilize these components for treating coughs, acting as laxatives, providing antiparasitic effects, and serving as antimicrobial agents. The seeds, in particular, are used to manage diarrhea, bronchitis, throat infections, and liver ailments. The economic contribution of *Garcinia cola* to both local and national markets is substantial, enhancing the living standards of those involved in its trade in rural and urban settings. In several developing countries, the trade of bitter cola is more profitable than that of other forest products, excluding timber, largely due to the favorable storage conditions for both fresh and dried[40].In Nigeria, where traditional industry employment is on the decline, individuals seeking alternative income sources frequently resort to the collection of non-timber forest products like bitter cola from nearby forests. The bark, seeds, and trunk of the Garcinia cola plant have historically been employed to treat throat infections, acute fevers, and respiratory issues, while the leaves are utilized for gastrointestinal ailments and as a remedy for typhoid fever. Thus, *Garcinia cola* seeds and other plant parts serve as both a medicinal resource and a source of income for local communities[41].

2.3 *Zingiber officinale* Roscoe

Scientific Classification of *Zingiber officinale*[42]

Kingdom: Plantae

Phylum: Tracheophyte

Class: Liliopsida

Order: Zingiberales

Family: Zingiberaceae

Genus: *Zingiberaceae*

Species: *Zingiber officinale*

Zingiber officinale is native to Southeast Asia and a member of the *Zingiberaceae* family. Common names for it include Hausa (Cithar), Igbo (Jinja), Yoruba (Atale, ata-ile), and English (Ginger). One of the healthiest and most delicious foods in the world, ginger is a crop that is grown all over the world[43]. The most important part of ginger that is eaten is the rhizome, which is the horizontal stem from which the roots grow. Found worldwide, it is also used in medicine and cooking[44]. It is closely related to cardamom, galangal, and turmeric. One of the most popular food seasonings in the world, ginger officinale is harvested when the

plant is 8 to 9 months old. Its hairy skin needs to be removed before consumption[45]. It is used as a spice and flavoring agent all over the world and has several health benefits, such as pharmacological, antioxidant, antibacterial, anti-inflammatory, antimutagenic, and hepatoprotective effects. *Zingiber officinale* can be used in a variety of ways, such as fresh, dried, pickled, crystallized, candied, powdered, or ground. Ginger is a popular nutraceutical that is frequently used as a flavoring, dietary supplement, and traditional culinary herb because of its tasty flavor and healthful nutrients[46].Functional foods and drinks like ginger tea, ginger ale, ginger juice, and ginger candy are frequently made from ginger and its processed products, including ginger powder and ginger slices[47].

Zingiber officinale belongs to the same family of plants as turmeric and cardamom. Ketones, particularly gingerols, which appear to be the primary constituent of ginger, are primarily responsible for its spicy scent. Additionally, ginger has been used for centuries by the indigenous peoples of Asia, particularly in China and India, as a spice and sweetener in local cuisine as well as a herbal remedy for a variety of ailments. In some Asian countries, ginger is also used as a herbal remedy for colds and to boost human immunity[48].

Ginger is thought to have therapeutic benefits, particularly in traditional Chinese, Indian, and Ayurvedic medicine. Because of its expectorant properties, which loosen and expel mucus, it is used as a cough remedy. *Zingiberaceae* is one of the most significant families in the plant world and is primarily used as raw materials for the production of various traditional medicines[49].Additionally, ginger is used to help with digestion, pain relief, nausea, vomiting, and poisoning. It is currently recognized to have anti-inflammatory, antioxidant, and antitumor properties. Numerous studies have demonstrated the effectiveness of ginger in the prevention and treatment of gastrointestinal, cardiovascular, respiratory, and neurological diseases[50]. *Zingiberaceae* is one of the most significant families in the plant

world and is primarily used as raw materials for the production of various traditional medicines[51].

Ginger, which is frequently used as a spice in cooking to enhance the flavor and aroma of food as well as the quality of preservation, comes in roughly two varieties that are sold at food markets. Varieties like "local" and "hybrid" ginger are referred to by indigenous peoples (suppliers and consumers). Ginger that has small rhizomes, thick brown skin, and yellow flesh or powder with a strong, pungent scent is referred to as "local" ginger. "Hybrid" ginger refers to a type that has big rhizomes, light yellow skin, off-white powder or flesh, and a milder scent[52].

The potential of ginger to treat different aspects of cardiovascular diseases has drawn attention, as have *in-vitro* and animal studies demonstrating the spice's anti-inflammatory, antioxidant, platelet-inhibiting, blood pressure-lowering, and hypolipidemic properties. Ginger's constituents are proposed as a possible novel class of platelet activation inhibitors that do not have the possible adverse effects of aspirin, which is typically used in this method. It was discovered that aspirin was more effective than gingerols and analogs at preventing the release and aggregation of platelets caused by arachidonic acid. Since ancient times, ginger has been used to treat respiratory disorders, including diabetes, asthma, and other ailments.

2.3.1 *Zingiber officinalle* and Healthy Ageing

Active and healthy ageing refers to the capacity of an individual to perform daily activities without being hindered by cognitive or physical impairments as well as chronic health conditions. Besides serving as a protective barrier against various external threats, the skin also plays significant aesthetic roles. Changes in the skin are among the most apparent indicators of ageing. As one ages, phenomena such as wrinkles, decreased elasticity, and skin

sagging become prevalent. Notably, extended exposure to ultraviolet (UV) rays from the sun is a key factor that contributes to the external signs of skin aging[53].A growing collection of research indicates that ginger may support healthy aging, decrease morbidity, and enhance healthy life expectancy. It is also thought that ginger can protect against, prevent, and even treat various diseases associated with aging by influencing the molecular pathways involved in their development. The enzyme elastase, which comes from fibroblasts, is known to play a role in wrinkle formation by reducing skin elasticity as a result of UV-B exposure. Ginger extract, previously demonstrated to inhibit elastase from fibroblasts, has been shown to prevent the loss of skin elasticity caused by UV-B when applied topically to the skin of mice and rats. In a separate study, individuals who applied a body cream containing ginger oil for four weeks experienced a decrease in signs of skin aging, likely due to the plant's antioxidant properties.

2.3.2 *Zingiber officinalle* for Oxidative Stress

Activation of inflammatory pathways and decreased production of antioxidant enzymes are linked to oxidative stress. Consequently, oxidative stress and persistent inflammation cause a variety of molecular and cellular alterations, such as mitochondrial dysfunction, stem cell loss, genomic instability, epigenetic modifications, and cell aging. Cellular aging and the emergence of age-related metabolic and degenerative diseases are significantly influenced by these physiological/pathological alterations[54].

The extraction solvent, extraction temperature and duration, and storage conditions are some of the variables that affect the antioxidant content and composition of plant extracts. Ginger extracts prepared with ethanol, methanol, and acetone solvents showed higher antioxidant activity than extracts made with water[55]. This implies that ginger retains its antioxidant activity even after being cooked at high temperatures, in contrast to other

vegetables that contain antioxidants. In particular, dried ginger tea has better antioxidant qualities than commercial tea. It is noteworthy that consuming ginger tea can effectively prevent and treat colds, coughs with sore throats, and inflammation[56].

2.3.3 Ginger for Nausea and Vomiting

Ginger helps reduce nausea and vomiting because of its strong antiemetic qualities. The most frequent and disagreeable side effects experienced by cancer patients receiving chemotherapy are nausea and vomiting. The success rate of treatment has not been sufficient, even though there are now effective drug and cytoplast combinations available[57].Ginger extract may be useful in treating chemotherapy-induced nausea and vomiting (IVNV), according to a number of studies conducted on both humans and animals [58].

Ginger has traditionally been used to treat gastrointestinal symptoms, and recent research has shown that ginger can effectively relieve nausea and vomiting. In a clinical trial, inhaling ginger oil can reduce the intensity of nausea and reduce vomiting two and six hours after nephrectomy in patients. In addition, treatment with dried ginger powder reduced episodes of intraoperative nausea in patients undergoing elective cesarean section. Ginger relieves nausea caused by anti-tuberculosis drugs and antiretroviral. Ginger can relieve morning sickness and vomiting and motion sickness, while recent studies have focused on ginger's preventive effect on postoperative and chemotherapy-induced nausea and vomiting[59].

2.3.4 Cardioprotective Activity of Ginger

Cardiovascular disease is considered one of the leading causes of premature death, with 17.9 million people dying every year. Dyslipidaemia and hypertension are known to be risk factors for cardiovascular diseases, including stroke and coronary artery disease. Several studies have shown that ginger can lower blood lipid levels and blood pressure, which helps protect

against cardiovascular disease. Intravenous administration of fresh ginger extract has been reported to lower blood pressure in rats under anaesthesia, and this activity has been attributed to its inhibitory effect on voltage-gated calcium channels.

Platelet aggregation is a well-established risk factor for coronary heart disease and stroke. More recently, the potent in vitro antiplatelet agent ginger has been shown to be stimulated by platelet aggregation stimulated by adenosine-5-diphosphate (ADP), bovine thrombin, and arachidonic acid compared to aspirin (positive control)[60].

Ginger protected against complications caused by hypertension by reducing the activity of platelet adenosine deaminase (ADA) and increasing adenosine levels, which prevented platelet aggregation and promoted vasodilation in hypertensive rats. In addition, ginger extract has shown Vasu protective effects on the coronary arteries of pigs by suppressing NO synthase and cyclooxygenase[61]. In addition, a cross-sectional study showed that the likelihood of hypertension and coronary heart disease decreased when the daily intake of ginger was increased. Overall, ginger has shown cardiovascular protective effects in relieving hypertension and improving dyslipidaemia, such as improving HDL-C, TC, LDL, TG and VLDL[62].

2.3.5 Ginger and COVID-19

Ginger's high concentration of antiviral compounds has demonstrated exceptional antiviral activity. The severe acute respiratory syndrome coronavirus (SARS-CoV-2) is the infectious respiratory disease that causes coronavirus disease 2019 (COVID-19)[63]. Since its discovery in 2019, the severe acute respiratory syndrome coronavirus (SARS-CoV-2) has spread throughout the world, causing the COVID-19 pandemic. Coronavirus disease 2019 (COVID-19) is an infectious respiratory disease. Infected elderly patients with comorbidities are particularly susceptible to death from the disease. Extensive research is being conducted

despite the lack of a proven cure or fully effective vaccine against COVID-19. Furthermore, because ginger extract has been shown to be beneficial in treating COVID-19-related conditions like sepsis, pneumonia, pulmonary fibrosis, and acute respiratory distress syndrome (ARDS), it can be used as an adjuvant treatment for the virus, according to a very recent publication. A study examined the possible application of ginger's antiviral qualities to fight SARS-CoV-2 infection by examining how ginger ligand compounds interact with major protease (MPro) and viral spike protein (S). The development of structure would therefore be a promising therapeutic approach[64].

2.3.6 Cytotoxicity Activity of *Zingiber officinalle*

With roughly 90.6 million deaths from cancer in 2018, it has been shown to be the leading cause of death[65]. Ginger has recently been extensively researched for its anticancer qualities against a variety of cancers, including breast, cervical, colorectal, and prostate cancers. In cancer, inhibition of proliferation and induction of apoptosis are examples of potential mechanisms of action. Ginger and its bioactive compounds have been shown in numerous studies to interfere with the processes that cause colorectal cancer. A dried ginger powder fraction high in polyphenols was found to inhibit the growth of gastric adenocarci and colorectal cancer cells in an *in-vitro* experiment[66].

Ginger's cytotoxic properties and underlying mechanisms in prostate cancer were assessed *In-vitro* and *In-vivo* by previous research.Through the downregulation of glutathie-S-transferase (GSTπ) and multidrug resistance (MRP1) protein expression, 6-gingerol, 10-gingerol, and 6-shogaol have been demonstrated to have an antiproliferative effect on human prostate cancer cells. Furthermore, 6-gingerol, 8-gingerol, 10-gingerol, and 6-shogaol, which are binary combinations of ginger phytochemicals, collectively suppressed the growth of PC-3

prostate cancer cells. Ginger's impact on naked athymian mice with human prostate cancer xenografts was investigated in an *In-vivo* study. Compared to a synthetic combination of 6-shogaol, 6-gingerol, 8-gingerol, and 10-gingerol, natural ginger extract demonstrated a 2–4 times greater inhibitory effect on tumor growth.Furthermore, it is possible that 6-shogaol is more significant than 6-gingerol and 6-paradol in lowering cell survival and triggering apoptosis in prostate cancer cells in both humans and mice.NF-κB signaling and signal converter and activator transcription 3 (STAT3) were the main ways it function [67].

2.3.7 Protective Effect of *Zingiber officinale* against Respiratory Disease

Zingiber officinale caused smooth muscle from the human respiratory system to relax noticeably and quickly. Ginger extracts can inhibit the growth of respiratory tract pathogens, including Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, and Haemophilus influenzae. Fresh ginger can inhibit the formation of plaques caused by the human respiratory syncytial virus (HRSV) in respiratory tract cell lines. Ginger can also be effective against rhinovirus. A diet rich in ginger reduced the levels of inflammatory cytokines. Ginger may help with asthma symptoms by modulating intracellular calcium in airway smooth muscle cells[68].

2.3.8 Side Effects of *Zingiber officinale*

Because *Zingiber officinale* has anticholinergic and antiserotonergic qualities, it directly affects the gastrointestinal system by increasing muscle tone and peristalsis. Ginger may intensify adverse effects associated with antiemetics that affect the central nervous system. Extracts from ginger, like those from onions and garlic, can prevent blood

clotting *in-vitro*. The side effects of ginger are quite rare; the most common ones are mild ones like diarrhea and heartburn. *In-vitro*, high dosages of ginger may improve gastric exfoliation and anti-prostaglandin activity[69].

2.3.9 Economic and Social Importance of Ginger

Grown commercially in tropical regions of Africa, Latin America, the Caribbean, and South and Southeast Asia, ginger is a significant economic crop. The demand for ginger is rising in North America and Western Europe, even though fresh ginger is mostly consumed in Asia. Ginger is an essential component of many cultures worldwide as a spice. It is thought to be necessary in many Asian dishes because of its distinct scent and sweet-spicy flavor.

In traditional medicine, ginger has also played a significant cultural role. It is thought that ginger has been grown in Asia for more than 5,000 years to treat a variety of common conditions, such as pain, inflammation, nausea, and vomiting. Ginger has been shown to have numerous therapeutic benefits by modern science, and many medical professionals now advise using it as a home remedy. As a result, ginger supplements are crucial to both the pharmaceutical and herbal medicine sectors. In addition to its use in the food and pharmaceutical industries, ginger is also used in the cosmetics industry as a fragrance and skin care ingredient in lotions, balms, and perfumes.

Farmers could use ginger as a sustainable source of income to help reduce poverty and generate income, and traders and exporters could use it to make money and help the government reduce the international trade deficit to some degree. Ginger not only generates income for its exporters, but it also creates jobs and produces goods for domestic consumption. E. chili sauces, dry sauces, snack cookies, and powder. People are drawn to the economic profitability of ginger production, and its mass production raises people's earning potential. Given its numerous applications and health advantages, it is

obvious that ginger deserves a place among spices of great culinary, economic, and social importance worldwide[70].

Ginger offers a unique opportunity because it reduces poverty, increases export earnings and the resulting foreign exchange additions, increases employment opportunities for women in processing, grading, and packaging, and increases market demand for processed fiberless ginger in both domestic and international markets.

2.4 *Aframomum melegueta*

Scientific Classification *Aframomum melegueta*[71]

Kingdom: Plantae

Phylum: Tracyofita

Class : Liliopsida

Order: Zingiberales

Family: Zingiberaceae

Genus: *Aframomum*

Species : *Aframomum melegueta*

Aframomum melegueta a member of the *Zingiberaceae* family of gingers, is known by a number of names, including Africa Kakulesi, Ginny pepper, Guinea pepper, Mbongo spice, Atare (in Yoruba), Chitta (Hausa), or Guinea pepper. Native to marshy areas along the coast of Nigeria in West Africa, *Aframomum melegueta* is a perennial

herbaceous plant. The pods, which are 5-7 cm long and contain numerous tiny, reddish-brown seeds, are formed from the purple tubular flowers. It is primarily found in Nigeria, Ghana, Liberia, Ivory Coast, and Togo[72].Aromatic ketones give the seeds a sharp, peppery flavor. This plant can reach a height of 1.5 m and has pink-orange upper flowers that can turn into fleshy, unpronounceable pods, as well as orange-colored lips. The trunk's short bark is spotted with scars from fallen leaves. The underside of the leaves has narrow veins, and they measure around 30 cm in length and 12 cm in width[73]. The leaves have a well-organized vascular system and measure, on average, 35cm in length and 15cm in width. The herb's fragrant flowers have an orange-pink top and an orange lip. Alligator pepper's fruits, leaves, and seeds are used to flavor traditional foods.

2.4.1 Antioxidant Properties of *Aframomum melegueta*

One of the primary health advantages of alligator pepper is its ability to act as an antioxidant. The seeds of alligator pepper are rich in phytochemicals, including terpenoids, alkaloids, flavonoids, tannins, cardiac glycosides, saponins, and phenolic compounds[74].Antioxidants are crucial in shielding the body from harm inflicted by free radicals, which are unstable molecules that can attack vital macromolecules, leading to cellular injury and disruption. They eliminate free radicals and offer defense against viruses, allergens, microbes, platelet clumping, tumors, and hepatotoxins, which can inflict chemical damage on the liver[75].

2.4.2 Antimicrobial Properties of *Aframomum melegueta*

Aframomum melegueta possesses antimicrobial qualities. It has phenolic compounds, which suggests it may aid in hindering bacterial growth. This plant can inhibit the proliferation of bacteria including *Salmonella typhi, Klebsiella pneumonia*, and *Staphylococcus aureus*, and

is utilized in the treatment of infectious illnesses like urinary tract infections caused by *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Staphylococcus saprophyticus*, *Proteus mirabilis*[76].

2.4.3 *Aframomum melegueta* for Special Treatment and Treatment of Skin Diseases

The seeds of *Aframomum melegueta* can be crushed and utilized to create mixtures for medicinal purposes. Alligator pepper is rich in tannins, which are known for their astringent properties and are highly effective for healing, addressing burns, and calming inflamed mucous membranes. These granules can assist in the regeneration of new tissue in the damaged area. Additionally, it is important to highlight that alligator pepper can also be beneficial in skin care. In traditional medicine, alligator pepper has been used to treat viral infections such as chickenpox, measles, and smallpox, which lead to skin rashes[77].

2.4.4 Treatment of Gastrointestinal Diseases

Alligator pepper could be beneficial for addressing gastrointestinal issues like stomach discomfort and diarrhoea. Certain individuals opt to nibble on spicy alligator pepper to alleviate gastrointestinal concerns. Consuming the seeds may also aid in relieving bloating, constipation, intestinal cramps, and worms in the gut[78].

2.5 Respiratory System

The respiratory system comprises organs, tissues, and muscles that facilitate breathing, including the central nervous system, chest wall, pulmonary circulation, and respiratory system. Key components of the respiratory system include the nasal passages, sinus cavities, mouth, throat (pharynx), larynx, trachea, diaphragm, lungs, bronchi, bronchioles, alveoli, and capillaries. Its primary function is to distribute oxygen throughout the body while filtering out carbon dioxide and other waste materials[79].The respiratory system enables the exchange of gases between the air and blood, as well as between blood and the body's countless cells. It encompasses the respiratory tract, pulmonary vessels, lungs, and respiratory muscles. While most organs in the respiratory system assist in air distribution, only the tiny alveoli and alveolar ducts shaped like grapes are responsible for the actual gas exchange. The respiratory system is an interconnected network of organs and tissues that facilitate the process of breathing, including the respiratory tract, lungs, and blood vessels[80].

The respiratory system can be categorized into two sections: the upper respiratory tract and the lower respiratory tract. The upper respiratory tract includes the nose, pharynx, and larynx,

with its organs situated outside the thoracic cavity. The lower respiratory tract comprises the trachea, lungs, and all parts of the bronchial tree (including the alveoli), with its organs found within the thoracic cavity. While the lungs are generally classified as part of the lower respiratory tract, they are occasionally treated as a distinct entity. They contain respiratory bronchioles, alveolar ducts, alveolar sacs, and alveoli. The internal surface of the respiratory system is lined with mucous membranes and is safeguarded by several immune defenses. Goblet cells in the respiratory epithelium release a layer of sticky mucus. The viscosity and acidity of this mucus serve to inhibit the adhesion of microbes to the underlying cells. Additionally, the respiratory tract has epithelial cells equipped with cilia. The beating cilia help to expel mucus and any trapped microbes, moving them towards the epiglottis to be swallowed. This process of removing microbes is known as the mucociliary escalatory effect and is a crucial mechanism that prevents inhaled microorganisms from advancing deeper into the lower respiratory tract[81].

2.6 Respiratory Tract Infections (RTIs)

Respiratory tract infections (RTIs) represent a significant concern in public health due to their extensive prevalence and the alarming rates of morbidity and mortality observed globally. These infections are characterized as diseases of infectious origin that affect the respiratory system. The clinical manifestations can vary widely, ranging from asymptomatic cases or mild infections to severe or even fatal outcomes. The severity of RTIs is influenced by a triad of factors: the infectious agent, environmental conditions, and the host's characteristics[82]. Typically, these infections present acutely, with symptoms emerging rapidly within hours to days post-infection, including fever, cough, sore throat, nasal congestion, dyspnea, wheezing, and/or respiratory distress. The epidemiological landscape of RTIs is dynamic, shaped by rapid sociodemographic shifts and the impacts of climate change[83].As some of the deadliest

infectious diseases globally, particularly among children and the elderly, RTIs are the leading cause of healthcare consultations and hospital admissions. They significantly contribute to the rising demand for medical evaluations in both outpatient and emergency settings, as well as an increase in antimicrobial use. Additionally, recent epidemiological findings reveal the profound impact of RTIs on life quality and expectancy, highlighting their serious implications for global public health[84].

Respiratory infections can range from minor upper respiratory tract infections (RTIs) or colds to more serious conditions like pneumonia or lower respiratory tract infections (IRLs). They are categorized based on their anatomical involvement and symptomatology. Colds, laryngitis, pharyngitis, acute rhinitis, acute rhinosinusitis, and acute otitis media are all considered upper respiratory tract infections (RTIs). Acute bronchitis, bronchiolitis, pneumonia, and tracheitis are examples of lower respiratory tract infections. Despite a general decline in global mortality, pneumonia continues to rank among the leading causes of death for children under five, accounting for 15% of all deaths in this age group. It is noteworthy that pneumonia contributes to lung function impairment and chronic sequelae, such as the emergence of asthma and chronic obstructive pulmonary disease in adulthood. There is agrowing needto comprehend the variables that influence the vulnerabilit y and severity of RTIs due to the possible long-term consequences of these infections at a young age. The underlying biological mechanisms are still unknown, despite the fact that many of these factors such as birth style, diet, exposure to smoke, premature birth, kindergarten attendance, and the presence of siblings have been identified [85].

Many members of the normal microflora of the respiratory system are opportunistic pathogens. To multiply and harm the host, they must first overcome the immune system of the respiratory tissues. Many mucosal pathogens produce virulence factors, such as adhesins involved in binding to host epithelial cells or polysaccharide capsules that allow microbes to escape phagocytosis.

Endotoxins in gram-negative bacteria can stimulate a strong inflammatory response that damages respiratory cells. André pathogens produce exotoxins, and others have the ability to survive in host cells. When a respiratory infection is diagnosed, it tends to clog the mucociliary escalator, limiting the body's ability to expel invading microbes, making it easier for pathogens to multiply and spread[86].

Respiratory tract infections (RTIs) have been viewed as originating from viral or bacterial sources, specifically "pathogens" like *Haemophilus influenzae, Streptococcus pneumoniae*, and respiratory syncytial virus (RSV). However, this focus on pathogens as the sole cause of RTIs appears to be outdated, given that asymptomatic "colonization" by the same bacteria and viruses is quite common. Recent research into the respiratory microbiota defined as the community of bacteria, viruses, archaea, and unicellular eukaryotes present on mucous membranes indicates that a more comprehensive microbial community plays a role in the causes of RTIs. Alongside the effects of the local microbiota, there is evidence suggesting that the gut microbiota may also influence the development and severity of RTIs through its immunomodulating effects. Collectively, this new information underscores our historical misunderstanding of the intricate mechanisms involved in the causes of RTIs[87].

