Antimicrobial and Antioxidant Activities of *Chrysopogon zizanioides* Root Extract against Some Human Pathogenic strains

Haymanot Tewelde

A Thesis Submitted to the Department of Applied Biology, School of Applied Natural Science

Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science in Applied Biology (Microbiology)

Office of Graduate Studies

Adama Science and Technology University

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Antimicrobial and Antioxidant Activity of *Chrysopogon zizanioides* Root Extract against Some Human Pathogenic strains and Its Bioactive Compound Characterization

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DECLARATION

I hereby declare that this Master Thesis entitled "Antimicrobial and Antioxidant Activities of *Chrysopogon zizanioides* Root Extract against Some Human Pathogenic strains" is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

Name of student	Signature	Date

RECOMMENDATION

We, the advisor(s) of this thesis, hereby certify that we have read the revised version of the thesis entitled "Antimicrobial and Antioxidant Activities of *Chrysopogon zizanioides* Root Extract against Some Human Pathogenic strains" prepared under our guidance by **Haymanot Tewelde** submitted in partial fulfillment of the requirements for the degree of Master's of Science in Applied Microbiology. Therefore, we recommend the submission of a revised version of the thesis to the department following the applicable procedures.

Major Advisor	Signature	Date
Co-advisor	Signature	Date

APPROVAL PAGE

We, the advisors of the thesis entitled "Antimicrobial and Antioxidant Activities of *Chrysopogon zizanioides* Root Extract against Some Human Pathogenic strains" and developed by **Haymanot Tewelde** hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

Major Advisor	Signature	Date
Co-advisor	Signature	Date

APPROVAL OF BOARD OF REVIEWERS

We, the undersigned, members of the Board of Examiners of the thesis by Haymanot Tewelde have read and evaluated the thesis entitled "Antimicrobial and Antioxidant Activities of *Chrysopogon zizanioides* Root Extract against Some Human Pathogenic strains" and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Microbiology.

Chairperson	Signature	Date
Reviewer 1	Signature	Date
Reviewer 2	Signature	Date

Final approval and acceptance of the thesis proposal is contingent upon the submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

Department Head	Signature	Date
School Dean	Signature	Date
Office of Postgraduate Studies, Dean	Signature	Date

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LIST OF ACRONYMS

ABTS	- 2, 2-azinobis (3-ethylbenzothiazoline- 6-sulfonic acid)
ASTM	- American Society for Testing and Materials
ATCC	- American Type Culture Collection
CC	- Column Chromatography
DPPH	- 2,2-diphenyl-1-picrylhydrazyl
EO	- Essential oil
EtOAc	- Ethyl acetate
FT IR	- Fourier-transform Infrared Spectroscopy
GC-MS	- Gas Chromatography and Mass Spectrometry
HPLC	- High Pressure Liquid Chromatography
MBC	- Minimum Bactericidal Concentration
MHA	- Muller Hinton Agar
MIC	- Minimum Inhibitory Concentration
NMR	- Nuclear Magnetic Resonance
TLC	- Thin Layer Chromatography
UV	- Ultraviolet

ABSTRACT

Chrysopogon zizanioides, vetiver or Khush, is a perennial bunchgrass of the family Poaceae. C. zizanioides is found to be effective against many infections from bacteria, fungi and protozoa. The aim of this study was to determine the antimicrobial and antioxidant activities of C. zizanioides root extract against selected human pathogenic microbial strains and to analyze its chemical composition. The root of C. zizanioides was washed, air dried, powdered and extracted by using petroleum ether, chloroform, and methanol. The chloroform extract was subjected to column chromatography over silica gel (60-200 mesh). The silica gel column chromatographic separation of the chloroform extract from fraction 27 afforded compound 1. The structures of the isolated compound were characterized using infra-red (IR) spectrum and NMR spectroscopic techniques (^{1}H NMR, ^{13}C NMR, DEPT-135). The C. zizanioides root was hydro distilled by using a Clevenger apparatus. The crude extract and isolated compound 1 of the plant were tested against Escherichia coli (ATCC 25923), Pseudomonas aeruginosa (ATCC 27823), Staphylococcus aureus (ATCC 25923), Streptococcus pyogenes (ATCC 11778) and Candida albicans (ATCC 10231). The crude extract and essential oil of C. zizanioides root were assessed for radical scavenging activity using DPPH assay. Antibacterial activities of the plant extracts were assessed using the agar disk diffusion method. The isolated compound was terpenoid.Gas chromatography and mass spectrometry analysis revealed the presence of a total of 19 chemical components. The petroleum ether extract at 200 mg/ml exhibited the maximum inhibition zone of 18.33±2.08 mm against S. pyogenes, while the chloroform and methanol extract at 200 mg/ml showed maximum inhibition zones of 19±1 mm against C. albicans. The isolated compound 1 exhibited maximum inhibition against S. aureus 12±0mm at a concentration of 10 mg/ml and C. albicans 17. 66±0mm. Among the studied crude samples chloroform extract at 500µg/mL showed the best radical scavenging activity with a % inhibition of 93.32 and an IC_{50} of 5.603. In the case of essential oil of C. zizanioides root radical scavenging activity % inhibition was 30.56 and its IC_{50} value was 255.88.The finding of this study showed that C. zizanioides root has considerable antimicrobial, and antioxidant properties, which should be further examined with particular attention to the mechanisms of antimicrobial activity.

Keywords: Column chromatography, Essential oil, Phytochemical

1. INTRODUCTION

1.1. Background

Antimicrobial agents play a crucial role in mitigating the worldwide impact of infectious diseases (Bhatia & Narain, 2010). Nevertheless, the rise and dissemination of multidrug-resistant (MDR) strains among pathogenic bacteria pose a considerable threat to public health, as the options for effective antimicrobial agents against bacterial infections become increasingly limited(*Boucher et al*., 2009; Giamarellou, 2010). Thus, the development of novel antimicrobial drugs is vital in light of the rapidly spreading evidence of resistant clinical isolates worldwide. However the history of newly introduced antimicrobial agent resistance emerging quickly and widely indicates that even new antimicrobial agent families will have a short shelf life (Coates *et al*., 2002; Marasini *et al*., 2015). Various beneficial plants have been discovered to be excellent sources of naturally occurring antimicrobial substances that may be useful in the control of these difficult bacterial illnesses (Iwu *et al*., 1999). The World Health Organization (WHO) considers medicinal plants to be the best source of many different kinds of drugs (Rodgers & Vaughan, 2002). Because of the phytochemicals produced in the plant's secondary metabolism, many plants have been utilized for their antibacterial capabilities (Medina et al., 2005; Romero *et al*., 2005). Numerous secondary metabolites, such as flavonoids, phenolic compounds, alkaloids, and tannins, are found in plants and have been shown to have antibacterial activity under *in vitro* condition (Djeussi *et al*., 2013; Duraipandiyan *et al*., 2006). Many biological activities, such as antibacterial, anti-inflammatory, and antioxidant qualities, have been shown using extracts from medicinal plants (Mehta *et al*., 2001).

Compounds with antimicrobial properties extracted from medicinal plants could hinder the proliferation of bacteria, fungi, viruses, and protozoa through mechanisms distinct from those employed by existing antimicrobials. Such compounds are anticipated to hold substantial clinical significance in addressing resistant microbial strains (Shankar *et al*., 2010). In the mid-1980s, the World Bank introduced *C. zizanioides*, a tall and enduring grass indigenous to India, specifically for the purpose of soil and water conservation. This grass is characterized by the formation of a spongy, extensively branched root system (Barad *et al*., 2013). The root serves as the main provider of the renowned *C. zizanioides* oil, a valuable stabilizer in the formulation of cosmetics and an essential ingredient in perfumes, agarbattis, soaps, soft drinks, and pan masala. Additionally, *C. zizanioides* essential oil offers various benefits such

as fortifying bones and addressing conditions like rheumatism, gout, arthritis, muscle aches, dryness, cramps, and dry skin (Balasankar *et al*., 2013).Historically, indigenous tribes in India have employed different components of *C. zizanioides* for the traditional treatment of a diverse range of cases including boils, burns, urinary tract infections, malarial fever, epilepsy, general fever, headaches, toothaches, weakness, rheumatism, and as an anti-helminthic remedy (Luqman, 2012).The main objective of this study is to evaluate the antimicrobial and antioxidant activities of *C. zizanioides* root, extracts, and isolated compound 1 against (ATCC 25923), (ATCC 27823), (ATCC 25923), (ATCC 11778) and (ATCC 10231).

1.2. Statement of the problem

Pathogenic microorganisms can induce diverse diseases by entering the body through various mechanisms. Antibiotics, whether natural or synthetic, are employed for treating these pathogens. Nevertheless, while synthetic antibiotics possess therapeutic benefits, they also have negative side effects on human health (H. H. Wang *et al*., 2006). Due to the adverse effects associated with synthetic antibiotics, scientists are exploring natural alternatives devoid of such drawbacks. Notably, the scientific community has shown interest in medicinal and aromatic plants traditionally employed by local communities to address various bacterial diseases. An example is *C. zizanioides* (Poaceae), commonly known as khus grass, which has been utilized in India since ancient times. The root extract is applied for alleviating headaches and toothaches, the leaf paste for cases like lumbago, sprains, and rheumatism, the stem decoction for treating urinary tract infections, the leaf juice as an anthelmintic, vapors for managing malarial fever, and the root ash for relief from acidity (Singh & Maheshwari, 1983). In Ethiopia there is no published research paper on the phytochemical analysis, antibacterial and antioxidant activity of *C. zizanioides.* The plant was selected for this study based on ethnomedical information by which local communities use it and its genus is rich sources of variety of classes of secondary metabolites with different pharmacological properties. Since *C. zizanioides* are utilized in society, this work aims to study these properties of the vetiver grass grown in Ethiopia to fulfil this knowledge gap.

1.3. Objective of the study

1.3.1. General objective

- The general objective of this study was to evaluate the antimicrobial and antioxidant activities of *C. zizanioides* extracts against some human pathogenic strains

1.3.2. Specific objective

- To screen the phytochemicals components of *C. zizanioides* root extracts.
- To isolate phytochemicals components of *C. zizanioides* root extracts by chromatographic techniques.
- To characterize the bioactive compounds obtained from the root of *C. zizanioides* using NMR, and FTIR.
- To extract essential oil from the plant using hydro distillation and analyze its components using GC-MS.
- To determine the antimicrobial activity of *C. zizanioides* root extracts, isolated compound against selected human pathogenic strains.

1.4. Scope of the study

The sampling area for this study was Wondo Genet Agricultural Research Center. The study was conducted at Adama Science and Technology University. This research was mainly focused on the antimicrobial and antioxidant activity of *C. zizanioides* root extracts on only some human diseases caused by bacterial and fungi species.

1.5. Significance of the study

This research is expected to:

- ✓ Provide antimicrobial activity information of *C. zizanioides* root extracts and bioactive compounds against selected microbial strains to provide scientific support.
- ✓ To provide scientific proof for claims of traditional uses of the plants to treat infectious diseases.
- ✓ Provide information on essential oils composition of *C. zizanioides* root extract.
- ✓ Provide baseline information for future related scientific studies on this plant.

2. LITERATURE REVIEW

2.1. Human pathogenic microorganisms

Microorganisms used in the study were *Escherichia coli* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27823), *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 11778) and *Candida albicans (*ATCC 10231).

In 1885, Theodor Escherich identified and described *E. coli* as rod-shaped, Gram-negative bacteria that belong to the Enterobacteriaceae family. The initial isolation of these bacteria occurred from infant stool samples (Escobar-Páramo *et al*., 2003). A few hours after birth, *E. coli* colonize and inhabit the gastrointestinal tract of infants. Due to numerous mutual advantages, humans and commensal *E. coli* strains live together harmoniously without any negative consequences. Nevertheless, in individuals with compromised gastrointestinal barriers or weakened immune systems, commensal *E. coli* can lead to illness (Tenaillon *et al.*, 2010) Significantly, specific *E. coli* strains play a role in diverse diseases, impacting both humans and animals on a global scale. There are nine pathovars identified in *E. coli* strains isolated from humans, contributing to conditions such as diarrheagenic and extraintestinal diseases (Nash *et al*., 2010). Of these, seven pathotypes have been described as enteric pathogenic *E. coli*, including Enteropathogenic *E. coli* (EPEC), Enterohaemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Diffusely adherent *E. coli* (DAEC), and a new pathotype, Adherent-Invasive *E. coli* (AIEC), causing mostly diarrhea and intestinal disorders. However, the EHEC pathotype has been implicated in extraintestinal diseases such as Hemolytic Uremic Syndrome (HUS) (Donnenberg, 2013).

P. aeruginosa is a prevalent opportunistic pathogen that falls under the *Pseudomonas* (Mackie & McCartney, 1989). *P. aeruginosa* is an obligate aerobic bacterium, characterized by its motility and rod-shaped structure, measuring approximately 0.6 x 2 µm. Gram-negative in nature, it typically appears individually, in pairs, or occasionally in short chains. On occasions, it emits a fragrance resembling sweetness, grapes, or corn (Al-Kobaisi, 2007). Its production of blue, yellow, or rust-colored pigments distinguishes it from most other gram-negative bacteria. The blue pigment, pyocyanin, is produced only by *P. aeruginosa.* Fluorescin, a yellow pigment that fluoresces under ultraviolet light is by *P. aeruginosa* and other free-living less pathogenic *Pseudomonas* species. Pyocyanin produced and fluorescin

combined produce a bright green color that diffuses throughout the medium (Ryan *et al*., 2004). *P. aeruginosa* thrives in temperatures ranging from 37–42 °C, and its ability to grow optimally at 42 °C distinguishes it from other *Pseudomonas* species. Unlike fermenting carbohydrates, it's common for many strains to oxidize glucose (Al-Kobaisi, 2007). *P. aeruginosa* commonly live soil, water, and vegetation and can be isolated from the skin, throat, and stool of healthy persons. They commonly inhabit hospital food, sinks, taps, mops and respiratory equipment.

