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The Antimicrobial Potential of Citrus Araniifolia Juice and Allum Cepa Extract on Gram Positive and Gram Negative Bacteria

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A B S T R A C T

The increasing prevalence of antimicrobial resistance has necessitated the search for alternative and natural sources of antimicrobial agents. Medicinal plants have gained considerable attention due to their availability, affordability, and reduced side effects compared to synthetic drugs. This seminar focuses on the evaluation of the antimicrobial potential of Citrus aurantiifolia (lime) and Allium cepa (onion) against selected Gram-positive and Gram-negative bacteria. Lime and onion are widely used in traditional medicine and are known to possess bioactive compounds such as flavonoids, phenolics, alkaloids, and sulfur-containing compounds, which contribute to their antimicrobial properties. The antimicrobial activity of their extracts is commonly assessed using standard microbiological techniques, including agar well diffusion and determination of zones of inhibition. Findings from existing studies indicate that both lime and onion extracts exhibit inhibitory effects against various pathogenic microorganisms, although the degree of activity varies depending on the organism and concentration of the extract. This study highlights the relevance of lime and onion as potential sources of natural antimicrobial agents and supports their possible application in managing microbial infections and combating antibiotic resistance.

Introduction

Medicinal plants have continued to play a significant role in healthcare systems across the world, particularly in developing regions where access to modern healthcare may be limited. These plants are rich sources of bioactive compounds such as alkaloids, flavonoids, tannins, phenols, and organic acids, which have been widely reported to exhibit antimicrobial, antioxidant, anti-inflammatory, and therapeutic properties. The increasing interest in plant-derived compounds has been driven by the growing prevalence of infectious diseases and the rising resistance of microorganisms to conventional antibiotics, which has become a major global health challenge (Mahady, 2005).

The search for alternative antimicrobial agents has therefore intensified, with particular attention given to

plants that are readily available, cost-effective, and traditionally used in medicine. Among such plants, *Citrus aurantifolia* (lime) and *Allium cepa* (onion) have been widely documented for their pharmacological significance and antimicrobial potential.

Citrus aurantifolia (lime) is a citrus fruit widely cultivated in tropical and subtropical regions and is known for its acidic juice and rich phytochemical composition. The juice contains bioactive constituents such as flavonoids, limonoids, alkaloids, and ascorbic acid, which contribute to its antimicrobial properties. These compounds have been reported to exert antibacterial effects by disrupting bacterial cell membrane integrity, causing leakage of cellular contents, denaturing proteins, and interfering with essential enzymatic and metabolic pathways. Several studies have demonstrated that citrus-based extracts exhibit inhibitory activity against both Gram-positive and Gram-negative bacteria, supporting their potential use as natural antimicrobial agents (Nzeako *et al.,* 2006; Oikeh *et al.,* 2016; Gyawali & Ibrahim, 2014).

In addition to its pharmacological relevance, *Citrus aurantifolia* has long been used in traditional medicine for the treatment of various disease conditions. It has been applied in managing diarrhea, sore throat, fever, respiratory tract infections, gastrointestinal disturbances, and skin infections. In some ethnomedicinal practices, lime juice is also used as a topical cleansing agent for wounds, where its acidic nature is believed to help inhibit microbial growth and promote wound healing by creating an unfavorable environment for bacterial proliferation (Nzeako *et al.,* 2006).

Similarly, *Allium cepa* (onion) is one of the most commonly consumed vegetables globally and has been extensively studied for its medicinal and antimicrobial properties. It contains a variety of bioactive compounds including sulfur-containing compounds, phenolics, and flavonoids such as quercetin, which are largely responsible for its antibacterial activity. These compounds have been shown to inhibit microbial growth through multiple mechanisms, including disruption of bacterial cell wall synthesis, inhibition of enzyme activity, induction of oxidative stress, and interference with nucleic acid replication and protein synthesis (Griffiths *et al.,* 2002).

Beyond its biochemical properties, *Allium cepa* has also been traditionally used in the management of several health conditions. It is commonly employed in treating cough, cold, flu, microbial infections, inflammation, and digestive disorders. Furthermore, onion extracts are traditionally applied to infected wounds, boils, and insect bites due to their perceived antibacterial and anti-inflammatory properties, which further supports its ethnomedicinal relevance (Lanzotti, 2006).

The emergence and rapid spread of antibiotic-resistant bacteria have become a major global health concern, significantly threatening the effectiveness of standard antimicrobial therapies. The misuse and overuse of antibiotics in human medicine, veterinary practice, and agriculture have accelerated the development of resistant strains of pathogenic microorganisms. As a result, infections that were once easily treatable have become difficult, costly, and in some cases life-threatening, thereby necessitating the urgent search for alternative antimicrobial agents, particularly from natural plant sources that may offer safer and more sustainable therapeutic options (Ventola, 2015; World Health Organization, 2020).

Among clinically important bacterial pathogens, *Escherichia coli* and *Staphylococcus aureus* are of major public health concern. *Escherichia coli* is a Gram-negative, rod-shaped bacterium commonly found in the intestinal tract of humans and animals. While many strains are harmless, pathogenic strains are associated with gastrointestinal infections, urinary tract infections, neonatal meningitis, and septicemia. Of major concern is its ability to acquire and transfer antimicrobial resistance genes through horizontal gene transfer, which contributes to its rapid adaptation and survival in different environments (Tenaillon *et al.,* 2010; Okeke *et al.,* 2007).

Similarly, *Staphylococcus aureus* is a Gram-positive bacterium that colonizes the skin and nasal passages of humans but is also a significant opportunistic pathogen responsible for a wide range of infections. These include skin and soft tissue infections, pneumonia, osteomyelitis, endocarditis, and bloodstream infections. The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) has further complicated treatment due to its resistance to multiple antibiotic classes, leading to increased morbidity, prolonged hospital stays, and higher healthcare costs (Lowy, 1998; Chambers & DeLeo, 2009).

The ability of both *Escherichia coli* and *Staphlylococcus aureus* to develop resistance, form biofilms, and survive

in harsh conditions makes them particularly important in clinical microbiology and infectious disease research. Their continued prevalence in both community and hospital settings highlights the need for continuous exploration of alternative antimicrobial strategies.

Given the reported antimicrobial properties of *Citrus aurantifolia* juice and *Allium cepa* extract, there is a need to scientifically evaluate their effectiveness against clinically relevant bacterial pathogens.

Escherichia coli

Escherichia coli is a Gram-negative, facultatively anaerobic, rod-shaped, non-spore-forming bacterium belonging to the family *Enterobacteriaceae*. It was first isolated by Theodor Escherich in 1885 and is a normal inhabitant of the gastrointestinal tract of humans and warm-blooded animals, where it contributes to intestinal homeostasis and vitamin synthesis. Most strains are harmless; however, pathogenic strains possess virulence factors that enable them to cause intestinal and extraintestinal infections.