Individuals can suffer from respiratory distress syndromes, common colds, influenza, and acute bronchitis due to these conditions. Diagnosing most of these patients' ailments is difficult because the symptoms and causes of upper respiratory tract infections often resemble one another.

Upper respiratory tract infections affect the large airways, pharynx, larynx, sinuses, and nasal passages. While generally benign and prevalent during winter months, URIs can significantly impair quality of life for several weeks. Acute upper respiratory tract infections include conditions like pharyngitis, laryngotracheitis, epiglottitis, and colds. In young children, epiglottitis and laryngotracheitis can pose serious risks, yet these infections are usually mild, aggravate temporarily, and eventually resolve without intervention. The following infectious agents are associated with URIs: viruses, bacteria, fungi, and mycoplasmas[88].

Specific infections target specific regions of the upper respiratory tract. Examples include rhinitis (inflammation of the nasal cavity), sinus infection (sinusitis or rhinosinusitis) (inflammation of the sinuses), cold (nasopharyngitis) (inflammation of the sinuses), pharyngitis (inflammation of the pharynx, cervix, tonsils, and pharynx), epiglottitis (inflammation of the upper larynx or epiglottis), laryngitis (inflammation of the larynx), laryngotracheitis (inflammation of both the larynx and trachea), and tracheitis (inflammation of the trachea). Upper respiratory tract infections are among the most common reasons people see a doctor, as they may lead to symptoms such as coughing, a runny nose, a sore throat, fatigue, and shortness of breath. Typically, URTIs occur when a virus or bacteria directly

invades the mucosa, or inner lining, of the upper respiratory tract. In order to penetrate the mucous membrane of the upper respiratory tract, pathogens like bacteria and viruses must navigate several physical and immune barriers[89].

URTIs are typically brought on by a direct invasion of the causative virus or bacteria into the mucosa, or inner lining, of the upper respiratory tract. Pathogens, such as bacteria and viruses, must overcome a number of physical and immunological barriers in order to enter the mucous membrane of the upper respiratory tract. Hoarseness or loss of voice, along with a dry cough, are more typical symptoms of lower upper respiratory tract infections, like laryngotracheitis. Other symptoms include rib pain from a severe cough, retching, and barking or whooping cough[90].

The upper respiratory tract has a diverse and abundant microbiota. The primary bacteria that infiltrate the sinuses and nasal passages are Firmicutes, Proteobacteria, and Actinobacteria.Along with varying numbers of bacteria, such as *Moraxella*, *Eikenella*, *Fusobacterium*, and *Prevotella species*, and some isolates of Candida fungi, the oropharynx also contains many of the same isolates that were discovered in the nose and sinuses. A large number of healthy people may also harbor upper respiratory tract pathogens without showing any symptoms. Up to 20% of people have *Staphylococcus aureus* in their nostrils. The pharynx can also become colonized by pathogenic strains of *Neisseria, Haemophilus*, and *Streptococcus*[91]. Typical symptoms of an upper respiratory infection include nasal congestion, runny nose (which can turn clear, white, or green), nasal

breathing, sneezing, sore throat or scratching, difficulty swallowing (odynophagia), malaise, and low-grade fever (more common in children). Some of the less frequent symptoms include foul-smelling alkali, a diminished sense of smell (hyposmia), headache, deprivation, sinus pain, watery and itchy eyes (conjunctivitis), vomiting, diarrhea, and pain[92].

Bacterial pharyngitis, specifically streptococcal tonisillitis resulting from a group A streptococcal infection, should be considered if symptoms persist and worsen after the initial week, particularly in the absence of a runny nose, cough, or conjunctivitis. The implementation of rapid diagnostic tests and the administration of suitable antibiotics are crucial due to the potential risk of rheumatic fever, particularly in pediatric populations. Epiglottitis represents an acute infection of the upper respiratory tract in children, characterized by a rapid onset of symptoms such as severe sore throat, a sensation of a lump in the throat, a hoarse voice, dry cough, intense pain during swallowing, and excessive drooling[93].

2.7 *Pseudomonas aeruginosa*

Taxonomical of *Pseudomonas aeruginosa*[94]

Kingdom: Bacteria

Phylum: Proteobakterie

Class :P Gamma roteobacteria

Order :Pseudomonadales

Family:Pseudomonadaceae

Genus: *Pseudomonas*

Species: *Pseudomonas aeruginosa*

2.7.1 Characteristics of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is characterized as an aerobic, non-spore-forming, rod-shaped, gram-negative with dimensions ranging from 0.5 to 0.8 µm in width and 1.5 to 3.0 µm in length. This organism functions as a facultative aerobe, capable of utilizing both aerobic and anaerobic respiration, with nitrates serving as the terminal electron acceptor during anaerobic processes. Additionally, *P. aeruginosa* can grow in anaerobic conditions using arginine,

although its fermentation capabilities are limited, typically resulting in very slow or negligible growth[95].

An organism can utilize over 100 organic molecules as potential sources of carbon and/or energy. As a prototroph, it is usually capable of growing in a medium with low salt concentrations, depending on a single source of carbon and energy. The organism's motility is enabled by a single polar flagellum, which generates thermal labile antigens known as H-antigens. The importance of antibodies against these antigens, aside from their role in serological classification, remains largely unknown. Clinical isolates frequently possess pili, which may act as antiphagocytic factors and assist in bacterial attachment, thereby facilitating colonization. Species are differentiated through biochemical tests and DNA hybridization analyses. Additionally, antisera targeting outer membrane lipopolysaccharides and outer membrane proteins exhibit cross-reactivity among different serovars[96].Various strains of *Pseudomonas aeruginosa* are known to produce pigments that exhibit diffuse coloration, specifically pyocyanin, which is characterized by a bluish-green hue, and pyoverdine, which displays a fluorescent yellow-green appearance. Certain strains may also present a mucous texture, attributed to the significant presence of a polysaccharide sheath, a phenomenon particularly observed in individuals suffering from cystic fibrosis. The pathogenicity of *Pseudomonas aeruginosa* is influenced by both cell-associated and secreted virulence factors. The cell-associated virulence factors encompass structures such as flagella, pili, adhesins, alginate, and lipopolysaccharides. In contrast, the secreted virulence factors include haemolysins, lipases, proteases, exotoxin A, and type III cytotoxins, namely ExoS, ExoT, ExoU, and ExoY[97].

Pseudomonas aeruginosa is one of the most dangerous bacterial pathogens and has been designated as a priority pathogen by the World Health Organization for the advancement of

new antibiotic therapies. The effectiveness of conventional antimicrobials, particularly antibiotics, is frequently compromised due to the organism's remarkable adaptability and inherent resistance to antibiotics, which contributes to elevated mortality rates[98].

Pseudomonas aeruginosa is a prevalent pathogen in patients with weakened immune systems, including those with cystic fibrosis, neutropenia, extensive burns, malignancies, AIDS, organ transplant recipients, and individuals with poorly managed diabetes mellitus, particularly in intensive care settings. Furthermore, patients with invasive devices, such as indwelling catheters or endotracheal tubes, are particularly vulnerable due to the bacterium's ability to develop biofilms that are often difficult to detect[99].

The antibiotic resistance of *Pseudomonas aeruginosa* is attributed to multiple mechanisms and a variety of virulence factors, which together elucidate the extensive range of infections it can induce and the growing difficulties in managing antimicrobial resistance. Among the recognized mechanisms of resistance are intrinsic antibiotic resistance, efflux systems, and enzymes that inactivate antibiotics. Intrinsic resistance involves the bacterium's ability to limit the entry of antimicrobials through its membrane. Efflux systems enable the organism to expel toxic substances from its interior. Additionally, some strains produce beta-lactamases and extended-spectrum beta-lactamases. The capacity of *Pseudomonas aeruginosa* to develop biofilms is another critical factor that enhances its resistance to antibiotics and helps it withstand host defense mechanisms[100].

The cell envelope of *Pseudomonas aeruginosa*, much like that of other gram-negative bacteria, is structured in three layers: the inner or cytoplasmic membrane, the peptidoglycan layer, and the outer membrane. The outer membrane contains a combination of phospholipids, proteins, and lipopolysaccharides (LPS). Interestingly, the LPS present in *Pseudomonas aeruginosa* is considered to be less toxic than that of other gram-negative rod-shaped bacteria[101].The lipopolysaccharide (LPS) composition of the majority of *Pseudomonas*

aeruginosa strains is characterized by the presence of heptose, 2-keto-3-deoxyoctonic acid, and hydroxylated fatty acids, alongside polysaccharide side chains and core structures. Recent studies indicate that a significant proportion of LPS derived from strains obtained from cystic fibrosis patients exhibit minimal or absent polysaccharide side chains (O antigen), a phenomenon that appears to be associated with the polyagglutination observed in these strains when exposed to type serum. Furthermore, *Pseudomonas aeruginosa* is known to produce various virulence factors. Notably, strains isolated from respiratory infections demonstrate a higher production of haemolysin compared to their environmental counterparts, implying that this glycolipid haemolysin may be instrumental in the pathogenesis of pulmonary infections caused by *Pseudomonas aeruginosa*[102].

2.7.2 Respiratory infection with *Pseudomonas aeruginosa*

Pseudomonas aeruginosa can be found in the upper respiratory tract, including the nasopharynx and paranasal sinuses. An opportunistic microorganism that represents the paradigm of chronic bacterial infections, where pathogens weaken a host's defenses and adapt and evolve in order to persist. *Pseudomonas aeruginosa* finds a favorable ecological niche in people with chronic airway inflammation, thus enabling it to perpetuate itself. The progressive increase in *P. aeruginosa*'s bacterial load has significant deleterious effects and can lead to chronic bronchial infection, characterized by increased inflammation, both locally and systemically, and an unfavorable clinical evolution [103]. The upper respiratory tract, including the nasopharynx and paranasal sinuses, has been identified as a potential reservoir for *P. aeruginosa*. Sinus complications, including chronic sinusitis, have been reported in CF patients. Furthermore, *P. aeruginosa* has been identified in these sinusitis samples and similarities between the isolates found in the paranasal sinuses and the lungs have been observed. *P. aeruginosa* isolates were recovered more frequently from the sinuses than

from bronchoalveolar lavage fluid of CF patients. It has been suggested that colonization of the upper reaches of the respiratory tract (including the nasopharynx and paranasal sinuses) allows *P. aeruginosa* to adapt to the anoxic environment present in the lower lung. Following this, adapted bacteria can descend along the respiratory tract and cause lung infection and chronic inflammation[104].

2.8 *Streptococcus pneumoniae*[105]

Taxonomical Classification of *Streptococcus pneumoniae*

Domain:Bacteria

Phylum: Firmicutes

Class: Diplococci

Order: Lactobacillale

Family: Streptococcaceae

Genus:*Streptococcus*

Species: *Streptococcus pneumoniae*

2.8.1 Characteristics of *Streptococcus pneumoniae*

Streptococcus pneumoniae are nonmotile, non-spore-forming, gram-positive, facultatively anaerobic bacteria[106]. These bacteria typically present as lance-shaped cocci, predominantly found in pairs (diplococci), although they may also exist as solitary cocci or in chains. The diameter of individual *Streptococcus pneumoniae* cells ranges from 0.5 to 1.25 µm. Notably, these pneumococcal cells lack protein M, a virulence factor that differentiates them from other streptococcal species.

The cell wall of these bacteria is composed of peptidoglycan and teichoic acid, with a significant proportion of teichoic acid associated with one of the three N-acetylmuramic acids[107]. An immunogenic polysaccharide capsule encases the cell wall, with its composition differing among strains. The cell membrane is also associated with lipid-derived teichoic acid. *Streptococcus pneumoniae* is estimated to have around 500 proteins on its surface, and many strains are equipped with pili. The polysaccharide sheath envelops the bacterium, and the classification of *Streptococcus pneumoniae* serotypes is based on the variations in their capsule[108].

Streptococcus pneumoniae is responsible for most nosocomial pneumonias.This organism poses a serious challenge to global public health, leading to significant morbidity and mortality rates. It exists as a commensal in the human respiratory tract, offering benefits while remaining non-pathogenic. The presence of this bacterium can be found in the nasopharynx of 5% to 90% of healthy individuals. *Streptococcus pneumoniae* comprises more than 100 recognized serotypes, with most having the potential to cause illness; nevertheless, only a limited number of serotypes are responsible for the majority of pneumococcal infections[109].The serotypes most frequently linked to penicillin resistance include 6A, 6B, 9V, 14, 19A, 19F, and 23F. These bacteria can be found in the nasopharynx of healthy individuals, with prevalence rates ranging from 5% to 90%. For pneumococci to induce disease, they must transition from the nasopharynx to infect various organs, including the lungs and other internal structures via the bloodstream. Pneumococcal infections pose significant health risks, potentially leading to conditions such as pneumonia, bronchitis, otitis media, sepsis, and meningitis. Streptococcus pneumoniae is classified as alpha-haemolytic,

indicating its ability to lyse red blood cells through the production of hydrogen peroxide (H_2O_2). This H_2O_2 production during bacterial infection can also inflict damage on DNA and result in cell death within the lungs. Symptoms of pneumococcal pneumonia typically encompass fever, chills, cough, respiratory distress, and chest pain[110].

2.8.2 Transmission of *Streptococcus pneumoniae*

Streptococcus pneumoniae is transmitted primarily through respiratory activities such as coughing and sneezing, as well as through oral contact among individuals, with aerosol microdroplets from the nasopharynx of carriers playing a significant role. Additionally, individuals with upper respiratory tract infections may experience transmission through self-vaccination[111].The transmission of bacteria is influenced by several factors, including clumping, peak respiratory tract infections, and the presence of pneumococcal disease in the host. While the transmission of *Streptococcus pneumoniae* is prevalent, actual infections are relatively rare due to the host's capacity to maintain asymptomatic states. The precise infectious dose of *S. pneumoniae* in humans remains undetermined; however, studies utilizing animal models indicate that as few as 10^4 bacteria can penetrate the blood-brain barrier, leading to conditions such as sepsis or pneumonia at higher bacterial counts of 10^7 and 10^8. The incubation period for *S. pneumoniae* typically ranges from one to three days, during which the bacteria colonize the mucous membranes of the nasopharynx and upper respiratory tract. Within the nasopharynx, *S. pneumoniae* competes with *Staphylococcus aureus* and *Haemophilus influenzae* for resources, facilitating their colonization. If S. pneumoniae proliferates unchecked in the lungs, meninges, or middle ear, it can lead to significant inflammation[112].

2.8.3 Epidemiology of *Streptococcus pneumoniae*

The World Health Organization has identified *Streptococcus pneumoniae* as a priority pathogen, underscoring the critical need for new antibacterial agents[113].This bacterium is implicated in various diseases, including acute otitis media, sinusitis, pneumonia, and bacterial meningitis. The age groups most at risk for *S. pneumoniae* infections are children younger than two years and adults over 65 years. It is one of the most prevalent pathogens affecting humans worldwide, with approximately 1.6 million deaths resulting from pneumococcal disease each year. *S. pneumoniae* is commonly found in the throat and nasal passages of children. The pneumococcus is responsible for more than one million deaths annually[114]. As an opportunistic member of the respiratory microbiota, *S. pneumoniae* has become a priority pathogen that quickly develops resistance to widely used antibiotics and circumvents current vaccination efforts. The bacteria are transmitted through airborne particles, particularly when an infected person coughs or sneezes, and are not spread via contaminated food or water. Most individuals exposed to these pathogens do not exhibit symptoms, as their immune systems effectively inhibit the bacteria from spreading to other areas of the body.

Individuals with compromised immune systems are at an increased risk of infections, as pathogens may migrate from the throat to various critical areas, including the lungs, bloodstream, sinuses, middle ear, or even the brain. Such infections can pose significant health risks. A weakened immune response may arise from conditions that directly impact immune function, such as HIV or AIDS, or as a consequence of immunosuppressive therapies following organ transplants or in the treatment of autoimmune disorders. While the overall rates of pneumococcal pneumonia remained relatively stable throughout the twentieth century, the advent of antibiotics has markedly decreased associated mortality rates.

In Western populations, the annual incidence of pneumonia is approximately 1%. Furthermore, *Streptococcus pneumoniae* serotypes have the capacity to exchange genetic material, leading to genomic recombination. This genetic exchange has contributed to the global emergence of antibiotic resistance, particularly among serotypes prevalent in pediatric populations[115].

The development of pneumococcal disease in pediatric populations is primarily influenced by several key factors, including the virulence of specific serotypes, the absence of type-specific humoral immunity, and the coexistence of viral respiratory infections. Streptococcus pneumoniae typically colonizes the upper respiratory tract and is a component of the normal nasopharyngeal flora in healthy children. The bacterium can disseminate to the middle ear, sinuses, and lungs, or it may enter the bloodstream, leading to potential deposits in the meninges and other sites. The occurrence of otitis media associated with pneumococci is linked to the recent acquisition of serotypes in the nasopharynx, particularly within a month, rather than prolonged colonization. This recent serotype acquisition is also correlated with the risk of invasive disease. It is important to note that not all pneumococcal serotypes exhibit the same level of invasiveness. The structure and quantity of capsule polysaccharides are critical determinants of virulence, with strains that produce higher levels of these polysaccharides likely being the most virulent. Once the bacteria enter the bloodstream, their encapsulation confers a protective advantage against host defense mechanisms by impeding neutrophil phagocytosis and complement-mediated bactericidal activity[116].

In recent years, *Streptococcus pneumoniae* has emerged as a crucial model organism for the exploration of bacterial cell division and morphogenesis. The shape of pneumococci is typically described as egg-shaped, resembling a rugby ball, and they reproduce through binary fission along successive parallel planes that are perpendicular to the cell's long axis.

A distinctive feature of pneumococci is their method of synthesizing the cell wall, particularly the peptidoglycan network, which is essential for maintaining the cell's shape and physical integrity, primarily from the mid-cell region. During infections of the lungs by *S. pneumoniae*, the immune response of the host is instrumental in influencing the infection's progression. This immune response is characterized by the release of a variety of cytokines and chemokines that are vital for attracting neutrophils, monocytes, and lymphocytes to the lungs, with neutrophils being critical for the eradication of the bacteria. However, as proper lung function is vital for the survival of mammals and humans, the immune response to *S. pneumoniae* must be carefully regulated to ensure that pathogens are effectively cleared while minimizing the risk of excessive inflammation and tissue damage. To fulfill this need, anti-inflammatory cytokines, such as interleukin-10, are produced during the infection to modulate the inflammatory response and restore immune homeostasis in the host[117].

2.9 *Staphylococcus aureus*

Taxonomical Classification of *Staphylococcus aureus*[118]

Domain: Bacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: *Staphylococococaceae*

Genus: *Staphylococcus*

Species: *Staphylococcus aureus*

2.9.1 Characteristics of *Staphylococcus aureus*

Staphylococcus aureus is classified as an aerobic, gram-positive bacterium with facultative anaerobic properties, which allows it to thrive in various environments. When cultured on nutrient-rich agar media, it produces relatively large colonies that can be identified by their yellow or white coloration. This yellow pigmentation is a result of carotenoid production by the organism. The name "*aureus*," which means gold in Latin, reflects this characteristic color. Observations under a light microscope, particularly after Gram's staining, reveal that these bacteria often appear in grape-like clusters. The term "*Staphylococcus*" is derived from Greek, meaning a cluster of grapes (Staphyle) and small berries (kokkos)[119].Utilizing a scanning electron microscope, it is observed that the cells are predominantly spherical and feature a smooth surface texture. The size of the cells is measured to be between 0.5 and 1.0 µM in diameter. Transmission electron microscopy reveals a substantial cell wall, a well-defined cytoplasmic membrane, and a cytoplasm that appears amorphous. Under laboratory conditions, these cells demonstrate optimal growth at a temperature of 37 °C, with a permissible range from 7 to 48 °C[120].

2.9.2 Etiology of *Staphylococcus aureus*

Staphylococcus aureus exhibits the ability to grow in both aerobic and anaerobic conditions (facultative) and thrives at temperatures ranging from 18 °C to 40 °C. Common biochemical tests for identification include catalase positivity (characteristic of all pathogenic *Staphylococcus species*), coagulase positivity (which differentiates *Staphylococcus aureus* from other *Staphylococcus species*), sensitivity to Novobiocin (used to differentiate it from *Staphylococcus saprophyticus*), and positive mannitol fermentation (which helps distinguish it from *Staphylococcus epidermidis*[121].

Staphylococcus aureus exhibits the ability to proliferate across a broad temperature range (7°C to 48.5°C, with an optimal range of 30°C to 37°C) and pH levels (4.2 to 9.3, with an optimal pH of 7 to 7.5). Notably, this bacterium demonstrates resilience, thriving and even reproducing under conditions of low temperature and elevated sodium chloride (NaCl) concentrations. Its resistance to high osmolarity, detergents, and alcohol can be attributed to its highly cross-linked peptidoglycan structure, although it is susceptible to inactivation through pasteurization or cooking processes[122].*Staphylococcus aureus* is recognized as one of the most notorious and prevalent bacterial pathogens, responsible for an indeterminate number of uncomplicated skin infections and potentially hundreds of thousands to millions of more serious, invasive infections worldwide each year. This bacterium is a primary contributor to pneumonia and various respiratory tract infections, as well as infections related to surgical sites, prosthetic joints, and the cardiovascular system, in addition to being a significant cause of nosocomial bacteraemia[123].

2.9.3 *Staphylococcus aureus* Virulence Factors

Staphylococcus aureus possesses a variety of virulence factors that enable it to act as a pathogen, leading to numerous infections in both humans and animals. These virulence factors facilitate the binding to host cells, the breakdown of immune defenses, tissue invasion, the onset of sepsis, and the emergence of toxin-mediated syndromes. Consequently, this underpins the persistence of staphylococcal infections, particularly in the absence of a robust immune response from the host[124].

Staphylococcus aureus functions as both a commensal organism and an opportunistic pathogen. In humans, the primary ecological niche for this bacterium is located within the anterior nostrils. Individuals who are nasal carriers of *Staphylococcus aureus* face an elevated risk of infection, particularly in healthcare environments. It is estimated that approximately

30% of the human population may be nasal carriers of this pathogen. Given that nasal carriage heightens the likelihood of surgical site infections, lower respiratory tract infections, and bloodstream infections in hospital settings, various strategies are being implemented to mitigate this risk[125].

2.9.4 *Staphylococcus aureus* Pathophysiology

Staphylococcus aureus is a prevalent bacterial pathogen in humans, responsible for a variety of infections. These include bacteremia, infectious endocarditis, and a range of skin and soft tissue infections such as childhood ulcers, folliculitis, carbuncles, cellulitis, and sunburned skin syndrome. Additionally, it can lead to osteomyelitis, septic arthritis, infections associated with prosthetic devices, pulmonary infections like pneumonia and empyema, gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections. The pathogenicity of *S. aureus* is influenced by the specific strains involved and the location of the infection, resulting in either invasive infections or diseases mediated by toxins. The pathophysiological mechanisms can differ significantly based on the particular type of *S. aureus* infection[126].

The evasion of the host immune response by bacteria involves several mechanisms, including the production of an antiphagocytic capsule, the sequestration of antibodies, and antigen masking through protein.

Other tactics include biofilm formation, intracellular survival, and the blockade of leukocyte chemotaxis. In infectious endocarditis, the binding of bacteria to extracellular matrix proteins and fibronectin is mediated by proteins associated with the bacterial cell wall, such as fibrinogen-binding proteins, agglutination factors, and teichoic acids.

Additionally, superantigens produced by *Staphylococcus*, particularly TSST-1, are critical virulence factors implicated in infectious endocarditis, sepsis, and toxic shock syndrome.

Staphylococcus aureus represents a significant pathogen in pulmonary infections, particularly in the context of nosocomial pneumonia. This bacterium is characterized by a diverse array of surface proteins that interact with cellular adhesive molecules, enabling it to adhere to and penetrate the lung epithelial cells. This interaction not only aids in evading the host's immune response but also promotes the establishment of chronic infections.

Research conducted *In-vitro* has demonstrated that the activation of the NF-κB (nuclear factor kappa light chain of activated B cells) signaling pathway in pulmonary epithelial cells infected with S. aureus results in heightened inflammatory responses, primarily through the upregulation of IL-8. Furthermore, the presence of intracellular *S. aureus* can induce apoptosis in these epithelial cells following an initial period of latency. In cases of necrotizing pneumonia, the virulence factors of *S. aureus* that contribute to lung cell apoptosis include pore-forming toxins such as Panton-Valentin leucocidin (PVL) and alpha-haemolysin[127].