Spread is from patient to patient through contact with fomites or by ingestion of contaminated food and water (Baron, 1996). *P. aeruginosa* is responsible for infections in both healthy individuals and those with compromised immune systems, either due to hospitalization or other underlying diseases. This bacterium is commonly linked to various human infections, including urinary tract infections, ventilator-associated pneumonia, surgical site infections, ear infections (external otitis, malignant external otitis), and skin and soft tissue infections, such as hot tub folliculitis and osteomyelitis. People with conditions that compromise their immune systems, such as HIV/AIDS, cystic fibrosis, chemotherapy-related neutropenia, and diabetes, face an elevated risk of infection and potential complications. (Trautmann *et al*., 2008). *P. aeruginosa* often exhibits resistance to numerous commonly prescribed antibiotics. While some strains remain susceptible to gentamicin, tobramycin, colistin, and amikacin, the emergence of resistant forms underscores the importance of conducting susceptibility testing (Baron, 1996).

S. aureus were members of the genus *Staphylococcus* (*staphylococci*) are Gram-positive cocci that tend to be organized in grape-like clusters (Ryan *et al*., 2004). *S. aureus* is a facultative anaerobe with an optimal growth temperature of 37 ºC and a preferred pH of 7.5. These bacteria exhibit a spherical shape, approximately 1cm in diameter, and organize into asymmetrical clusters. In liquid cultures, one can observe single cocci, pairs, tetrads, and chains. In their early stages, these cocci stain prominently as Gram-positive, but as they age, many cells transition to a Gram-negative state. *Staphylococci* lack motility and spore-forming capabilities (Al-Kobaisi 2007). On nutrient agar, after 24 hours of aerobic incubation at 37ºC, the colonies measure 1 – 3mm in diameter. They exhibit a smooth, shiny surface, a complete edge, and an opaque pigmented appearance. Typically, the pigmentation appears golden, with variations in shades such as orange, yellow, and cream across different strains. When cultured

on MacConkey agar, the colonies are generally small to medium in size and display a pink or pink-orange color (Mackie & McCartney, 1989).

S. aureus forms initially white colonies that gradually transition to a buff-golden hue over time, a characteristic that aligns with the species epithet aureus, meaning golden. While not universal, a considerable number of strains exhibit a border of clear β-hemolysis surrounding the colony (Ryan *et al*., 2004).

Staphylococci are widely prevalent inhabitants of both humans and animals. They primarily reside on the skin, particularly in moist regions like the anterior nares (nose), axilla, and groin. A significant proportion of individuals, ranging from one-third to three-quarters, carry these organisms at any given time. *Staphylococcal* infections are a global occurrence, and recently, highly virulent or antibiotic-resistant strains have rapidly spread across extensive geographical areas. The bacteria can persist in the air, on objects, or in dust for days, leading to the potential contamination of environments, including hospitals, with ongoing transmission over extended periods. Some individuals may shed the organism more prolifically than others. *Staphylococcal* infections can be acquired from either internal (endogenous) or external (exogenous) sources (Lenart-Boroń *et al*., 2017).

Staphylococcus aureus causes severe infections of the skin, soft tissues, bone, lung, heart, brain or blood (Lenart-Boroń *et al*., 2017). This encompasses pneumonia, bacteremia resulting in subsequent pneumonia and endocarditis, osteomyelitis resulting from bacteremia, and septic arthritis, observed in pediatric patients and individuals with a background of rheumatoid arthritis. Illnesses induced by *Staphylococcal* toxins encompass scalded skin syndrome and toxic shock syndrome (Al-Amin *et al*., 2009). A positive β-lactamase test can anticipate resistance to penicillin G, with around 90% of *S. aureus* strains exhibiting β-lactamase production. Resistance to nafcillin, as well as oxacillin and methicillin, is found in about 35% of *S. aureus* and roughly 75% of S. epidermidis isolates (Al-Kobaisi, 2007). Alternative antibiotics for resistant organisms (e.g. MRSA) include vancomycin, erythromycin and gentamicin. Some strains become resistant to multiple antibiotics (Lenart-Boroń *et al*. 2017).

S. pyogenes is a Gram-positive bacterium belonging to the *Streptococcus* genus, which is aerotolerant. *Streptococci* are spherical bacteria, and the species name originates from Greek terms meaning 'a chain' (streptos) of berries (coccus) (Latinized from kokkos) and pus (pyo) forming (genes). This nomenclature reflects the tendency of streptococcal cells to form chains

of round cells (refer to the image), and infections caused by this bacterium often result in the formation of pus. The primary distinguishing factor between *Staphylococcus spp*. and *Streptococcus spp*. lies in the catalase test, where Staphylococci are catalase-positive, whereas streptococci are catalase-negative (Ryan *et al*., 2004). *S. pyogenes* can be cultured on fresh blood agar plates under ideal conditions; it has an incubation period of 1 to 3 days (Aziz *et al*., 2010).

S. pyogenes typically inhabits the throat, genital mucosa, rectum, and skin. Of healthy individuals, 1% to 5% has throat, vaginal, or rectal carriage. In healthy children, such carriage rate varies from 2% to 17%. There are four methods for the transmission of this bacterium: inhalation of respiratory droplets, skin contact, and contact with objects, surface, or dust that is contaminated with bacteria or, less commonly, transmission through food. Such bacteria can cause a variety of diseases such as streptococcal pharyngitis, rheumatic fever, rheumatic heart disease, and scarlet fever. The number of pharyngitis cases is higher in children when compared with adults due to exposures in schools, nurseries, and as a consequence of lower host immunity. Such cases *Streptococcal pharyngitis* occurs more frequently from December to April (later winter to early spring) in seasonal countries, possibly due to changing climate, behavioral changes or predisposing viral infection. Disease cases are the lowest during autumn (Ferretti *et al*., 2016).

S. pyogenes is the cause of many human diseases, ranging from mild superficial skin infections to life-threatening systemic diseases (Ryan *et al.,* 2004). Infections usually initiate in the throat or skin and are notably identified by a rash resembling strawberries. Mild cases of *S. pyogenes* infections encompass conditions like pharyngitis (commonly known as strep throat) and localized skin infections like impetigo. Erysipelas and cellulitis are characterized by the bacterial multiplication and lateral spread of S. pyogenes in the deeper layers of the skin. This bacterium is also present in neonatal infections (Baucells *et al*., 2016).

C. albicans is opportunistic pathogenic yeast (Gow and Yadav 2017).That is a common member of the human gut flora and belonging to the genus *Candida*. *C. albicans* is frequently used as a typical organism for fungal pathogens (Kabir *et al*., 2012). It is often referred to as a dimorphic fungus because it has the ability to grow as both yeast and filamentous cells. However, it displays various morphological phenotypes beyond this dual growth form (Kadosh, 2019). *C. albicans* was traditionally believed to be exclusively diploid, lacking a haploid stage. Contrary to this belief, it has been discovered that *C. albicans* can indeed exist

in a haploid stage, and in addition to that, it can also exist in a tetraploid stage. The tetraploid stage is achieved through the mating of diploid *C. albicans* cells when they are in the opaque form (Hickman *et al*., 2013). The diploid genome is estimated to be around 29 Mb in size, with nearly 70% of the protein-coding genes still awaiting characterization (Kabir *et al*., 2012).

2.1.1. Antibiotic resistance

Antibiotics are mainly used both in human and veterinary medicine to insure human and animal health worldwide. Beside medical therapy, antibiotics have also been used to improve aquaculture and agricultural production. However, the emergence of resistant bacteria to commonly used effective antibiotics, resulted in the need for stronger drugs and more costly therapy. New forms of antibiotic resistance can even easily crossing with remarkable speed across international boundaries and spread between continents. World health leaders have described antibiotic resistant microorganisms as "nightmare bacteria" that "pose a catastrophic threat" to people in every country in the world (Wang *et al*., 2012). Some studies on bacterial resistance have shown that there is a huge diversity of resistance mechanisms, in which the distribution and interaction is mostly complex and unknown. However, there are varieties of biochemical and physiological mechanisms that are responsible for the development of antibiotic resistance. The mechanism of resistance may be evolution of either genetically inherent or the result of the microorganism being exposed to antibiotics. Most of the antibiotic resistance has emerged as a result of mutation or through transfer of genetic material between microorganisms. Several of various recent studies revealed that almost 400 different bacteria have demonstrated about 20,000 possible resistant genes (Davies & Davies, 2010). The resistances that evolve within bacteria that affect animals have the potential to affect humans. Zoonosis of the resistant strains is able to occur, posing a risk to human health. People who are employed at farms or food animal production facilities are at a higher risk of infection with a resistant strain of bacteria (Sarmah *et al*., 2006).

2.2.2. The use of Natural Products as Antimicrobials

Natural products (NPs) make up a heterogeneous group of chemical entities that possess diverse biological activities with various uses in fields such as human and veterinary medicine, agriculture and industry. Molecules from the secondary metabolism of animals, vegetables, bacteria and fungi are classified as NPs, which are not crucial for the producer's

survival under laboratory conditions, but which give him a clear advantage over his competitors in his native habitat (Katz & Baltz, 2016). Since the discovery of penicillin, more than 23,000 new NPs have been characterized, many of which have proven to be valuable tools in the field of pharmacology, herbicides, insecticides and more (Bérdy, 2012).

One of the main sources of antimicrobial NPs is plants. Plant organisms make up most of the biosphere on planet Earth, whose biomass accounts for a percentage greater than 80% of the total biomass (Bar-On *et al*., 2018). Since their appearance, plants have survived, evolved and adapted to all types of ecosystems and adverse conditions. This adaptive process has led them to develop complex and effective defense systems against external aggressions: predators, abiotic stress and, of course, infections. Being sessile organisms that cannot escape their threats, plants have developed a splendid chemical arsenal in the form of secondary metabolites capable of coping with the most dangerous pathogens(Muthamilarasan & Prasa, 2013). Humanity has made use of the medicinal properties of plants for thousands of years. There is evidence that in the year 5000 BC. the Sumerians already used thyme for its beneficial health properties (Muthamilarasan & Prasad, 2013).

2.2. The concept of medicinal plant

Medicinal plants, designated as plants utilized for sustaining health or addressing health issues, play integral roles in diverse medical systems, including both allopathic and traditional approaches worldwide. Even individuals exclusively accustomed to allopathic medicine are likely to have some dependence on medicinal plants, given that 20-25% of prescribed drugs are derived from plants (Chukwuemerie *et al*., 2022). As per the World Health Organization, 80% of the global population depends exclusively or predominantly on traditional remedies for their healthcare needs (Ma *et al*., 2022). There is speculation suggesting that the substantial reliance on medicinal plants extends to over two billion individuals (Lambert *et al*., 1997).

2.3. Vetiver Grass in Ethiopia

The precise origin of vetiver grass in Ethiopia remains uncertain, as certain sources suggest its introduction in 1971 by a British agronomist affiliated with the Jimma Research Center (JRC) during a coffee intensification program (Alemu *et al*., 2014), Some sources suggest that Ethiopian coffee researchers, who had traveled to India for education, brought vetiver grass into Ethiopia, while others claim it was introduced by an Indian scientist who worked as an

expatriate at the Jimma Research Center. However, the central point across all accounts is that vetiver grass is not native to Ethiopia. Its introduction aimed primarily to delineate different coffee estates and manage the spread of the toxic grass known as Cynodon dactylon. Furthermore, vetiver grass was first introduced to Ethiopia around the mid-1980s, initially at the Jimma Research Station and then to nearby coffee state farms and NGOs for mulching and soil erosion conservation purposes.

In 1984, vetiver grass was first deployed beyond the JRC to government coffee sites and demonstration areas by Menschen für Menschen (MfM), a German-based NGO, to utilize it for mulching and controlling soil erosion. Over the subsequent years, vetiver grass was disseminated to various districts including Illubabor, Debrezeit, and the Holetta Research Center, primarily to address soil erosion issues (Ayano, 2015). During the 1990s, the widespread dissemination of vetiver grass extended to every corner of the country, encompassing the Bale zone and reaching the Sinana Agricultural Research Center (Eshetu *et al*., 2017), Somali region (Eshetu *et al*., 2017), Wolayta, Gonder, Tigray (Lavania 2004; Maffei 2002).

2.4.Taxonomic distribution ,traditional uses and Chemical composition of *C. zizanioides*

C. zizanioides, a versatile and globally utilized grass, originates from India and falls under the Poaceae family. It is cultivated in diverse landscapes such as plains, river banks, marshy areas, and lower hills, thriving at elevations up to 1200m (Balasankar *et al*., 2013). This plant is extensively found in tropical and subtropical regions, with India being a particularly notable habitat. While it can thrive in various soil types, well-drained soil is optimal for its robust growth. The annual growth rate is most favorable in regions with ample rainfall and temperatures ranging from 22 to 43°C. Cultivation during the monsoon season is optimal, although it is also practiced in early January. Different parts of the plant, especially the roots, are utilized for medicinal purposes. The oil extracted from *C. zizanioides* is employed for treating inflammation and infections (Figure 1), and it holds commercial significance in the fields of perfumery and aromatherapy (Nantachit *et al.*, 2010).

In addition to its distinct fragrance, *C. zizanioides* exhibits diverse biological activities, including antioxidant properties (Luqman *et al*., 2009), antibacterial (Luqman *et al*., 2005), and anti-inflammatory properties (Aravind *et al*., 2013), making it beneficial in aromatherapy

(Devprakash *et al*., 2011). Numerous tribal communities in the subcontinent employ different components of the grass to address various health issues, including boils, burns, epilepsy, fever, scorpion stings, snakebites, and mouth sores. The root paste is applied for alleviating headaches and toothaches, the leaf paste for cases like lumbago, sprains, and rheumatism, the stem decoction for treating urinary tract infections, the leaf juice as an anthelmintic, vapors for managing malarial fever, and the root ash for relief from acidity (Jain, 1991; Singh *et al*., 1983).