In the laboratory, *Escherichia coli* ferments lactose, producing pink colonies on MacConkey agar and a characteristic metallic green sheen on Eosin Methylene Blue (EMB) agar. Most strains are motile due to peritrichous flagella and are catalase-positive but oxidase-negative, characteristics useful in identification (Cheesbrough, 2006). Pathogenic strains are classified into different pathotypes, including Enteropathogenic (EPEC), Enterotoxigenic (ETEC), Enterohemorrhagic (EHEC), Enteroinvasive (EIEC), Enteroaggregative (EAEC), Diffusely Adherent (DAEC), Adherent-Invasive (AIEC), and Extraintestinal Pathogenic *E. coli* (ExPEC).

Escherichia coli is widely distributed in water, soil, food, sewage, and animal faeces, making it an important indicator of faecal contamination and environmental sanitation. The emergence of multidrug-resistant strains, including extended-spectrum β-lactamase (ESBL)-producing and carbapenem-resistant *Escherichia coli*, has become a major public health concern because it limits treatment options and increases morbidity and mortality. Owing to its medical importance and increasing antimicrobial resistance, *Escherichia coli* was selected as one of the test organisms in this study to evaluate the antibacterial activity of *Citrus aurantiifolia* juice and *Allium cepa* extract.

Morphology/Structure of *Escherichia coli*

Escherichia coli is a Gram-negative rod measuring approximately 1.1–1.5 µm in width and 2.0–6.0 µm in length. It possesses a thin peptidoglycan layer located between the inner cytoplasmic membrane and an outer membrane containing lipopolysaccharide (LPS), phospholipids, lipoproteins, and porins, all of which contribute to its structural integrity and pathogenicity. Most strains are motile due to peritrichous flagella, while some pathogenic strains possess a polysaccharide capsule (K antigen), especially the K1 capsule associated with neonatal meningitis. The organism also possesses fimbriae and pili that facilitate adhesion to host tissues and biofilm formation, enhancing colonization and resistance to host defenses. In the laboratory, *Escherichia coli* forms smooth cream colonies on nutrient agar, pink colonies on MacConkey agar, and metallic green colonies on EMB agar. It is catalase-positive, oxidase-negative, indole-positive, methyl red-positive, and citrate-negative, characteristics commonly used for identification (Cheesbrough, 2006). These structural features contribute to its survival, pathogenicity, and antimicrobial resistance.

Diseases/Symptoms Associated with *Escherichia coli*

Although *Escherichia coli* normally forms part of the intestinal flora, pathogenic strains can cause a variety of intestinal and extraintestinal diseases. Their ability to produce toxins, adhere to host tissues, and evade immune responses enables them to cause infections ranging from mild diarrhoea to severe systemic illnesses. The major diseases associated with *Escherichia coli* include:

Urinary Tract Infection (UTI)

Urinary tract infection (UTI) is the most common extraintestinal infection caused by *Escherichia coli*. Uropathogenic *Escherichia coli* accounts for approximately 70–95% of uncomplicated community-acquired UTIs and about half of hospital-acquired cases worldwide. Infection occurs when bacteria from the gastrointestinal tract ascend through the urethra into the bladder and may spread to the kidneys if untreated. Common symptoms include dysuria, urinary frequency, urgency, haematuria, fever, and flank pain. Women, pregnant women, catheterized patients, diabetics, and immunocompromised individuals are at greater risk. Increasing antimicrobial resistance among uropathogenic

Escherichia coli has made prompt diagnosis and susceptibility testing increasingly important.

Gastroenteritis

Gastroenteritis caused by pathogenic *Escherichia coli* is a major public health problem, particularly in developing countries. The disease is caused by pathotypes such as Enterotoxigenic (ETEC), Enteropathogenic (EPEC), Enterohemorrhagic (EHEC), Enteroinvasive (EIEC), and Enteroaggregative (EAEC) *Escherichia coli*. Infection is usually acquired through contaminated food or water and presents with diarrhoea, abdominal cramps, vomiting, fever, and dehydration. Severe infections caused by EHEC may result in haemolytic uraemic syndrome due to Shiga toxin production. Proper hygiene, safe water, and food safety practices remain important preventive measures.

Septicemia

Escherichia coli septicemia is a life-threatening bloodstream infection that commonly arises from untreated urinary tract, abdominal, or biliary infections. Extraintestinal pathogenic strains possess virulence factors that enable survival in the bloodstream and evasion of host immunity (Russo & Johnson, 2003; Poolman & Wacker, 2016). Clinical features include fever, chills, hypotension, confusion, and septic shock in severe cases. Neonates, elderly individuals, hospitalized patients, and immunocompromised persons are particularly susceptible. The emergence of multidrug-resistant and ESBL-producing *Escherichia coli* has complicated treatment, making early diagnosis and antimicrobial susceptibility testing essential.

Mode of Transmission of *Escherichia coli*

Escherichia coli is transmitted mainly through the faecal–oral route by ingestion of contaminated food or water. Other routes include person-to-person contact, exposure to contaminated recreational water, contact with infected animals, and cross-contamination during food preparation. Extraintestinal infections such as UTIs usually result from self-contamination, where intestinal *Escherichia coli* spreads to the urinary tract. Poor sanitation, inadequate hygiene, and contaminated medical equipment also contribute to transmission. Preventive measures include proper hand washing, safe drinking water, thorough cooking of food, environmental sanitation, and adherence to infection control practices (World Health Organization, 2022; Centers for Disease Control and Prevention, 2024).

Pathogenicity of *Escherichia coli*

Pathogenicity refers to the ability of certain strains of *Escherichia coli* to cause disease through the production of virulence factors such as adhesins, capsules, lipopolysaccharides, toxins, iron acquisition systems, and biofilms.

These virulence factors enable the organism to colonize host tissues, evade immune defenses, obtain nutrients, and damage host cells. While intestinal strains mainly cause diarrhoeal diseases, extraintestinal strains are responsible for urinary tract infections, septicemia, and neonatal meningitis.

Morphology/Structure of *Staphylococcus aureus*

Staphylococcus aureus consists of Gram-positive spherical cells measuring about 0.5–1.5 μm in diameter and arranged in irregular grape-like clusters after Gram staining (Tong *et al.,* 2015). Its cell wall is composed mainly of a thick peptidoglycan layer with teichoic and lipoteichoic acids, which provide structural support, facilitate adhesion, and stimulate inflammatory responses (Otto, 2014). The organism is non-motile and non-spore-forming but possesses numerous surface proteins known as MSCRAMMs that promote attachment to host tissues and implanted medical devices (Foster *et al.,* 2014). Some strains also produce a polysaccharide capsule and extracellular slime layer, both of which enhance resistance to phagocytosis and contribute to biofilm formation, making infections more persistent (Otto, 2008).

On nutrient agar, colonies are smooth, circular, convex, opaque, and golden-yellow because of the carotenoid pigment staphyloxanthin. On blood agar, many strains produce β-haemolysis, while on Mannitol Salt Agar they ferment mannitol, producing yellow colonies surrounded by yellow zones. Biochemically, *Staphylococcus aureus* is catalase-positive, coagulase-positive, and oxidase-negative, characteristics routinely used for laboratory identification (Cheesbrough, 2006; Tong *et al.,* 2015). These structural features contribute greatly to its survival, pathogenicity, and antimicrobial resistance.