Furthermore, *Staphylococcus aureus* is frequently associated with secondary bacterial pneumonia, particularly during seasonal influenza outbreaks. During the 2009 A/H1N1 influenza pandemic, bacterial co-infections complicated nearly one-third of influenza cases in the United States, with *S. aureus* identified as the predominant pathogen, responsible for 27% of cases among critically ill children and adults.

Staphylococcus aureus possesses a notable virulence factor known as coagulase, which is crucial for biofilm formation in infections caused by this bacterium. Coagulase interacts with host prothrombin to create active staphylotome complexes that facilitate the conversion of soluble monomer fibrinogen into insoluble fibrin, thereby triggering coagulation damage.

This mechanism has been utilized in clinical microbiology laboratories as a coagulase test for the identification of various *Staphylococcus species*[128].

2.10 *Klebsiella pneumoniae*

Taxonomical classification of *Klebsiella pneumoniae*[129]

Domain: Bacteria

Phylum: Proteobakterie

Class: Gammaproteobacteria

Order: Enterobacterales

Family: Enterobacteriaceae

Genus: *Klebsiella*

Species: *Klebsiella pneumoniae*

2.10.1 *Klebsiella pneumonia*

Klebsiella pneumoniae is a member of the Enterobacteriaceae family, characterized as a gram-negative, rod-shaped, non-spore-forming, encapsulated, and non-motile bacterium. This organism is a ubiquitous facultative anaerobe, commonly found as part of the normal flora in the oral cavity, skin, intestines, and feces of roughly 5% of the human population. It is recognized as an opportunistic pathogen responsible for a diverse range of diseases in humans[130].*Klebsiella pneumoniae* is responsible for nearly one-third of all gram-negative infections, which include various medical conditions such as urinary tract infections, cystitis, pneumonia, surgical site infections, endocarditis, and sepsis.

Furthermore, it is linked to critical health issues such as necrotizing pneumonia, pyogenic liver abscess, and endogenous endophthalmitis. Infections caused by this organism are often

associated with high rates of mortality and morbidity, resulting in elevated hospitalization rates and considerable healthcare expenditures[131].

Klebsiella pneumoniae forms colonies of pink mucous membranes on MacConkey's agar. They are widely distributed in nature and occur as commensal in the intestines of humans and animals, as well as saprophytes in the soil. The virulence of bacteria is ensured by a wide range of factors that can lead to infection and antibiotic resistance. The body's polysaccharide sheath is an important virulence factor and allows bacteria to escape opsonophagagocytosis and serum destruction by the host organism. The second virulence factor is lipopolysaccharides, which cover the outer surface of the gram-negative bacteria. Pyisning of lipopolysaccharides releases an inflammatory cascade in the host organism and is one of the main culprits in the consequences of sepsis and septic shock. Another virulence factor, fimbria, allows the body to bind to host cells. Siderophore are another virulence factor that the body needs to induce infection in hosts. Siderophore extract iron from the host to allow the spread of the infecting organism. These microorganisms can cause pneumonia, acute intestinal infections, urogenital infections, conjunctivitis, meningitis, and sepsis in lambs [132]. *Klebsiella* infection can also develop as a secondary infection associated with a viral infection, which can also lead to an increase in the number of deaths.

In order for pathogenic microorganisms to survive in habitat conditions, they must acquire certain properties, including the formation of biofilm. As a result, they acquire the ability to resist factors of natural forces, such as certain macro-organisms and antimicrobials of various origins[133].

Most diseases of the digestive and respiratory systems in animals are caused by *K. pneumoniae.* These diseases (such as gastritis, enteritis, hepatitis and pneumonia) can be acute, subacute and chronic; As a result, they lead to an increase in animal mortality. The

bacterium has a capsule that serves as resistance to the environment and the action of disinfectants and many antibiotics, making it deadly. It has a complex antigenic structure and contains capsule and somatic antigens and endotoxins; Some strains can produce exotoxins[134]. This capsule envelops the entire cell surface, explains the great appearance of the organism during Gram staining and provides resistance to many of the host's defence mechanisms. Members of the genus *Klebsiella* usually express 2 types of antigens on the surface of their cells. The first is lipopolysaccharide (O antigen); the other is a capsule polysaccharide (antigen K). Both antigens contribute to pathogenicity. There are approximately 77 K antigens and 9 O antigens. The virulence of all serotypes appears to be similar. Three species of the genus *Klebsiella* are associated with the disease in humans: *Klebsiella pneumoniae, Klebsiella oxytoca* and *Klebsiella granulomatis*. The organisms previously known as *Klebsiella ozaenae* and *Klebsiella rhinoscleromatis* are considered non-fermenting subspecies of *K pneumoniae* with characteristic clinical manifestations[135].

2.10.2 *Klebsiella pneumonia* Epidermiology

Klebsiella pneumoniae primarily resides in humans, serving as its principal reservoir. Studies indicate that between 5% and 38% of the general population excretes this organism in their feces, while its presence in the nasopharynx is observed in approximately 1% to 6% of individuals. Globally, *Klebsiella pneumonia* is responsible for around 11.8% of all cases of hospital-acquired pneumonia, with a significantly higher incidence of nearly 15% in developing nations. Patients infected with *K. pneumoniae* frequently exhibit a poor prognosis, as the mortality rate remains alarmingly high, ranging from 30% to 50% even with the best available treatment options[136] . The primary sources of infection are the gastrointestinal tract of the patient and the hands of healthcare personnel, which can contribute to the occurrence of nosocomial infections. Notably, elevated colonization rates have been observed among

individuals of Chinese descent and those with chronic alcohol use. In hospitalized individuals, the prevalence of *K. pneumoniae* carriers significantly exceeds that found in the general population. Research indicates that carrier rates can reach as high as 77% in the stool samples of hospitalized patients, a figure that correlates with the quantity of antibiotics administered[137].

K. pneumoniae-related pneumonia can be classified into two categories: community-acquired and hospital-acquired pneumonia. While hospital-acquired pneumonia is a relatively frequent diagnosis, infections caused by *K. pneumoniae* are comparatively uncommon. In Western nations, it is estimated that *K. pneumoniae* accounts for approximately 3-5% of pneumonia cases acquired outside the community, whereas in developing regions, such as Africa, this figure may rise to about 15%.

Globally, *K. pneumoniae* is implicated in roughly 11.8% of all hospital-acquired pneumonia cases. Among patients with ventilator-associated pneumonia, *K. pneumoniae* is responsible for 8% to 12% of cases, in contrast to 7% in non-ventilated patients. The mortality rate for individuals with alcoholism and sepsis ranges from 50% to 100% [138].

2.11 Antibiotics

Antibiotics are characterized as natural products or their derivatives. The discovery of penicillin marked the beginning of a substantial number of antibiotics being isolated from numerous microorganisms, with new antibiotics continually being developed and introduced into clinical settings each year. These essential drugs are pivotal in life preservation and are recognized as some of the most transformative medical interventions, significantly impacting

both life expectancy and quality of life[139].Prior to the advent of antibiotics, infections were responsible for over fifty percent of mortality rates. Antibiotics are essential in combating bacterial infections; however, the emergence of antibacterial resistance has significantly impacted both the healthcare and pharmaceutical industries, leading to considerable socio-economic repercussions. An antibiotic is defined as a chemical substance produced by living organisms, typically microorganisms, that exhibits harmful effects on other microorganisms. These substances are predominantly synthesized by soil-dwelling microorganisms, which likely utilize them as a mechanism to regulate the proliferation of competing microbial populations in complex environments such as soil. Notable microorganisms that generate antibiotics beneficial for disease prevention or treatment include various bacteria and fungi.

The spectrum of antibiotic activity varies widely; some antibiotics exhibit high specificity, while others, like tetracyclines, are effective against a broad range of bacterial species. This broad-spectrum efficacy is particularly advantageous in managing mixed infections and in situations where there is insufficient time to conduct susceptibility testing[140].

Certain antibiotics, including semi-synthetic penicillin and quinolones, are suitable for oral administration, while others necessitate intramuscular or intravenous delivery. Antibiotics can be categorized based on their spectrum of activity, which refers to the range of bacteria they target. They are classified as narrow-spectrum, broad-spectrum, or extended-spectrum agents. Narrow-spectrum antibiotics, such as penicillin G, predominantly target gram-positive bacteria. In contrast, broad-spectrum antibiotics like tetracyclines and chloramphenicol are effective against both gram-positive and some gram-negative bacteria. Extended-spectrum antibiotics are those that, through chemical modification, can also act on additional types of

bacteria, particularly gram-negative strains. The distinction between gram-positive and gram-negative bacteria is based on the structural differences in their cell walls, with gram-positive bacteria possessing a thick peptidoglycan layer and gram-negative bacteria having a thinner peptidoglycan layer[141].Antibiotics represent one of the most significant advancements in medical pharmacology, fundamentally altering both mortality and quality of life. Prior to the advent of antibiotics, infectious diseases accounted for over fifty percent of all fatalities. The positive impact of antibiotics extends beyond individual patients; their efficacy also plays a crucial role in curtailing the spread of infections among populations, thereby providing substantial public health advantages in disease prevention at the community level[142].

2.11.1 Antibiotic Effectiveness

The efficacy of an antibiotic is contingent upon its administration at adequate concentrations to rapidly and effectively inhibit the proliferation of the infectious microorganism. Meeting these requirements frequently necessitates the use of high dosages, ranging from hundreds of milligrams to several grams. Consequently, the toxicological and pharmacological limits for antibiotics are markedly distinct from those of pharmaceutical agents in other medical fields, which are typically administered at considerably lower doses. Furthermore, the physicochemical characteristics of antibiotics diverge from those that influence human biological molecules[143].For more than 3.5 billion years, bacteria have existed on Earth, developing advanced mechanisms to resist toxins, including antibiotics. These mechanisms involve distinctive membranes, efflux systems that efficiently eliminate toxic compounds from the cell, and the ability to proliferate rapidly while adapting to various physiological

states, such as biofilms and persistent forms, which often exhibit diminished metabolic activity and contribute to antibiotic tolerance. As a result, established criteria for assessing the physical properties of effective drug candidates, such as the 'rule of five', are not suitable for antibiotic discovery.

Furthermore, there is a lack of consistent general guidelines regarding the properties of antibiotics, due to the physiological, genomic, and biochemical diversity among gram-negative and gram-positive bacteria, mycobacteria, and even within specific genera like *Pseudomonas*. The interplay of microbial, physiological, and structural attributes, along with the distinct physicochemical characteristics of antibiotics, complicates the quest for broad-spectrum drugs. The necessity for broad-spectrum antibiotics is evident, as clinicians frequently lack knowledge of the specific infectious agent when prescribing treatment, making these versatile drugs essential against prevalent pathogens[144].

2.11.2 Antibiotic Mechanisms of Action

The action of antibiotics is mediated through various mechanisms. A prominent category of these drugs, known as β-lactam antibiotics, operates by inhibiting the synthesis of the bacterial cell wall. The construction of the bacterial cell wall involves multiple steps: the initial assembly of wall components, their transport through the cell membrane to the site of growth, the subsequent assembly within the wall, and the final crosslinking of the fibers that constitute the wall material. Antibiotics that impede cell wall synthesis have a targeted impact on both of these critical phases[145].The alteration in the cell wall and morphology of the organism ultimately leads to bacterial cell death. Antibiotics like aminoglycosides, chloramphenicol, erythromycin, and clindamycin function by inhibiting protein synthesis in bacteria. While the fundamental mechanism of protein synthesis in bacteria and animal cells is analogous, the specific proteins produced differ. Selectively toxic antibiotics take

advantage of these distinctions to attach to bacterial proteins or impede their activity, thereby obstructing the production of new proteins and the proliferation of bacterial cells[146].

Antibiotics such as polymyxin B and polymyxin E interact with the phospholipids present in bacterial cell membranes, thereby compromising their selective barrier function. This disruption permits the leakage of vital cellular macromolecules, ultimately resulting in cell death. However, due to the presence of similar or identical phospholipids in human cells, these antibiotics exhibit a degree of toxicity. In contrast, certain antibiotics like sulphonamides act as competitive inhibitors of folic acid synthesis, a crucial precursor in the synthesis of nucleic acids.

Sulphonamides inhibit folic acid production by mimicking para-aminobenzoic acid, an intermediate that is enzymatically converted into folic acid. The structural resemblance between sulphonamides and para-aminobenzoic acid leads to competitive inhibition at the enzyme level. This interaction is reversible upon the removal of sulphonamides, resulting in the inhibition of microbial growth without necessarily causing cell death. Additionally, the antibiotic Rifampicin disrupts RNA synthesis in bacteria by binding to a specific subunit of the bacterial RNA polymerase. The binding affinity of Rifampicin for the bacterial enzyme is significantly greater than that for the corresponding human enzyme, ensuring that therapeutic doses do not adversely affect human cells[147].

2.11.3 Antibiotic Resistance

The term bacterial resistance denotes the ability of bacterial cells to resist the inhibitory or lethal effects of antibiotics. The inappropriate and excessive use of these medications

significantly contributes to the rise of bacterial resistance. This resistance manifests as a reduction in the susceptibility of bacteria to the harmful (bactericidal) or growth-suppressing (bacteriostatic) effects of antibiotics. In cases where a resistant bacterial strain prevails in an infection, the resulting condition may become untreatable and pose a serious threat to life[148].

Since the advent of antibiotic treatment, one of the persistent issues has been the emergence of bacterial resistance to these drugs. Antibiotics are capable of eradicating nearly all harmful bacteria in a patient; however, a small number of bacteria that are genetically predisposed to resist the effects of the drug may survive.

These resilient bacteria can reproduce and disseminate their resistance traits to other bacteria through gene exchange mechanisms. As the more susceptible bacteria are eliminated or significantly reduced by the antibiotics, the resistant strains begin to thrive. This situation results in bacterial infections in humans that are resistant to one or more antibiotics that would normally be effective. The misuse and overuse of antibiotics further facilitate the proliferation of such resistance. It is projected that by 2050, multidrug resistance could lead to an increase of 10 million deaths each year[149].

Over the years, advancements in biological screening, phytochemical separation, and clinical trials involving medicinal plants have unveiled the knowledge embedded in ancient herbal remedies. Traditional medicine has demonstrated efficacy in addressing ailments associated with bacterial infections and oxidative stress. Since the late 20th century, antibiotic resistance has emerged as a significant challenge for humanity. Consequently, there has been a marked increase in the demand for novel antimicrobials capable of effectively targeting resistant pathogens. Traditional methodologies for discovering new antimicrobials have proven

insufficient in light of the rapid evolution of resistance mechanisms. The rise of multidrug-resistant bacteria has further complicated the accessibility and cost-effectiveness of numerous antibiotics that are currently in use globally. The excessive, inappropriate, and indiscriminate application of antibiotics has contributed to the rise of antimicrobial resistance, thereby diminishing the effectiveness of many existing therapeutic agents[150].

This new trend is worrying and is considered by the World Health Organisation to be perhaps the most pressing problem for medical science. As a result, there is a growing demand for the development of new antimicrobials that can reduce the use of antibiotics and counter the development of resistance.

This has led researchers to isolate and identify new bioactive chemicals from plants that counteract microbial resistance, also taking into account that approximately 50% of current drugs and nutraceuticals are natural products and their derivatives. Medicinal plants are an almost unlimited source of bioactive substances and their use as antimicrobials has been used in various ways[151].

Natural antimicrobials can function independently or synergistically with antibiotics to enhance their efficacy against various microbial strains. Given that the antimicrobial properties of numerous medicinal plants remain largely uninvestigated, researchers are dedicating more efforts to the discovery of novel, effective, and rapidly developing therapeutic options[152].

The four main forms of antibiotic resistance

1- Natural resistance (intrinsic, structural) :In this form of resistance, the ineffectiveness of antibiotics is not a result of acquired resistance but rather stems from the inherent structural characteristics of the bacteria. This phenomenon is referred to as intrinsic resistance, where certain microorganisms possess structural attributes that render them incompatible with the

action of antibiotics, or where the antibiotics themselves lack the necessary properties to effectively target the bacteria. For instance, Gram-negative bacteria exhibit natural insensitivity to vancomycin due to the inability of this antibiotic to penetrate their outer membrane. Likewise, bacteria that are L-shaped and lack a cell wall, such as Ureaplasma and Mycoplasma, demonstrate intrinsic resistance to beta-lactam antibiotics[153].

2. Acquired resistance :The development of antibiotic resistance in bacteria is influenced by changes in their genetic characteristics. Notably, bacteria that have acquired resistance may paradoxically show heightened sensitivity to certain antibiotics.

This phenomenon is often linked to larger genetic structures, such as plasmids and transposons, which exist outside the chromosomal framework. Chromosomal resistance typically arises from random mutations that alter the bacterial chromosome, often triggered by specific physical and chemical stimuli. These mutations can lead to modifications in the bacterial cell structure, resulting in decreased permeability to antibiotics or lower concentrations of the drug within the cell. In contrast, extrachromosomal resistance is associated with genetic material that can be disseminated through plasmids, transposons, and integrons. Plasmids are particularly significant as they often carry genes that produce enzymes that neutralize the effects of antibiotics. Bacterial cells can maintain genetic material, including resistance genes and plasmids, through various mechanisms such as transduction, transformation, conjugation, and transposition. The antibiotic resistance genes found on chromosomes or plasmids are typically organized within different integration groups or integrons, where recombination processes are frequently observed [154].

3. Cross-resistance:This concept pertains to the resistance exhibited by specific microorganisms against a certain antibiotic, particularly those that function through

analogous or related mechanisms, and concurrently display resistance to other antibiotics. Such resistance is frequently noted among antibiotics that possess a similar structural composition, as seen in the cases of erythromycin, neomycin, and canamycin, or in the context of cephalosporins and penicillin. Additionally, cross-resistance can manifest between distinct groups of antibiotics, as illustrated by the resistance observed between erythromycin and lincomycin, which may originate from chromosomal or non-chromosomal factors [155].

4. Multidrug-resistant and other forms of resistance:Multidrug-resistant strains typically refer to pathogens that exhibit resistance to multiple antibiotics, rendering them ineffective in controlling bacterial infections with a single therapeutic agent. The misuse of antibiotics in clinical settings has significantly contributed to the emergence of these multidrug-resistant pathogenic bacteria. There are two primary mechanisms through which bacteria can develop multi-resistance. The first involves the acquisition of various genes that confer resistance to specific antibiotics, often located on R-plasmids. The second mechanism includes enhanced expression of efflux pumps, enzymatic degradation of antibiotics, and alterations in the target sites of these drugs, all of which can contribute to the development of multi-resistance[156].

2.11.4 Mechanisms of Action of Antimicrobials

The antimicrobial efficacy of the reagent can be primarily ascribed to two mechanisms: the chemical disruption of the synthesis or functionality of essential bacterial components and/or the evasion of established antibacterial resistance mechanisms. Nevertheless, bacteria have the capacity to develop resistance to a range of antimicrobials due to selective pressure or by acquiring resistance traits from neighboring microbial populations[157].

The mechanism described below is consistent with known antimicrobials

i. **Bacterial protein biosynthesis**:The inhibition of protein synthesis by targeting the ribosomal subunits of bacteria is a highly effective method for addressing bacterial infections. Antibiotics such as macrolides, tetracyclines, aminoglycosides, and oxazolidinones utilize this mechanism to exert their antibacterial properties.

Specifically, amikacin binds irreversibly to the 16 S rRNA and the RNA-binding protein S12 located in the 30 S subunit of the prokaryotic ribosome. This binding modifies the ribosome's conformation, hindering its ability to accurately interpret mRNA codons and disrupting the interaction with the tRNA anticodon's wobble base[158].

ii. **Inhibition of nucleic acid synthesis**:DNA gyrase is a crucial enzyme involved in the synthesis, replication, repair, and transcription of bacterial DNA. As such, it represents a significant target for antibacterial compounds, including nalidixic acid and fluoroquinolones like ciprofloxacin. The mechanism of action of ciprofloxacin involves the inhibition of topoisomerase type II (DNA gyrase) and topoisomerase IV, both of which are essential for the separation of bacterial DNA, ultimately leading to the disruption of cell division[159].

iii. **Biosynthesis of cell walls**:Bifunctional enzymes, notably transglucosylases and transpeptidases, play a crucial role in the formation of bacterial cell walls and serve as effective targets for bactericidal antibiotics, including penicillin, cephalosporins, and vancomycin. These antibiotics interfere with the peptide substrate located within the peptide layer, thereby hindering the enzymatic activities essential for cell wall synthesis. Specifically, vancomycin impedes the cell wall synthesis in gram-positive bacteria. This large hydrophilic

compound forms hydrogen bonds with the D-alanil-d-alanine terminal residues found in N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) peptides. By binding to the D-Ala-D-Ala motif, vancomycin prevents the formation of the extensive NAM and NAG polymers that are vital for the structural integrity of the bacterial cell wall[160].

iv. **Destruction of the bacterial cell wall**: Antibiotics such as polymyxins exhibit the ability to bind to the lipid constituents of lipopolysaccharides, instigating structural alterations via phospholipid exchange. This mechanism can induce osmotic imbalances, which may result in the swift demise of bacterial cells. In particular, Polymyxin B affects the permeability of the bacterial outer membrane by attaching to negatively charged regions of the lipopolysaccharide layer, which is electrostatically attracted to the positively charged amino groups present in the cyclic peptide structure. This interaction leads to a destabilization of the outer membrane. Additionally, the fatty acid component penetrates the hydrophobic domains of the cytoplasmic membrane, thereby disrupting its structural integrity. This disruption facilitates the release of cellular components and hampers cellular respiration[161].

2.11.5 The Need for Innovative Antibiotics

The increasing clinical demand for effective antibiotics can be attributed to the global rise of multidrug-resistant organisms (MDRs). In the mid-20th century, the introduction of antibiotics marked a significant advancement in medical treatment. During this period, the majority of pathogens responsible for infections were typically sensitive to antibiotic therapies, notwithstanding the natural resistance exhibited by certain organisms, such as

gram-negative bacteria with their outer membranes or pathogens like M. tuberculosis that produce β-lactamases. However, through the processes of evolution and natural selection, antibiotic resistance has become increasingly prevalent. This phenomenon can be primarily traced to two factors: mutations during DNA replication that result in the emergence of drug-resistant strains, which are then propagated vertically within bacterial populations, and the horizontal transfer of resistance genes among bacteria[162].

DNA polymerase exhibits a low failure rate, estimated between 10^-9 and 10^-11, which is crucial for the faithful replication of the bacterial genome. However, in the context of a large bacterial population—often comprising millions of cells at an infection site—and their rapid generation times, which can range from minutes to hours, the accumulation of mutations is an unavoidable consequence. If these mutations yield a competitive edge in the presence of antibiotics, either by altering the drug's target affinity or by increasing the activity of efflux pumps that eliminate the antibiotic from the bacterial cell, such mutants can swiftly proliferate, leading to resistance and the failure of treatment strategies[163].Mutations are significant contributors to clinical resistance against specific antibiotic classes, particularly fluoroquinolones, and in certain organisms that infrequently engage in horizontal gene transfer, such as Mycobacterium tuberculosis. Furthermore, pathogens that were once predominantly found in healthcare or agricultural settings characterized by high antibiotic usage are increasingly being detected in the general population. For instance, methicillin-resistant *Staphylococcus aureus* has become more prevalent outside clinical environments. Additionally, prolonged armed conflicts have exacerbated the issue of antibiotic resistance. The rise of multidrug-resistant Acinetobacter baumannii during the conflicts in Iraq and Afghanistan exemplifies a growing clinical challenge faced by hospitals globally. This

escalation in multidrug resistance is not a recent phenomenon; it is a direct consequence of antibiotic utilization. Considering the vast number of microbial genomes estimated at 10^30 bacteria on Earth the intense selective pressure exerted by antibiotics renders the emergence of resistance, when physiologically advantageous, nearly unavoidable[164].

2.12 Plant Phytochemical Composition

The realm of medicinal plants encompasses a variety of bioactive organic compounds, known as phytochemicals, which are instrumental in defending against prevalent chronic diseases linked to metabolic or genetic dysfunctions, as well as infectious diseases. These phytochemicals are found in an array of plant-derived products, including grains, vegetables, and fruits. They are chemical entities produced by plants that possess biological activity and may confer health benefits. Phytochemicals serve to protect plants from ultraviolet light, herbivorous animals, insects, and pathogens. Furthermore, many of these compounds function as pigments, contributing to the diverse and vibrant colors of fruits and vegetables, exemplified by the orange coloration in carrots and the blue in blueberries[165].