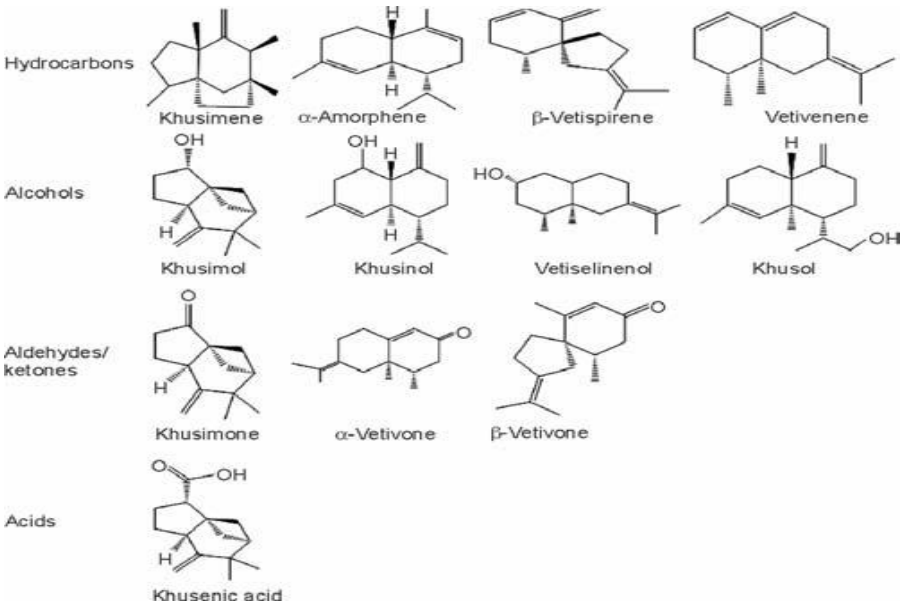


Figure 2: Structure of *C. zizanioides* oil constituents (Chahal *et al.*, 2015).

Figure 2: *C. zizanioides* plants (Picture taken by Haymanot Tewelde; Wendo Genet, Ethiopia 01/05/2023G.C).

2.5. Antimicrobial activity of the root of *C. zizanioides*

The evaluation of the ethanolic root extract of *C. zizanioides* antibacterial effectiveness is determined through the measurement of the zone of inhibition. Within this extract, flavonoids, terpenoids, and tannins exhibit antimicrobial properties. The study involves four bacterial strains, including two gram-positive bacteria (*S. aureus and B. subtilis*) and two gram-negative bacteria (*P. aeruginosa and E. coli*). The research findings reveal that flavonoids demonstrate antimicrobial effects specifically against gram-negative bacteria by binding to microbial cell membranes and disrupting their functionality. Tannins, present in the root extract, exhibit antimicrobial activity through interactions with microbial enzyme adhesion, cell transport proteins, and the formation of complexes with polysaccharides. Consequently, tannins are identified as active constituents in the ethanolic root extract of *C. zizanioides*, contributing to its observed antibacterial activity *in vitro* (Balasankar *et al*., 2013).

2.6. Antioxidant activity of the root of *C. zizanioides*

In a living organism, the plant extract from *C. zizanioides* demonstrates antioxidant properties by effectively neutralizing free radicals. Free radicals are implicated in various diseases as they induce DNA damage and lipid peroxidation. The ethanolic root extract of *C. zizanioides* is employed to assess diverse antioxidant activities, including its capability to reduce oxidation, capture superoxide anion radicals, and exhibit overall total antioxidant capacity (Zahoor *et al*., 2018).

2.7. *C. zizanioides* for Soil and water conservation

Soil erosion and water runoff bring devastating effects on the environment and economy. Most of the time, soil erosion occurs due to severe flooding in the winter season or due to strong wind or flash floods in summer (Council, 1993). Most of these occur in the developing nations due to poor infrastructures and unfortunately during natural disasters such as earthquake and hurricane. On the other hand, population growth has increased the energy demand for cooking and household activities. This demand is met through forest fuels such as fuelwood and charcoal leading to deforestation in several developing countries. Though forest fuel use can mitigate climate change compared to fossil fuel, risk of soil erosion is high in case of deforestation, without counting a possible negative carbon balance. In addition,

climate change has altered the pattern of rainfall, storm and drought around the world that has an impact on soil erosion. Furthermore, development of infrastructures such as residential buildings, dams and industries has influence on soil erosion. During soil erosion, vast amount of soil and water is displaced from the fertile fields to rivers that need to be cleaned (Mekonnen *et al*., 2015).

2.8. *C. zizanioides* for Phytoremediation

Phytoremediation is a cost-effective solar energy-driven technique that involves utilizing green plants to purify contaminated soil, sludge, sediments, natural groundwater, and surface water. Plants, including vetiver, have the ability to assimilate, accumulate, or even break down various types of pollutants, ranging from heavy metals to organic wastes. Vetiver has demonstrated significant potential as a phytoremediation tool. The documented forms of phytoremediation involving the vetiver plant primarily include the accumulation of contaminants in its root and shoot areas, known as phytoextraction or phytoaccumulation (Danh *et al*., 2009; Das *et al*., 2010; Kede *et al*., 2017; Meyer *et al*., 2017); (2) phytostabilizazion – plants are capable of reducing the mobility and transport of heavy metals by keeping them in the rhizosphere microenvironment (Bolan et al. 2011; Danh et al. 2009; Phusantisampan *et al*., 2016); and (3) phytodegradation – once the contaminant molecules are taken by the plant, vetiver is able to break them down throughout the metabolic pathways of the plant (Das *et al*., 2015).

2.9. Techniques of isolation and purification of bioactive molecules from plants

The purification and isolation of bioactive compounds from plants may represent a newly developed technique in recent years (Altemimi *et al*., 2015). This contemporary method aligns with the emergence and accessibility of numerous advanced bioassays, offering precise techniques for the isolation, separation, and purification of bioactive compounds. In the pursuit of identifying bioactive compounds, the objective is to employ an effective method that can rapidly and specifically screen source materials for various bioactivities like antioxidant, antibacterial, or cytotoxicity (Mulinacci *et al*., 2004). *in vitro* methods are favored over in vivo assays due to the higher costs, extended durations, and ethical considerations associated with animal experiments. The complexity of isolating and characterizing specific bioactive molecules is compounded by several factors. These include

the diverse plant tissues that may yield distinct compounds, as well as the varied chemical structures and physicochemical properties exhibited by the bioactive phytochemicals. Consequently, defining definitive procedures or protocols for the isolation and characterization of these compounds becomes a challenging task (SaraJliJa *et al*., 2012). The initial phases of isolating and characterizing a bioactive phytochemical involve the crucial steps of selecting and collecting plant materials. Subsequently, ethnobotanical information is gathered to pinpoint potential bioactive molecules. Extracts are then generated using diverse solvents to isolate and purify the active compounds responsible for the observed bioactivity. Techniques like column chromatography are applied for the isolation and purification of these bioactive compounds. Notably, to expedite the purification process, advanced methods such as High-Pressure Liquid Chromatography (HPLC) have been developed. The final identification of the purified compounds is accomplished through various spectroscopic techniques, including UV-visible, infrared (IR), nuclear magnetic resonance (NMR), and mass spectroscopy (SaraJliJa *et al*., 2012).

2.9.1. Fourier transform infrared (FTIR) spectroscopy

Infrared spectroscopy, commonly referred to as Fourier transform infrared (FTIR) spectroscopy, is widely employed in diverse fields. Its applications span from the analysis of small molecules or molecular complexes to the examination of cells or tissues (Levin & Bhargava, 2005).

FTIR offers several benefits when compared to alternative spectroscopic techniques: (1) it allows for the simultaneous recording of spectra across a broad spectral range at any desired resolution, known as the multiplex advantage; (2) it boasts high optical throughput due to the use of a circular aperture instead of a narrow slit, a feature referred to as the Jacquinot advantage; and (3) it enables precise optical calibration through the utilization of a frequency-stabilized reference laser for determining the optical path difference between the two light beams, known as the Connes advantage. These advantages make it possible for FTIR to accurately measure small spectral changes by employing the differences between spectra recorded under specific conditions, a method known as FTIR difference spectroscopy. While FTIR was initially applied primarily to the study of small inorganic molecules, over the past two decades, its scope has expanded to include biological molecules, such as for static and dynamic studies of protein secondary structure (Surewicz *et al*., 1993), of enzymes (Alben ,1996), of nucleic acids(Liquier, 1996) of lipids (Lewis, 1996) and glycolipids (Brandenburg

and Seydel 1996). The incorporation of time-resolved (tr)FTIR difference spectroscopy enables the identification of the molecular reaction mechanism in proteins, particularly in the case of bacteriorhodopsin (Gerwert, 1993; GERwERT, 1990; Nabedryk, 1996; Nabedryk, 1996; Remy & Gerwert, 2003).

2.9.2. Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography is widely applicable across various fields, with its primary focus on the separation and examination of complex mixtures, including essential oils, hydrocarbons, and solvents1-3. Utilizing detectors like the flame ionization detector and the electron capture detector, both characterized by high sensitivities, gas chromatography can accurately quantify substances even at extremely low concentrations. Subsequently, its second significant application area encompasses pollution studies, forensic investigations, and general trace analysis. Given its simplicity, sensitivity, and efficiency in separating mixture components, gas chromatography stands out as a crucial tool in the realm of chemistry. It finds extensive use in both quantitative and qualitative analyses of mixtures, compound purification, and the determination of thermochemical constants such as heat of solution and vaporization, vapor pressure, and activity coefficients (De Fátima *et al*., 2006; Kaushik *et al*., 2002; Lal & Verma, 2006; Marston, 2007; Milne & Beamish, 1999). Understanding the chemical components of plants is valuable not only for uncovering potential therapeutic compounds but also for identifying new reservoirs of economically significant phytochemicals used in synthesizing intricate chemical substances. Additionally, this knowledge is instrumental in unveiling the true significance of traditional remedies. Higher plants remain vital sources of bioactive compounds, playing a crucial role in preserving human health. Existing reports on green plants provide a wealth of effective chemotherapeutic agents that are non-phytotoxic, exhibit greater systemic effects, and are easily biodegradable (Di Donato *et al*., 2008; Siebert & Hildebrandt, 2008; Standard, 2006).

2.9.3. Nuclear Magnetic Resonance Spectroscopy

Over the last decade, metabolomics has emerged as a significant area within the realms of plant sciences and natural products chemistry (Hall, 2006; Rochfort, 2005; Seger & Sturm , 2007; Sumner *et al*., 2003; Verpoorte *et al*., 2007; Ward *et al*., 2007). The primary aim of metabolomics is to comprehensively analyze all the metabolites within an organism, both qualitatively and quantitatively. This endeavor aims to offer a detailed metabolic snapshot of

a living organism under specific conditions. Achieving this goal is highly ambitious, given the intricate complexity of the plant metabolome. For instance, a single part of a plant, like tobacco leaves, has already been reported to contain approximately 3,000 metabolites, highlighting the immense challenge of this task (Wahlberg & Enzell, 1987). Metabolomics seeks to thoroughly examine all the metabolites within an organism, providing a detailed understanding of its metabolism both qualitatively and quantitatively. This ambitious objective is particularly challenging due to the intricate complexity of the plant metabolome. As an example, a singular part of a plant, such as tobacco leaves, has been documented to contain around 3,000 metabolites, underscoring the formidable nature of this undertaking.

paraphrase this At the same time, these metabolites are all different regarding their polarity, chemical behavior, stability and concentration, which make the analysis of all metabolites in one single experiment extremely difficult. In view of this, instead of aiming to analyze all individual metabolites quantitatively and qualitatively, a more realistic and suitable approach will be to have a general view of all the metabolites present in an organism under certain conditions. In this context, NMR is a very suitable method to carry out such an analysis because it allows the simultaneous detection of diverse groups of secondary metabolites (flavonoids, alkaloids, terpenoids and so on) besides abundant primary metabolites (sugars, organic acids, amino acids and so on). Moreover, in an NMR spectrum, signals are proportional to their molar concentration, making the direct comparison of concentrations of all compounds possible, without the need for calibration curves of each individual compound. In other words, it reflects the real molar levels of metabolites present in a plant. In addition, NMR is a very useful technique for structure elucidation. Using various two-dimensional NMR measurements, many signals can be identified often without the need for further fractionation of the extract

Simultaneously, these metabolites exhibit variations in terms of polarity, chemical properties, stability, and concentration, making it exceedingly challenging to analyze all of them comprehensively in a single experiment. Given this complexity, instead of striving for the quantitative and qualitative analysis of each individual metabolite, a more pragmatic and feasible approach is to gain a broad understanding of all metabolites within an organism under specific conditions. In this context, NMR proves to be a highly suitable method for such analysis, as it enables the simultaneous detection of diverse groups of secondary metabolites (flavonoids, alkaloids, terpenoids, etc.) alongside abundant primary metabolites (sugars, organic acids, amino acids, etc.). Furthermore, NMR spectra provide signals proportional to

their molar concentrations, facilitating the direct comparison of compound concentrations without the necessity for calibration curves for each individual compound. Essentially, it reflects the actual molar levels of metabolites present in a plant. Additionally, NMR serves as a valuable technique for structure elucidation, as numerous signals can be identified through various two-dimensional NMR measurements, often eliminating the need for further fractionation of the extract (Choi *et al*., 2010).