Diseases/Symptoms Associated with *Staphylococcus aureus*

Staphylococcus aureus is an opportunistic pathogen capable of causing diseases ranging from mild skin infections to severe systemic illnesses. Infection occurs when the organism enters the body through cuts, wounds, burns, surgical sites, or medical devices. The severity of disease depends on the virulence of the infecting strain and the immune status of the host (Tong *et al.,* 2015; Taylor & Unakal, 2023). The major diseases associated with *Staphylococcus aureus* include:

Skin and Soft Tissue Infections

Skin and soft tissue infections are the most common diseases caused by *Staphylococcus aureus*. The organism enters through cuts, burns, surgical wounds, or abrasions and produces enzymes and toxins that promote tissue destruction (Tong *et al.,* 2015). Common infections include boils, carbuncles, folliculitis, impetigo, cellulitis, abscesses, and infected wounds. Symptoms include redness, swelling, pain, warmth, pus formation, and sometimes fever. Delayed treatment may allow the infection to spread into deeper tissues or the bloodstream. Methicillin-resistant *Staphylococcus aureus* (MRSA) has made treatment increasingly difficult because of multidrug resistance (Chambers & DeLeo, 2009).

Bacteremia and Septicemia

Bacteremia is the presence of *Staphylococcus aureus* in the bloodstream, while septicemia is a severe bloodstream infection accompanied by systemic inflammation. The organism usually enters the blood through infected wounds, catheters, surgery, or skin infections and may spread to organs such as the heart, lungs, bones, and kidneys (Tong *et al.,* 2015; Holland *et al.,* 2014). Common symptoms include fever, chills, hypotension, rapid heartbeat, confusion, and weakness. Severe cases may progress to septic shock, multiple organ failure, and death if not treated promptly. Early diagnosis using blood culture and appropriate antimicrobial therapy are essential, particularly with the increasing prevalence of MRSA (Otto, 2014; Holland *et al.,* 2014).

Materials and Methods: Methods of Extraction of *Citrus aurantiifolia*

The extraction of bioactive compounds from *Citrus aurantiifolia* depends on the part of the plant being investigated and the intended purpose of the study. The fruit juice may be used directly as an aqueous extract, while the peel and leaves are commonly extracted using solvents such as ethanol, methanol, acetone, or water. Various extraction methods including maceration, Soxhlet extraction, hydrodistillation, cold pressing, and mechanical expression have been employed to obtain phytochemicals and essential oils from the plant (Azwanida, 2015; Pathan *et al.,* 2020).

For antimicrobial studies involving fresh lime juice, aqueous extraction is the most commonly employed method because it preserves naturally occurring organic acids such as citric acid and ascorbic acid, together with flavonoids and other water-soluble phytochemicals. In this method, freshly harvested lime fruits are washed thoroughly, cut aseptically, and the juice is extracted manually or mechanically. The juice is filtered through sterile muslin cloth or filter paper to remove pulp and debris before being used directly or diluted to the required concentrations for antimicrobial evaluation (Oikeh *et al.,* 2016).

The efficiency of extraction depends on several factors including fruit maturity, storage conditions, extraction technique, and handling procedures. Freshly extracted juice generally possesses higher concentrations of biologically active compounds than stored juice because prolonged storage may result in oxidation and degradation of important phytochemicals, thereby reducing antimicrobial activity (Pathan *et al.,* 2020).

Antimicrobial Activity of Citrus *aurantifolia*

The antimicrobial activity of *Citrus aurantifolia* has been extensively investigated because of its long-standing use in traditional medicine for the treatment of infectious diseases. Numerous studies have demonstrated that different parts of the plant, including the fruit juice, peel, leaves, and essential oils, possess inhibitory activity against a broad spectrum of pathogenic microorganisms. These include Gram-positive bacteria, Gram-negative bacteria, fungi, and certain viruses. The antimicrobial properties of *Citrus aurantifolia* are primarily attributed to the synergistic action of flavonoids, phenolic compounds, citric acid, essential oils, tannins, and other bioactive phytochemicals present within the plant (Oikeh *et al.,* 2016; Pathan *et al.,* 2020).

Several investigators have reported that fresh lime juice exhibits appreciable antibacterial activity against

important human pathogens such as *Escherichia coli, Staphylococcus aureus, Salmonella enterica, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, and Bacillus subtilis.* The acidic nature of the juice, together with the presence of antimicrobial phytochemicals, contributes to bacterial inhibition by disrupting cell membranes, denaturing cellular proteins, altering membrane permeability, inhibiting enzyme activity, and interfering with DNA replication and cellular metabolism (Oikeh *et al.,* 2016; Pathan *et al.,* 2020).

Research has also shown that the essential oils obtained from *Citrus aurantifolia* possess stronger antimicrobial activity than some crude extracts because they contain concentrated volatile compounds capable of penetrating bacterial cell membranes. These essential oils cause structural damage to microbial cells, resulting in leakage of intracellular materials, disruption of energy production, and eventual bacterial death. In addition to antibacterial activity, the essential oils have demonstrated antifungal effects against species of *Candida, Aspergillus*, and other medically important fungi (Ghasemi *et al.,* 2009).

The antimicrobial effectiveness of *Citrus aurantifolia* depends on several factors including the concentration of the extract, extraction method, plant part used, microbial species tested, duration of exposure, and environmental conditions. Generally, higher extract concentrations produce larger zones of inhibition, indicating increased antimicrobial activity. Gram-positive bacteria are often more susceptible than Gram-negative bacteria because the outer membrane present in Gram-negative organisms acts as an additional barrier that restricts the penetration of many antimicrobial compounds (Pathan *et al.,* 2020).
The growing problem of antimicrobial resistance has renewed scientific interest in medicinal plants such as *Citrus aurantifolia* as potential alternatives to conventional antibiotics. Plant-derived antimicrobial agents are considered promising because they are widely available, relatively inexpensive, biodegradable, and often associated with fewer adverse effects. Furthermore, the presence of multiple phytochemicals with different mechanisms of action reduces the likelihood of rapid resistance development by microorganisms. These characteristics have encouraged continued research into the antimicrobial potential of *Citrus aurantifolia* against multidrug-resistant bacterial pathogens (World Health Organization, 2023; Oikeh *et al.,* 2016).

The inclusion of *Citrus aurantifolia* in the present study is based on these documented antimicrobial properties. Evaluating the antibacterial activity of fresh lime juice against *Escherichia coli* and *Staphylococcus aureus* will provide additional scientific evidence regarding its effectiveness and may contribute to the development of affordable plant-based antimicrobial agents for managing bacterial infections, particularly in regions where access to conventional antibiotics is limited.

Allium cepa

Allium cepa L., commonly known as onion, is a herbaceous biennial plant belonging to the family *Amaryllidaceae* (formerly *Alliaceae*). It is one of the oldest cultivated vegetable crops in the world and is widely grown for culinary, nutritional, and medicinal purposes. The plant is believed to have originated in Central Asia before spreading to Europe, Africa, and other parts of the world through trade and cultivation. In Nigeria, *Allium cepa* is extensively cultivated and consumed because of its characteristic flavour, nutritional value, and numerous therapeutic properties. For centuries, onions have been used in traditional medicine for the treatment of infections, wounds, cough, fever, hypertension, diabetes mellitus, and inflammatory conditions (Griffiths *et al.,* 2002).