Phytochemicals represent a vital area of investigation in both medical and nutritional sciences, as many exhibit properties that are antioxidant, anticarcinogenic, neuroprotective, or anti-inflammatory. Although more than 10,000 different phytochemicals have been recognized, only a small subset has been thoroughly examined. These compounds are categorized based on their chemical structures, and a variety of phytochemicals present in plants are essential for maintaining health[166]. The practice of utilizing herbal remedies, whether on their own or in conjunction with traditional medicine, has become prevalent in the management of

diseases worldwide, notably in developing countries like Nigeria. This phenomenon is largely due to the low cost and local availability of these herbal options. The distribution of these medicinal plants is influenced by climatic conditions that determine the growth and survival of different species. While many of these plants are predominantly used for culinary purposes, their medicinal use is still in its infancy. However, there is a growing commitment to explore their potential for creating more effective and safer therapeutic agents[167].

Phytochemicals are natural compounds that occur in edible plants like fruits, vegetables, seeds, nuts, and grains, and they have been found to offer health benefits including antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. The bioactivity of a phytochemical refers to its ability to encourage positive changes in human health (such as decreasing heart disease risk) or influencing a health-related biomarker (like reducing LDL levels). This bioactivity is contingent on the concentration of the phytochemical in its active form within the target tissues. Consequently, it can be affected by factors such as plasma protein binding, metabolism in the liver, and processes of excretion[168].

Phytochemical constituents play a crucial role in the survival and optimal functioning of plants. They serve as a defense mechanism against herbivores, pathogens, and competing species; they also regulate growth processes, such as delaying seed germination until conditions are favorable. Additionally, phytochemicals influence pollination, fertilization, and the dynamics of the rhizosphere. From the perspective of the plant itself, these compounds are beneficial. However, their inherent biological properties can adversely affect other organisms, leading to outcomes that may be deemed detrimental or even catastrophic, as they can result in the death or significant harm to these entities. Human curiosity often gravitates towards the negative aspects of nature, making the study of phytochemicals with pronounced harmful effects more appealing[169].

Phytochemicals are non-nutritive compounds that exhibit biological activity and are synthesized by plants through their metabolic processes. These compounds are naturally occurring in a variety of foods, including fruits, vegetables, seeds, nuts, whole grains, legumes, dark chocolate, and tea, and have been linked to a decrease in food consumption and the prevention of numerous chronic diseases.

Although the number of identified and isolated phytochemicals is relatively small, it is estimated that tens of thousands exist within the plant kingdom. The beneficial effects of phytochemicals on human and animal health are attributed to their ability to alter gut microbiota, specifically by fostering the growth of certain beneficial bacterial populations known as probiotics[170].The category of endosymbionts includes yeasts, lactobacillus, lactic acid bacteria, and bacilli, which are integral to the gastrointestinal metabolism of both humans and animals. In relation to micro- and macronutrients, the bioavailability of phytochemicals is considerably low in the human body, largely due to their complex chemical structures and their classification as xenobiotics during metabolism. This limited absorption results in longer retention times in the gut, where phytochemicals can exert a beneficial influence on gut ecology[171].

Phytochemicals can be categorized into two main groups: primary and secondary metabolites, depending on their roles in plant metabolism. Primary metabolites are crucial for the survival of plants and encompass carbohydrates, amino acids, proteins, lipids, as well as purines and pyrimidine nucleic acids. In contrast, secondary metabolites are byproducts of plant cellular processes, synthesized through metabolic pathways that originate from primary metabolism[172].The chemical constituents in question are recognized for their antiviral, antifungal, and antibiotic properties, which play a vital role in safeguarding plants against

various pathogens. Furthermore, these compounds serve as essential agents that absorb ultraviolet radiation, thereby mitigating potential light-induced damage to foliage.

The significant biological activity exhibited by plant secondary metabolites has led to their utilization in traditional medicine for centuries, with the therapeutic benefits of plants largely attributed to these molecules. Additionally, different tissues and organs within medicinal plants may possess unique medicinal properties that vary according to developmental stages. In contemporary contexts, these metabolites are linked to important sectors, including pharmaceuticals, cosmetics, and fine chemicals[173].

The classification of secondary metabolites in plants is primarily based on their biosynthetic origins, which can be divided into three major categories: a) nitrogenous compounds, including alkaloids, glycosylates, and cyanogenic glycosides; b) phenolic compounds, such as phenylpropanoids and flavonoids; and c) terpenes. Notably, several bioactive constituents, including alkaloids, flavonoids, sterols, organosulfur compounds, peptidoglycans, amino acids and their derivatives, saponins, terpenoids, and carotenoids, are found in abundance within the plant kingdom[174].

i. Alkaloids have provided distinctive lead compounds for medicinal applications. Characterized by their basic nature, these compounds exhibit solubility in water under acidic conditions and in lipids when in basic or neuronal environments. They are essential not only in human healthcare but also in the body's intrinsic defense mechanisms. Approximately 20% of the identified secondary metabolites in plants consist of alkaloids. In the plant kingdom, alkaloids serve as a defense

mechanism against herbivores and play a role in regulating growth. From a therapeutic perspective, alkaloids are primarily recognized for their narcotic, cardioprotective, and anti-inflammatory properties. Notable examples of alkaloids utilized in clinical practice include morphine, strychnine, quinine, ephedrine, and nicotine[175].

Approximately 20% of low-value plant species contain alkaloids, making their production, including biotechnological approaches, mining, and processing, critical areas for ongoing research and development. An example of this is the genetic engineering of alkaloid biosynthesis pathways to increase the yield of alkaloid[176].

Alkaloids serve a protective function for certain plants, safeguarding them against specific insect threats. These compounds are characterized by the presence of a nitrogenous ring and are synthesized by a diverse array of organisms, including bacteria, fungi, plants, and animals. They belong to a broader category of natural products, often referred to as secondary metabolites. With over 12,000 distinct nitrogenous cyclic compounds identified, alkaloids are prevalent in approximately 20% of plant species[177].

Prominent families that contain these compounds include Papaveraceae, Acanthaceae, Apocynaceae, and Solanaceae. Some alkaloids have been recognized for their cardioprotective properties, as they can lower cholesterol levels and exhibit antioxidant and anti-inflammatory effects. The most prevalent types of alkaloids include propane, purine, acridone, indole, imidazole, and morphine[178].

Among the various alkaloids, caffeine stands out as a compound found in coffee, tea, and numerous other drinks, celebrated for its stimulating effects and possible enhancements to cognitive abilities and athletic performance. Theobromine, another alkaloid, is present in chocolate and various food items, and it has been linked to potential improvements in

cardiovascular health, including the possibility of lowering blood pressure. Nicotine, an alkaloid sourced from tobacco and other botanical species, is notorious for its addictive nature and detrimental health consequences, yet some studies propose that it may offer therapeutic benefits for certain neurological conditions, such as Parkinson's disease[179].

ii. Organic sulphur compounds

The plant-derived sulfur compounds most commonly recognized for their role in the prevention of cardiovascular diseases include garlic, onions, leeks, and cruciferous vegetables such as broccoli, cabbage, cauliflower, and green cabbage. The predominant sulfur compound present in cruciferous vegetables is sulforaphane, an isothiocyanate that exists in the plant as glucoraphanin, which is in an inactive state. The conversion of glucoraphanin to sulforaphane is facilitated by the enzyme tyrosinase. The enzyme responsible for converting glucoraphanin into sulforaphane is known as tyrosinase. This enzyme becomes active when the vegetables are chopped and cooked at temperatures not exceeding 140°C; otherwise, it remains inactive unless the plant structure is damaged[180].

iii.Among the various phytochemicals, polyphenols constitute the largest classification. A substantial and growing body of research supports the notion that phytochemicals can effectively alleviate inflammation, impede the growth of cancerous cells, lower the synthesis of carcinogens, influence gene expression and intracellular hormone signaling, strengthen the immune response, minimize DNA damage, counteract oxidative damage to cells, and activate insulin receptors[181].Polyphenols, which are compounds featuring phenolic rings, are also referred to as phenolic compounds. Examples of these include anthocyanins responsible for the purple coloration of grapes, isoflavones and phytoestrogens found in soybeans, and tannins that provide tea with its characteristic astringency. These compounds are extensively

found in various plant sources, such as vegetables, fruits, grains, coffee, tea, and wine. The category of food polyphenols encompasses a broad spectrum of naturally occurring heterogeneous substances that are characterized by hydroxylated phenyl fractions.

Typically, polyphenols are classified into two primary categories: flavonoids and non-flavonoids, based on their structural complexity, the types and numbers of substituent groups, and the total count of phenolic rings[182].Nonflavonoids are represented by compounds like phenolic acids, stilbene, curcumin, tannins, lignans, and coumarin. Although polymerization and esterification are the most common alterations, a significant number of polyphenols are present in glycosylated forms in plants. In a broader context, polyphenols, much like xenobiotics, are assimilated into the host's body after they are consumed. This leads to a lower bioavailability in comparison to macro- and micronutrients[183].

a. Flavonoids:As the largest subgroup within the polyphenol family, flavonoids comprise more than 6,000 identified compounds sourced from plants. The most significant among these are those that impart color to flowers and other plant parts, playing an essential role in the mitigation of free radicals. Flavonoids can be classified into seven distinct subgroups, with each subgroup containing unique compounds that exhibit various biological functions[184].

b. Flavones:Various foods, such as rutabaga, tea, oranges, wheat germ, onions, cilantro, and chamomile, contain flavanones. Citrus fruits are recognized as the most abundant source of flavanones in human nutrition, while aromatic herbs like mint and tomatoes provide these compounds in lesser amounts. Although flavanones are consumed in relatively small quantities, they play a crucial role in the diet, particularly because citrus fruits and their juices are widely enjoyed worldwide. Naringenin and hesperetin, the most prevalent flavonones in

grapefruit and sweet oranges, are primarily responsible for the distinctive bitter taste associated with these fruits[185].

c. Flavanols: The flavanol group includes several compounds, such as quercetin, kaempferol, isorhamnetin, and myricetin, which are commonly found in green leaves, fruits, and grains. Among these, quercetin and kaempferol have garnered considerable attention in research concerning their effects on genetically modified organisms (GMOs). Fruits and vegetables are rich in quercetin, with typical daily consumption in Western diets estimated to be between 0 and 30 mg, influenced by the intake of these foods. Notable sources of quercetin include berries, apples, onions, and various greens[186].

d. Curcumin:Curcumin is classified as a member of the polyphenol subgroup, with the rhizome of *Curcuma longa* identified as the most abundant source of this compound, which finds applications in culinary practices and traditional medicine [187].

e. Anthocyanins:Anthocyanins are a subgroup of polyphenols that exhibit vibrant pigmentation and are soluble in water, enabling their absorption without the need for accompanying dietary fats. These compounds have been linked to a range of health benefits, including improvements in cardiovascular function, management of metabolic diseases, cancer prevention, and reduction of inflammation. The striking red, purple, and blue shades produced by anthocyanins are particularly noteworthy, as they are found in both edible and ornamental flowers. Notable food sources rich in anthocyanins encompass berries, red spinach, various red greens, purple corn, purple sweet potatoes, grapes, and purple carrots[188].

iv. Glycosides:Glycosides are predominantly found in plants and are defined as compounds that consist of one or more sugar molecules linked to a non-carbohydrate component, which

may include alcohols, phenolic compounds, or complex structures like steroid nuclei. These substances are widely distributed among medicinal plants and are recognized as important sources of therapeutic agents that typically exhibit lower toxicity and minimal side effects. Natural glycosides are often the key active ingredients in various plant species[189].

Their pharmacological properties are increasingly recognized, particularly for their anti-inflammatory, anti-cancer, immune-modulating, and vascular-protective effects. Glycosides function as essential molecules in biological systems, with many plants storing bioactive compounds in an inactive glycosidic form. These can be enzymatically hydrolyzed to release sugars and activate valuable chemicals. Additionally, in both animals and humans, toxins are frequently conjugated to sugar molecules to facilitate their elimination from the organism[190].

i. Cyanogenic Glycosides

Cyanogenic glycosides, which are derived from amino acids, are found in more than 2,500 plant species and are prevalent across 100 flowering plant families. The toxicity associated with these compounds arises from the release of hydrogen cyanide. These glycosylates, containing sulfur and nitrogen, are synthesized by certain plants and remain chemically stable under typical environmental conditions. Non-protein amino acids, resembling protein amino acids in structure, are primarily involved in the plant's defense against stressors and act as crucial mediators in response to abiotic influences. Furthermore, low molecular weight amines, which are naturally occurring nitrogenous substances in plants, are responsible for various biological effects, including their role as significant precursors to hormones[191].

v. Terpenoids

Terpenoids represent the most prevalent category of secondary metabolites found in plants, primarily synthesized in flowers, vegetative tissues, and roots. These compounds are known for their diverse biological activities, which contribute to the reduction of total cholesterol, triglycerides, and LDL cholesterol, as well as the regulation of blood pressure. Carbohydrates,

generated through the process of photosynthesis in plants, serve as a crucial source of energy and carbon, forming the foundational structure of organic compounds and storage materials[192].

Terpenes constitute a broad and diverse group of organic compounds generated by many plant species, especially trees, and are often characterized by their strong fragrances, which may offer protective benefits. These compounds are the primary ingredients in resin and turpentine, with the term "terpene" deriving from "turpentine." Additionally, terpenes are crucial precursors in the biosynthetic processes of nearly all living organisms; for example, steroids are derived from the triterpene squalene. When terpenes undergo chemical modifications, such as oxidation or rearrangement of their carbon framework, the resulting substances are typically classified as terpenoids. Both terpenes and terpenoids are key constituents of essential oils in numerous plant and flower species. The application of essential oils is prevalent in the culinary arts as natural flavor enhancers, in the fragrance industry, and within the realms of traditional and alternative medicine, particularly aromatherapy. The development of synthetic alternatives and derivatives of natural terpenes and terpenoids has significantly enriched the array of flavors available for use in perfumes and food additives. The distinctive aromas of rose and lavender are primarily due to the presence of monoterpenes, while terpenoids encompass a broad and diverse category of lipids that are naturally occurring. These compounds are structured from one or more isoprene units. Sesquiterpenes, diterpenes, and monoterpenes are derived from specialized anatomical structures, including lysogenic glands, cellular schizogonic glands, and ducts. The fragrant characteristics of plant terpenoids are widely exploited, with notable compounds such as salvinorin-A, camphor, menthol, and terpenoids like citral being investigated for their potential biological effects[193].

vi. The saponin: Saponins represent a significant category of secondary metabolites prevalent throughout the plant kingdom. These phytochemicals are commonly found in a variety of vegetables, legumes, and herbs.

Notably, saponins are present in numerous medicinal plants, including citrus juice, mango leaves (*Mangifera indica L.*), avocado (*Persea americana*), the leaves and roots of *Leucas aspera L.*, the root of *Rhamnus prinomides*, as well as the bitter leaf (*Vernonia amygdalina*) and its stem bark. Certain plant species exhibit variable saponin content depending on the extraction solvent used, and their distribution is not uniform across all plant parts. For instance, saponins have been identified in the leaves of *Bersama abyssinica* and *Dioscorea alata*, as well as in the roots of *Gilbertii* and flaxseed[194].

7. Carotenoids: Carotenoids constitute another important class of phytochemicals found in a wide array of fruits and vegetables, including carrots, tomatoes, and leafy greens[195]. These compounds are recognized for their antioxidant properties and may offer potential advantages for ocular health. Here are some examples of carotenoids:

a.Beta-carotene -This carotenoid is prevalent in a variety of orange and yellow fruits and vegetables, including carrots, sweet potatoes, and pumpkins. Research indicates that beta-carotene possesses antioxidant properties and may offer potential advantages for ocular health and cancer prevention[196].

b.Lycopene : Found predominantly in tomatoes and other red fruits and vegetables, lycopene is another significant carotenoid. Studies suggest that it exhibits antioxidant properties and may contribute to the prevention of prostate cancer

c.Lutein and zeaxanthin :These carotenoids are commonly present in numerous green leafy vegetables, such as spinach. Evidence suggests that lutein and zeaxanthin may provide

benefits for eye health and could play a role in protecting against age-related macular degeneration[197].

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Chapter Three

Methodology

3.1 Collection, Indentification and Processing of Plant Samples

Fresh seeds of *Garcinia cola*, rhizomes of *Zingiber officinale*, and pods of *Aframomum melegueta* were collected from Bode Market in Ibadan, Oyo State, and placed into sterile containers. The identification and verification of these plant materials were conducted at the Forestry Research Institute of Nigeria (FRIN), where they were subsequently deposited in the forestry herbarium with the following voucher numbers: FHL-114184 for *Garcinia cola*, FHL-114185 for *Zingiber officinale*, and FHL-114183 for *Aframomum melegueta*. The pods of *Aframomum melegueta* were opened to extract the seeds, which were then thoroughly washed with distilled water. After washing, all seeds were separated and stored in airtight sample bottles for experimental use, following air-drying at room temperature and grinding into a fine powder using an EMEL (EN311) mill. The research utilized analytical-grade chemical reagents.

3.2 Collection of the Test Organisms

Bacterial Isolates from upper respiratory tract infection were obtained from the Medical Microbiology Laboratory, Leadcity Hospital, Ibadan, provided pure stock cultures of the pathogenic bacterial isolates. The pathogenic bacterial were *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aerouguinosa* and *Streptococcus pneumonia*

3.3 Sterilization of Glassware and Non-glassware

All glass and non-glass items underwent a comprehensive cleaning process involving liquid detergent, followed by rinsing with distilled water. Subsequently, they were drained, dried, wrapped in aluminum foil, and subjected to sterilization in a hot air oven at a temperature of 160°C for a duration of 2 hours. The inoculating wire loop and cork borer (8 mm) were re-

sterilized by immersion in ethanol and then subjected to a flame from an alcohol lamp both prior to and following their application[1].

3.4 Preparation of Nutrient Media

In accordance with the manufacturer's guidelines, 28 grams of nutrient agar powder were accurately measured using a sensitive balance (Mettler Toledo FA2104A). This agar powder was then dissolved in 1000 milliliters of distilled water within a 1000 ml conical flask. The flask's opening was sealed with a cotton plug that had been covered in aluminum foil. The solution was thoroughly mixed and subsequently homogenized in a water bath set at 80°C for a duration of 15 minutes. Upon completion of the homogenization process, the conical flask containing the nutrient agar was removed from the water bath, wrapped in aluminum foil, and subjected to autoclaving for 15 minutes at a temperature of 121°C and a pressure of 15 psi[2]. After autoclaving, the mixture was allowed to cool to approximately 47-50°C before being transferred into sterile petri dishes to solidify[3].

3.4.1 Preparation of MacConkey Agar Media

According to the manufacturer's instructions,55.37 grams of MacConkey agar powder were weighed using a precision balance (Mettler Toledo FA2104A). This powder was dissolved in 1000 milliliters of distilled water within a 1000 ml conical flask. The flask was then sealed with a cotton plug that had been wrapped in aluminum foil. The solution was mixed thoroughly and subsequently homogenized in an 80°C water bath for 15 minutes. After the homogenization process, the conical flask was taken out of the water bath, wrapped in aluminum foil, and autoclaved for 15 minutes at 121°C under a pressure of 15 psi. The mixture was allowed to cool to a temperature range of 47-50°C before being poured into sterile petri dishes to solidify[4].

3.4.2 Preparation of Simmon Citrate Agar

Following the specifications provided by the manufacturer, 24.28 grams of simmons citrate agar powder were precisely weighed using a Mettler Toledo FA2104A sensitive balance. This powder was then dissolved in 1000 milliliters of distilled water within a 1000 ml conical flask. The flask was sealed with a cotton plug that was covered in aluminum foil to prevent contamination. The solution was mixed thoroughly and then homogenized in an 80°C water bath for 15 minutes. After the homogenization, the conical flask was taken out of the water bath, wrapped in aluminum foil, and autoclaved for 15 minutes at 121°C under a pressure of 15 psi. The mixture was subsequently cooled to a temperature of approximately 47-50°C before being poured into sterile petri dishes to allow for solidification[6].

3.5 Validity of Test Organisms

Organisms were cultivated in Petri dishes employing the streak plate technique following a 48-hour incubation period on nutrient agar slants stored in a refrigerator at approximately 4°C. Subsequently, they were streaked onto selective agar plates. To assess the validity and viability of the tested bacteria, the Gram staining procedure, along with various biochemical test including catalase, starch hydrolysis, oxidase, urease, indole, and citrate tests, was conducted on each isolate.

3.5.1 Gram Staining

Gram Staining Procedures

On a clean grease free slide one drop of normal saline was added to a picked colony and bacteria smear was made on a slide.The smear was heat fixed on the slide and then flood theslide with Crystal violet stain to the bacteria for 30 seconds and rinse off. Lugol iodine was used a mordant. Decolorize with acetone and counterstain with safranin, the slide was examined under a microscope and procedure was repeated for all the test isolate.

3.5.2 Biochemical test

Catalase Test

A Smear of bacterial colony was placed onto a clean glass slide, a drop 3% hydrogen peroxide was added to the bacteria and observe for bubbles immediately

Results

For organisms that are catalase positive bubbles form immediately and organisms that are catalase negative did not form bubbles after 20 seconds

Coagulase Test

On a clean grease free slide,two drops of plasma was drops, inoculum of the organisms was added and emulsified.The slide was gently rocked for 10 seconds to observe for clumping in the plasma. For organisms that are coagulase positive clumping form immediately and organisms that are coagulase negative did not clump bubbles after 20 seconds.

Starch Hydrolysis

Pure culture of bacteria was streak on a sterile starch agar plate, incubate at 35–37°C for up to 48 hours, plate was flooded with Gram iodine and then observe for a clear zone around the bacterial growth. For organisms that are starch hydrolysis positive a clear, colorless zone around the bacterial growth and organisms that are negative no clearing around the bacterial growth, and the medium turns blue

Oxidase Test

Impregnated oxidase strip was put on a clean petri dish, a sterile inoculatind loop was used to pick up a well-isolated colony of test bacteria from fresh culture and make a smear on the oxidase strip. It was observed for 60 seconds for colour change from initial colour. For organisms that are oxidase positive purple colour indicate

Urease Test

Inoculate a urea agar slant with a pure culture of bacteria,Incubate the tube at 35–37°C and observe the tube for a color change. For organisms that are urease positive a bright pink or magenta color develops within 15 minutes to 24 hours and negative no color change

Citrate Test

A small amount of bacteria was streak onto the slant of the citrate agar using a sterile inoculating loop Incubate the tube at 37°C then the color of the slant was observed.And the medium turns blue, for organism that are citrate positive, no color change for organism that are citrate negative

3.6 Antimicrobial Susceptibility Tests

3.6.1 Preparation of the Plant Extract Dilution

The plant extract was prepared using cold maceration extraction method. A mechanical shaker set to 220 rpm was used to extract the *Garcinia cola*, *Zingiber officinale* rhizome, and *Aframomum melegueta* for 48 hours. The samples were subsequently weighed at 200 g each of *Garcinia cola* blended seeds, *Zingiber officinale* rhizome, and *Aframomum melegueta* seeds using a balance. The plants samples were then separately dissolved in 1000 ml of different extraction solvents, namely Ethanol, Petroleum ether, and Water. The mixtures were allowed to sit for a duration of 48 hours, during which they were stirred at regular intervals to

facilitate extraction. The solvents were filtered after concentrations using a rotary evaporator at 40°C to produce the extracts, which were then filterer using Whatman No. 1 filter paper.

The concentrations for the antimicrobial analysis were determined using a designated concentration formula:

$C_1V_1 = C_2V_2$

Where C_1 = Initial concentration
C_2 = Final concentration
V_1 = Initial Volume
V_2 = Final Volume[7]

a) 10g ofundiluted extract converted to milligrams = 10 g x 1,000 = 10,000 mg
Initial concentration = 10000mg
100ml
100 mg/ml = initial concentration

b) C_1 = 100mg/ml
C_2 =?
V_1 = 10ml
V_2 = 15ml

$$C_2 = \frac{C_1V_1}{V_2}$$

$$C_2 = \frac{100mg/ml \times 10ml}{15ml}$$

$$C_2 = \frac{100mg/ml}{15}$$

C_2= 66.7mg/ml = concentration of 1st dilution

c) C_1 = 100mg/ml
C_2= ?
V_1 = 10ml
V_2 = 20ml

$$C_2 = \frac{C_1V_I}{V_2}$$

$$C_2 = \frac{100mg/ml \times 10ml}{20ml}$$

$$C_2 = \frac{1000mg}{20ml}$$

C_2 = 50mg/ml = concentration of 2nd dilution

d) $C_1 = 100mg/ml$

$C_2 = ?$

$V_1 = 10ml$

$V_2 = 25ml$

$$C_2 = \frac{C_1V_1}{V_2}$$

$$C_2 = \frac{100 \times 10}{25ml}$$

$$C_2 = \frac{1000mg}{25ml}$$

$C_2 = 40mg/ml$ = concentration of 3rd dilution

Following this, the concentrated filtrates were diluted to various concentrations, starting from 100 mg/mL, 66.7 mg/mL, 50 mg/mL, 40 mg/mL, and 33.3 mg/mL. The antimicrobial properties of these extracts were subsequently assessed against the bacterial isolates *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae*.