2.9.4. UV-Visible Spectroscopy

UV-Visible Spectroscopy involves the study of how a sample responds to light. When a monochromatic beam of light passes through sample, certain amount of light may be absorbed and the remaining is transmitted through the sample (Upstone, 2000)It is a technique based on the measurement on depletion of electromagnetic radiation by absorbing substance. The depletion electromagnetic radiation is resulted from the reflection, scattering, absorption, or interference. The spectral range of this radiation is approximately around 190-800nm (Passos &Saraiva, 2019) A distinct spectrum is obtained by the absorption of ultraviolet or visible light by chemical compounds. The basis of spectroscopy involves the interaction of light with matter. When a matter absorbs light, it may experience excitation and de-excitation, which may result in the formation of spectrum. Electromagnetic wave strikes a material resulting in various phenomenon such as transmission, absorption, reflection, as well as scattering. Principle involved in Ultraviolet-Visible spectroscopy is electronic transition which describes the excitation of an electron from ground state to the excited state. (Pate *et al*., 2022) When a monochromatic light passes through the sample, it may absorb light in ultraviolet or visible range. The molecules present in sample may experience change in their electronic state. The electrons will be promoted from ground state orbital (Lower energy) to excited state orbital (Higher energy) by obtaining the energy from the light.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The roots of *C. zizanioides* were gathered from Wondo Genet Agricultural Research Center, situated in the Sidama region of southern Ethiopia (Figure 2). The district is positioned 270 km south of Addis Ababa, 14 km southeast of Shashemene, and 34 km eastward from Hawassa. Geographically, the district is located at 7°19'N and 38°38'E, with an elevation of 1780 meters above sea level (Figure 3**)**. The region experiences a mean annual minimum rainfall of 709 mm and a maximum of 2062 mm. The average maximum and minimum temperatures in the district are 26°C and 12°C, respectively. Wondo Genet exhibits a bimodal rainfall pattern, with short rains occurring from March to May and long rains from July to Octobe (Shanku, 2022).

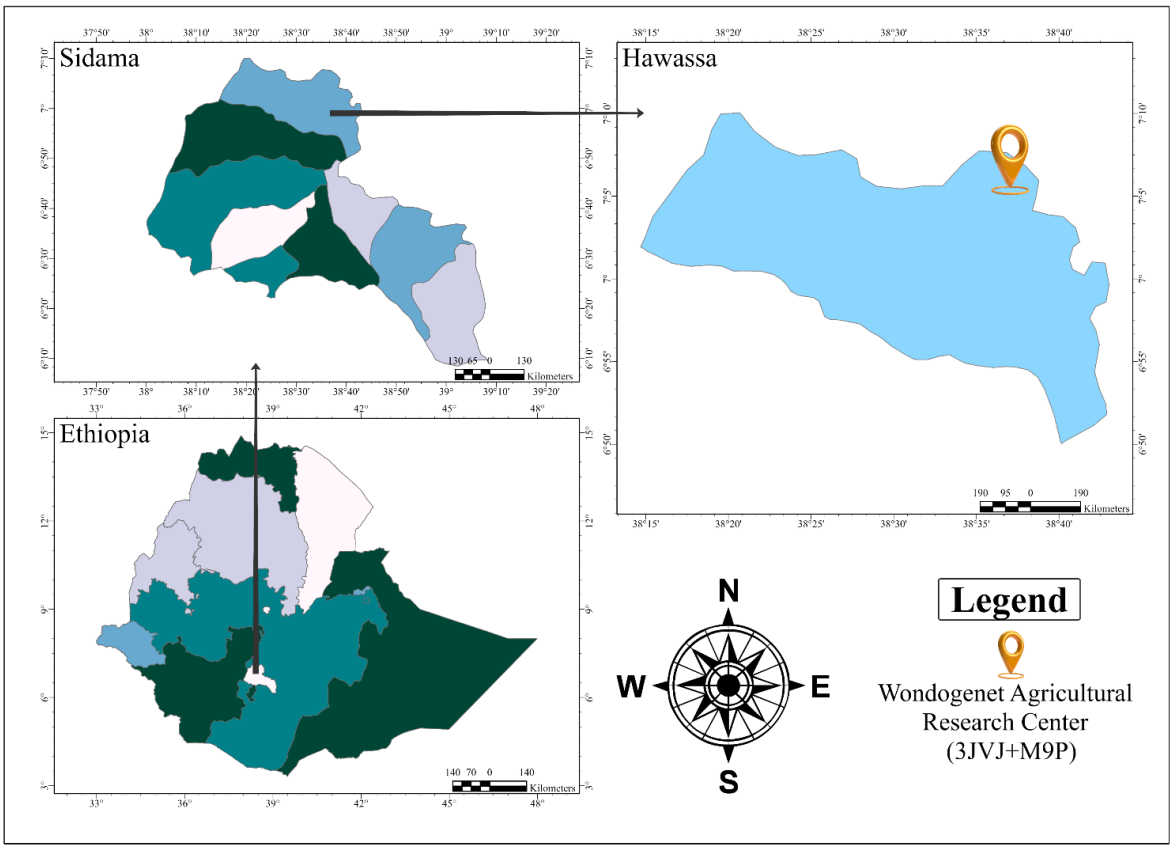


Figure 4: Map of study area.

3.2. Sample Collection and Identification

A 500g of *C. zizanioides* root sample was collected randomly at the age of 11 month from Wondo Genet Agricultural Research Center. The plant material was identified and voucher specimen number HY-002 was deposited at the National Herbarium of Ethiopia, Addis Ababa University, Ethiopia.

3.3. Apparatuses and instruments

Pre-coated aluminum TLC sheet silica gel 60 F254 (Merck), TLC chamber, capillary tube, glass column, waring commercial laboratory blender (Torrington, CT, USA), suction filtration and Petri plates, rotary evaporator (Rotary vacuum), incubator (Binder B28, Germany), autoclave (tuttnauer 3150EL, Israel), UV- lamp cabinet (UVP Chromato-Vue C-70G, Analytik Jena, USA), UV-Vis spectrophotometer (Cecil CE4001 UV/VIS, Cambridge, England), Spectrum 65 FT-IR (PerkinElmer, 4000-400 cm^{-1}), EI GC-MS spectrometry (7890B and 5977A, Agilent Technologies, USA), Brucker), ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectroscopy were employed.

3.3.1. Chemicals

The chemicals and reagents used in this research were: petroleum ether, ethyl acetate, *n*-hexane, chloroform, methanol, Mueller-Hinton Agar 9 BD (Becton, Dickinson and Company), silica gel, Mueller-Hinton broth BD (Becton, Dickinson and Company), Saboured Dextrose agar (Merck KGaA), Saboured Dextrose broth (Merck KGaA),Wagner's reagent, distilled water, ferric chloride, vanillin, DMSO, normal saline and other chemicals.

3.4. Preparation of plant extract

In this study, the solid liquid extraction technique was employed as a general extraction method. Total extraction of plant material was prepared by mixing the dried and grounded *C. zizanioides* root with organic solvents. The dried root under shade for three days was ground using electric grinder and the root was weighed using an electrical balance. The ground plant material (0.5 kg) was extracted with absolute petroleum ether (2.5 L).Residue left was further extracted with 2.5 L chloroform (100%) and methanol (100%) respectively and macerated at room temperature by placing on a shaker (Labnet International,USA) at 110 rpm for 48 hr.

Each solvent fraction was filtered using Whatman No. 1 filter paper. The filtrate was concentrated using rotary evaporator and kept in beaker (Figure 4).

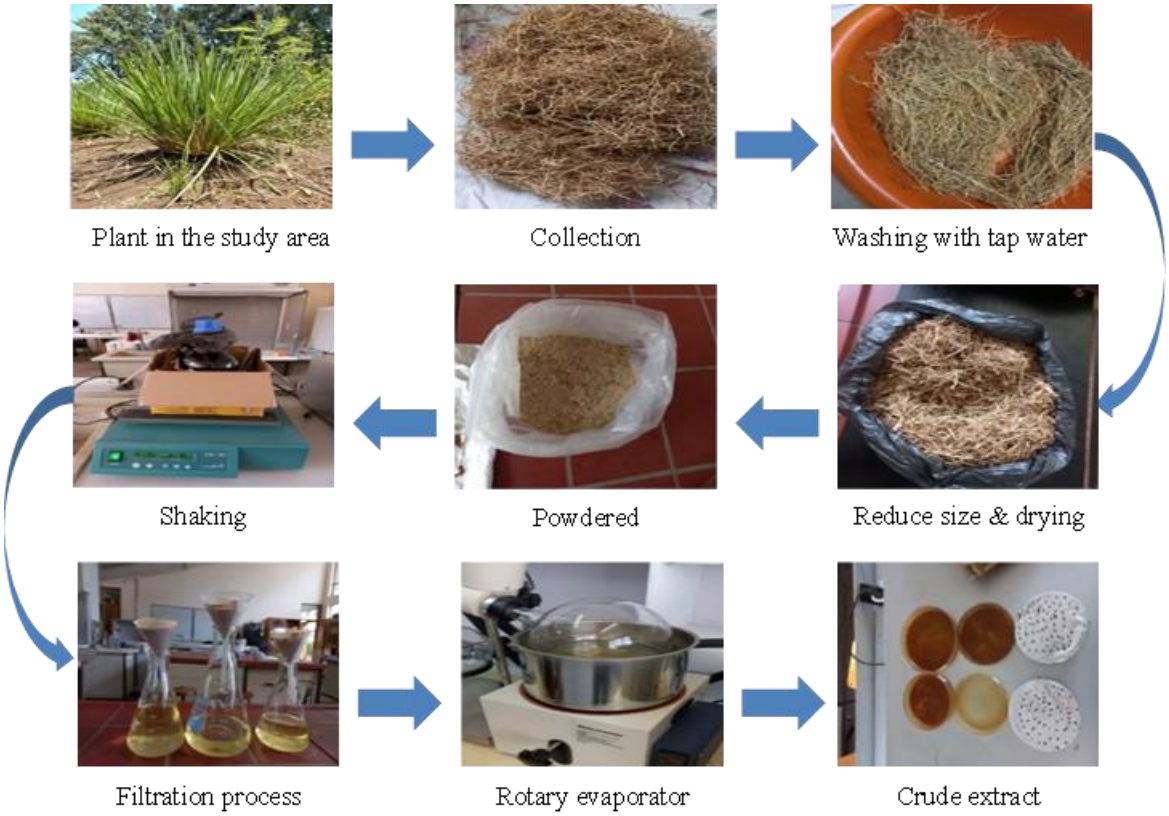


Figure 4: The extraction processes.

3.5. Phytochemicals detection/analysis of crude extract

The preliminary screening of phytochemical constituents of crude extract of the *C. zizanioides* root was done for the investigation of secondary metabolites compounds such as alkaloids, flavonoids, phenols, saponins, steroid, tannins and terpenoids by using the standard procedures.

3.5.1. Test for steroid (Lieberman-Burchard test)

A 0.5 g of the root crude extract was dissolved in 0.5 ml dichloromethane (100%) to give a dilute solution and then 0.5 ml of acetic anhydride (100 %) was added, followed by three drops of concentrated sulphuric acid (98%). A blue-green coloration indicates the presence of steroids (MacNee, 2005).

3.5.2. Test for terpenoid (Salkowski test)

A 0.5 g/ml of root crude extracts was mixed with 1 ml of chloroform (100 %). Then 1 ml of concentrated sulphuric acid 98 % was added to form a layer. A reddish-brown coloration at the interface indicated the presence of terpenoids (Takaidza *et al*., 2018).

3.5.3. Test for saponins (Frothing test)

Saponins were tested by dissolving about 0.5 g/ml of the root crude extract in a test tube containing 3ml of hot distilled water and then the mixture was shaken vigorously for one minute and persistent foaming that indicates the presence of saponins (Solanki *et al.,* 2019).

3.5.4. Test for flavonoids (Cyanidin test)

One-half gram (0.5 g/ml) of the root crude extract was dissolved in methanol and 2 ml of concentrated hydrochloric acid 98 % was added. A spatula full of magnesium turnings was added and the mixture was observed for effervescence. A brick-red coloration observed indicates the presence of flavonoids (Christen, 2000).

3.5.5. Test for tannins (Ferric chloride test)

A 0.5 g/ml of the root crude extract was dissolved and added to a tube containing 20 ml of boiling distilled water and then boiled for an hour. A few drops of ferric chloride (10 %) were added and allowed to stand for proper color development. A blue-black coloration indicates the presence of tannins (Alebiosu & Yusuf, 2015).

3.5.6. Test for alkaloids (Wagner's test)

A 0.5 g/ml of the root crude extract was treated with 3 drops of Wagner's reagent (Iodine 1.27 gm in Potassium Iodide 2.54 gm). The formation of brown/reddish precipitate indicates the presence of alkaloids(Sunil & Vinod, 2019).

3.5.7. Test for phenols (Ferric chloride Test)

A 0.5 g/ml of the root crude extract was treated with 3-4 drops of ferric chloride (10%) solution. The formation of bluish-black color indicates the presence of phenols (Joshi *et al*., 2013).

3.6. Isolation of compounds

3.6.1. Column chromatography

The chloroform extract (3g) was adsorbed with silica gel (3g) and subjected to silica gel column chromatography (150 g silica gel). The elution of the extract was done with an increasing gradient of solvent systems (increasing gradient of n-hexane in ethyl acetate followed by ethyl acetate in methanol and finally methanol) (Table 1). Silica gel was used as the stationary phase while varying solvent combinations as the mobile phase, and the purity of all fractions was analyzed with TLC. Further purification of isolated compounds was done using repeated column chromatography. The pure compounds were submitted for NMR analysis and biological activity tests to elucidate their structures and examine their biological activities, respectively. Fraction 27 afforded Compound 1 (13 mg) white compound with single spot-on TLC (eluted with 60% hexane in EtOAc) **(**Appendix 3).