Allium cepa is a monocotyledonous plant characterized by a shallow fibrous root system, cylindrical hollow leaves, a short compressed stem, and an underground bulb composed of concentric fleshy scales. The bulb serves as the primary storage organ and contains numerous bioactive compounds responsible for the plant's medicinal activities. The outer scales are usually dry and papery, while the inner scales are succulent and rich in nutrients and phytochemicals. Depending on the variety, onion bulbs may be white, yellow, or red in colour and vary considerably in size and pungency (Griffiths *et al.,* 2002).

The medicinal properties of Allium cepa are attributed to its diverse phytochemical constituents, including flavonoids, phenolic acids, organosulfur compounds, saponins, tannins, alkaloids, glycosides, and essential oils. Among these, the sulfur-containing compounds such as allicin, cepaenes, thiosulfinates, disulfides, and trisulfides are considered the principal bioactive constituents responsible for the antimicrobial activity of onion. Flavonoids such as quercetin and kaempferol also contribute significantly to its antioxidant, anti-

inflammatory, antimicrobial, and anticancer properties (Slimestad *et al.,* 2007).

Allium cepa has attracted considerable scientific interest because of its broad spectrum of biological activities. Studies have demonstrated that extracts obtained from the bulb, leaves, and outer scales possess antibacterial, antifungal, antiviral, antiparasitic, antioxidant, anti-inflammatory, antihyperglycaemic, antihypertensive, cardioprotective, and immunomodulatory properties. These pharmacological effects have encouraged the use of onion in both traditional medicine and modern biomedical research as a potential source of naturally occurring therapeutic compounds (Griffiths *et al.,* 2002).

Several studies have reported that *Allium cepa* extracts exhibit antibacterial activity against clinically important microorganisms including *Escherichia coli, Staphylococcus aureus, Salmonella enterica, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, and Bacillus subtilis*. The antimicrobial activity is believed to result from the synergistic effects of organosulfur compounds, flavonoids, phenolic compounds, and other phytochemicals, which disrupt bacterial cell walls, inhibit essential enzymes, interfere with nucleic acid synthesis, alter membrane permeability, and ultimately suppress bacterial growth (Rose *et al.,* 2005).

The increasing prevalence of antimicrobial resistance has renewed scientific interest in *Allium cepa* as a potential alternative source of antimicrobial agents. The plant is inexpensive, readily available, and widely consumed, making it an attractive candidate for the development of plant-based antimicrobial preparations. Consequently, *Allium cepa* was selected for the present study to evaluate the antimicrobial activity of its extract against *Escherichia coli* and *Staphylococcus aureus*. The findings may provide additional scientific evidence supporting the traditional medicinal use of onion and contribute to the search for effective alternative therapies against antibiotic-resistant bacterial pathogens.

Methods of Extraction of *Allium cepa*

Extraction of the bioactive constituents of *Allium cepa* is an important step in evaluating its medicinal and antimicrobial properties. Various extraction methods have been employed depending on the phytochemicals of interest, including aqueous extraction, ethanolic extraction, methanolic extraction, Soxhlet extraction, and maceration. Ethanol is among the most widely used solvents because it efficiently extracts both polar and moderately non-polar phytochemicals, including flavonoids, phenolics, and sulfur-containing compounds responsible for the antimicrobial activity of onion (Azwanida, 2015; Handa *et al.,* 2008).

For ethanolic extraction, fresh onion bulbs are washed, sliced, oven-dried, and pulverized into fine powder before being soaked in ethanol for several days with intermittent shaking. The mixture is then filtered, and the solvent is removed by evaporation to obtain a concentrated crude extract. The crude extract may subsequently be dissolved in Dimethyl Sulfoxide (DMSO) or another suitable solvent to prepare different concentrations for antimicrobial susceptibility testing (Handa *et al.,* 2008).

The effectiveness of the extraction process depends on factors such as solvent polarity, extraction time, temperature, particle size of the plant material, and storage conditions. Appropriate extraction procedures ensure maximum recovery of the biologically active constituents responsible for the antimicrobial activity of *Allium cepa* (Azwanida, 2015).

Antimicrobial Activity of *Allium cepa*

The antimicrobial activity of *Allium cepa* has been widely investigated because of its long history of use in traditional medicine for the treatment of infectious diseases. Previous studies have demonstrated that extracts prepared from onion bulbs possess inhibitory activity against a broad spectrum of Gram-positive and Gram-negative bacteria as well as several fungal species. The antimicrobial properties of onion are mainly attributed to the presence of organosulfur compounds, flavonoids, phenolic compounds, tannins, and other phytochemicals that act either individually or synergistically to suppress microbial growth (Rose *et al.,* 2005).

Several investigators have reported that *Allium cepa* extracts inhibit the growth of clinically important bacterial pathogens including *Escherichia coli, Staphylococcus aureus, Salmonella enterica, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, and Bacillus subtilis*. The antibacterial activity results from disruption of bacterial cell membranes, inhibition of protein and nucleic acid synthesis, inactivation of essential metabolic enzymes,

and increased leakage of intracellular constituents. These mechanisms ultimately impair bacterial growth and may result in bacterial cell death (Rose *et al.,* 2005; Griffiths *et al.,* 2002).

The antimicrobial effectiveness of *Allium cepa* depends on several factors, including the extraction solvent, concentration of the extract, onion variety, duration of exposure, and the susceptibility of the test organism. Higher concentrations generally produce greater zones of inhibition, indicating stronger antibacterial activity.

Variations in extraction methods may also influence the concentration of bioactive compounds present in the final extract and consequently affect antimicrobial efficacy.

In addition to its direct antibacterial effects, *Allium cepa* contains strong antioxidant and anti-inflammatory activities that may enhance its therapeutic value during the management of infectious diseases. The antioxidant constituents protect host tissues from oxidative damage associated with inflammation, while the anti-inflammatory compounds help reduce tissue injury and promote healing. These combined pharmacological properties further support the medicinal importance of onion extracts (Slimestad *et al.,* 2007).

The growing challenge of multidrug-resistant bacterial infections has stimulated renewed interest in medicinal plants such as *Allium cepa* as potential sources of novel antimicrobial compounds. Because onion is inexpensive, readily available, and generally regarded as safe for human consumption, it represents an attractive candidate for the development of alternative antimicrobial therapies. Therefore, evaluating the antibacterial activity of *Allium cepa* extract against *Escherichia coli and Staphylococcus aureus is i*mportant in determining its potential usefulness as a natural antimicrobial agent and in providing scientific validation for its traditional medicinal applications.

Materials and Methods

Study Area

The tomato samples for this study were collected from Mile 3 Market located in Port Harcourt city. This sampling site was selected because it is a major commercial hub where produce is exposed to varying ambient temperatures and handling practices.

The extraction of plant materials, isolation of *Escherichia coli and Staphylococcus aureus*, and subsequent antimicrobial susceptibility testing were conducted at the Microbiology Laboratory of Rivers State University, Nkopulu-Oroworukwi, Port Harcourt.