3.6.2 Preparation of the Combined Extract Dilution

The procedure commenced with the precise weighing of 100 g each of *Garcinia cola* blended seeds, *Zingiber officinale* rhizome, and *Aframomum melegueta* seeds and another 100g of the other plants using a balance. These combined plants were then dissolved in 1000 ml of different extraction solvents, namely Ethanol, Petroleum ether, and Water. The mixtures were allowed to sit for a duration of 48 hours, during which they were stirred at regular intervals to facilitate extraction. Following this, the concentrated filtrates were diluted to various concentrations, 100 mg/ml, 66.7 mg/ml, 50 mg/ml, 40 mg/ml, and 33.3 mg/ml. The antimicrobial properties of these extracts were subsequently assessed against the bacterial

isolates *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae*

3.6.3 Determination of Anti-bacterial Activities of Plant Extract against Test Pathogens

The test organisms were evenly distributed across the surface of solidified nutrient agar plates using a glass spreader that adhered to the MacFarland standard. A sterile cork borer measuring 6 mm was employed to create five wells in a nutrient agar plate, with the central well designated as a control. The extracts were introduced into the wells using a syringe and needle, while for the control, the central well was filled solely with the solvent. This setup allows the extract within the gel cavity to diffuse into the solid medium, thereby inhibiting the growth of the inoculated microorganisms in accordance with the efficacy of the extract's constituents. This inhibition was evidenced by the formation of a distinct circular zone surrounding the well containing the extract and zone of inhibition was measured with a transparent sterile rular.

3.6.4 Determination of Minimum Inhibitory Concentration (MIC) of *Garcinia cola*, *Zingiber officinale* and *Aframomum melegueta*

Minimum Inhibitory Concentration (MIC) of the extracts were determined by agar dilution.The MIC was obtained using double fold dilution. Different dilution of the extracts were prepared to give final concentration in the range of 100mg/mL ,50mg/mL ,25mg/mL ,12 .5mg/mL . 2mL of each dilution was mixed with 18mL of Nutrient Agar poured into petri-dishes and allow to set. The agar was streaked with the different bacterial isolates and incubated at 37C for 48hrs. The plates were then examined for

the presence or absence of growth. The minimum concentration that completely inhibited the organisms regarded as the MIC of the respective extracts.

3.6.5 Determination of Minimum Bactericidal Concentration (MBC)

The lowest bactericidal concentration of the plants extract was determined by sub culturing the test tubes from minimum inhibitory concentration tubes that showed no growth on nutrient agar and incubated for 24hours at 37°C. Plates containing new solidified nutrient agar were streaked with inoculum from tubes with no turbidity in the MIC setup and incubated at 37°C for 24hrs.

The plate with the least concentration of the extract from MIC that showed no growth was recorded as MBC[9].

3.6.6 Antibiotic Susceptibility Testing

The antibiotic susceptibility of bacterial isolates was evaluated using the disc diffusion method, which involved the application of antibiotic discs targeting both gram-positive and gram-negative bacteria. Each pathogenic isolate from the nutrient agar slants was streaked onto nutrient agar plates. Afterward, sterile forceps were employed to aseptically position the antibiotic discs onto the agar surface of each Petri dish. The plates were then incubated at 37°C for 24 hours. Upon completion of the incubation period, the zones of inhibition produced by the bacterial isolates were analyzed. The diameters of these zones were measured in millimeters using a transparent ruler, providing a quantitative assessment of antibiotic susceptibility. The patterns of antibiotic susceptibility, known as antibiograms, were derived from the observed zones of inhibition on the agar plates[10].

3.7 Phytochemical Detection of Extracts from Seeds of *Garcinia kola*, Rhizome of Zingiber officinale and Seeds of *Aframomum melegueta*

The seeds of *Garcinia cola*, the rhizome of *Zingiber officinale*, and the seeds of *Aframomum melegueta* underwent phytochemical screening to identify their phytochemical constituents. This assessment was carried out following standardized methodologies. The phytochemicals analyzed comprised saponins, tannins, steroids, terpenoids, anthraquinones, flavonoids, alkaloids, and flavotanins.

3.7.1 Phytochemical Qualitative Test

Standard procedures were employed to conduct Phytochemical tests on the extract in order to identify its constituents [11].

3.7.2 Tannin Test

In this particular experiment, one gram of extract was subjected to heating in 20 ml of water, followed by filtration. The introduction of several drops of 0.1 percent ferric chloride resulted in the observation of a green or blue-black coloration, indicating the presence of tannins.

3.7.3 Phlobatannin Test

The formation of a red precipitate, observed when 2 ml of extract from each plant sample was heated with 1% aqueous hydrochloric acid, served as evidence for the presence of phlobatannins[12].

3.7.4 Saponin Test

In a regulated water bath, 5 ml of the extract was mixed with 20 ml of distilled water and then filtered. To create a stable and long-lasting foam, 10 ml of the filtrate was combined with 5 ml of distilled water and vigorously stirred. Subsequently, the froth was quickly agitated with

three drops of olive oil, leading to the formation of an emulsion, which confirmed the presence of saponin[13].

3.7.5 Flavonoid Test

The procedure involved the addition of three milliliters of a 1% aluminum chloride solution to 5 milliliters of each extract, resulting in a yellow coloration that suggested flavonoid presence. This mixture was then treated with 5 milliliters of a dilute ammonia solution, followed by the introduction of concentrated sulfuric acid (H_2SO_4). The yellow color was observed to vanish upon standing, which confirms a positive test for flavonoids[14].

3.7.6 Steroid Test

Two millilitres of acetic anhydride were introduced to two millilitres of extract from each sample, after which two millilitres of H_2SO4 were added. The indication of steroid presence is marked by a transition in color from purple to blue or green[15].

3.7.7 Terpenoid Test (Salkowski test)

In the experimental procedure, five millilitres of each extract were meticulously mixed with two millilitres of chloroform and three millilitres of concentrated sulfuric acid (H_2SO4). The appearance of a reddish-brown hue at the interface signified the existence of terpenoids[16].

3.7.8 Cardenolides and Cardiac Glycosides Test (Keller-Kilani Test)

Each extract, measuring five millilitres, was combined with two millilitres of glacial acetic acid that contained one millilitre of ferric chloride solution. The mixture was subsequently reduced by the addition of one millilitre of pure sulfuric acid. The formation of a brown ring at the interface suggests the presence of a cardenolide deoxysugar, thereby indicating the

existence of cardenolides. Additionally, a green-violet ring observed beneath the brown ring in the acetic acid layer confirms the positive identification of glucosides.

3.7.9 Alkanoids

In a steam bath, one milliliter of the extract was combined with 5 mL of a 1 percent aqueous solution of hydrochloric acid and subsequently filtered while hot. A volume of 1 mL from the filtrate was then subjected to treatment with a few drops of Mayer's reagent (potassium mercuric iodide solution), Wagner's reagent (iodine solution in potassium iodide), or Dragendorff's reagent (a solution of bismuth and potassium iodide). The presence of alkaloids is indicated by the formation of a cream-colored precipitate with Mayer's reagent and a reddish-brown precipitate when using Wagner's and Dragendorff's reagents[17].

3.7.10 Anthraquinone

Five millilitres of extract were combined with ten millilitres of benzene, filtered, and 5 millilitres of 10% NH3 solution were added to the filtrate. The mixture was mixed, and the presence of anthraquinones was indicated by the presence of a pink, red, or purple colour in the ammoniacal (lower) phase[18].

3.7.11 Chalcones

Ammonia solution (2 mL) was added to 5 mL of plant extract from each portion.The formation of a reddish colour confirmed the presence of chalcones.

3.7.12 Phenol

Five millilitres of the extract were pipetted into a 30 ml test tube, followed by ten millilitres of distilled water. 2 mL of ammonium hydroxide solution and 5 mL of concentrated amyl alcohol were added as well, and the mixture was allowed to react for 30 minutes. The appearance of a bluish-green tint was seen as positive for phenol[19].

3.8 Quantitative Determinations of Phytochemical

3.8.1 Tannin

A 0.20g sample was placed in a 50 ml beaker. 20 mL of 50% methanol was added, wrapped in parafilm, and placed in a 77-80°C water bath for 1 hour. I was carefully stirring to achieve a uniform mixture. The extract was quantitatively filtered through Whatman No. 41 double-layer filter paper into a 100 mL volumetric flask, followed by 20 mL water, 2.5 mL Folin-Denis reagent, and 10 mL 17 percent Na_2CO_3. The marking mixture has been created with well-mixed water and set aside for 20 minutes; the bluish-green hue will appear after 20 minutes. The 0-10 ppm tannin working standard solutions were processed in the same manner as the previous 1 mL sample[20]. The absorbance of Tannic acid standard solutions and samples was measured using a Spectronic 21D spectrophotometer at 760nm after colour development. Tannin percentage was computed as follows:

$$\text{Tannin percentage} = \frac{\text{Sample absorbance X gradient factor X dilution factor}}{\text{Sample weight X 10000}}$$

3.8.2 Alkaloid

To make a homogenous paste, a finely powdered 2g sample was weighed into a 100 ml beaker and 20 ml of 80 percent pure alcohol was added. The liquid was transferred to a 250ml flask, and 100ml of alcohol and 1g of magnesium oxide were added. The mixture was digested in a boiling water bath for 1.5 hours with intermittent stirring under an air reflux condenser. A tiny Büchner funnel was used to filter the heated mixture. The residue was

reintroduced to the flask and digested for 30 minutes with 50 cc of alcohol before being evaporated and hot water added to restore the lost alcohol.After removing all of the alcohol, 3 drops of 10% HCL were added.

After a few minutes, the flask was filtered through dry filter paper, and 10 ml of the filtrate was transferred to a separatory funnel, where the alkaloids were violently agitated out with five successive amounts of chloroform. The resulting residue was diluted in 10 ml of hot distilled water and transferred to a Kjeldahl tube with 0.20 g of sucrose, 10 ml of H2SO4 Conc., and 0.02 g of selenium for digestion in a colourless solution to calculate percent N using the Kjeldahl distillation method[21]. The resulting nitrogen percentage is transformed into a percentage of total alkaloid by multiplying it by 3.26.

Total alkaloid as a percentage = N X 3.26

Alkaloids percentage = percent N X 3.26

3.8.3 Flavonoids

To prevent lumping, 0.5g of finely ground sample was weighed into a 100 mL beaker and 80 mL of 95 percent ethanol was added and agitated with a glass rod. Whatman No. 1 was used to filter the mixture. Filter into a 100 mL volumetric flask and top up with ethanol to volume. 1 ml of the extract was pipetted into a 50 ml volumetric flask, four drops of concentrated HCl were pipetted in using a dropper pipet, and 0.5 g of magnesium shavings were added to create a magenta red tint. From a 100 ppm stock solution, a 0-5 ppm flavonoid standard solution was generated and similarly treated with magnesium chips and HCL as a sample. A Jenway V6300 digital spectrophotometer was used to measure the red-magenta colour absorbance of the sample and standard solutions at 520 nm. The formula is used to calculate the proportion of flavonoids[22].

Sample absorbance X gradient factor X dilution factor
Sample weight X 10000

3.8.4 Saponin

A finely pulverised sample (1g) was weighed into a 250 ml beaker, followed by 100 ml of isobutyl alcohol. To achieve equal mixing, the slurry was shook for 5 hours using a UDY shaker. The mixture was then filtered through Whatman No. 1 filter paper into a 100 mL beaker before being mixed with 20 mL of 40% saturated magnesium carbonate solution. The resulting saturated MgCO3 combination was filtered again through Whatman No. 1 filter paper to achieve a clear colourless solution. 1ml of the colourless solution was pipetted into a 50ml volumetric flask, followed by 2ml of 5% FeCl3 solution brought up to volume with distilled water. It was let to stand for 30 minutes to acquire the blood red colour. A saponin stock solution was used to make standard saponin solutions ranging from 0 to 10 ppm. Standard solutions were similarly handled with 2 mL of 5% $FeCl_3$ solution as the prior sample (1mL)[23]. After colour development, the absorbance of the sample and saponin reference solutions was measured on a Jenway V6300 spectrophotometer at wavelength

= Sample absorbance X gradient factor X dilution factor
Sample weight X 10000

3.8.5 Glycosides

An extract volume of 10 millilitres was pipetted into a 250 ml conical flask. To this, 50 mL of chloroform was added, and the mixture was subjected to stirring on a Vortex mixer for one hour. The resulting solution was then filtered into a 100 ml conical flask, where 10 ml of pyridine and 2 ml of a 2% sodium nitroprusside solution were introduced, followed by vigorous stirring for 10 minutes. Subsequently, 3 mL of a 20% NaOH solution was added to produce a brownish-yellow hue. Glycoside standards with concentrations from 0 to 5 mg/ml

were prepared from a 100 mg/ml glycoside stock solution. The 0-5 mg/ml standard pool was treated identically to the initial sample. The absorbance of both the sample and the standards was recorded using a Spectronic 21D digital spectrophotometer at a wavelength of 510 nm[24]

$$\frac{\text{Sample absorbance X gradient factor X dilution factor}}{\text{Sample weight X 10000}}$$

3.8.6 Steroids

In a dry bottle, six millilitres of Liebermann Burchard's reagent were added, and the absorbance was measured at 620 nm with a Spectronic 21D digital spectrophotometer. Standard steroids were created from a 100 mg/ml stock solution, resulting in concentrations ranging from 0 to 4 mg/ml, and were treated in the same way as the previous sample. The percentage of steroids was calculated using the appropriate formula[25]:

The percentage of steroids was calculated using the following formula:

$$\frac{\text{Sample absorbance X gradient factor}}{\text{Sample weight X 10000}}$$

3.8.7 Phlobatanin

A sample extract weighing 0.5 g was placed in a 50 ml beaker. Subsequently, 20 mL of 50% methanol was introduced, and the beaker was sealed with parafilm before being immersed in a water bath maintained at a temperature of 77-80°C for a duration of one hour. After thorough mixing, the solution was filtered using Whatman No. 1 filter paper into a 50 ml volumetric flask, where it was rinsed with aqueous methanol and brought to the desired volume with distilled water. To prepare the final solution, 1 mL of the sample extract was transferred into a 50 mL volumetric flask, followed by the addition of 20 mL of water, 2.5

mL of Folin-Dennis reagent, and 10 mL of sodium carbonate. This mixture was allowed to react for 20 minutes, resulting in a completely homogeneous solution[26].

$$\frac{\text{Sample absorbance X gradient factor X dilution factor}}{\text{Sample weight X 10000}}$$

3.8.8 Anthraquinones

A 0.5 g sample was accurately weighed and introduced into a 250 ml beaker, where 60 ml of benzene was subsequently added. The mixture was stirred using a glass rod to eliminate any lumps. The solution was then filtered through Whatman No. 1 filter paper into a 100 ml volumetric flask. From this, 10 ml of the filtrate was pipetted into a separate 100 ml volumetric flask, to which 0.2 percent zinc dust was added, followed by 50 ml of hot 5 percent NaOH solution. The mixture was heated for five minutes, ensuring it did not reach boiling, and was then filtered and rinsed with water. To produce a red hue, the filtrate was boiled again with an additional 50 ml of 5 percent NaOH[27]:

$$\frac{\text{Sample absorbance X gradient factor X dilution factor}}{\text{Sample weight X 10000}}$$

3.8.9 Terpenes

A 0.5 g sample was placed into a 50 ml conical flask, to which 20 ml of a 2:1 mixture of chloroform and methanol was added. The mixture was agitated thoroughly and allowed to stand for a period of 15 minutes. Following this, the mixture was centrifuged for another 15 minutes. The supernatant was removed, and the precipitate was washed with an additional 20 ml of the chloroform-methanol mixture before undergoing centrifugation again. The resulting precipitate was then dissolved in 40 ml of a 10% sodium dodecyl sulfate solution. A terpene stock solution, with a concentration of 100 mg/l, was obtained from Sigma-Aldrich Chemicals, USA[28].

Sample absorbance X gradient factor X dilution factor
Sample weight X 10000

3.8.10 Phenol Determination

To prevent the occurrence of lumps, a sample weighing 0.20 g was placed in a 50-ml beaker, followed by the addition of 20 ml of acetone. The resulting mixture was homogenized for a duration of one hour. Subsequently, the mixture was filtered through Whatman No. 1 filter paper into a 100-mL volumetric flask, rinsed with acetone, and then brought to volume with distilled water, ensuring thorough mixing. A volume of 1 mL of the sample extract was transferred into a 50 mL volumetric flask, to which 20 mL of water was added, followed by the addition of 3 mL of phosphomolybdic acid and 5 mL of 23% Na2CO3, ensuring complete mixing. The solution was then diluted to the mark with distilled water and allowed to stand for 10 minutes to develop a bluish-green color.

A phenol standard was prepared from a 100 mg/l phenol solution sourced from Sigma-Aldrich in the United States. The absorbance of both the sample and the phenol standard was measured using a digital spectrophotometer at a wavelength of 510 nm. The proportion of phenol is computed as follows[29]:

Sample absorbance X gradient factor X dilution factor
Sample weight X 10000[17]

3.4 Method of Data Analysis

All measurements were done in triplicates for each microorganism. All collected data were entered into a computer and analyzed using the statistical package for the social sciences (SPSS) version 23. The results were displayed in tables and percentages. Values were reported as Mean ± Standard Deviation (SD). ANOVA tests were performed using GenStat

software to determine significant differences in extracts. Statistical comparisons were separated using Duncan's multiple range tests with Least Significant Differences (LSD) considered at $P \leq 0.05$[30].

Endnotes

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Chapter Four

Results and Discussions of the Findings

4.1 Results of Findings

In this research work, the antibacterial activity of *Garcinia cola, Aframomum melegueta* and *Zingiber officinale Roscoe* plant was tested against selected respiratory tracts pathogenic bacteria, which are *Klebsiella pneumonia, Pseudomonas aeuroginosa, Streptococcus Pneumonia* and *Staphylococcus aureus*. The Qualitative and Quantitative photochemical constituents of the extract of *Garcinia cola, Aframomum melegueta* and *Zingiber officinale* were analysed.

The qualitative phytochemical analysis of the *Garcinia cola, Aframomum melegueta* and *Zingiber officinale* extract using Ethanol, Petroleum ether and Aqueous are show in Table 4.1 and 4.2 Tennin, Saponin, alkaloid, flavonoid, terpenoid, chalcones and steroids were the phytochemical detected in varied concentration in the *Garcinia cola, Aframomum melegueta* and *Zingiber officinale*. Glycosides, and phcnol were detected in the ethanol, petroleum ether

and aqueous extracts. Aqueous, Ethanol and Petroleum ether extracts contain small amount of terperiod.

The qualitative analysis of the phytochemical screening of *Garcinia cola, Aframomum melegueta* and *Zingiber officinale Roscoe* shows that *Garcinia cola, Aframomum melegueta* and *Zingiber officinale Roscoe* has all phytochemical constituents which includes Alkaloid, Saponin, Steroid, flavonoids, glycosides, terpenoid, phenolic and tannins. This study shows that *Garcinia cola,* has all phytochemical constituents which includes Alkaloid, Saponin, flavonoids, glycosides, phenolic and tannins except Steroid and terpenoid. it shows that *Zingiber officinale Roscoe* has all phytochemical constituents which includes Alkaloid, Steroid, flavonoids, terpenoid, glycosides, phenolic and tannins except Saponin and it also shows that *Aframomum melegueta* has all phytochemical constituents which includes Alkaloid, Steroid, flavonoids, terpenoid, glycosides, phenolic and Saponin except tannins. it shows that *Zingiber officinale Roscoe* has all phytochemical constituents which includes Alkaloid, Steroid, flavonoids, terpenoid, glycosides, phenolic and tannins except Saponin and it also shows that *Aframomum melegueta* has all phytochemical constituents which includes Alkaloid, Steroid, flavonoids, terpenoid, glycosides, phenolic and Saponin except tannins. It shows that *Zingiber officinale Roscoe* and *Garcinia cola* has all phytochemical constituents which includes Alkaloid, Tannins , Flavonoids, Terpenoid, Glycosides, Phenolic and Saponin except Steroid and it also shows that *Aframomum melegueta* and *Zingiber officinale Roscoe* has all phytochemical constituents which includes Alkaloid, Flavonoids, Glycosides except Steroid, Saponin, Terpenoid, Tannins and Phenolic. it also shows that *Aframomum melegueta ,Zingiber officinale Roscoe* and *Garcinia cola* has all phytochemical constituents which includes Alkaloid, Tannins, Flavonoids, Phenolic ,Glycosides except Steroid, Saponin and Terpenoid.

The quantitative phytochemical analysis of the *Garcinia cola, Aframomum melegueta* and *Zingiber officinale* extract using Ethanol, Petroleum ether and Aqueous are show in Table 4.3.The quantitative phytochemical test results were presented in the study revealed that the quantitative amount of phytochemicals in medicinal plants are the primary components responsible for the plants pharmacological potentials. Therefore, the plants used for treating upper respiratory tracts infections may be due to significant amount of phytochemicals. The alkaloid content ranged from 0.135– 0.286mg/mL,tannin content ranged from 0.52 - 0.799,saponin content ranged from 0.30 - 0.273, flavonoid content ranged from 0.019 - 0.195, terpenoid content ranged from 0.019 - 0.195, glycosides content ranged from 0.042 - 0.171 and steroid 0.018-0.0157 respectively. Phenol is not significant, hence does not record any value in this study.

The quantitative phytochemical analysis of the combined *Garcinia cola, Aframomum melegueta* and *Zingiber officinale* extract using Ethanol, Petroleum ether and Aqueous are show in Table 4.4.The quantitative phytochemical composition of combined *Garcinia cola*, *Aframomum melegueta* and *Zingiber officinale* extract using ethanol, water and petroleum ether respectively. The tammin content ranged from 0.032 – 0.096. The saponin alkaloid and flavonoid concentration ranged from 0.092 – 0.192mg/mL, 0.028 – 0.327mg/mL, and 0.017 – 0.286mg/mL. the highest saponin alkaloid and flavonoid was recorded in ethanol combined *Aframomum melegueta* and *Zingiber officinale* , petroleum ether combined *Garcinia cola* and *Zingiber officinale* and ethanol combined *Garcinia cola* and *Aframomum melegueta* extracts respectively. The terpenoid and glycosides and phenol content ranged from 0.0115 – 0.241mg/mL, 0.024 – 0.172mg/mL and 0.015 – 0.115mg/mL, Non of phytochemicals was detected in aqeous of combined *Garcinia cola, Aframomum melegueta* and *Zingiber officinale* extracts. The highest was detected in combined ethanol *Garcinia cola, Aframomum melegueta* and *Zingiber officinale* extracts.

4.1: Qualitative Phytochemical Analysis of *Garcinia cola, Aframomum melegueta* and *Zingiber officinale* in Petroleum ether, Ethanol and Aqueous.