Table 1: Summary of fraction of chloroform extract.

Solvent system	**Ratio**	**Fractions**	**Solvent system**	**Ratio**	**Fractions**
n-hexane	10	F1	EtOAc: MeOH	9:01	F59-F72
n-hexane: EtOAc	9:01	F2-F3	"	8.5:1.5	F73-F79
"	8.5:1.5	F4-F8	"	8:02	F78-F83
"	8:02	F9-F14	"	7:03	F84-F89
"	7:03	F15-F19	"	6:04	F90-F91
"	6:04	F20-F25	"	5:05	F92-F97
"	5:05	F26-F28	"	4:06	F98-105
"	4:06	F29-F34	"	3:07	F106-F110
"	3:07	F35-F40	"	2:08	F111-F117
"	2:08	F41-f46	"	1:09	F118-119
"	1:09	F47-F52	MeOH	10	F120-125

EtOAc	10	F53-F58

3.6.2. TLC analysis

Frequently, TLC analysis is used to facilitate the identification of present compounds. These readings include the distance covered by the solvent and the distances traveled by individual spots. Once the solvent front approaches the top of the plate, the plate was taken out of the beaker. Before evaporation occurs, the positions of the solvents were marked by lead with another line (Kumar *et al*., 2013).

3.7. Essential Oil Extraction

A 100 g *C. zizanioides* (root) was powdered using an electrical grinder (Ririhong,Germany) and extracted using hydro distillation for 3 hr in a Clevenger apparatus (3.3 borosilicate glass, India). The essential oil was collected, dried over anhydrous Na_2SO_4 (Acros Organics filtered), concentrated using a rotary evaporator, and kept in a refrigerator at -4 °C until further analysis.

3.8. GC-MS analysis of Essential oil extract

The analysis of the essential oil (EO) extracted from *C. zizanioides* roots was conducted through GC/MS utilizing an Agilent 7000 Series Triple Quad gas chromatograph linked to a mass spectrometer (GC/MS,USA). The gas chromatograph was equipped with an Elite 5MS (5% diphenyl/ 95% dimethylpolysiloxane) capillary column (30 x 0.25um ID x 0.25 um df). The GC-MS detection employed an electron ionization system with an ionization energy of 70eV. Helium gas (99.999%) at a constant flow rate of 1ml/min and an injection volume of 2 µl served as the carrier gas (split ratio of 30:1). The injector temperature was set at 250°C, and the ion source temperature was maintained at 200°C. The oven temperature was programmed, starting from 110°C (isothermal for 2 min), increasing at a rate of 109 °C/min to 120 °C, followed by a 5 °C/min increase to 280 °C, and concluding with a 9-min isothermal period at 280 °C. The relative percentage of each component was determined by comparing the average peak area to the total areas, and Turbo Mass software was used for processing the mass spectra and chromatograms. GC/MS-MS mass spectra were analysed using the National Institute of Standard and Technology database (NIST), which encompasses more than 62,000

samples. The spectrum of the unknown components was stored in the NIST library (Ezhilan & Neelamegam, 2012).

3.9. Antimicrobial assay against C. zizanioides root extracts

3.9.1. Test microorganisms

Microorganisms selected for this study were two Gram-negative *Escherichia coli* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27823), and two Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 11778) and one fungus *Candida albicans* (ATCC 10231) which were obtained from Oromia regional laboratory, Adama.

3.9.2. Inoculum preparation and standardization

The McFarland is a chemical solution composed of barium chloride and sulfuric acid, leading to the formation of a fine precipitate known as barium sulfate. McFarland is thoroughly shaken and distributed into test tubes, mirroring those used for the inoculum suspension preparation. It is crucial to shake well before each use to ensure an even distribution of barium sulfate in the solution. A 0.5 McFarland standard is recommended for antimicrobial susceptibility testing. To prepare this standard, 0.05 ml of $BaCl_2$ (1.17% w/v $BaCl_2$, $2H_2O$) is mixed with 9.95 ml w/v H_2SO_4 in distilled water. This standardization process aims to approximate the microbial quantity in a liquid suspension by comparing its turbidity with the established standard. (Hudzicki, 2016; Mukhtar & Mohammed, 2017).

3.9.3. Inoculum preparation for bacteria

On Muller Hinton agar (beef extract, acid casein hydrolysate, starch and agar), each bacterial species was subcultured and incubated for 24 hours at 37°C. Three to five fresh colonies were selected to create the inoculum suspension, which was then incubated for the entire night. Next, a sterile saline 0.87% solution was added to dilute the fresh cultures, and they were completely vortexed. This was done again and again until the turbidity 0.5 McFarland criterion was met, which is equivalent to 1.5×10^8 CFU/ml for bacterial inoculum suspension (Andrews 2001; Wiegand & Hancock, 2008).

3.9.4. Fungi Inoculum preparation

On Saboured Dextrose agar (Merck KGaA), the fungus (ATCC 10231) was subcultured and incubated for 48 hrs at 27°C. Using a sterile inoculating loop, three to five fresh *C. albicans* cultures were selected for the inoculum suspension. The colonies were then transferred to a test tube filled with saline solution and vigorously vortexed. Then, the turbidity of the fungal strains was adjusted to 1×10^6 cells (Andrews, 2001; Wiegand *et al*., 2008)

3.10. Antimicrobial evaluation of *C. zizanioides* root crude and isolated compound

Disk diffusion assay was used to determine the antimicrobial activity of petroleum ether, chloroform, methanol extracts, and isolated compound 1 of *C. zizanioides* root on five selected pathogenic microbial strains. An overnight culture of bacteria in MHB and overnight cultures of fungi in SDB was diluted to adjust to 0.5Mcfarland standard as mentioned above. Microbial inoculum suspensions were taken by sterile cotton swab and streaked on sterile Muller Hinton agar and SDA plate for bacterial strain and *C. albicans* respectively. A 200mg/ml stock solution of each crude extract was prepared by dissolving 0.4g of petroleum ether, chloroform, and methanol extracts in 2 ml of 10% DMSO. Two-fold serial dilutions (200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml) of each crude extract were formed for disk diffusion assay. A 10 mg/ml stock solution of isolated compound 1 was prepared by dissolving 0.02 g of isolated compound 1 in 2 ml of 10% DMSO. Two-fold serial dilution 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml) were formed from this stock solution. Approximately, 6 mm diameter of Whatman No1 filter paper was prepared by using a puncher and sterilized by an autoclave for this test. These sterile 6 mm discs were soaked in each two-fold serial concentration of crude extract and isolated compound 1 for 20 minutes. Sterile paper discs soaked in 10 μg/ml ciprofloxacin and 10 mg/ml ketoconazole standard antibiotic solutions were used as positive control. Whereas discs impregnated with DMSO were used as a negative control. Next, all these discs were placed by using sterile forceps on sterile agar plates (MHA and SDA) which spread with a fresh culture of microbial strains (bacteria strains and *C. albicans*) in the laminar flow cabinet on their labeled location and the discs into the plate. Then, plates were inverted and incubated for 24hr at 37°C and 27°C for 48 hr for bacterial and fungus growth respectively. After the end of incubation time, the inhibition zone of microbes by microbial agents was measured by using a transparent rural

millimeter scale. All the tests were conducted in triplicated to minimize test error and the average of three measurements was used to represent the results.

3.11. Determination of minimum inhibitory concentration (MIC)

The determination of the minimum inhibitory concentration (MIC) was carried out through the broth dilution method. For bacterial and fungal MIC determination, the initially adjusted microbial suspension was diluted to concentrations of 1.5×10^8 CFU/ml and 1×10^6 CFU/ml, respectively. Stock solutions of each crude root extract of *C. zizanioides* were prepared at 400 mg/ml, followed by two-fold serial dilutions (200, 100, 50, 25, 12.5, 6.25 mg/ml) in 25 ml beakers. Similarly, a 40 mg/ml solution of isolated compound 1 was prepared, and two-fold serial dilutions were created (10, 5, 2.5, 1.25, and 0.625 mg/ml). Using a micropipette, 100 microliters of each crude extract and isolated compound were transferred into test tubes containing 5ml of Mueller Hinton Broth (MHB) and Sabouraud dextrose Broth (SDB), each marked with their respective concentrations. Subsequently, 10 microliters of inoculum adjusted to 0.5 McFarland of selected pathogenic microbes (*S. aureus, S. pyogenes, P. aeruginosa, E. coli, and C. albicans*) were added to each marked test tube, following a slightly modified version of the previously adopted method. Two test tubes served as negative controls (broth with inoculum) and positive controls (broth only) for each tested microbe. Finally, all the test tubes were incubated at 37 ^{0}C for 24 hr for bacteria and 27 ^{0}C for 48 hr for fungus to observe their growth (Koeth et al., 2015). After the end of the incubation period, the lowest concentration which inhibited microbial growth was recorded as MIC value determination by visual observation for each crude extract and isolated compound 1. The minimum concentration with low microbial turbidity was taken as MIC value.

3.12. Determination of MBC and MFC

The assessment of minimum bactericidal and fungicidal concentrations followed the determination of MIC values. Subculturing aliquots from test tubes without observable bacterial and fungal growth, as determined in the MIC assessment, was conducted on the surfaces of Muller Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) plates. Each subculture underwent a24-hrs incubation at 37°C for bacterial growth and a 48-hrs incubation at 27°C for fungal growth. After the incubation period, the lowest concentration of each plant extract and isolated compound 1 that prevented the growth of bacterial and fungal cells were

identified as the Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) values, respectively (Akinduti *et al*., 2019).

3.12. Antioxidant activity of *C. zizanioides*

The method used to determine the free radical scavenging activity of the extracts relies on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Deng et al., 2017) with slight modification. The root extract was dissolved in four methanol containing vials to give a concentration of 500, 250, 125, and 65.5 µg/ml. similarly, the essential oil of *C. zizanioides* root was dissolved in four methanol containing vials to give a concentration of 100, 50, 25, and 12.25 µg/ml. Ascorbic acid was used as a positive control, while sample-free DPPH solution in methanol was as a negative control. The solution of DPPH (0.004% mg/ml) in methanol was prepared, and 3 ml of the solution was mixed with 1 ml of extract solution at various concentrations immediately and incubated for 30 min at room temperature to complete the reaction. The absorbance of the samples was measured at 527 nm using spectrophotometer. Radical scavenging activity was expressed as the inhibition percentage (IP) of free radicals and calculated using the following formula (Yen & Duh, 1994).

$$\textbf{Percentage inhibition activity } = \frac{[(\boldsymbol{A0} - \boldsymbol{A1})]}{\boldsymbol{A0}} \boldsymbol{x}\ \mathbf{100}$$

where A0 is the absorbance of the control, and A1 is the absorbance of the extract/ standard. The inhibition curves were prepared and IC_{50} values were obtained.

3.13. Method of data analysis

The experimental results were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data was subjected to SPSS 16 to one-way analysis of variance (ANOVA), and the results were presented using tables and graphs. P values less than 0.05 were considered statistically significant. Microsoft Excel 2010 statistical package was used for all analyses.

4. RESULTS AND DISCUSSION

4.1. Extract yield

Five hundred grams of powdered roots of *C. zizanioides* were extracted using 2.5 L of petroleum ether, chloroform, and methanol respectively by maceration methods and yielded 1.06%, 1.88%, and 3.64% crude extracts respectively (Figure 5). The yield (1.06%) from petroleum ether extraction was lower than the yield obtained from methanol extract (3.64%). This is because the root of *C. zizanioides* contains polar compounds in larger amount than non-polar ones.

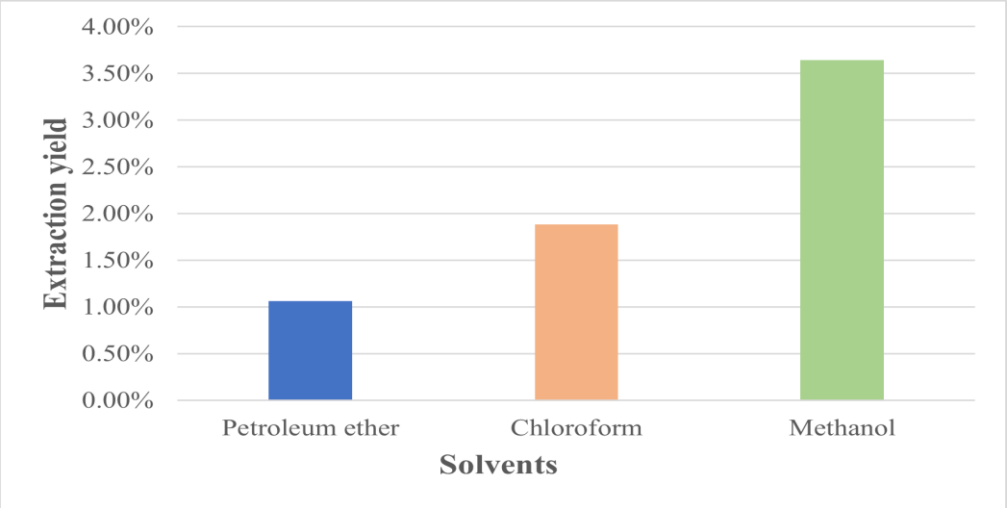
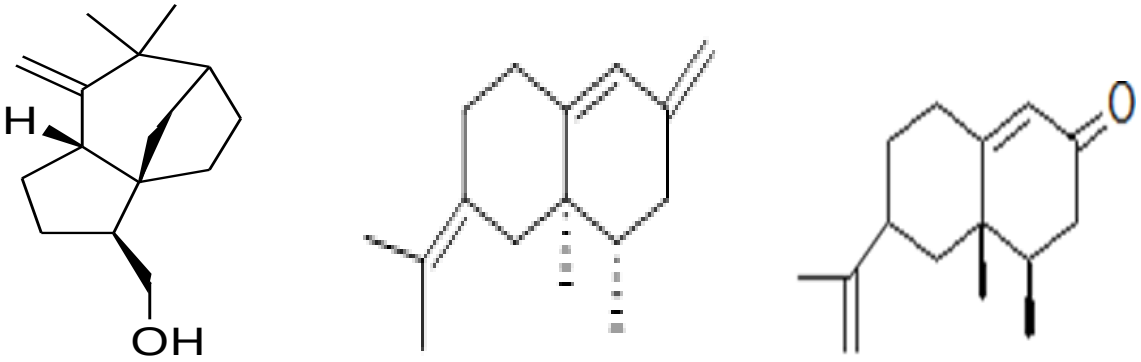


Figure 5: Percentage of yields of extracts obtained from the root of *C. zizanioides*.