Materials and Equipment

The following materials and equipment were used during this study:

i. Petri dishes: Sterile glass or disposable plastic plates used for culturing microorganisms on solid media.
ii. Conical (Erlenmeyer) flasks: Used for preparing culture media, mixing reagents, and extracting plant materials.
iii. Beakers: Used for measuring, mixing, and heating solutions and reagents.
iv. Test tubes: Used for holding culture media, bacterial suspensions, and biochemical test reagents.
v. Test tube rack: Used to hold test tubes securely during incubation and laboratory procedures etc.

Collection of Samples

Fresh onion bulbs (*Allium cepa*) and mature lime fruits (*Citrus aurantiifolia*) were purchased from Mile 3 Market, Port Harcourt, Rivers State. Six Tomato fruits; three fresh and three spoilt, were also purchased, each from a different stall at the market. The samples were transported to the Microbiology Laboratory in clean sterile polythene bags for extraction.

Sterilization of Materials

All materials used during this study were sterilized using standard microbiological procedures. Glassware, including test tubes, conical flasks, beakers, and measuring cylinders, were thoroughly washed with detergent, rinsed with distilled water, air-dried, and sterilized in a hot air oven at 160°C for 1 hour. Pipettes were dried, placed in a canister, and sterilized in the hot air oven. Test tubes were covered with aluminium foil before sterilization, while inoculating loops were sterilized by dipping them in 70% ethanol and flaming over a Bunsen burner before and after use.

Culture media were sterilized by autoclaving at 121°C for 15 minutes under 15 psi pressure. Sterile disposable Petri dishes were used throughout the study and discarded after use. Laboratory benches, the inoculation hood (where available), and other working surfaces were

disinfected with 70% ethanol before and after laboratory procedures. Personal protective equipment (PPE) was worn throughout the experiment to minimize contamination and ensure laboratory safety.

Media Preparation

The culture media used in this study were prepared according to the manufacturer's instructions. Appropriate quantities of the dehydrated media were weighed and dissolved in 1 litre of distilled water in a conical flask plugged with cotton wool. The media were heated with gentle agitation until completely dissolved and sterilized by autoclaving at 121°C for 15 minutes under 15 psi pressure. After sterilization, the media were allowed to cool to approximately 45–50°C before being aseptically poured into sterile Petri dishes and allowed to solidify (Cheesbrough, 2006).

Eosin Methylene Blue (EMB) Agar

Eosin Methylene Blue (EMB) agar was prepared according to the manufacturer's instructions. The medium was used for the selective isolation and differentiation of Escherichia coli and other Gram-negative enteric bacteria. Thirty-six grams (36 g) of the dehydrated medium were weighed and dissolved in 1 litre of distilled water in a conical flask plugged with cotton wool. The medium was heated with gentle agitation until completely dissolved and sterilized by autoclaving at 121°C for 15 minutes under 15 psi pressure. After sterilization, the medium was allowed to cool to approximately 45–50°C before being aseptically poured into sterile Petri dishes. The plates were allowed to solidify and dried before use.

Nutrient Agar

Nutrient agar was prepared according to the manufacturer's instructions. The medium was used for the isolation, cultivation, subculturing, and enumeration of heterotrophic bacteria. Twenty-eight grams (28 g) of the dehydrated medium was weighed and dissolved in 1 litre of distilled water in a conical flask plugged with cotton wool. The medium was sterilized by autoclaving at 121°C for 15 minutes under 15 psi pressure. After cooling to approximately 45–50°C, it was mixed thoroughly, aseptically poured into sterile Petri dishes, allowed to solidify, and the plates were dried before use.

Mannitol Salt Agar (MSA)

Mannitol Salt Agar (MSA) was prepared according to the manufacturer's instructions. The medium was used for the selective isolation and differentiation of Staphylococcus aureus. One hundred and eleven grams (111 g) of the dehydrated medium were weighed and dissolved in 1 litre of distilled water in a conical flask plugged with cotton wool. The medium was heated with gentle agitation until completely dissolved and sterilized by autoclaving at 121°C for 15 minutes under 15 psi pressure. After cooling to approximately 45–50°C, the medium was aseptically poured into sterile Petri dishes, allowed to solidify, and the plates were dried before use.

Microbiological Analysis

Isolation of Escherichia coli and Staphylococcus aureus

The tomato specimen was first subjected to a two fold serial dilution. Approximately 1g of each tomato sample was aseptically weighed, macerated using a sterile mortar and pestle, and homogenized in 9mL of sterile normal saline to obtain the stock suspension.

Serial dilution was carried out, after which 0.1 mL aliquots of the appropriate dilutions were inoculated onto Eosin Methylene Blue (EMB) agar for the isolation of *Escherichia coli* and Mannitol Salt Agar (MSA) for the isolation of *Staphylococcus aureus* using the spread plate technique. The inoculated plates were incubated at 37°C for 24–48 hours. Colonies exhibiting characteristic morphological features were selected and subcultured repeatedly on nutrient agar to obtain pure cultures.

Organisms were identified through conducting series of biochemical test such as indole, oxidase, catalase, coagulase, methyl red, Voges-Proskauer, citrate utilization, motility and sugar fermentation tests and confirmed using ABIS online identification tool.

Gram Staining

A loop full drop of distilled water was dropped on a clean grease free glass slide, the inoculating loop was sterilized by dry heat i.e. flaming it until it was red hot. The loop was allowed to cool and a little portion of the isolate was picked and smeared in the drop of distilled

water on the slide, making the smear a thin one and was allowed to air dry. Afterwards the smear was heat fixed with gentle heat by passing it over flame. The smear was flooded with 1% Crystal violet for 60 seconds and was rinsed with water, and was again flooded with Lugol's Iodine for 60 seconds and washed off with water. The slide was decolorized with 75% alcohol and was washed off almost immediately in other to avoid over decolorization, the slide was counter stained with Safranin for 60 seconds, rinsed with tap water, and smeared glass slides were air- dried. The slides were viewed under the light microscope, using the 100x objective lens (Oil immersion lens). The bacteria cells that appeared pink or red were the Gram-negative either cocci or rod, and those that appeared purple or blue were the Gram positive bacteria. (Cheesbrough, 2006).

Biochemical Test

Biochemical tests are fundamental tools in diagnostic microbiology used to identify bacterial species based on their metabolic capabilities. (Cappuccino & Welsh, 2017). These tests detect the presence or absence of specific enzymes or metabolic pathways which produce characteristic end-products or changes in the growth medium etc.

Results and Discussion

Bacterial Population

The microbial population of the fresh and spoiled tomato samples cultured on Eosin Methylene Blue (EMB) agar and Nutrient Agar (NA) showed the presence of bacterial contaminants in all six tomato samples. EMB counts ranged from $2.4 \pm 0.21 \times 10^2$ CFU/g (F1) to $7.9 \pm 0.51 \times 10^2$ CFU/g (S3), while Nutrient Agar counts ranged from $4.8 \pm 0.34 \times 10^2$ CFU/g (F1) to $9.2 \pm 0.61 \times 10^2$ CFU/g (S3). The spoiled tomato samples (S1–S3) generally recorded higher bacterial populations on both EMB and Nutrient Agar than the fresh tomato samples (F1–F3), indicating a greater microbial load associated with spoilage. Sample S3 recorded the highest bacterial population on both media, whereas F1 recorded the lowest bacterial population.