Phytochemicals	Petroleum ether			Ethanol			Aqueous		
	G	**A**	**Z**	**G**	**A**	**Z**	**G**	**A**	**Z**
Alkaloid	-	+	-	-	+	++	-	+	+
Tannins	-	-	+	+	-	+	+	-	-
Saponnis	-	-	-	++	+	-	+	++	-
Terperoid	-	-	+	-	+	+	-	+	+
Steroid	-	-	-	-	+	++	-	-	-
Flavonoid	-	+	++	++	+	+	+	++	+
Glycosides	-	-	-	+	+	++	+	+	+
Phenolic	-	-	+	+	-	+	-	+	-

Source: Authors Field Work, 2024

Key: - = Nil + = Moderate ++ = Heavy

G : *Garcinia cola*

A : *Aframomum melegueta*

Z : *Zingiber officinale*

Table 4.2: Qualitative Phytochemical Analysis of *Garcinia cola* and *Aframomum melgueta*, *Aframomum melegueta* and *Zingiber officinale* AND *Garcinia cola*,and *Zingiber officinale*

Phytochemicals	Petroleum ether				Ethanol				Aqueous			
	GA	**AZ**	**GZ**	**GAZ**	**GA**	**AZ**	**GZ**	**GAZ**	**GA**	**AZ**	**GZ**	**GAZ**
Alkaloid	+	-	+	+	+	++	-	++	+	-	-	-
Tannins	-	-	+	-	+	-	-	++	-	-	-	-
Saponnis	-	-	-	-	-	-	+	-	-	-	-	-
Terperoid	-	-	++	-	-	-	-	+	-	-	++	-
Steroid	-	-	-	-	-	+	-	+	-	-	-	-
Flavonoid	-	-	-	+	++	++	+	+	-	+	+	-
Glycosides	-	+	-	+	-	+	-	+	-	+	+	-
Phenolic	-	-	-	-	+	-	+	++	-	-	-	-

Source: Author's Field Work, 2024

Key: - = Nil + = Moderate ++ = Heavy

GA: *Garcinia cola* and *Aframomum melegueta*

AZ: *Aframomum melegueta* and *Zingiber officinale*

GZ: *Garcinia cola* and *Zingiber officinale*

GAZ: *Garcinia cola, Aframomum melegueta* and *Zingiber officinale*

Table 4.3: Quantitative Phytochemical Analysis of *Aframomum melegueta* ,*Zingiber officinale Roscoe* and *Garcinia cola* in Petroleum ether, Ethanol and Aqueous.

Phytochemicals	Petroleum ether			Ethanol			Aqueous		
	G	A	Z	G	A	Z	G	A	Z
Alkaloid	0.000	0.286	0.286	0.000	0.000	0.000	0.000	0.135	0.135
Tannins	0.085	0.00	0.40	0.52	0.0	0.069	0.799	0.00	0.00
Saponnis	0.000	0.84	0.000	0.86	0.273	0.000	0.30	0.91	0.000
Terperoid	0.0000	0.0056	0.019	0.0000	0.0000	0.195	0.0000	0.0073	0.0073
Steroid	0.0000	0.157	0.0000	0.0000	0.018	0.030	0.0000	0.0000	0.0000
Flavonoid	0.625	0.0119	0.000	0.0017	0.0018	0.158	0.0024	0.582	0.0024
Glycosides	0.000	0.042	0.000	0.159	0.000	0.153	0.145	0.149	0.171
Phenolic	0.000	0.000	0.000	0.000	0.000	0.153	0.0000	0.0000	0.0000

Source: Author's Field Work, 2024

G : *Garcinia cola*

A : *Aframomum melegueta*

Z : *Zingiber officinale*

Table 4.4: Quantitative Phytochemical Analysis of *Garcinia cola* and *Aframomum melgueta, Aframomum melegueta* and *Zingiber officinale,Garcinia cola,*and *Zingiber officinale* AND *Garcinia cola, Aframomum melequeta and Zingiber officinale*

Phytochemicals	Petroleum ether				Ethanol				Aqueous			
	GA	AZ	GZ	GAZ	GA	AZ	GZ	GAZ	GA	AZ	GZ	GAZ
Alkaloid	0.086	0.028	0.038	0.068	0.042	0.286	0.000	0.327	0.135	0.000	0.000	0.000
Tannins	0.00	0.096	0.096	0.067	0.032	0.000	0.000	0.000	0.00	0.000	0.00	0.000
Saponnis	0.000	0.092	0.192	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Terperoid	0.0000	0.0000	0.0000	0.0115	0.0000	0.0000	0.0241	0.0000	0.0000	0.0000	0.0000	0.0000
Steroid	0.0065	0.0000	0.0000	0.0059	0.0000	0.0065	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Flavonoid	0.0000	0.0025	0.0125	0.0042	0.288	0.0017	0.017	0.0124	0.0000	0.0024	0.0038	0.0000
Glycosides	0.000	0.000	0.000	0.115	0.000	0.114	0.000	0.113	0.000	0.024	0.172	0.000
Phenolic	0.000	0.054	0.064	0.000	0.015	0.000	0.000	0.115	0.0000	0.0000	0.0000	0.000

Source: Author's Field Work, 2024

GA: *Garcinia cola* and *Aframomum melegueta*

AZ: *Aframomum melegueta* and *Zingiber officinale*

GZ: *Garcinia cola* and *Zingiber officinale*

GAZ: *Garcinia cola, Aframomum melegueta* and *Zingiber officinale*

The results of the zone of inhibition of *Garcinia cola* extract against respiratory pathogenic bacteria are shown in Table 4.5. The antibacterial activity of *Garcinia cola* extracts against selected upper respiratory tract pathogens at 100mg/mL,66.7 mg/mL,50 mg/mL of the extracts, the antibacterial activity ranged from 9.1±3.61–14.4 ± 1.5mm. at 100mg/mL, ethanol extracts had the highest activity against *Klebsiella pneumonia* at 14.4 ± 1.5mm and lowest activity against *Streptococcus pneumonia* at 12.8±2.43mm. Petroleum ether extract had the highest activity against *Staphylococcus aureus* at 12.1 ±0.05mm and lowest activity against *Streptococcus pneumonia* at 10.1±2.44mm. At 66.7mg/mL of the extract, ethanol extract had the highest activity against *Klebsiella pneumonia* at 13.7 ± 1.2mm and *Pseudomonas aeuroginosa* 12.7±0.04mm and lowest activity against *Streptococcus pneumonia* at 10.1±2.44mm. At 40mg/mL of the extract concentration none of the isolates were susceptible to the extract. The Aqueous extracts were not reactive against any of the bacterial isolates.Antibacterial activity concentrations in this table are significantly different from each other and slightly better than *Zingiber officinalle* and combination of *Zingiber officinalle* and *Aframomum melegueta.*

Table 4.5: Antibacterial Activity of *Garcinia cola* Extract on Selected Upper Respiratory Tracts Microorganisms in Human

			Antibacterial Activity/Extract Concentration (mg/mL)				
	Isolates of Bacteria	Extraction Solvent	100	66.7	50 40		Control
1	*Pseudomonas aeuroginosa*	Ethanol	13.1±0.05[b]	12.7±0.04[a]	11.4±0.55[a] -		-
		Petroleum ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
2	*Streptococcus pneumonia*	Ethanol	12.8±2.43[b]	10.1±2.44[a]	9.1±3.61[a]	-	-
		Petroleum ether	10.1±0.05[a]	-	-	-	-
		Aqueous	-	-	-	-	-
3	*Klebsiella pneumonia*	Ethanol	14.4 ± 1.5[c]	13.7 ± 1.2[a]	12.8±1.5[a]	-	-
		Petroleum ether	-	-	-		-
		Aqueous	-	-	-	-	-
4	*Staphylococcus aureus*	Ethanol	13.5±1.5[b]	11.1 ± 2.2[a]	9.8 ± 3.2[a]	-	-
		Petroleum ether	12.1 ±0.05[b]	-	-	-	-
		Aqueous	-	-	-	-	-

Source: Authors Field Work, 2024

Key: n-3: mean ± (standard error) = negative; stock = 200g of extract + 1000ml of solvent, 66.7mg/ml = 10ml of extract + 5ml of solvent, 50mg/ml = 10ml of extract + 10ml of solvent, 40mg/ml = 10ml of extract + 15ml of solvent and control. - = Nill

Values with different superscripts in the column differ significantly for the variety and the extraction solvent. While values with same superscripts in the column do not differ significantly for the variety and the extraction solvent.

a-no significant different between means p values >0.05

b-significant different between means p values <0.05

c-significant different between means p values <0.05

The results of the zone of inhibition of *Aframomum melegueta* extract against respiratory pathogenic bacteria are shown in Table 4.6. The antibacterial activity of *Aframomum melegueta* extracts against the selected upper respiratory tracts pathogen at 100mg/mL,66.7 mg/mL,50 mg/mL of the extracts, the antibacterial activity ranged from 11.1 ± 0.05– 24.1±0.05mm. At 100mg/mL, ethanol extracts had the highest activity against *Pseudomonas aeuroginosa* at 24.1±0.05mm and lowest activity against *Staphylococcus aureus* at 19.5 ± 1.12mm. Petroleum ether extract had the highest activity against Staphylococcus aureus at 12.5± 0.60mm and was not active against any of the other pathogens . At 66.7mg/mL of the extract, ethanol extract had the highest activity against *Streptococcus pneumonia* at 15.8±2.02mm and *Pseudomonas aeuroginosa* 15.4±2.28mm and lowest activity against *Klebsiella pneumonia* at 11.8±0.55 mm. Petroleum ether was not active against any of the pathogens. At 50mg/mL of the extract, ethanol extract had the highest activity against *Pseudomonas aeuroginosa* at 14.8±3.05mm and *Staphylococcus aureus* at 13.1± 0.05mm and lowest activity against *Klebsiella pneumonia* at 11.1± 0.05mm. Petroleum ether was not active against any of the pathogens. At 40mg/mL of the extract concentration none of the isolates were susceptible to the extract. The Aqueous extracts were not reactive against any of the bacterial isolates. Minimum inhibitory concentrations in this table are significantly different from each other and slightly better than *Zingiber officinalle* and *Garcinia cola* combination of *Zingiber officinalle* and *Garcinia cola*

Table 4.6: Antibacterial Activity of *Aframomum Melegueta* **On Selected Upper Respiratory Tracts Microorganisms In Human**

			Antibacterial Activity/Extract Concentration (mg/mL)				
	Isolates of Bacteria	Extraction Solvent	100	66.7	50	40	Control
1	*Pseudomonas aeuroginosa*	Ethanol	24.1±0.05^{bc}	15.4±2.28^{c}	14.8±3.05^{a}	-	-
		Petroleum ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
2	*Streptococcus pneumonia*	Ethanol	22.8 ± 1.09^{b}	15.8±2.02^{a}	12.1 ± 0.05^{a}	-	-
		Petroleum ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
3	*Klebsiella pneumonia*	Ethanol	22.1± 0.05^{bc}	11.8 ±0.55^{a}	11.1 ± 0.05^{a}	-	-
		Petroleum ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
4	*Staphylococcus aureus*	Ethanol	19.5 ± 1.12^{c}	15.1 ±0.05^{b}	13.1 ± 0.05^{a}	-	-
		Petroleum ether	12.5 ± 0.60^{b}	-	-	-	-
		Aqueous	-	-	-	-	-

Source: Authors Field Work, 2024

Key: n-3: mean ± (standard error) = negative; stock = 200g of extract + 1000ml of solvent, 66.7mg/ml = 10ml of extract + 5ml of solvent, 50mg/ml = 10ml of extract + 10ml of solvent, 40mg/ml = 10ml of extract + 15ml of solvent and control. - = nill
Values with different superscripts in the column differ significantly for the variety and the extraction solvent. While values with same superscripts in the column do not differ significantly for the variety and the extraction solvent.

a-no significant different between means p values >0.05

b-significant different between means p values <0.05

c-significant different between means p values <0.05

The results of the zone of inhibition of *Zingiber officinale Roscoe* extract against respiratory pathogenic bacteria are shown in Table 4.7. The antibacterial activity of *Zingiber officinale Roscoe* extracts against selected upper respiratory tract pathogens at 100mg/mL,66.7 mg/mL,50 mg/mL of the extracts, the antibacterial activity ranged from 4.1±3.55–24.5±1.12mm. At 100mg/mL, ethanol extracts had the highest activity against *Staphylococcus aureus* 24.5± 1.12mm and *Klebsiella pneumonia* at 22.1±0.05mm and lowest activity against *Pseudomonas aeuroginosa* at 13.8±0.60mm. Petroleum ether extract had the highest activity against *Streptococcus pneumonia* at 11.4±0.57mm and lowest activity against *Staphylococcus aureus* 9.5± 1.12mm.At 66.7mg/mL of the extract, ethanol extract had the highest activity against *Staphylococcus aureus* at 14.8±1.18mm and *Klebsiella pneumonia* at 13.1± 1.67mm and lowest activity against *Pseudomonas aeuroginosa* at 11.8±0.60mm. Petroleum ether was active against *Streptococcus pneumonia* at 10.8±0.60mm and was not active against any other pathogens. At 50mg/mL of the extract, ethanol extract had the highest activity against *Klebsiella pneumonia* at 11.5± 2.25mm and *Staphylococcus aureus* at 10.8±0.51mm and lowest activity against *Streptococcus pneumonia* at 6.1±0.05mm and *Pseudomonas aeuroginosa* at 4.1±3.55mm. Petroleum ether was not active against any of the pathogens. At 40mg/mL of the extract concentration none of the isolates were susceptible to the extract. The Aqueous extracts were not reactive against any of the bacterial isolates. Minimum inhibitory concentrations in this table are slightly significantly different from each other and shows little better antibacterial activity than *Garcinia cola*

Table 4.7: Antibacterial Activity of *Zingiber Officinale Roscoe* Extract On Selected Upper Respiratory Tracts Microorganisms

			Antibacterial Activity/Extract Concentration (mg/mL)				
	Isolates of Bacteria	Extraction Solvent	100	66.7	50	40	Control
1	*Pseudomonas aeuroginosa*	Ethanol	13.8±0.60 [a]	11.8±0.60 [a]	4.1±3.55[a]	-	-
		Petroleum ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
2	*Streptococcus pneumonia*	Ethanol	14.4±1.57[b]	12.5±1.47 [a]	6.1±0.05[a]	-	-
		Petroleum ether	11.4±0.57 [a]	10.8 ±0.60 [a]	-	-	-
		Aqueous	-	-	-	-	-
3	*Klebsiella pneumonia*	Ethanol	22.1± 0.05[c]	13.1± 1.67[b]	11.5± 2.25[a]	-	-
		Petroleum ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
4	*Staphylococcus aureus*	Ethanol	24.5 ± 1.12[bc]	14.8 ±1.18[a]	10.8 ±0.51[a]	-	-
		Petroleum ether	9.5 ± 1.12[a]	-	-	-	-
		Aqueous	-	-	-	-	-

Source: Author's Field Work, 2024

Key: n-3: mean ± (standard error) = negative; stock = 200g of extract + 1000ml of solvent, 66.7mg/ml = 10ml of extract + 5ml of solvent, 50mg/ml = 10ml of extract + 10ml of solvent, 40mg/ml = 10ml of extract + 15ml of solvent and control. - = Nill

Values with different superscripts in the column differ significantly for the variety and the extraction solvent. While values with same superscripts in the column do not differ significantly for the variety and the extraction solvent.

a-no significant different between means p values >0.05

b-significant different between means p values <0.05

c-significant different between means p values <0.05

The results of the zone of inhibition of *Garcinia cola and Aframomum melegueta* extract against respiratory pathogenic bacteria are shown in Table 4.8. The antibacterial activity of *Garcinia cola and Aframomum melegueta* extracts against selected upper respiratory tract pathogens at 100mg/mL,66.7 mg/mL,50 mg/mL of the extracts, the antibacterial activity ranged from 9.4±0.55 – 24.2± 1.73mm. At 100mg/mL, ethanol extracts had the highest activity against *Staphylococcus aureus* 24.2± 1.73mm and *Klebsiella pneumonia* at 23.1± 1.67mm and lowest activity against *Streptococcus pneumonia* at 12.1±0.05mm. Petroleum ether extract had the highest activity against *Staphylococcus aureus* at 13.4±1.58mm and lowest activity against *Streptococcus pneumonia* at 9.4±0.55mm.At 66.7mg/mL of the extract, ethanol extract had the highest activity against *Klebsiella pneumonia* at 19.8±1.58mm and *Streptococcus pneumonia* at 15.2±0.51mm and lowest activity against *Pseudomonas aeuroginosa* at 12.5±0.51mm. Petroleum ether was active against *Staphylococcus aureus* at 12.5±1.57mm and was not active against any other pathogens. At 50mg/mL of the extract, ethanol extract had the highest activity against *Streptococcus pneumonia* at 12.8±1.18mm and *Staphylococcus aureus* at 13.1±1.78mm and lowest activity against *Klebsiella pneumonia* at 11.1± 0.05mm and *Pseudomonas aeuroginosa* at 9.8±0.60mm. Petroleum ether was active against *Staphylococcus aureus* at 9.4±0.55mm and was not active against any other pathogens. At 40mg/mL of the extract concentration none of the isolates were susceptible to the extract. The Aqueous extracts were not reactive against any of the bacterial isolates. Minimum inhibitory concentrations in this table are slightly significantly different from each other and shows better antibacterial activity than single plants used.

Table 4.8: Antibacterial Activity of *Garcinia cola* and *Aframomum Melegueta* **Extract On Selected Upper Respiratory Tract Microorganisms**

	Antibacterial Activity/Extract Concentration (mg/mL)						
	Isolates of Bacteria	Extraction Solvent	100	66.7	50	40	Control
1	*Pseudomonas aeuroginosa*	Ethanol	15.1±0.05[a]	12.5±0.51[a]	9.8±0.60[a]	-	-
		Petroleum ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
2	*Streptococcus pneumonia*	Ethanol	18.1±2.05[b]	15.2±0.51[a]	12.8±1.18[a]	-	-
		Petroleum ether	12.1±0.05[a]	-	-	-	-
		Aqueous	-	-	-	-	-
3	*Klebsiella pneumonia*	Ethanol	23.1 ± 1.67[b]	19.8 ±1.58[b]	11.1 ± 0.05[a]	-	-
		Petroleum ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
4	*Staphylococcus aureus*	Ethanol	24.2 ± 1.73[c]	14.5 ±2.04[a]	13.1 ±1.78[a]	-	-
		Petroleum ether	13.4 ±1.58[a]	12.5 ±1.57[a]	9.4±0.55[a]	-	-
		Aqueous	-	-	-	-	-

Source: Author's Field Work, 2024

Key: n-3: mean±(standard error) = negative; stock = 200g of extract + 1000ml of solvent, 66.7mg/ml = 10ml of extract + 5ml of solvent, 50mg/ml = 10ml of extract + 10ml of solvent, 40mg/ml = 10ml of extract + 15ml of solvent and control. - = Nill

Values with different superscripts in the column differ significantly for the variety and the extraction solvent. While values with same superscripts in the column do not differ significantly for the variety and the extraction solvent.

a-no significant different between means p values >0.05

b-significant different between means p values <0.05

c-significant different between means p values <0.05

The results of the zone of inhibition of *Garcinia cola* and *Zingiber officinale Roscoe* extract against respiratory pathogenic bacteria are shown in Table 4.9. The antibacterial activity of *Garcinia cola* and *Zingiber officinale Roscoe* extracts against selected upper respiratory tract pathogens at 100mg/mL,66.7 mg/mL,50 mg/mL, 40mg/mL of the extracts, the antibacterial

activity ranged from 3.06±2.70 – 23.23±0.05mm. At 100mg/mL, ethanol extracts had the highest activity against *Staphylococcus aureus* 23.23±0.05mm and *Pseudomonas aeuroginosa* at 16.1±0.05mm and lowest activity against *Streptococcus pneumonia* at 13.8±2.25mm. Petroleum ether extract had the highest activity against *Staphylococcus aureus* at 17.2± 5.08 mm and lowest activity against *Streptococcus pneumonia* at 11.16±0.05mm. At 66.7mg/mL of the extract, ethanol extract had the highest activity against *Staphylococcus aureus* at 20.1±0.05mm and *Pseudomonas aeuroginosa* at 12.1±0.05mm and lowest activity against *Streptococcus pneumonia* at 10.4±4.01mm. Petroleum ether was active against *Staphylococcus aureus* at 11.8± 2.85mm and lowest activity against *Streptococcus pneumonia* at 9.4± 1.18mm. At 50mg/mL of the extract, ethanol extract had the highest activity against *Staphylococcus aureus* at 13.1± 0.05mm and *Pseudomonas aeuroginosa* at 10.7±0.57mm and lowest activity against *Klebsiella pneumonia* at 10.4±4.01mm and *Streptococcus pneumonia* at 8.8±2.85mm. Petroleum ether had the highest activity against *Staphylococcus aureus* at 8.1± 2.58mm and lowest against *Streptococcus pneumonia* at 3.06±2.70mm. At 40mg/mL of the extract concentration none of the isolates were susceptible to the extract. The Aqueous extracts were not reactive against any of the bacterial isolates. Minimum inhibitory concentrations in this table are slightly significantly different from each other and shows better antibacterial activity than single plants used.

Table 4.9: Antibacterial Activity of *Garcinia Cola* And *Zingiber Officinale Roscoe* Extract on Selected Upper Respiratory Tracts Microorganisms

			Antibacterial Activity/Extract Concentration (mg/mL)				
	Isolates of Bacteria	Extraction Solvent	100	66.7	50	40	Control
1	*Pseudomonas aeuroginosa*	Ethanol	16.1±0.05[b]	12.1±0.05[a]	10.7±0.57[a]	-	-

		Petroleum ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
2	*Streptococcus pneumonia*	Ethanol	13.8±2.25[b]	10.4±4.01[a]	8.8±2.85[a]	-	-
		Petroleum ether	11.16±0.05[a]	9.4 ± 1.18[a]	3.06±2.70[a]	-	-
		Aqueous	-	-	-	-	-
3	*Klebsiella pneumonia*	Ethanol	15.8 ± 0.57[b]	12.5 ±0.51[a]	10.4± 0.55[a]	-	-
		Petroleum ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
4	*Staphylococcus aureus*	Ethanol	23.23±0.05[bc]	20.1±0.05[b]	13.1 ± 0.05[a]	-	-
		Petroleum ether	17.2 ± 5.08[b]	11.8 ± 2.85[a]	8.1 ± 2.58[a]	-	-
		Aqueous	-	-	-	-	-

Source: Author's Field Work, 2024

Key: n-3: mean±(standard error) = negative; stock = 200g of extract + 100ml of solvent, 66.7mg/ml = 10ml of extract + 5ml of solvent, 50mg/ml = 10ml of extract + 10ml of solvent, 40mg/ml = 10ml of extract + 15ml of solvent and control. - = Nill

Values with different superscripts in the column differ significantly for the variety and the extraction solvent. While values with same superscripts in the column do not differ significantly for the variety and the extraction solvent.

a-no significant different between means p values >0.05

b-significant different between means p values <0.05

c-significant different between means p values <0.05

The results of the zone of inhibition of *Aframomum melegueta* and *Zingiber officinale Roscoe* extract against respiratory pathogenic bacteria are shown in Table 4.10. The antibacterial activity of *Aframomum melegueta* and *Zingiber officinale Roscoe* extracts against the test upper respiratory tracts pathogen at 100mg/mL,66.7 mg/mL,50 mg/mL of the extracts, the antibacterial activity ranged from 7.06±6.14 – 20.2± 4.35mm. At 100mg/mL, ethanol extracts had the highest activity against *Klebsiella pneumonia* at 20.2± 4.35mm and *Staphylococcus aureus* 14.5± 1.18mm and lowest activity against *Pseudomonas aeuroginosa* at 7.06±6.14

mm. Petroleum ether extract had the highest activity against *Streptococcus pneumonia* at 14.2±1mm and lowest activity against *Staphylococcus aureus* 12.5±0.60mm. At 66.7mg/mL of the extract, ethanol extract had the highest activity against *Klebsiella pneumonia* at 15.1±0.05mm and *Pseudomonas aeuroginosa* at 12.4±0.57mm and lowest activity against *Streptococcus pneumonia* at 11.1±0.95mm. Petroleum ether was not active against any other pathogens. At 50mg/mL of the extract, ethanol extract had the highest activity against *Klebsiella pneumonia* at 8.06± 6.98mm and lowest activity against *Pseudomonas aeuroginosa* at 7.06±6.14mm. Petroleum ether was not active against any of the pathogens. At 40mg/mL of the extract concentration none of the isolates were susceptible to the extract. The Aqueous extracts were not reactive against any of the bacterial isolates.It has the lowest mean antibacterial activity compared to other,these combination of extracts is significantly less effective than other plant extracts.