4.2. Essential oil of *C. zizanioides*

The essential oils of *C. zizanioides* root (yield of 0.89% (v/w)) was analyzed by GC-MS **(**Table 2 & Figure 6) and it was found that khusimol (21.62%) was the major constituent followed by α-vetivone (9.31%), nootkatone (9.23%), sequentially (Figure 6). The findings of the present work are with a good agreement with that of (Soidrou *et al*., 2020) who reported khusimol (25.60%), as a major component of *C. zizanioides* root. The comparison with most recent literature concerning the chemical composition of essential oil of *C. zizanioides* root contain khusimol percentage found (21.62%) is higher than the values recorded in other studies(9.33%) (Kusuma *et al*., 2017),16.25% (Chahal *et al*., 2015), (12.21%) (Pripdeevech *et al*., 2006),12.77% (Samaan *et al*., 2022). In our sample, the amount of α-vetivone was

(9.31%),but in essential oil analyzed by Andreea and co-workers α-vetivone was not detected (David *et al*., 2019). The amount of nootkaton (9.23%) which is lower than the one found by (Kusuma *et al*., 2017) (22.64%).



Khusimol **(1)** *α-vetivone* **(2)** *Nootkatone* **(3)**

Figure 6: Structures of the compounds 1-3 isolated from essential oil of *C. zizanioides* root.

The amount of nootkatone (9.23%), is greater than the one found by (Bharathi Raja *et al*., 2018) (0.57%), but the result was comparable to the values reported previously (Soidrou *et al.,* 202*0*).

The chemical composition of *C. zizanioides* oil from different locations has been studied by different researchers. In Turkey, major constitutes were kusinol, β-vetivenene and dehydro-aromadendrene (Chahal *et al*., 2015), in Brazil, China, India, Java, Madagascar, Mexico, Reunion and Salvador β-vetisprene, khusimol, vetiselineol and α-vetivone were major compounds (Filippi *et al*., 2013). The literature mentioned in the above reports are in good agreement with the results obtained in this work. In Bangladeshi *C. zizanioides* root essential oil is rich in cyclocitrinol and myrcenol (Bhuiyan *et al*., 2008). In our sample all the above compounds were not detected.

Table 2: GC-MS analysis result of essential oil of *C. zizanioides*.

Compound Name	**RT**	**Formula**	**%**
Isophorone	16.878	$C_{12}H_{18}O$	2.91
Bornyl acetate	17.138	$C_{15}H_{22}$	1.17
Borneol	17.306	$C_{15}H_{26}O$	4.20
Bisabolol	18.444	$C_{15}H_{26}O$	4.58
β-Guaiene	18.767	$C_{15}H_{24}$	1.96
Myrcene	19.044	$C_{15}H_{24}O$	7.11
Cannabidiol	19.154	$C_{15}H_{24}O$	1.92
Caryophyllene oxide	19.373	$C_{15}H_{22}O$	2.93
Perillyl alcohol	19.449	$C_{15}H_{24}O$	3.83
Isoledene	19.604	$C_{15}H_{26}O$	3.59
Sativeneol	20.35	$C_{15}H_{24}O$	6.85
Khusimol	20.736	$C_{15}H_{24}O$	21.62
Valerenic acid	21.112	$C_{15}H_{22}O_2$	1.53
α-vetivone	21.533	$C_{15}H_{22}O$	9.31
Santalic Acid.	21.851	$C_{15}H_{22}O_2$	4.31

Perillone	22.042	$C_{15}H_{22}O$	3.70
Isovaleraldehyde	22.232	$C_{15}H_{22}O$	4.09
Nootkatone	22.498	$C_{15}H_{22}O$	9.23
Dihydroactinidiolide	23.382	$C_{15}H_{22}O$	0.73

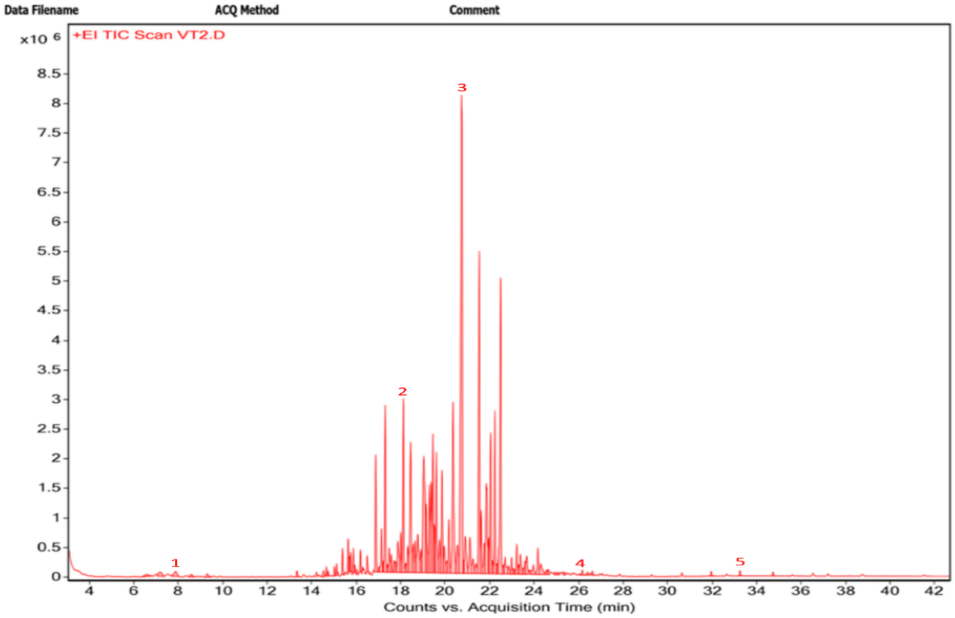


Figure 7: GC of Essential oil of C. zizanioides root 1: Khusimol (21.62%); 2: α-Vetivone (9.31%);3: nootkatone (9.23%);4: Myrcene (7.11%); 5: Sativeneol (6.85%).

4.3. Phytochemicals analysis

Results of preliminary phytochemical analysis revealed the presence of saponins, steroids, and terpenoids in all *C. zizanioides* petroleum ether, chloroform, and methanol extracts (Table 3). Phenols were detected only in methanol extract. None of the plant extracts tested had shown the presence of tannins. The availability and concentration of phytochemicals in plants are affected by seasonal fluctuations

Plant derived substances have recently become a great interest owing to the versatile applications. Medicinal plants and herbs represent rich sources of bio-resources for traditional medicine, modern medicine, pharmaceutical intermediates, and chemical compounds used in the synthesis of drugs (Muthukrishnan & Manogaran, 2018). Accordingly a study done by Devi *et al*., (2010) showed that *C. zizanioides* is recognized for containing flavonoids, alkaloids, terpenoids, saponins, tannins, and phenols. These compounds, whether acting individually or in combination, demonstrate antimicrobial properties. A study conducted by Muthukrishnan & Manogaran, (2018) demonstrated that he screening analysis of *C. zizanioides* through the use of different solvents exhibited indicated the existence of carbohydrate, protein, alkaloids, as well as tannins and phenols in both the methanolic and aqueous extracts.

The potency of the plant extracts depends on the solvent used for extraction. This may be due to the degree of solubility of the bioactive constituents. It has been documented that different solvents have diverse solubility capacities for other phytochemicals (Takazawa *et al*., 1982; Nenaah &Ahmed, 2011). Variations in the composition of the secondary metabolites might be due to the difference in their genotypes, climatic conditions in the respective locality where the plants were grown, and also to the plant parts processed and extracted (Onuminya *et al*., 2017; Dewijanti *et al*., 2019).

Flavonoids exhibit potent antimicrobial properties, possibly attributed to their capacity to form complexes with extracellular and soluble proteins, as well as their ability to interact with the bacterial cell wall. Flavonoids with greater lipophilicity may disrupt microbial membranes (Snigdha *et al*., 2013).

Table 3: phytochemical detection results of *C. zizanioides* root extract.

Phytochemical Screening	**Extracts**		
	Petroleum ether	**Chloroform**	**Methanol**
Alkaloids	_	+	+
Flavonoids	_	+	+
Saponins	+	+	+
Phenol	_	_	+
Tannin	_	_	_
Terpenoids	+	+	+
Steroids	+	+	+

Key: + shows presence, -shows absence

4.4. Characterization of compound

In the present work, Compound 1 was isolated from the chloroform root extract of *C. zizanioides.* A comprehensive description of this compound is outlined as follows. Compound 1 was acquired in the form of white solids, exhibiting an Rf value of 0.53 (eluted with a mixture of 60% hexane in ethyl acetate).

4.4.1. Compound 1

Compound 1 (13mg) was obtained as a white solid with an R_f value of 0.53 (eluted with 60% hexane in EtOAc).^{1}H NMR (400 MHz, DMSO) data of compound 1 displayed signals attributable to six methyl groups at δH 0.88 (s, Me-15), 1.01 (s, Me-14), 1.20 (s, Me-12), 1.39 (s, Me-16), 1.39 (s, Me-17), and 2.19 (s, Me-of acetyl group). A signal at 5.74 (t) is due to the presence of olefinic proton. A proton signal at 2.5 s indicated the presence of methoxy group proton (Table 3). ^{13}C NMR (101 MHz, DMSO) and DEPT 135 data of the indicted the presence of a quaternary carbonyl carbon at δC 169.1, olefinic CH at δC 124.6 (C-1), olefinic quaternary carbon at δC 142.9 (C-10), oxygenated quaternary SP^3 carbons at δC 64.1 (C-11) and 79.6 (C-13) and methoxy carbon at δC 56.4 (Table 4 & Figure 8).

Table 4: ^{1}H and ^{13}C NMR spectral data of compound 1.

Position	**Compound 1**		(Yukiko *et al*., 2016) **,** Figure 7	
	^{1}H (J)	^{13}C	^{1}H	^{13}C
1	5.74 (t)	124.6	2.5, 2.5	121.0
2	2.1 (m)	25.7		25.7
			17.8,7.5,5.1,2.5	
3	1.6, 1.35 (m)	29.3		26.6
4	1.55	31.1		40.4
5		31.4		39.2
6	1.38, 1.14	41.8	12.8, 2.7	42.8
			12.8	
7		71.2		66.4

8	1.5, 1.25	40.7	13.0, 4.6, 2.6, 2.4	31,7
			15.9,13.0,4.6	
9	2.42, 2.3	29.4	14.4,14.1,2.8,1.7,1.4	31.6
			14.4,4.5,2.8	
10		142.9		141.2
11		64.1		66.5
12	1.2	15.3		17.6
13		79.6	11.4	65.2
			11.4	
14	1.01	19.0		18.1
15	0.88	17.1		15.7
16	1.39	29.3		
17	1.39	29.3		
$CH_3-\underset{\underset{O}{\|}}{C}-$	2.19	20.9		
$CH_3-\underset{\underset{O}{\|}}{C}-$	-	169.1		
OCH_3	2.5 s	56.4		

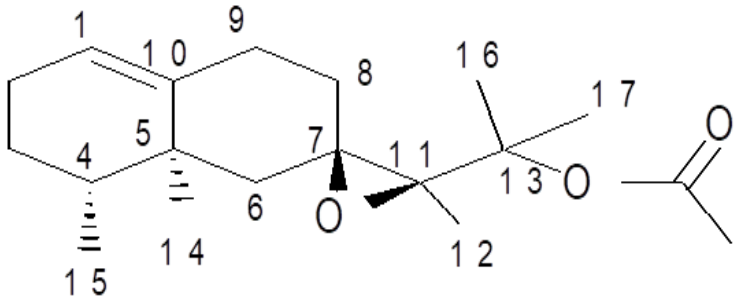


Figure 8: Chemical structure of the proposed compound 1.

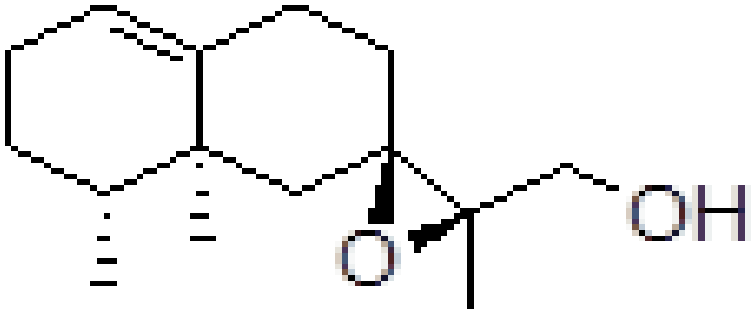


Figure 9: The chemical structure reported by (Yukiko *et al*., 2016).

The infra-red (IR) spectrum of compound 1 (Figure 10) showed bands in the region of 4000 and 500 cm^{-1}. The band due to C-H stretching at 2919.01 $cm^{1}$and 2852.03, and carbonyl group (C=O) at 1708.20 ,1657.58 cm^{-1} and the band at 2000 cm^{-1} is accounted to C-H stretching of aromatic compound.