The p-values for EMB (0.024) and NA (0.013) were less than 0.05, indicating that there was a statistically significant difference in bacterial populations between the fresh and spoiled tomato samples. This suggests that spoilage was associated with a marked increase in microbial contamination, with Nutrient Agar yielding higher bacterial counts than EMB due to its ability to support the growth of a wider range of bacteria.

Table 4.1 presents the bacterial population of fresh and spoiled tomato samples cultured on Eosin Methylene Blue (EMB) agar and Nutrient Agar (NA). On EMB agar, spoiled tomato sample S3 recorded the highest bacterial population (7.9 ± 0.51), whereas fresh tomato sample F1 recorded the lowest (2.4 ± 0.21). On Nutrient Agar, S3 also recorded the highest bacterial population (9.2 ± 0.61), while F1 had the lowest (4.8 ± 0.34). Overall, the spoiled tomato samples (S1–S3) exhibited higher bacterial populations than the fresh tomato samples (F1–F3) on both culture media. Statistical analysis showed a significant difference ($p < 0.05$) between the bacterial populations of the fresh and spoiled tomato samples on both EMB and Nutrient Agar, as indicated by the different superscript letters and the p-values of 0.024 and 0.013, respectively.

Colonial, Morphological and Biochemical Characterization of the Bacterial Isolates

The results of the characterization of the bacterial isolates revealed their colonial and morphological characteristics such as colour, elevation, margin, form, and opacity. A total of 20 bacterial isolates were recovered from the six tomato samples and subjected to Gram staining and biochemical characterization. Of the 20 bacterial isolates recovered, 9 were Gram-negative while 11 were Gram-positive. Based on the colony morphology, Gram reaction and the objectives of the study, six representative isolates (three Gram-negative and three Gram-positive) were selected for biochemical characterization. The three Gram-negative isolates were confirmed as Escherichia coli, while the three Gram-positive isolates were confirmed as Staphylococcus aureus. The confirmed Escherichia coli isolates were Gram-negative rods, oxidase-negative, catalase-positive, indole-positive, methyl red-positive, Voges–Proskauer-negative, citrate-negative, motile, and demonstrated acid and gas production during glucose and lactose fermentation.

The confirmed Staphylococcus aureus isolates were Gram-positive cocci, catalase-positive, coagulase-positive, oxidase-negative, indole-negative, methyl red-negative, Voges–Proskauer-positive, citrate-positive, non-motile, and fermented mannitol and lactose with acid production. The confirmed isolates were subsequently

used for antimicrobial susceptibility testing.

Distribution of *Escherichia coli* and *Staphylococcus aureus* Isolates Recovered from Tomato Samples

Distribution of *Escherichia coli* Isolates Recovered from Tomato Samples

A total of 9 Gram-negative bacterial isolates were recovered from the tomato samples. Of these, 3 (33.3%) were confirmed as *Escherichia coli*. One isolate (33.3%) was recovered from fresh tomatoes, while two isolates (66.7%) were recovered from spoilt tomatoes (Table 4.3).

Distribution of *Staphylococcus aureus* Isolates Recovered from Tomato Samples

A total of 11 Gram-positive bacterial isolates were recovered from the tomato samples. Of these, 3 (27.3%) were confirmed as *Staphylococcus aureus*. All three confirmed *Staphylococcus aureus* isolates (100%) were recovered from spoilt tomatoes, while none was isolated from fresh tomatoes (Table 4.4).

Across all three *Escherichia coli* isolates, the 100% and 50% concentrations of aqueous *Citrus aurantiifolia* juice produced high antibacterial activity, with all isolates remaining susceptible. Antibacterial activity gradually decreased at 25%, 12.5% and 6.25% concentrations, while no inhibition was observed in the control. This indicates that aqueous *Citrus aurantiifolia* juice retained strong antibacterial activity against *Escherichia coli*, with effectiveness progressively declining as the extract concentration decreased.

Across all three *Escherichia coli* isolates, the 400 mg/mL concentration of ethanolic Allium cepa extract produced high antibacterial activity, while 200 mg/mL and 100 mg/mL generally produced moderate activity. Lower concentrations (50, 25 and 12.5 mg/mL) showed reduced antibacterial activity, although all isolates remained susceptible. Compared with aqueous *Citrus aurantiifolia* juice, the ethanolic *Allium cepa* extract produced smaller zones of inhibition across all concentrations, indicating comparatively lower antibacterial potency against *Escherichia coli*.

Across all three *Staphylococcus aureus* isolates, the 100% and 50% and 25% concentrations of aqueous *Citrus aurantiifolia* juice produced high antibacterial activity, with all isolates remaining highly susceptible. Moderate antibacterial activity was observed at 12.5% and 6.25% concentrations, while the 3.125% concentration produced no antibacterial activity. No inhibition was observed in the control. These findings indicate that aqueous *Citrus aurantiifolia* juice maintained strong antibacterial activity against *Staphylococcus aureus*, with antibacterial effectiveness gradually decreasing as the concentration of the extract decreased.

Across all three *Staphylococcus aureus* isolates, the 400 mg/mL concentration of ethanolic *Allium cepa* extract produced moderate antibacterial activity, while antibacterial activity declined progressively at 200, 100, 50, 25 and 12.5 mg/mL concentrations, with most isolates showing low susceptibility at the lower concentrations. Compared with aqueous *Citrus aurantiifolia* juice, ethanolic *Allium cepa* extract produced smaller zones of inhibition across all concentrations, indicating comparatively lower antibacterial activity against *Staphylococcus aureus*.

Antibacterial Activity of *Citrus aurantiifolia* Juice and Ethanolic *Allium cepa* Extract Against *Escherichia coli*

The antibacterial activities of *Citrus aurantiifolia* juice and ethanolic *Allium cepa* extract against the confirmed *Escherichia coli* isolates are presented in Tables 4.5and 4.6. The results showed that both extracts possessed antibacterial activity against the test organism, with the degree of inhibition decreasing as the concentration of each extract decreased.

The aqueous *Citrus aurantiifolia* juice exhibited greater antibacterial activity than the ethanolic *Allium cepa* extract at all concentrations tested. At the highest concentration (100%), *Citrus aurantiifolia* juice produced inhibition zones ranging from 23–25 mm, with a mean inhibition zone of 24.0 ± 1.0 mm, whereas the highest concentration of the ethanolic *Allium cepa* extract (400 mg/mL) produced inhibition zones ranging from 14–16 mm, with a mean inhibition zone of 15.0 ± 1.0 mm. As the concentrations decreased, the inhibition zones produced by both extracts also decreased. No zone of inhibition was observed at 3.125% lime juice concentration, while the lowest onion concentration (12.5 mg/mL) produced a mean inhibition zone of 6.0 ± 1.0

mm. The negative control produced no inhibitory effect against the test organism. Overall, *Citrus aurantiifolia* juice demonstrated stronger antibacterial activity against *Escherichia coli* than the ethanolic *Allium cepa* extract.

Antibacterial Activity of *Citrus aurantiifolia* Juice and Ethanolic *Allium cepa* Extract Against *Staphylococcus aureus*

The antibacterial activities of *Citrus aurantiifolia* juice and ethanolic *Allium cepa* extract against the confirmed *Staphylococcus aureus* isolates are presented in Tables 4.7 and 4.8. Both extracts inhibited the growth of the test organism, although the inhibitory activity varied with extract concentration.