Table 4.10: Antibacterial Activity of *Aframomum Melegueta* And *Zingiber Officinale Roscoe* **Extract On Selected Upper Respiratory Tracts Microorganisms**

			Antibacterial Activity/Extract Concentration (mg/mL)				
	Bacteria isolates	Extraction Solvent	100	66.7	50	40	Control
1	*Pseudomonas aeuroginosa*	Ethanol	14.1±0.05[a]	12.4±0.57[a]	7.06±6.14[a]	-	-
		Petroleum ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
2	*Streptococcus pneumonia*	Ethanol	12.5±0.60[b]	11.1±0.95[a]	-	-	-
		Petroleum ether	14.2±1[a]	-	-	-	-

		Aqueous	-	-	-	-	-
3	*Klebsiella pneumonia*	Ethanol	20.2 ± 4.35[b]	15.1 ±0.05[a]	8.06 ± 6.98[a]	-	-
		Petroleum ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
4	*Staphylococcus aureus*	Ethanol	14.5 ± 1.18[b]	11.4 ± 0.55[a]	-	-	-
		Petroleum ether	12.5 ± 0.05[b]	-	-	-	-
		Aqueous	-	-	-	-	-

Source: Author's Field Work, 2024

Key: n-3: mean ±(standard error) = negative; stock = 200g of extract + 1000ml of solvent, 66.7mg/ml = 10ml of extract + 5ml of solvent, 50mg/ml = 10ml of extract + 10ml of solvent, 40mg/ml = 10ml of extract + 15ml of solvent and control. - = nill

Values with different superscripts in the column differ significantly for the variety and the extraction solvent. While values with same superscripts in the column do not differ significantly for the variety and the extraction solvent.

a-no significant different between means p values >0.05

b-significant different between means p values <0.05

c-significant different between means p values <0.05

The results of the zone of inhibition of *Garcinia cola*, *Aframomum melegueta* and *Zingiber officinale Roscoe* extract against respiratory pathogenic bacteria are shown in Table 4.11. The antibacterial activity of *Garcinia cola*, *Aframomum melegueta* and *Zingiber officinale Roscoe* extracts against selected upper respiratory tracts pathogen at 100mg/mL,66.7 mg/mL,50 mg/mL of the extracts, the antibacterial activity ranged from 12.1±0.05– 26.1±0.05mm. At 100mg/mL, ethanol extracts had the highest activity against *Staphylococcus aureus* 26.1±0.05mm, *Streptococcus pneumonia* at 25.8±0.55mm and *Pseudomonas aeuroginosa* at 24.5±3.78mm and lowest activity against *Klebsiella pneumonia* at 21.1±0.05mm. Petroleum

ether extract had the highest activity against Staphylococcus aureus at 25.125.1±0.05mm and lowest activity against *Streptococcus pneumonia* 15.2± 0.05mm.

At 66.7mg/mL of the extract, ethanol extract had the highest activity against *Pseudomonas aeuroginosa* at 20.1±3.05mm and *Streptococcus pneumonia* at 17.1±0.57mm and lowest activity against *Staphylococcus aureus* at 15.1±0.05mm. Petroleum ether was active against *Staphylococcus aureus* at 18.5±1.4mm and was not active against any other pathogens. At 50mg/mL of the extract, ethanol extract had the highest activity against *Pseudomonas aeuroginosa* at 18.5±2.85mm and *Streptococcus pneumonia* at 15.1±0.05mm and lowest activity against *Staphylococcus aureus* at 12.1±0.05mm. Petroleum ether was active against *Staphylococcus aureus* at 13.38±2.13mm and was not active against any other pathogens. At 40mg/mL of the extract concentration none of the isolates were susceptible to the extract. The Aqueous extracts were not reactive against any of the bacterial isolates.It has the highest antibacterial activity. It is significantly more effective than other extracts and combinations.

Table 4.11: *Garcinia cola, Aframomum melegueta* And *Zingiber officinale* Extract's Antibacterial Activity Against Selected Upper Respiratory Tracts Pathogenic Microorganisms

			Antibacterial Activity/Extract Concentration (mg/mL)				
	Bacteria Isolates	Extraction Solvent	100	66.7	50	40	Control
1	*Pseudomonas aeuroginosa*	Ethanol	24.5±3.78^{bc}	20.1±3.05^{b}	18.5±2.85^{b}	-	-
		Petroleum ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
2	*Streptococcus pneumonia*	Ethanol	25.8±0.55^{b}	17.1±0.57^{b}	15.1±0.05^{a}	-	-
		Petroleum ether	15.2± 0.05^{b}	-	-	-	-
		Aqueous	-	-	-	-	-

3	*Klebsiella pneumonia*	Ethanol	21.1±0.05^{b}	16.8±1.15^{a}	14.1±0.05^{a}	-	-
		Petroleum ether	-	-	-	-	-
		Aqueous	-	-	-	-	-
4	*Staphylococcus aureus*	Ethanol	26.1±0.05^{bc}	15.1±0.05^{b}	12.1±0.05^{a}	-	-
		Petroleum ether	25.1±0.05^{b}	18.5±1.4^{b}	13.38±2.13^{a}	-	-
		Aqueous	-	-	-	-	-

Source: Author's Field Work, 2024

Key: n-3: mean ± (standard error) = negative; stock = 200g of extract + 1000ml of solvent, 66.7mg/ml = 10ml of extract + 5ml of solvent, 50mg/ml = 10ml of extract + 10ml of solvent, 40mg/ml = 10ml of extract + 15ml of solvent and control. - = Nill

Values with different superscripts in the column differ significantly for the variety and the extraction solvent. While values with same superscripts in the column do not differ significantly for the variety and the extraction solvent.

a-no significant different between means p values >0.05

b-significant different between means p values <0.05

c-significant different between means p values <0.05

The present study evaluated the minimum inhibitory concentrations (MICs) of Petroleum ether and ethanol, Aqueous extracts of *Aframomum melegueta*, *Garcinia cola*, and *Zingiber officinale* against four clinical bacterial isolates: *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Streptococcus pneumoniae*. The degree of bacterial growth inhibition was recorded as No Growth (NG), Little Growth (LG), or Growth (G) across concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL.

Both Aqueous and Petroleum ether extracts of all three plant samples showed no significant antibacterial activity against any of the tested bacterial strains. Across all concentrations, all isolates (*Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Streptococcus pneumoniae*) demonstrated consistent growth (G). This suggests that the

petroleum ether extracts lacked effective non-polar phytochemicals capable of exerting bacteriostatic or bactericidal effects. The result may also reflect the limited solubility or extraction efficiency of petroleum ether for antimicrobial compounds, especially those targeting Gram-negative bacteria with complex outer membrane

In contrast, the ethanol extracts exhibited significant antibacterial activity, particularly against Gram-positive organisms. For *Staphylococcus aureus*, complete inhibition (NG) was observed at all concentrations with the *Aframomum melegueta* and *Garcinia cola* ethanol extracts, indicating a MIC ≤12.5 mg/mL. The *Zingiber officinale* ethanol extract also showed complete inhibition at 100, 50, and 25 mg/mL, with little growth (LG) at 12.5 mg/mL, suggesting its MIC lies between 12.5 and 25 mg/mL.

Klebsiella pneumoniae showed complete inhibition (NG) at all concentrations with *Aframomum melegueta* and *Garcinia cola* extracts, while *Zingiber officinale* extract caused little growth at the lowest concentration (12.5 mg/mL), indicating relatively high efficacy across all three extracts. Similarly, *Pseudomonas aeruginosa* was inhibited by *Aframomum melegueta* and *Garcinia cola* extracts at all concentrations, with some partial growth (LG) observed for *Garcinia cola* and *Zingiber officinale* at lower doses, suggesting moderate activity.

For *Streptococcus pneumoniae* , *Aframomum melegueta* extract showed the most potent activity, with no growth at all concentrations. *Garcinia cola* and *Zingiber officinale* extracts exhibited complete inhibition at 100 and 50 mg/mL, and partial inhibition (LG) at 25 and 12.5 mg/mL, indicating MIC values around 50 mg/mL.

Overall, the ethanol extracts demonstrated superior antibacterial activity compared to petroleum ether and aqeous extracts, supporting the hypothesis that polar solvents such as ethanol are more effective in extracting bioactive phytochemicals with antimicrobial

properties. The difference in activity may also be attributed to the chemical nature and polarity of active constituents present in the plant materials. Ethanol-extracted compounds appeared more potent against Gram-positive bacteria, though notable inhibition of Gram-negative organisms (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) was also observed, especially with *Aframomum melegueta* extracts.

These findings underscore the therapeutic potential of ethanol extracts of *Aframomum melegueta*, *Garcinia cola*, and *Zingiber officinale*, particularly against antibiotic-resistant Gram-positive pathogens. Further phytochemical analysis and purification of active compounds are recommended to identify the bioactive constituents responsible for the antimicrobial effects.

For the various combinations of *Aframomum melegueta*, *Garcinia cola*, and *Zingiber officinale* plant extracts prepared in petroleum ether and ethanol, using the Minimum Inhibitory Concentration (MIC) method. Results were interpreted based on growth responses, where No Growth (NG) indicates strong antimicrobial activity, Limited Growth (LG) indicates partial inhibition, and Growth (G) indicates minimal or no inhibitory effect.

Among the petroleum ether combinations, *Aframomum melegueta* & *Garcinia cola* showed the most consistent antibacterial activity, producing NG against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pnuemonia* across all tested concentrations. *Klebsiella*, however, displayed greater resistance, with LG observed at intermediate concentrations and full Growth at the lowest (12.5 mg/mL), indicating a higher MIC threshold. The *Garcinia cola* & *Zingiber officinale* and *Zingiber officinale* & *Aframomum melegueta* combinations also demonstrated notable activity, particularly at higher concentrations, but showed decreasing efficacy as the concentration declined. This trend highlights a dose-dependent response in the inhibition of bacterial growth.

Interestingly, ethanol-based combinations exhibited broader and more consistent antibacterial effects compared to their petroleum ether counterparts. The *Aframomum melegueta* & *Garcinia cola* and *Zingiber officinale* & *Aframomum melegueta* ethanol extracts maintained NG across all tested concentrations for all four bacterial strains, suggesting the presence of potent ethanol-soluble bioactive compounds. The *Garcinia cola* & *Zingiber officinale* combination was similarly effective, with strong inhibition observed across concentrations, especially against *Staphylococcus aureus* and *Streptococcus pnuemonia*. This enhanced efficacy in ethanol-extracted combinations may be attributed to the solvent's ability to extract a wider range of polar and moderately polar antimicrobial compounds.

Across all extracts, Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pnuemonia*) were generally more susceptible than Gram-negative species (*Klebsiella pnuemonia* and *Pseudomonas aeruginosa*), which aligns with known structural differences. The outer membrane of Gram-negative bacteria can restrict the penetration of antimicrobial agents, often requiring higher doses or more active compounds for effective inhibition.

Overall, these findings demonstrate that plant extract combinations, particularly when extracted in ethanol, exhibit synergistic and potent antibacterial activity. The consistent NG results observed even at low concentrations suggest potential for therapeutic applications. These results support the hypothesis that certain plant extract combinations can act synergistically to inhibit bacterial growth more effectively than individual extracts alone.

Future research should focus on identifying the active constituents responsible for the observed antimicrobial effects, evaluating their mechanisms of action, and exploring their efficacy in vivo. Additionally, evaluating the potential cytotoxicity of these combinations will be essential for developing safe, plant-based antimicrobial therapies.

The Minimum Bactericidal Concentration (MBC) assay was conducted to determine the lowest concentration of various plant extracts capable of killing selected bacterial isolates. The extracts tested included Petroleum Ether, Ethanol, and aqueous extracts of *Garcinia cola*, *Zingiber officinale*, and *Aframomum melegueta*. Bacterial strains assessed were *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Streptococcus pneumoniae*.

The petroleum ether extract of *Garcinia cola* and *Zingiber officinale* exhibited strong bactericidal activity across all concentrations tested (100 mg/mL to 3.12 mg/mL), indicated by growth inhibition symbols ('T' for turbidity and 'G' for growth). Notably, *Zingiber officinale* showed consistent activity down to 6.25 mg/mL for *Staphylococcus aureus* and *Streptococcus pneumonia*, highlighting its potent antibacterial effect. In contrast, *Pseudomonas aeruginosa* exhibited resistance at lower concentrations, reflecting its known intrinsic resistance mechanisms.

Ethanol extracts demonstrated variable activity. The ethanol extract of *Garcinia cola* and *Zingiber officinale* showed effectiveness primarily at higher concentrations (100–25 mg/mL),

with no turbidity ('NT') observed in many wells, indicating bactericidal effects. However, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* remained largely resistant to ethanol extracts, consistent with their outer membrane barrier against polar compounds.

Among the tested organisms, *Staphylococcus aureus* was the most susceptible to all extracts, while *Pseudomonas aeruginosa* displayed the highest level of resistance, particularly to ethanol-based preparations. These findings suggest that non-polar components present in petroleum ether extracts may contribute more significantly to the bactericidal properties of these plants.

In summary, *Zingiber officinale* and *Garcinia cola* demonstrated broad-spectrum bactericidal activity, especially in petroleum ether extracts, indicating their potential as sources of natural antimicrobial agents. However, further studies including compound isolation and mechanism elucidation are needed to substantiate their therapeutic potential.

The investigation into the bactericidal efficacy of combination extracts revealed insightful interactions between plant components, highlighting potential synergistic or additive effects. Combinations such as *Aframomum melegueta* / *Garcinia cola*, *Garcinia cola*/ *Zingiber officinale*, and *Zingiber officinale* / *Aframomum melegueta* were evaluated using different solvents: petroleum ether and ethanol.

The petroleum ether extract of the *Garcinia cola*/ *Zingiber officinale* combination demonstrated the most promising activity, showing bactericidal effects down to 6.25 mg/mL against *Staphylococcus aureus* and *Streptococcus pnuemonia*, as evidenced by the absence of turbidity (denoted as "NT"). This indicates that the lipophilic compounds in these plants might interact synergistically, enhancing antimicrobial potency. Similarly, the *Zingiber officinale* / *Aframomum melegueta* petroleum ether extract exhibited moderate efficacy, particularly against *Streptococcus pneumoniae*,while showing reduced activity against

Klebsiella pneumoniae and *Pseudomonas aeruginosa*, organisms that are typically more resistant to plant-based treatments.

Ethanol extracts of the combinations revealed limited bactericidal activity, particularly at lower concentrations. While *Aframomum melegueta /Garcinia cola* showed some activity at higher concentrations (100–25 mg/mL), the efficacy dropped significantly at more dilute levels. This trend reinforces previous observations that ethanol extracts generally have reduced effectiveness compared to petroleum ether extracts, possibly due to the poor solubility of key antimicrobial phytochemicals in polar solvents.

Pseudomonas aeruginosa remained the most resistant across all combinations and solvents, a finding consistent with its well-documented multidrug resistance mechanisms and robust outer membrane barrier. In summary, the petroleum ether extracts of plant combinations, particularly *Garcinia cola/Zingiber officinale*, exhibited enhanced bactericidal effects, suggesting possible synergism. These findings support the potential of using plant extract combinations to overcome resistance and broaden antimicrobial spectra.

Table 4.12: Minimum Inhibitory Concentration (MIC) of *Garcinia cola* Extract on Selected Upper Respiratory Tracts Microorganisms in Human

		Antibacterial Activity/Extract Concentration (mg/mL)					
	Isolates of Bacteria	Extraction Solvent	100	50	25	12.5	Control
1	*Pseudomonas aeuroginosa*	Ethanol	NG	NG	NG	NG	-
		Petroleum ether	G	G	G	G	-
		Aqueous	-	-	-	-	-
2	*Streptococcus pneumonia*	Ethanol	NG	NG	LG	LG	-
		Petroleum ether	G	G	G	G	-
		Aqueous	-	-	-	-	-
3	*Klebsiella pneumonia*	Ethanol	NG	NG	NG	NG	-
		Petroleum ether	G	G	G	G	-
		Aqueous	-	-	-	-	-
4	*Staphylococcus aureus*	Ethanol	NG	NG	NG	NG	-
		Petroleum ether	G	G	G	G	-
		Aqueous	-	-	-	-	-

Source: Authors Field Work, 2024

KEY: G:Growth, NG: No Growth, LG: Little Growth,

Table 4.13: Minimum Inhibitory Concentration (MIC) of *Aframomum melegueta* Extract on Selected Upper Respiratory Tracts Microorganisms in Human

		Antibacterial Activity/Extract Concentration (mg/mL)				
	Isolates of Bacteria	Extraction Solvent	100	50	25 12.5	Control
1	*Pseudomonas aeuroginosa*	Ethanol	NG	NG	NG NG	-
		Petroleum ether	G	G	G G	-
		Aqueous	-	-	- -	-
2	*Streptococcus pneumonia*	Ethanol	NG	NG	NG NG	-
		Petroleum ether	G	G	G G	-
		Aqueous	-	-	- -	-
3	*Klebsiella pneumonia*	Ethanol	NG	NG	NG NG	-
		Petroleum ether	G	G	G G	-
		Aqueous	-	-	- -	-
4	*Staphylococcus aureus*	Ethanol	NG	NG	NG NG	-
		Petroleum ether	G	G	G G	-
		Aqueous	-	-	- -	-

Source: Authors Field Work, 2024

KEY: G:Growth, NG: No Growth, LG: Little Growth,

Table 4.14: MIC Activity of *Zingiber officinale* Extract on Selected Upper Respiratory Tracts Microorganisms in Human

		Antibacterial Activity/Extract Concentration (mg/mL)					
	Isolates of Bacteria	Extraction Solvent	100	50	25	12.5	Control
1	*Pseudomonas aeuroginosa*	Ethanol	G	G	G	G	-
		Petroleum ether	G	G	G	G	-
		Aqueous	-	-	-	-	-
2	*Streptococcus pneumonia*	Ethanol	NG	NG	LG	LG	-
		Petroleum ether	G	G	G	G	-
		Aqueous	-	-	-	-	-
3	*Klebsiella pneumonia*	Ethanol	NG	NG	NG	NG	-
		Petroleum ether	G	G	G	G	-
		Aqueous	-	-	-	-	-
4	*Staphylococcus aureus*	Ethanol	NG	NG	NG	NG	-
		Petroleum ether	G	G	G	G	-
		Aqueous	-	-	-	-	-

Source: Authors Field Work, 2024

KEY: G:Growth, NG: No Growth, LG: Little Growth,

Table 4.14B: Minimum Inhibitory Concentration (MIC) Activity of Petroleum Ether *Garcinia cola* and *Aframomum melgueta, Aframomum melegueta* and *Zingiber officinale,Garcinia cola*,and *Zingiber officinale* AND *Garcinia cola*, *Aframomum melequeta* and *Zingiber officinale* Extract on Selected Upper Respiratory Tracts Microorganisms

	Antibacterial Activity/Extract Concentration (mg/mL)															
	Aframomum melegueta & Garcinia cola				*Garcinia cola & Zingiber officinale*				*Zingiber officinale & Aframomum melegueta*				*Aframomum melegueta, Garcinia cola & Zingiber officinale*			
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5
S.a	NG	NG	LG	G	NG	NG	LG	G	NG	LG	LG	LG	NG	NG	LG	G
K.p	NG	NG	LG	G	NG	NG	LG	G	NG	LG	LG	LG	NG	NG	LG	G
P.a	NG	NG	LG	G	NG	NG	LG	G	NG	LG	LG	LG	NG	NG	LG	G
S.p	NG	NG	LG	G	NG	NG	LG	G	NG	LG	LG	LG	NG	NG	LG	G

Source: Authors Field work,2024

Keys: G:Growth ,NG:No Growth, LG:Little Growth

S.a: Staphylococcus aureus

K.p:Klebsiella pneumonia

P.a:Pseudomonas aeruginosa

S.p:Streptococcus pnuemonia

Table 4.14C: Minimum Inhibitory Concentration (MIC) Activity of Ethanol *Garcinia cola* and *Aframomum melgueta, Aframomum melegueta* and *Zingiber officinale,Garcinia cola,*and *Zingiber officinale* AND *Garcinia cola, Aframomum melequeta and Zingiber officinale* Extract on Selected Upper Respiratory Tracts Microorganisms in Human

	Antibacterial Activity/Extract Concentration (mg/mL)															
	Aframomum melegueta & Garcinia cola				*Garcinia cola & Zingiber officinale*				*Zingiber officinale & Aframomum melegueta*				*Aframomum melegueta, Garcinia cola & Zingiber officinale*			
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5
S.a	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
K.p	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
P.a	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
S.p	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG

Source: Authors Field work,2024

Keys: G:Growth ,NG:No Growth, LG:Little Growth

S.a: Staphylococcus aureus

K.p:Klebsiella pneumonia

P.a:Pseudomonas aeruginosa

S.p:Streptococcus pneumonia

Table 4.15: Minimum Bactericidal Concentration (MBC) of *Garcinia cola* Extract on Selected Upper Respiratory Tracts Microorganisms in Human

			Antibacterial Activity/Extract Concentration (mg/mL)						
	Isolates of Bacteria	Extraction Solvent	100	50	25	12.5	6.25	3.12	Control
1	*Pseudomonas aeuroginosa*	Ethanol	NT	NT	NT	NT	T		T
		Petroleum ether	T	T	T	T	T		T
		Aqueous	T	T	T	T	T		T
2	*Streptococcus pneumonia*	Ethanol	NT	NT	NT	NT	T		T
		Petroleum ether	T	T	T	T	T		T
		Aqueous	-	-	-	-	-		-
3	*Klebsiella pneumonia*	Ethanol	NT	NT	NT	NT	T		T
		Petroleum ether	T	T	T	T	T		T
		Aqueous	-	-	-	-	-	-	-
4	*Staphylococcus aureus*	Ethanol	NT	NT	NT	NT	T	T	-
		Petroleum ether	T	T	T	T	T	T	-
		Aqueous	-	-	-	-	-		-

Source: Authors Field Work, 2024

KEY: T: Turbid, NT: Not Turbid

Table 4.15B: Minimum Bactericidal Concentration (MBC) of *Aframomum melegueta* Extract on Selected Upper Respiratory Tracts Microorganisms in Human

			Antibacterial Activity/Extract Concentration (mg/mL)						
	Isolates of Bacteria	Extraction Solvent	100	50	25	12.5	6.25	3.15	Control
1	*Pseudomonas aeuroginosa*	Ethanol	NT	NT	NT	NT	T	T	-
		Petroleum ether	T	T	T	T	T	T	-
		Aqueous	-	-	-	-			-
2	*Streptococcus pneumonia*	Ethanol	NT	NT	NT	NT	T	T	-
		Petroleum ether	T	T	T	T	T	T	-
		Aqueous	-	-	-	-	-	-	-
3	*Klebsiella pneumonia*	Ethanol	NT	NT	NT	NT	T	T	-
		Petroleum ether	T	T	T	T	T	T	-
		Aqueous	-	-	-	-			-
4	*Staphylococcus aureus*	Ethanol	NT	NT	NT	NT	T	T	-
		Petroleum ether	T	T	T	T	T	T	-
		Aqueous	-	-	- -		-	-	-

Source: Authors Field Work, 2024

KEY: T: Turbid, NG: Not Turbid

Table 4.15C: Minimum Bactericidal Concentration (MBC) Activity of *Zingiber officinale* Extract on Selected Upper Respiratory Tracts Microorganisms in Human

			Antibacterial Activity/Extract Concentration (mg/mL)						
	Isolates of Bacteria	Extraction Solvent	100	50	25	12.5	6.25	3.15	Control
1	*Pseudomonas aeuroginosa*	Ethanol	NT	NT	NT	T	T	T	-
		Petroleum ether	T	T	T	T	T	T	-
		Aqueous	-	-	-	-			-
2	*Streptococcus pneumonia*	Ethanol	NT	NT	T	T	T	T	-
		Petroleum ether	T	T	T	T	T	T	-
		Aqueous	-	-	-	-			-
3	*Klebsiella pneumonia*	Ethanol	NT	NT	NT	NT	T	T	-
		Petroleum ether	T	T	T	T	T	T	-
		Aqueous	-	-	-				-
4	*Staphylococcus aureus*	Ethanol	NT	NT	NT	NT	T	T	-
		Petroleum ether	T	T	T	T	T	T	-
		Aqueous	-	-	-	-	-	-	-

Source: Authors Field Work, 2024

KEY: T: Turbid, NG: Not Turbid

Table 4.16: Minimum Inhibitory Concentration (MIC) Activity of ETHANOL *Garcinia cola* and *Aframomum melgueta, Aframomum melegueta* and *Zingiber officinale,Garcinia cola,*and *Zingiber officinale* AND *Garcinia cola, Aframomum melequeta and Zingiber officinale* Extract on Selected Upper Respiratory Tracts Microorganisms in Human

	Antibacterial Activity/Extract Concentration (mg/mL)		
	Aframomum melegueta & Garcinia cola	*Garcinia cola & Zingiber officinale*	*Zingiber officinale & Aframomum melegueta*	*Aframomum melegueta, Garcinia cola & Zingiber officinale*
	100 50 25 12.5 6.25 3.12	100 50 25 12.5 6.25 3.12	100 50 25 12.5 6.25 3.12	100 50 25 12.5 6.25 3.12
S.a	NT NT NT NT T T	NT NT NT NT T T	NT NT NT NT T T	NT NT NT NT T T
K.p	NT NT NT NT T T	NT NT NT NT T T	NT NT NT NT T T	NT NT NT NT T T
P.a	NT NT NT NT T T	NT NT NT NT T T	NT NT NT NT T T	NT NT NT NT T T
S.p	NT NT NT NT T T	NT NT NT NT T T	NT NT NT NT T T	NT NT NT NT T T

Source: Authors Field work,2024

Keys: T:Turbid ,NT:Not Turbid

S.a: Staphylococcus aureus

K.p:Klebsiella pneumonia

P.a:Pseudomonas aeruginosa

S.p:Streptococcus pneumonia

Table 4.16B: Minimum Inhibitory Concentration (MIC) Activity of PETROLEUM ETHER *Garcinia cola* and *Aframomum melgueta, Aframomum melegueta* and *Zingiber officinale,Garcinia cola,*and *Zingiber officinale* AND *Garcinia cola, Aframomum melequeta and Zingiber officinale* Extract on Selected Upper Respiratory Tracts Microorganisms in Human

Antibacterial Activity/Extract Concentration (mg/mL)																							
Aframomum melegueta & Garcinia cola						*Garcinia cola & Zingiber officinale*						*Zingiber officinale & Aframomum melegueta*						*Aframomum melegueta, Garcinia cola & Zingiber officinale*					
100	50	25	12.5	6.25	3.12	100	50	25	12.5	6.25	3.12	100	50	25	12.5	6.25	3.12	100	50	25	12.5	6.25	3.12
S.a	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
K.p	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
P.a	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T	T
S.p	T	T	T	T	T	T																	

Source: Authors Field work,2024

Keys: T:Turbid ,NT:Not Turbid

S.a: Staphylococcus aureus

K.p:Klebsiella pneumonia

P.a:Pseudomonas aeruginosa

S.p:Streptococcus pneumonia

The antibiotics susceptible test results are presented in table 4.8. The Susceptibility testing of multiple antibiotic disc against four bacterial isolates *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeuroginosa* and *Streptococcus pneumonia)* revealed varying levels of resistance and sensitivity, based on the Clinical Laboratory Standards Institute (CLSI) reference values. Rocephin was effective against *Staphylococcus aureus* (16 mm),*Klebsiella pneumonia* (18 mm) and *Pseudomonas aeuroginosa* (17 mm) while Ciprofloxacillin had an effect on *Staphylococcus aureus* (16 mm), *Streptococcus pneumonia* (12 mm) Amoxacillin also had no effect on other organisms except Staphylococcus aureus (15 mm), gentamicin had effect on *Staphylococcus aureus* (15 mm), and *Streptococcus pneumonia* (17 mm) also pefloxacin had an effect on *Staphylococcus aureus* (18 mm), *Pseudomonas aeuroginosa* (16 mm) and *Klebsiella pneumonia* (17 mm) also erythromycin had an effect on *Staphylococcus aureus* (15 mm),*Streptococcus pnuemonia* (16 mm) and *Klebsiella pneumonia* (16 mm) and, streptomycin had an effect on *Staphylococcus aureus* (12mm), *Streptococcus pneumonia* (15 mm).Tarivid had an effect *Staphylococcus aureus* (13 mm), *Pseudomonas aeruginosa* (17mm) .Septrin had no effect on all bacterial isolates except *Klebsiella pneumonia* (17).Finally, Esperfloxacin had an effect on *Staphylococcus aureus* (19 mm), *Klebsiella pneumonia* (14 mm).