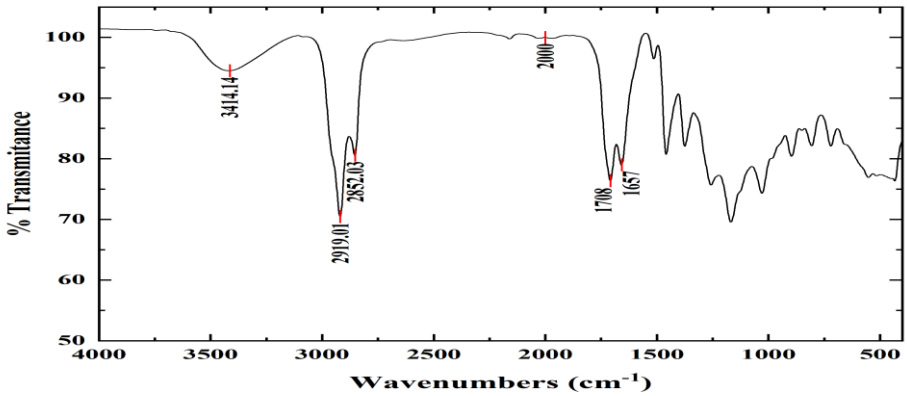


Figure 10: The IR spectrum of compound 1.

4.5. Biological activity of crude extract and isolated compound of *C. zizanioides* root

4.5.1. Antibacterial activity of *C. zizanioides* root extracts and CZO

The extract (petroleum ether, chloroform, methanol,) and isolated compound 1 were tested for their antibacterial and antifungal activity in different concentrations against human pathogens. The mean zone of inhibition values indicated that all the plant extracts and isolated compound 1 exhibited antibacterial and antifungal activities ranging from 7.00 mm to 19 mm (Table 5). The antimicrobial activity increased in a dose dependent manner.

Among the extracts the petroleum ether extract showed maximum zone (18.33±2.08) of inhibition against ATCC 11778. While the chloroform extract showed maximum inhibition (17±1 mm) against *P. aeruginosa*, and methanol showed good activity (15.33±0.57 mm) against *S. pyogenes* at concentration of 200mg/mL compared to ciprofloxacin with 11.33±0.80, 20.84±0.80 and 11.33±0.80 mm, respectively at a concentration of 10 µg/ml (Table 5). The isolated compound 1 (Appendix 2) showed maximum inhibition (12±0 mm) against *S. aureus* at a concentration of 10 mg/ml. In this study, isolated compound 1 showed high antimicrobial activity at lower concentration than crude extract.

In another study (Soni and Dahiya, 2015), methanol extract of *C. zizanioides* demonstrated the most significant activity against both *S. aureus* (15±0.12 mm) and *S. typhi* (13.9±0.08 mm). Clinical isolates of *S. aureus, E. coli,* and *P. aeruginosa* exhibited susceptibility to *C. zizanioides* (Soni & Dahiya, 2015). This may support the findings of this study in terms of the antibacterial effect.

Phytochemical investigations indicate that plants possessing antimicrobial properties typically harbor bioactive components, including tannins, flavonoids, alkaloids, and saponins. (Chukwuka *et al*., 2011). Polyphenols in plants include flavonoids, phenolic acids, stilbenes, and lignans (Manach et al., 2004). Similar investigations have also verified the occurrence of tannins, phenolic compounds, cardiac glycosides, flavonoids, alkaloids, and saponins in extracts from the *C. zizanioides* plant (De Zoysa *et al*., 2019). In the present study phytochemical analysis of *C. zizanioides* root extract showed the presence of the above chemicals which can exert antimicrobial activity through different mechanisms. Preliminary research suggests that flavonoids have the potential to alter allergens, viruses, and carcinogens, making them potential biological "response modifiers." *In vitro* studies demonstrate that flavonoids also possess antiallergic, anti-inflammatory, antimicrobial, anticancer, and antidiarrheal properties (Spencer, 2008). Saponins seem to stimulate the immune system (Xu *et al*., 1996). Therefore, presence of polyphenolic compounds, alkaloids, tannins, and saponin may contribute to the antimicrobial activity of the above plant.

The antimicrobial potential of *C. zizanioides* roots was investigated, revealing that the ethanolic extract exhibits greater activity compared to other extracts. This heightened activity is attributed to its abundance of active constituents, including alkaloids, flavonoids, essential oil, terpenoids, tannins, saponins, and phenols. These components can exert their effects individually or in synergy (Ates & Turgay, 2003).

C. zizanioides root extracts and isolated compound 1 showed better activity on Gram-positive bacteria than Gram-negative bacteria. The variations in structurally components between Gram-positive and Gram-negative bacteria may account for the differences in their susceptibility to antibacterial agents. The structural elements of lipopolysaccharides in the outer phospholipid membrane of Gram-negative bacteria contribute to the impermeability of their cell walls to antimicrobial substances. In contrast, Gram-positive bacteria possess an outer peptidoglycan layer that renders their cell walls more permeable to antimicrobial agents compared to the lipopolysaccharide layer in Gram-negative bacteria. Consequently, the structural complexity of the cell walls in Gram-negative bacteria surpasses that of Gram-positive bacteria. As a result, Gram-negative bacteria demonstrate lower susceptibility to antimicrobial substances compared to Gram-positive bacteria (Chanda & Baravalia, 2010).

As indicated in (Table 5), petroleum ether extract of *C. zizanioides* root show that the zones of inhibition for ATCC 11778 were significantly higher ($p<0.05$) than those observed against ATCC 27823, ATCC 25923, ATCC 25923. Similarly, the chloroform extracts of *C. zizanioides* root show that the zones of inhibition against *P. aeruginosa* were significantly higher than ($p<0.05$) *S. pyogenes, S. aureus, E. coli* at the same concentration (200mg/ml). Methanol extract of *C. zizanioides* root shows less antibacterial activities against all bacterial pathogen except *S. pyogenes.*

4.5.2. Antifungal activity of *C. zizanioides* root

The extract (petroleum ether, chloroform, methanol) and isolated compound were tested for their antifungal activity in different concentrations against *C. albicans* and compared to ketoconazole as the positive control and DMSO was used as a negative control. The mean zone of inhibition values indicated that all the plant extracts (Appendix 1) and isolated compound 1 exhibited antifungal activities ranging from 10.00 to 19 mm (Table 5). Activity increased in a dose dependent manner.

Among the extracts the chloroform and methanol extract showed maximum zone of inhibition 19±1 mm at 200 mg/ml concentration. While the petroleum ether extract showed 17.66±0.57 inhibition on *C. albicans* at concentration 200 mg/ml (Figure 10). The isolated compound 1 showed 17.66±0.57 mm inhibition on *C. albicans* at a concentration of 10mg/ml compared to ketoconazole 18±1.15 mm this result reveals compound 1 showed good antifungal activity at lower concentration than the crude extract. The chloroform and methanol extracts of *C.*

zizanioides root show that the zones of inhibition against *C. albicans* were significantly higher than ($p<0.05$) petroleum ether root extract of *C. zizanioides* at 200mg/ml (Table 5).

In another study(Jayashree *et al*., 2011) ethanolic extract of *C. zizanioides* is known to possess flavonoids, alkaloids, terpenoids, saponins, tannins and phenols which either individually or combination exert antifungal activity. Another research showed that ethanolic extract of *C. zizanioides* inhibited *A. niger, A. clavatus* and *C. albicans.* Flavonoids are found to be effective antimicrobial substance against a wide range of microorganisms, probably due to their ability to complex with extra cellular and soluble proteins; more lipophilic flavonoids may disrupt microbial membrane. Even though tannins was not found in this study; the antifungal properties of tannins may be associated with their capacity to deactivate enzymes involved in microbial adhesion and transport proteins within the cell envelope. Additionally, tannins have the ability to form complexes with polysaccharides (Kannan, Ramadevi, and Hopper 2009). In the present study phytochemical analysis of *C. zizanioides* root extract showed the presence of the above phytochemicals which can exert antifungal activity through different mechanisms. The plant extract containing tannin exhibited antifungal properties (Natarajan *et al*., 2005). This study indicated that root extract and isolated compound possess antifungal activity and can be exploited as an ideal treatment for future herbal drug contaminant for eliminating fungal spread.

The oil from *C. zizanioides* demonstrated extensive natural fungicidal effects against various pathogens. The antifungal activity of altered sesquiterpenoid products present in *C. zizanioides* oil was assessed against two phytopathogenic fungi, namely *A. alternata* (responsible for early blight in tomato and potato) and *F. oxysporum* (causing wilt in tomato), using the technique of spore germination inhibition. Among the tested compounds, khusinodiol monobrosylate was identified as an effective antifungal agent against both fungi (Dikshit, 1984). Antifungal properties were observed in both North and South Indian *C. zizanioides* oils when tested against *R. solani*. The fungicidal efficacy of South Indian *C. zizanioides* oil was marginally superior to that of the North Indian oil (Dubey *et al*., 2010).

The antifungal effectiveness of *C. zizanioides* was additionally assessed against specific pathogenic microorganisms, with fluconazole serving as a positive control. Among the evaluated fungal cultures, *A. niger* showed the greatest average inhibition zone of 32 mm against extracts from both leaves and roots of *C. zizanioides* (Sangeetha & Stella, 2012).

Furthermore, compound 1 at concentration of 10 mg/mL showed strong activity 17.66±0.57 mm against ATCC 10231 compared to ketoconazole 18±1.15 mm µg/mL concentration. The negative control (DMSO) showed no inhibitory activity against *C. albicans*.

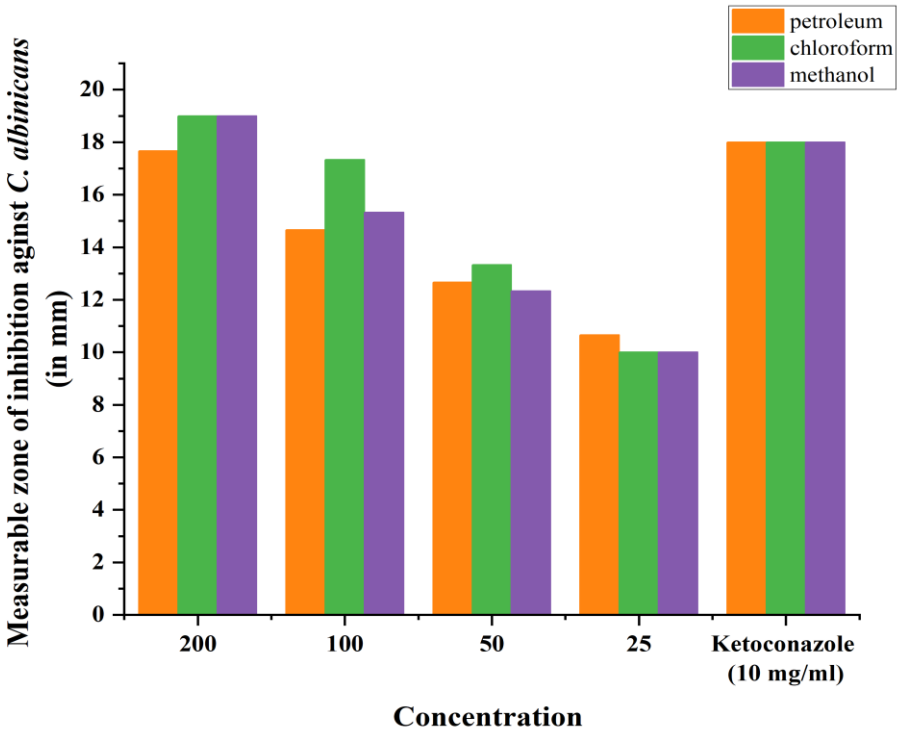


Figure 11: The antifungal activity of root extract of *C. zizanioides*.

Table 5: Antibacterial and antifungal activities of extracts and isolated compound from *C. zizanioides*.