Citrus aurantiifolia juice exhibited greater antibacterial activity than the ethanolic *Allium cepa* extract throughout the study. At 100% concentration, *Citrus aurantiifolia* juice produced inhibition zones ranging from 25–27 mm, with a mean inhibition zone of 26.0 ± 1.0 mm. In comparison, the highest concentration of ethanolic *Allium cepa* extract (400 mg/mL) produced inhibition zones ranging from 12–14 mm, with a mean inhibition zone of 13.0 ± 1.0 mm. The antibacterial activity of both extracts decreased progressively as the concentrations were reduced. No inhibition was observed at the lowest concentration of *Citrus aurantiifolia* juice (3.125%), while the lowest concentration of ethanolic *Allium cepa* extract (12.5 mg/mL) produced a mean inhibition zone of 2.7 ± 0.6 mm. The negative control produced no zone of inhibition.
Overall, *Staphylococcus aureus* was more susceptible to *Citrus aurantiifolia* juice than to the ethanolic *Allium cepa* extract under the conditions of this study

The present study demonstrated that fresh and spoilt tomatoes sold in the study area harboured bacteria of public health significance, including *Escherichia coli* and *Staphylococcus aureus*. Out of the 20 bacterial isolates recovered from six tomato samples, three isolates were confirmed as *Escherichia coli*, while three isolates were confirmed as *Staphylococcus aureus*. Two of the *Escherichia coli* isolates were recovered from spoilt tomatoes, whereas one was isolated from fresh tomatoes. In contrast, all three *Staphylococcus aureus* isolates were recovered from spoilt tomatoes. The occurrence of *Escherichia coli* in fresh tomatoes suggests contamination before or after harvest through irrigation water, contaminated soil, handling, transportation or marketing. The isolation of *Staphylococcus aureus* only from spoilt tomatoes may be attributed to increased bacterial multiplication resulting from deterioration of tomato tissues and poor handling during storage. Similar observations have been reported by Beuchat (2002), Olaimat and Holley (2012), and Arah *et al.,* (2015), who identified poor hygiene and post-harvest handling as major sources of microbial contamination of fresh vegetables.

The confirmed *Escherichia coli* isolates were identified by their characteristic Gram-negative, motile, rod-shaped morphology together with positive reactions for indole, methyl red and catalase tests and negative reactions for oxidase, Voges-Proskauer and citrate utilization tests. Likewise, the confirmed *Staphylococcus aureus* isolates were identified as Gram-positive cocci arranged in clusters that were positive for catalase, coagulase and citrate utilization tests but negative for oxidase and indole tests. These biochemical characteristics agree with the standard descriptions of the organisms reported by Cheesbrough (2010) and Bergey's Manual of Systematic Bacteriology (Whitman *et al.,* 2015), confirming the accuracy of the identification procedures employed in this study.

The antibacterial evaluation showed that both aqueous *Citrus aurantiifolia* juice and ethanolic *Allium cepa* extract inhibited the growth of *Escherichia coli* and *Staphylococcus aureus*.

However, aqueous *Citrus aurantiifolia* juice consistently produced larger zones of inhibition than the ethanolic *Allium cepa* extract against both bacterial species at all concentrations tested. This indicates that lime juice possessed greater antibacterial activity than onion extract under the conditions of the present study. This agreed with findings reported by Pathan *et al.,* (2012), who observed marked antibacterial activity of *Citrus aurantiifolia* against foodborne bacterial pathogens.

Table.1 Bacterial population

Isolate code	EMB (10^{-2})	NA (10^{-2})
F1E1	2.4 ± 0.21^{a}	4.8 ± 0.34^{a}
F2E2	3.8 ± 0.32^{a}	5.6 ± 0.41^{a}
F3E3	4.5 ± 0.28^{a}	6.3 ± 0.29^{a}
S1M1	5.7 ± 0.46^{b}	7.5 ± 0.52^{b}
S2M2	6.8 ± 0.39^{b}	8.4 ± 0.43^{b}
S3M3	7.9 ± 0.51^{b}	9.2 ± 0.61^{b}
P - value	0.024	0.013

Keys
F1E1 – FRESH TOMATO SAMPLE 1, *Escherichia coli* Isolate 1
F2E2 – FRESH TOMATO SAMPLE, 2 *Escherichia coli* Isolate 2
F3E3- FRESH TOMATO SAMPLE, 3 *Escherichia coli* Isolate 3
S1M1 – SPOILT TOMATO SAMPLE 1, *Staphylococcus aureus* Isolate 1
S2M2 – SPOILT TOMATO SAMPLE 2, *Staphylococcus aureus* Isolate 2
S3M3 – SPOILT TOMATO SAMPLE 3, *Staphylococcus aureus* Isolate 3

Table.2 Biochemical Test Results

Isolate code	Catalase	Oxidase	Citrate	Indole	MR	VP	Lactose	Mannitol	Glucose	Coagulase
F1E1	+	+	-	+	+	-	AG	A	AG	-
F2E2	+	+	-	+	+	-	AG	A	AG	-
F3E3	+	+	-	+	+	-	AG	A	AG	-
S1M1	+	-	+	-	+	+	A	A	AG	+
S2M2	+	-	+	-	+	+	A	A	AG	+
S3M3	+	-	+	-	+	+	A	A	AG	+

Keys
MR – Methyl- Red
VP- Vogues – Proskaeur
F1E1 – FRESH TOMATO SAMPLE 1, *Escherichia coli* Isolate 1
F2E2 – FRESH TOMATO SAMPLE, 2 *Escherichia coli* Isolate 2
F3E3- FRESH TOMATO SAMPLE, 3 Escherichia coli Isolate
S1M1 – SPOILT TOMATO SAMPLE 1, *Staphylococcus aureus* ISOLATE 1
S2M2 – SPOILT TOMATO SAMPLE 2, *Staphylococcus aureus* ISOLATE 2
S3M3 – SPOILT TOMATO SAMPLE 3, *Staphylococcus aureus* ISOLATE 3
A – Acid produced
AG- Acid and Gas produced
N – No fermentation

Table.3 Distribution of *Escherichia coli* isolates from Tomato Samples

Source of tomato samples	Number of Gram negative isolates	Number of *Escherichia coli* isolates	Distribution of confirmed isolates (%)
Fresh tomatoes	1	1	33.3
Spoilt tomatoes	8	2	66.7
Total	9	3	100.0

Table.4 Distribution of *Staphylococcus aureus* isolates from Tomato Samples

Source of tomato samples	Number of Gram positive isolates	Number of *Staphylococcus aureus* isolates	Distribution of confirmed isolates (%)
Fresh tomatoes	0	0	0
Spoilt tomatoes	11	3	100.0
Total	11	3	100.0

Table.5 Measured zones of inhibition of *Citrus aurantiifolia* Juice Against *Escherichia coli*