However, Ampliclox, Augmentin Chloramphenicol and Zinnacef had no effect against all organisms tested.

Table 4.17: Antibiotic Susceptibility Pattern against the Test Bacterial Isolates

				Isolates used			
S/N	**Antibiotics**	**Code**	**Conc. (µg)**	***S. aureus***	***S. pneumonia***	***K.pneumoniae***	***P. aeruginosa***
1	Perfloxacin	PEF	10	18m (R)	0	17m (R)	16m (S)
2	Gentamycin	CN	10	15 (R)	12 (I)	0	0
3	Ampiclox	APX	30	0	0	0	0
4	Zinnacef	Z	20	0	0	0	0
5	Amoxacilin	AM	30	15 (R)	0	0	0
6	Roceptin	R	25	16 (R)	0	18 (S)	17 (R)
7	Cirpoflaxin	CPX	10	16 (R)	12 (I)	0	0
8	Streptomycin	S	30	12 (S)	0	0	0
9	Septrin	SXT	30	0	0	17 (R)	0
10	Erythromycin	E	10	15 (S)	12 (R)	16 (S)	16 (I)
11	Chloramphenicol	CH	30	0	0	0	0
12	Sparfloxacin	SP	30	15 (S)	0	18 (R)	17 (R)
13	Augmentin	AU	10	0	0	0	0

14	Tarivid	OFX	30	13 (R)	0	0	15 (R)

Source: Author's Field Work, 2024

S- (Sensitive), I- (Intermediate), R- (Resistance)

4.2 Discussions of Findings

The practice of utilizing herbs and other botanical substances for medicinal purposes is common among rural communities in both developing and developed countries. Traditional plant-based therapies have been utilized for thousands of years to treat and prevent diseases in humans, highlighting their historical significance as precursors to modern medicine. Furthermore, in regions where contemporary medical practices are prevalent, including industrialized nations, many individuals still turn to traditional medicine. The arsenal of drugs available to combat highly resistant bacteria is restricted, and it is increasingly observed that bacteria can develop resistance to several drugs concurrently[1].Bacteria exhibit rapid evolutionary changes, leading to the swift development of resistance mechanisms. In recent years, there has been a notable increase in antibiotic usage in low- and middle-income nations. The World Health Organization has recognized antibiotic resistance as one of the three most critical public health challenges of the current century. The predominant pathogens responsible for upper respiratory tract infections are Gram-positive and Gram-negative bacteria. Given the concerning rate of drug resistance observed in many of these pathogens, there is a pressing need for alternative therapeutic agents derived from natural sources rather than synthetic compounds to effectively treat respiratory tract infections[2]. The findings from this study highlight the antimicrobial potential of the three medicinal plants used which are *Aframomum melegueta*, *Zingiber offinale* and *Garcinia cola* extracts against bacterial isolates from upper respiratory tracts infections. The growth of some Gram negative (*Pseudomonas aeruginosa*,*Klebsiella pnuemonia*) and some Gram positive (*Staphylococcus aureus,*

Streptococcus pnuemonia) isolates was suppressed by the petroleum ether, ethanolic and Aqueous extracts made from the *Aframomum melegueta*, *Zingiber offinale* and *Garcinia cola* plant.

The qualitative analysis of phytochemical screening in Table 4.1 reveals that *Aframomum melegueta*, *Zingiber offinale* and *Garcinia kola* are rich in terpenoids, flavonoids, glycosides, steroid, phenols, alkaloids, tannins and saponons particularly in the ethanol extracts and these compounds are widely known in the literature for their antimicrobial activities and its being linked to the inhibition of bacterial cell wall. Qualitative analysis in Table 4.2 reveals that the plants in various combination showed higher concentration of terpenoids, flavonoids, phenols, alkaloids, tannins and saponons particularly in the ethanol and petroleum ether extracts, which further corroborate their antimicrobial efficacy and effect on narrow and broad spectrum organisms. The observed potency of these phytochemicals aligns with other studies, which suggest that flavoids and tannins can damage bacterial cell wall and interrupt enzyme activities.

Tables 4.1 and 4.2 highlight the phytochemical contents of the various solvent extracts which all the three (3) plants used are rich in alkaloid,flavonoids, terpenoids, phenols and tannins particularly in petroleum ether and ethanol extracts.

Quantitative analysis showed that *Garcinia cola* petroleum ether contained the highest concentration of these bioactive compound with flavonoid 0.625mg/ml, Tannins 0.085. *Aframomum melegueta* with Alkaloid 0.286mg/ml, saponnis 0.84mg/ml, terperoid 0.056mg/ml, steroid 0.017mg/ml, glycosides 0.042mg/ml and *Zingiber officinale* petroleum ether contain Alkaloid 0.286mg/ml, Tannins 0.40mg/ml, terpenoid 0.019 while *Garcinia cola* ethanol extracts contain tannins 0.52mg/ml, saponnis 0.86mg/ml, flavonoid 0.0017mg/ml, glycosides 0.159mg/ml, *Aframomum melegueta* ethanol extracts Saponnins 0.273mg/ml,flavonoid 0.0018mg//ml, steroid 0.018mg/ml, *Zingiber officinale* ethanol extract

tannins 0.069mg/ml,terperoid 0.195mg/ml, steroids 0.30mg/ml, flavonoid 0.158mg/ml, glycosides 0.153mg/ml, phenolic 0.153mg/ml, *Garcinia cola* Aqueous extract tannins 0.799, saponnins 0.30mg/ml, flavonoid 0.0024mg/ml,glycosides 0.145mg/ml, *Aframomum melegueta* aqueous extracts contains alkaloid 0.135mg/ml, saponnins 0.91mg/ml, terperiod 0.0073mg/ml,flavonoid 0.582mg/ml and glycosides 0.149mg/ml while Zingiber officinale aqueous extracts contain alkaloid 0.135mg/ml, terperoid 0.0073mg/ml, flavonoid 0.0024mg/ml and glycoside 0.175mg/ml.

To this end, the study evaluated the phytochemical,antioxidant and anti-microbial properties of *Aframomum melegueta*, *Zingiber offinale* and *Garcinia kola* to establish their usefulness in traditional medicine. Using petroleum ether as the extraction solvent, it was found to have 50% potency against the isolates used, particularly some of the gram positive bacteria. Furthermore, it has been found to only have antiseptic but not bactericidal properties, i.e. a narrow spectrum of activity. Ethanol extraction solvent is found to have 80% potency against isolated bacteria, making ethanol a broad spectrum gram-positive and gram-negative bacterium.

Previous report has shown *Aframomum melegueta* ,*Zingiber officinale Roscoe* and *Garcinia cola* antimicrobial activities by past researcher who demonstrated the medicinal with pathogenic bacteria.Research indicates that individual plant species can harbor multiple bioactive constituents that hold considerable biological importance[3].

Previous research conducted on *Aframomum melegueta* has sparked interest in its possible health implications, particularly due to the phenolic constituents found in its seeds. The methanolic extract of *Aframomum melegueta* seeds revealed a significant concentration-dependent anti-adhesive effect against Staphylococcus aureus in a lung carcinoma cell line. Furthermore, the n-butanol and chloroform fractions were also found to possess considerable anti-adhesive activity against *Staphylococcus aureus*[4].

Past research concerning *Garcinia cola* aimed to investigate the antimicrobial efficacy of seed extracts against clinical isolates of *Streptococcus pyogenes* in Zaria, Nigeria. The findings indicated significant antimicrobial activity, especially with the n-butanol and aqueous extracts. The minimum inhibitory concentration (MIC) for the n-butanol extract was found to be

0.31 mg/ml, while the minimum bactericidal concentration (MBC) was recorded at 0.63 mg/ml[5].

Ethanol is an excellent solvent extractor in minimum inhibitor concentrates on pathogenic bacteria or otherwise treatment of pathogenic infection as it was documented to has the highest MIC on pathogenic bacteria. This result confirms ethanol as excellent solvent in other medicinal plants past studies from *Crypotolepis sanguinolenta*[6] and *Acacia dealbata,*[7] and in *Olea europaea*[7].

This study does not align with a study was conducted to test for the antimicrobial activity of *Garcinia cola* against some microbial organisms, namely: *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The paper disc method was used to determine the inhibitory effect of Garcinia kola nut on the test microorganisms. The zones of inhibition for *Staphylococcus aureus* ranged between 0.5 to 5mm for the ethanol preparation of Garcinia kola nut while that of *E. coli* ranged between 0.3 to 3.2mm. The ethanol preparation of *Garcinia cola* nut was found to exhibit more significant inhibitory action against test organisms than the aqueous preparations of *Garcinia cola* because in this present study ethanolic extract of *Garcinia cola* was more effective than the aqeous extract[8].

The present study also revealed that ethanolic and petroleum extracts from *Aframomum melegueta* ,*Zingiber officinale Roscoe* and *Garcinia cola* were more effective against the bacteria isolates, unlike the aqueous extracts from *Aframomum melegueta* ,*Zingiber officinale*

Roscoe and *Garcinia cola*, which have little or no effect on the bacterial isolates of upper respiratory tracts infection.

Qualitative and Quantitative phytochemical analysis of the various concentrations of ethanol, petroleum ether and aqueous extracts for *Aframomum melegueta* ,*Zingiber officinale Roscoe* and *Garcinia cola* revealed the presence of all the various phytochemicals studied at various concentration[9]. The difference in phytochemical constituents may be attributed to the various concentrations which might have made the phytochemicals readily available in the higher concentration extracts. This finding corresponds to a report that phytochemicals are secondary constituents of plant which are responsible for biological actions and some have also been reported to possess anti-oxidative potentials[10]. The increasing in concentration tends to increase the mean zone of inhibition obtained with various microorganism. The findings align with previous research indicating that increased concentrations of the plant extract correspond to enhanced antimicrobial activities.The least concentration as well as the highest concentration was statistically significant, against specific microorganisms in ethanolic, methanol and aqueous extract[11]. This is to the fact that the petroleum ether, ethanolic and aqueous extract contain phytochemicals such as tannins, saponins, alkaloids, flavounid, terpenoid, glycosides, phenol and steroids which may be responsible for this effect. This supports the finding of a study in which it was reported that the *Aframomum melegueta* ,*Zingiber officinale Roscoe* and *Garcinia cola* phytoconstituents (terpenoids, saponins, tannins, glycosides, and flavonoids) have antimicrobial and antioxidant properties. The antibiotics susceptible test results of the bacteria isolates were all resistant to synthetic antibiotics except some which were susceptible, this is similer to the finding that explained the pathogen is multiding resistant (MDR) when it is resistant to more antibiotics at any given time[12].

Endnotes

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Conclusion and Recommendation

5.1 Summary of Finding

The study's finding show the *in-vitro* antibacterial efficacy of extracts of *Garcinia Cola, Aframomum melegueta* and *Zingiber officinale Roscoe* with ethanol, petroleum ether, and aqueous solution were tested on *Streptococcus pneumonia, Klebsiella pneumoniae, Staphylococcus aureus,* and *Pseudomonas aeuroginosa*. The ethanolic extracts of the *Aframomum melegueta* and *Garcinia cola* were quite effective against all of the bacterial isolates tested. This confirms the antibacterial properties of plant extracts. The antibacterial action justifies the traditional use of *Garcinia Cola, Aframomum melegueta* and *Zingiber officinale Roscoe* in the treatment of various bacterial infections

The results of this study indicated a significant comparism in the effectiveness of *ginger (Zingiber officinale*) to *Garcinia kola* (bitter cola) and to *Aframomum melegueta* (alligator pepper).The study revealed that *Aframomum melegueta* is more effective against all the tested isolates as compared to *Zingiber officinale* and *Garcinia kola.*

This study revealed the minimum inhibitory concentration of solvent fractions *Zingiber officinale* (ginger), *Garcinia kola* (bitter cola) and *Aframomum melegueta* (alligator pepper) on selected upper respiratory tract pathogens.The analysis revealed that the ethanol extract produced a more significant zone of inhibition for each bacterial strain assessed, leading to its classification as the optimal solvent for extraction. *Garcinia Cola, Aframomum melegueta* and *Zingiber officinale Roscoe* extracts have inhibitory properties similar to conventional antibiotics.

In this study, Ethanoic extract of *Garcinia cola* and *Aframomum melegueta* is more effective on all tested isolates than *Garcinia cola+Zingiber officinale* and *Aframomum melegueta +Zingiber officinale.*The results of this study also revealed the effectiveness of *Garcinia cola*

+*Aframomum melegueta*+ *Zingiber officinale as it* is more effective on all the tested isolates than the others

This study explored the phytochemical analysis of *Garcinia Cola, Aframomum melegueta* and *Zingiber officinale Roscoe* revealed the presence of alkanoids, saponins, tannins, phlobatannins, steroids, and terpenoids. The study also reveals the phytochemical analysis of the plants at various concentrations and combinations. It can also indicate that these plants contains more phytochemicals properties when combined together than when used single.

5.2 Conclusion

This study reveals that *Garcinia Cola, Aframomum melegueta* and *Zingiber officinale Roscoe* have antibacterial characteristics and can thus be used to make antimicrobial medicines.

The study also explores the effectiveness of these plants singling at different concentrations and combined at different concentrations which indicates that medical plants can be used together for better effectiveness.

The phytochemical examination conducted on various plant species identified the presence of terpenoids, alkaloids, saponins, tannins, steroids, and phlobatannins. It can be inferred that these phytochemicals likely contribute to the antimicrobial properties observed in the extracts of these plants. Furthermore, the plants demonstrate a range of pharmacological activities, including antimicrobial, antidiabetic, anti-inflammatory, immunosuppressive, and anticancer effects. The phytochemical analysis of each plant indicates that they possess diverse medicinal attributes.The indiscriminate use of drugs, particularly antibiotics, should be discouraged; sick patients should be urged to see a doctor in a hospital/clinic, take certain tests, and medicines should be completed in accordance with the test result and the medicine's dose.

5.3 Recommendations

1.Further research is required to explore the medicinal properties of Garcinia Cola, Aframomum melegueta, and Zingiber officinale Roscoe, particularly focusing on the antimicrobial activities associated with the extracts from the stems and leaves of these plants, in addition to identifying their phytochemical constituents.

2.There is a pressing need for additional investigation into the medicinal attributes of Garcinia Cola, Aframomum melegueta, and Zingiber officinale Roscoe, with particular emphasis on their antimicrobial properties in various combinations and the characterization of their phytochemical components.

3.It is essential for researchers across diverse medical disciplines to conduct additional studies on a variety of plants, exploring different combinations and concentrations.

4.It is imperative to enhance research initiatives within the fields of pharmacy and medicine to effectively isolate, identify, and purify the bioactive elements present in the plant. This research is crucial for assessing the antibacterial properties of the plant extracts. The findings could pave the way for the development of new antimicrobial substances that may prove effective against the growing threat of antibiotic-resistant bacteria, which poses a serious challenge worldwide..

5.4 Contribution to Knowledge

This study has established a better understanding on the antimicrobial efficacy of *Garcinia Cola, Aframomum melegueta* and *Zingiber officinale Roscoe* on respiratory tracts infections especially the tested isolates which are *Streptococcus pneumonia, Klebsiella pneumoniae, Staphylococcus aureus,* and *Pseudomonas aeuroginosa.* This study provides insights into potential uses of the medicinal plants used as they can serve as alternatives of complements to synthetic antibiotics, especially given the rise in antibiotics resistance. Understanding the

active compounds in these plants that are responsible for their antimicrobial properties could lead to the development of new drugs or supplements targeting upper respiratory tracts pathogens. The study provide scientific validation for traditional medicinal uses of *Garcinia Cola, Aframomum melegueta* and *Zingiber officinale Roscoe* in treating respiratory tracts infections and supporting their integration into modern medical practices. The research explore whether these plants work better in various combination than individually and vice-versa, contributing to the understanding of synergistic effects in antimicrobial therapy. The study has shed light on how effective these plants can be and their impact on the economy of a nation.

5.5 Suggested Areas for Future Research

This study explored the *in-vitro* comparative antimicrobial efficacy of *Garcinia Cola, Aframomum melegueta* and *Zingiber officinale Roscoe* on upper respiratory tracts pathogens which are *Streptococcus pneumonia, Klebsiella pneumoniae, Staphylococcus aureus,* and *Pseudomonas aeuroginosa.* The study also evaluate the combination of the efficacy *of Zingiber officinale* (ginger), *Garcinia kola* (bitter cola) and *Aframomum melegueta* (alligator pepper) in various combinations and concentration on selected upper respiratory tract pathogens using various solvents.

Research can also be done to examine the most effective solvents that can used to extract the medical properties of the plants or other locally available solvents that can be used instead of the chemical produced ones. Investigation should be done on better methods for extracting active antimicrobial compounds from plants and incorporating them into the production of newer, safer, and possibly less expensive medications.

Further research can also be done to harness more medicinal properties of *Garcinia Cola, Aframomum melegueta* and *Zingiber officinale Roscoe* especially the antimicrobial activities

of these plants in various combinations, as well as the identification of its phytochemical components.

Massive public awareness efforts are needed to raise public knowledge of the developing trend of antibiotic resistance, its causes, and prevention in order to limit its incidence.

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Bio-data

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Nationality: Nigerian

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B. Educational Background with Dates

Educational Institutions Attended with Dates and Qualification

Primary School Leaving Certificate
Oba Moshod Community Primary School Ota, Ogun State 2002-2007

Junior Secondary School Certificate
Ebenezer Baptist Hight School Iwo, Osun State 2007-2010

West African Senior School Certificate
Unity Schools Ejigbo, Osun State 2010-2013

Bachelor of Science (Microbiology)
Leadcity University, Ibadan Oyo State 2014-2018

Masters in Medical Microbiology
Leadcity University, Ibadan Oyo State 2022-Present

C.Work Experience with Dates

Leadcity University, Ibadan Oyo State

Laboratory Technologist **March 2021-Present**

- Established and managed quality assurance protocols for laboratory operations.
- Conducted specimen preparation and analysis utilizing a range of instruments, including histological and molecular diagnostic techniques.
- Executed complex testing procedures by preparing specimens for evaluation and ensuring the integrity of assay quality.
- Conducted and documented analyses that included microbiological, serological, and chemical assessments of samples obtained from both clinical and environmental contexts.
- Formulated and verified buffers, proprietary reagents, calibrators, and controls before initiating laboratory testing and analysis requests

Sales Executive **2019-2020**
Fabils Electrical and Electronics Work, Ibadan Oyo state

- Developed and maintained courteous and effective working relationship
- Increased sales by offering advice on purchases and promoting additional products

Laboratory Technologist **2017-2017**
Omega Medical Diagnostic Centre Ota, Ogun State (Industrial Training)

- Demonstrated a solid understanding of research methodologies and their application within a commercial context.
- Operated laboratory equipment with minimal oversight, while making and documenting observations, performing calculations, and collecting data, as well as assisting in its preparation for evaluation.

- Possessed comprehensive knowledge of relevant protocols, processes, and equipment, ensuring adherence to Environmental Health & Safety (EHS) standards.
- Maintained awareness of airborne and contact allergens, alongside extensive experience in working with diverse microorganisms, including the cultivation of microbial cultures

Biology Teacher (SSS 2) **2018-2019**
National Youth Service Corps (NYSC), Bauchi State
Government Day Secondary School, Dagauda

- Individualized lesson plans and educational materials were meticulously prepared to align the biology curriculum with the unique needs of each student
- I also designed resources for laboratory activities, homework assignments, and handouts to support the learning process
- A positive and encouraging relationship with students was cultivated, which contributed to fostering a greater appreciation for biology and scientific inquiry

Publication

Adekunle Odunayo Adejuwon,**Grace Oyatunde**,Victoria Anatolyvna,Abiola Mohammed Adeosun,Olubunmi Sharon Obayemi.The Antimicrobial Efficacy and Phytochemical Analysis of the Stem Bark of *Azadirachta indica* (Neem)

Dissertation

The Antimicrobial Efficacy and Phytochemical Analysis of the Stem Bark of Azadirachta (B.Sc thesis) was published on Researchgate, and submitted to the Department of Microbiology, Lead City University, Ibadan. June 2018.

Conference Presentation

OYATUNDE Grace Temitope, Bukola Ayodeji Bamkefa, Bakare C. Omonike,Phytochemical Analysis and *In-vitro* Comparative Evaluation of Antimicrobial Efficacy of *Garcinia cola, Aframomum melegueta,* and *Zingiber officinale Roscoe* against Selected Upper Respiratory Tracts Pathogens. Faculty of Natural and Applied Science Abstracts. Oral Presentation delivered at the FASCON 4th International Conference, Lead City University Ibadan,Oyo State. October 2024.

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Signature Date

University Compliance Certification

This is to certify that the thesis of Grace Temitope OYATUNDE with Matric number LCU/PG/003080, at the Department of Biological Sciences, Faculty of Basic Medical and Applied Sciences, Lead City University, Ibadan, Nigeria is in full compliance with the University approved form and style.

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Signature Date