Isolate code	100%	50%	25%	12.5%	6.25%	3.125%	Control%
F1E1	24mm	19mm	16mm	12mm	8mm	0mm	0mm
S1E1	23mm	18mm	14mm	11mm	9mm	0mm	0mm
S2E2	25mm	20mm	15mm	13mm	10mm	0mm	0mm
Mean+ S.D	24.0±1.0 mm	19.0±1.0 mm	15.0±1.0 mm	12.0±1.0 mm	9.0±1.0 mm	0mm	0mm

Susceptibility Classification of *Escherichia coli* Isolates by Concentration
(≥15 mm = High, 10–14 mm = Moderate, 1–9 mm = Low, 0 mm = Resistant)

Table.6 Measured zones of inhibition of Ethanolic *Allium cepa* Extract Against *Escherichia coli*

Isolate code	400 g/ml	200 g/ml	100 g/ml	50 g/ml	25 g/ml	12.5 g/ml	Contro g/mll
F1E1	16mm	14mm	13mm	11mm	9mm	7mm	0mm
S1E1	15mm	13mm	11mm	10mm	8mm	6mm	0mm
S2E2	14mm	12mm	12mm	9mm	7mm	5mm	0mm
Mean + S.D	15.0±1.0 mm	13.0±1.0 mm	12.0±1.0 mm	10.0±1.0 mm	8.0±1.0 mm	6.0±1.0 mm	0mm

Susceptibility Classification of *Escherichia coli* Isolates by Concentration
(≥15 mm = High, 10–14 mm = Moderate, 1–9 mm = Low, 0 mm = Resistant)

Table.7 Measured zones of inhibition of *Citrus aurantiifolia* Juice Against *Staphylococcus aureus*

Isolate codes	100%	50%	25%	12.5%	6.25%	3.125%	Control%
S1M1	27mm	20mm	18mm	15mm	13mm	0mm	0mm
S2M2	25mm	18mm	17mm	14mm	12mm	0mm	0mm
S3M3	26mm	19mm	19mm	16mm	14mm	0mm	0mm
Mean + S.D	26±1.0 mm	19.0±1.0 mm	18±1.0 mm	15±1.0 mm	13±1.0 mm	0mm	0mm

Susceptibility Classification of *Staphylococcus aureus* Isolates by Concentration
(≥15 mm = High, 10–14 mm = Moderate, 1–9 mm = Low, 0 mm = Resistant)

Table.8 Zone of inhibition of Ethanolic *Allium cepa* Extract Against *Staphylococcus aureus*

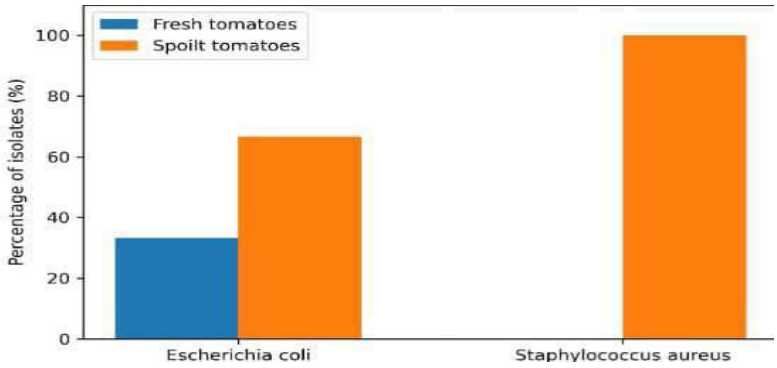
Isolate codes	400g/ml	200g/ml	100g/ml	50g/ml	25g/ml	12.5g/ml	Control
S1M1	13mm	11mm	9mm	8mm	7mm	3mm	0mm
S2M2	12mm	10mm	8mm	7mm	6mm	2mm	0mm
S3M3	14mm	12mm	9mm	9mm	8mm	3mm	0mm
Mean + S.D	13.0±1.0 mm	11.0±1.0 mm	8.7±1.0 mm	8.0±0.6 mm	7.0±1.0 mm	2.7±0.6mm	0mm

Susceptibility Classification of *Staphylococcus aureus* isolates by Concentration
(≥15 mm = High, 10–14 mm = Moderate, 1–9 mm = Low, 0 mm = Resistant)

Figure 2.2 *Citrus aurantiifolia*

Figure.2 *Allium cepa*

Figure.4 Percentage Distribution of *Escherichia coli and Staphylococcus aureus* isolates Recovered from Fresh and Spoilt Tomato Samples



Both plant extracts exhibited a clear concentration-dependent antibacterial effect. The largest inhibition zones were observed at the highest concentration, while the inhibition zones progressively decreased as the extracts were diluted. This concentration-dependent pattern agrees with previous studies and is most likely due to the greater quantity of bioactive phytochemicals present in the undiluted extracts. Higher concentrations provide more active compounds capable of diffusing through the agar medium, penetrating bacterial cells and disrupting essential cellular processes. As the extracts become diluted, the amount of active compounds available to inhibit bacterial growth decreases, resulting in smaller inhibition zones. Similar concentration-dependent antibacterial activity has been reported by Burt (2004).

The greater antibacterial activity exhibited by aqueous *Citrus aurantiifolia* juice may be attributed to its abundance of citric acid, flavonoids, limonoids, tannins and other phenolic compounds. These phytochemicals have been reported to disrupt bacterial cell membranes, alter membrane permeability, interfere with enzyme activity and cause leakage of intracellular components, ultimately inhibiting bacterial growth. Although ethanolic Allium cepa extract also demonstrated antibacterial activity due to sulphur-containing compounds such as allicin together with flavonoids and phenolic compounds, its inhibitory effect was lower than that of lime juice throughout the study. Similar mechanisms have been described by Griffiths *et al.,* (2002) and Rose *et al.,* (2005).

Both *Escherichia coli* and *Staphylococcus aureus* were susceptible to the two plant extracts. Nevertheless, aqueous *Citrus aurantiifolia* juice consistently produced greater inhibition against both organisms than ethanolic *Allium cepa* extract. This suggests that lime juice possesses stronger antibacterial potential against these common foodborne bacteria and may therefore be a more effective natural antimicrobial agent for reducing bacterial contamination of tomatoes.

Taken together, the findings of the present study demonstrate that both aqueous *Citrus aurantiifolia* juice and ethanolic *Allium cepa* extract possess concentration-dependent antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* isolated from tomato samples. The antibacterial activity of both extracts increased with increasing concentration, with aqueous *Citrus aurantiifolia* juice consistently producing larger zones of inhibition than ethanolic *Allium cepa* extract against both test organisms. The isolation of *Escherichia coli* from both fresh and spoilt tomatoes and *Staphylococcus aureus* from spoilt tomatoes further confirms that tomatoes can serve as vehicles for potentially pathogenic bacteria when handled or stored under poor hygienic conditions. These findings suggest that aqueous *Citrus aurantiifolia* juice has greater potential as a natural antibacterial agent for reducing bacterial contamination of tomatoes, while ethanolic *Allium cepa* extract may also serve as a useful complementary antimicrobial agent. Overall, the study highlights the importance of maintaining proper hygiene during cultivation, harvesting, transportation, storage and marketing of tomatoes in order to reduce microbial contamination and minimize the risk of food-borne diseases associated with contaminated fresh produce.

Author Contributions

M. I. Robinson: Investigation, formal analysis, writing—

original draft. A. K. Ndaguba: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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