Measurable zone of inhibition of petroleum extract					
Concentration (mg/mL)	ATCC 25923	ATCC 27823	ATCC 11778	ATCC 25923	ATCC 10231
200	10.66±1.15	16±2.64	18.33±2.08	18±1	17.66±0.57
100	10.33±0.57	12±2.64	14±3.60	10±1	14.66±0.57
50	8±1	8.33±0.57	11.33±2.08	7.33±0.57	12.66± 0.57
25	7±1	7±1	7±1	6±0	10.66±0.57
Ciprofloxacin 10 µg/ml	16.90±0.80	20.84±0.80	11.33±0.80	19.16±0.58	-
Ketoconazole 10 mg/ml	-	-	-		18±1.15
DMSO	0	0	0	0	0

Measurable zone of inhibition of chloroform extract					
Concentration (mg/mL)	ATCC 25923	ATCC 27823	ATCC 11778	ATCC 25923	ATCC 10231
200	12±1	17±1	14.66±1.52	15.66±2.51	19±1
100	9.66±0.57	14.33±3.05	11.66±0.57	14.66±0.57	17.33±0.57
50	8.66±0.57	14.33±3.05	10±1	12.66±1.52	13.33±1.52
25	7.66±0.57	9.66±0.57	7.66±1.15	9.66±0.57	10±0
Ciprofloxacin 10 µg/ml	16.90±0.80	20.84±0.80	11.33±0.80	19.16±0.58	-

Ketoconazole 10 mg/ml	-	-	-		18±1.15
DMSO	0	0	0	0	0

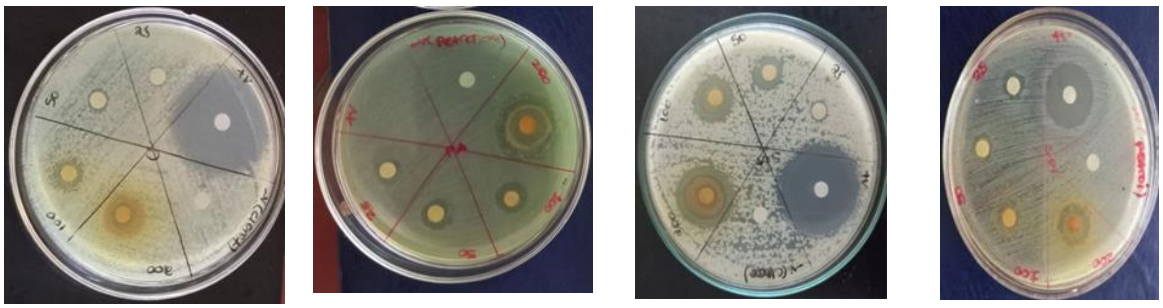
Measurable zone of inhibition of methanol extract

Concentration (mg/mL)	ATCC 25923	ATCC 27823	ATCC 11778	ATCC 25923	ATCC 10231
200	13.66±2.08	10.66±0.57	15.33±0.57	13.66±2.08	19±1
100	9.33±1.15	9±1	11.66±0.57	10±1	15.33±1.52
50	9.33±0.57	8.33±1.15	9.66±0.57	9±1	12.33±0.57
25	8±1	7±0	7.66±1.15	7±0	10±0
Ciprofloxacin 10 µg/ml	16.90±0.80	20.84±0.80	11.33±0.80	19.16±0.58	-
Ketoconazole 10 mg/ml	-	-	-	-	18±1.15
DMSO	0	0	0	0	0

Measurable zone of inhibition of compound 1

Concentration (mg/mL)	ATCC 25923	ATCC 27823	ATCC 11778	ATCC 25923	ATCC 10231
10	10.66±1.52	10.33±0.57	11±0.81	12±0	17.66±0.57
5	9 ±1	9.33±0.57	10.33±0.47	10.66±0.57	15.33±0.57
2.5	8±0	8.66±0.57	8.33±0.47	8.66±0.57	14.66±0.57
1.25	7±0	7±0	6.66±0.47	7.33±0.57	12.66±0.57
Ciprofloxaci	16.90±0.	20.84±0.80	11.33±0.80	19.16±0.58	-

n 10 µg/ml	80				
Ketoconazole 10 mg/ml	-	-	-	-	18±1.15
DMSO	0	0	0	0	0



E. coli ***P. aeruginosa*** ***S. aureus*** ***S. pyogenes***

Figure 12: Inhibition zone of *C. zizanioides* root crude extract on tested bacterial strains.

4.5.3. Minimum inhibitory concentration (MIC)

In the present study, the MIC value obtained from *C. zizanioides* crude extract, and isolated compound 1 showed adifference in the inhibitory concentrations against the test bacteria and *C. albicans*. The MIC value of root extracts (Table 6) and isolated compound1 (Table 7) showed strong activity at the lowest concentration that inhibited bacterial and fungus growth after 24 hr for bacteria and 48hr for fungus after incubation at 37°C.The MIC value were 50mg/mL for the chloroform extract (except ATCC 25923and ATCC 10231.which were inhibited at 25&12.5 mg/ml respectively).The isolated compound 1 MIC (Table 8) was 1.25 mg/ml except for ATCC 11778 & ATCC 27823 (2.5 mg/ml). The

MIC values of *C. zizanioides* for *E. cloacae*, *E. faecalis*, *E. coli* and *P. vulgaris* was 15.63, 31.25, 15.63 and 15.63 µg/ml (Efe, 2019). This finding disagrees with current study.

Table 6: The Minimum Inhibitory Concentration (MIC) of root extracts of *C. zizanioides*.

Plant extracts	**Minimum Inhibitory Concentration (in mg/ml)**				
	ATCC 25923	ATCC 27823	ATCC 11778	ATCC 25923	ATCC 10231
Petroleum ether	50	50	25	50	12.25
Chloroform	50	50	50	25	12.25
Methanol	25	50	50	25	50.00

4.5.4. The Bactericidal Concentration and Minimum Fungicidal Concentration

The MBC was validated by the absence of bacterial growth in the nutrient agar medium streaked with inhibition zones matching to the tested strains lowest MIC. Methanol extract showed potentially bactericidal activity against the tested pathogenic bacteria with MBC of 50 mg/ml against *E coli* and *S. aureus* and at 100 mg/ml against *P aeruginosa and S pyogenes*). In the case of chloroform, the MBC for *P. aeruginosa, S. pyogenes* and, *E. coli* bacteria was 100 mg/ml, while the MBC for *S. aureus* was 50 mg /ml. The MBC of petroleum ether extract for both *S. aureus and P. aeruginosa* was found to be 100 mg/ml. This means that the lowest concentration of petroleum ether that could completely kill all these bacteria was 100 mg/ml. The isolated compound1 showed MBC activity at 10 mg/ml in all the tested bacteria except *S. aureus*. The MIF result for isolated compound was 2.5mg/ml which was highly sensitive, and its MIC reached 1.25mg/ml.

Table 7: The MBC and MFC of crude extracts of *C. zizanioides*.

Extracts	**MBC and MFC (in mg/ml)**				
	ATCC 25923	ATCC 27823	ATCC 11778	ATCC 25923	ATCC 10231
Petroleum ether	200	100	50	100	50
Chloroform	100	100	100	50	50

Methanol	50	100	100	50	100

Table 8: The MIC**,** MBC, MFC of isolated compound 1.

Isolated compound	ATCC 25923		ATCC 27823		ATCC 11778		ATCC 25923		ATCC 10231	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
	1.25	10	2.5	10	2.5	10	1.25	5	1.25	2.5

4.5.5. Antioxidant activity of *C. zizanioides*

The DPPH radical scavenging activities (%) of crude extracts and essential oil were 65.32, 93.32, 70.84, 30.56 % for the petroleum ether, chloroform, and methanol (500μg/ml) and (100 μg/ml) oil extracts respectively (Table 9 and Figure 13). It was observed that the DPPH scavenging activity increased with increasing concentration of the samples in the assay. For the various concentrations, chloroform extract exhibited the highest percent inhibition of the DPPH as compared to the other extracts and essential oil. Ascorbic acid, a positive control, showed a percent scavenging of 97.6, 97.3, 97.1, 97% at concentrations of 500, 250, 125, 65.5 μg/ml respectively (Table 9 and Figure 13). Plant tissues are abundant in phenolic compounds, including tannins, phenolic acids, and flavonoids. Polyphenols are one of the major antioxidant chemicals. These substances exhibit a variety of biological properties, such as antioxidant properties (Chen & Ho, 1997; Kumaran, 2006; Rubió *et al*., 2013).

In the present study phytochemical analysis of *C. zizanioides* root extract showed the Polyphenols which can exert antioxidant activity through different mechanisms. In another study methanolic extract of *C. zizanioides* in the concentration range from (200,400,600,800& 1000 μg/ml) showed higher antioxidant potential by ABTS radical scavenging method (Muthukrishnan & Manogaran, 2018). The current study disagrees with this finding. The chemical makeup of essential oils, especially the presence of compounds with radical-functioning components, is generally responsible for the anti-oxidant properties of *C. zizanioides* oil (Bouhdid *et al*., 2008; Chun *et al*., 2005). In the present study the essential oil showed relatively lower antioxidant activity than other studies. The complexity of their chemical compositions is difficult to attribute the antioxidant activity of essential oil to one or another molecule. It is clearly evident that every single molecule, major and minor, significantly affects the activity of essential oils (Bakkali *et al*., 2008; W. Wang *et al*., 2008).

Based on data in (Table 9) the lowest IC_{50} value was shown by chloroform extract (5.603), followed by methanol extract (133.35). The IC_{50} value is a parameter widely used to measure the antioxidant activity of test samples. It is calculated as the concentration of antioxidants needed to decrease the initial DPPH concentration by 50 %. Thus, Low IC_{50} values correspond to a higher antioxidant capacity (Rivero-Cruz *et al*., 2020). Since high IC_{50} values correspond to a low antioxidant capacity, compared to previous research studies done on the antioxidant activity of petroleum ether extract and essential oil *C. zizanioides* of the current study result 196.33 & 255.88 (Appendix 4) showed low antioxidant activity.

Table 9: Antioxidant activities of crude extract from root of *C. zizanioides*.

Concentration (µg/ml)	**Control**	**Petroleum ether**		**Chloroform**		**Methanol**		**Ascorbic acid**	
		A	**%RSA**	**A**	**%RSA**	**A**	**%RSA**	**A**	**%RSA**
500	1.677	0.578	65.326	0.112	93.32	0.489	70.84	0.04	97.61
250	1.677	0.799	52.355	0.345	79.43	0.789	52.95	0.045	97.31
125	1.677	0.866	48.36	0.567	66.189	0.876	47.76	0.048	97.13
65.5	1.677	0.977	41.741	0.876	47.763	0.899	46.39	0.05	97.018
IC_{50}		196.33		5.603		133.35			

RSA= Radical scavenging activity

Figure 13: DPPH radical scavenging activity (%) of petroleum ether, chloroform, methanol extracts of *C. zizanioides* root and control (ascorbic acid).

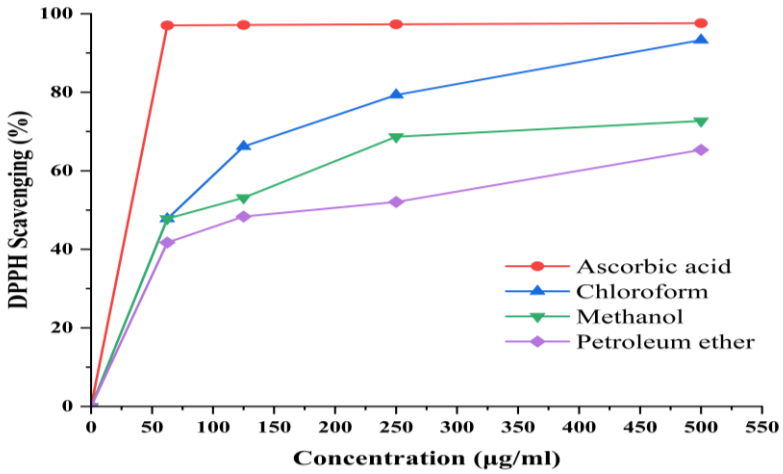


Table 10: Antioxidant activities of *C. zizanioides* essential oil.

Concentration	**Control**	**Essential oil**		**Ascorbic acid**	
(µg/ml)		**A**	**%RSA**	**A**	**%RSA**
100	1.024	0.711	30.56	0.025	97.55
50	1.024	0.811	20.80	0.025	97.55
25	1.024	0.822	19.72	0.027	97.36
12.5	1.024	0.911	11.03	0.029	97.16
IC_{50}		255.88			

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In the present study, extraction of *C. zizanioides* root (0.5kg) was extracted using 2.5L of petroleum ether, chloroform, and methanol respectively by maceration methods and yielded 1.06%,1.88%,3.64 % crude extracts respectively. Phytochemical screening tests conducted on *C. zizanioides* root crude extracts showed the presence of saponins, and steroids, terpenoids in all *C. zizanioides* petroleum ether, chloroform, and methanol extracts. Phenol was detected only in methanol extract. None of the plant extracts tested had shown the presence of tannins. The chloroform crude extract of the plant subjected to column chromatography and yielded compound 1 and characterized by NMR, and FTIR spectroscopy technique.

GC-MS analysis of essential oils from root of *C. zizanioides* revealed a total of 19 chemical components. It was found that khusimol (21.62%) was the major constituent followed by α-vetivone (9.31%), nootkatone (9.23%), sequentially.All the crude and isolated compound of *C. zizanioides* root extract had promising antibacterial and antifungal activity against *E. coli* (ATCC 25923), *P. aeruginosa* (ATCC 27823) and *S. aureus* (ATCC 25923), *S. pyogenes* (ATCC 11778), and *C. albicans* (ATCC 10231).

Among the extracts the petroleum ether extract showed maximum zone of inhibition on *S. pyogens* (18.33±2.08) at concentration 200 mg/mL compared to ciprofloxacin 11.33±0.80 a concentration of 10 µg/ml. The isolated compound 1 showed high inhibition on *S. aureus* 12±0 mm at a concentration of 10 mg/ml. The chloroform and methanol extract showed 19±1 mm inhibition on *C. albicans* at a concentration 200 mg/ml. The isolated compound 1 showed 17.66±0.57 inhibition on *C. albicans* at a concentration of 10 mg/ml compared to ketoconazole 18±1.15 a concentration of 10 mg/ml.

Crude extract and essential oil of *C. zizanioides* root were tested for their free radical scavenging activities in the DPPH scavenging assay. The DPPH radical scavenging activities (%) of crude extracts and essential oil were 65.32, 93.32, 70.84, 30.56 % for the petroleum ether, chloroform, and methanol (500 µg/ml) and (100 µg/ml) oil extract respectively. It was observed that the DPPH scavenging activity increased with increasing concentration of the samples in the assay.

The radical scavenging activity of *C. zizanioides* crude extracts (petroleum ether, methanol, chloroform) and essential oil was assessed at concentrations of 500 µg/mL for the extracts and 100 µg/mL for the essential oil. The percentages of radical scavenging activity (%RSA) were determined as 65.32, 93.32, 70.84, and 30.56% for petroleum ether, methanol, chloroform extracts, and essential oil, respectively. The positive standard, ascorbic acid, exhibited a % RSA of 97.60 at 500 µg/mL, Notably, the essential oil demonstrated a % RSA of 30.56 contrasting with ascorbic acid's 97.55 at 100 µg/ml. These results validate the historical use of the plant for treating infectious diseases caused by Gram-positive and Gram-negative bacterial strains as well as *C. albicans*.

5.2 Recommendation

These observations promote further research work with biological activity on antifungal and antibacterial activity of *C. zizanioides* oil and anticancer and cytotoxicity, antidiabetic on crude extracts as well as isolated compounds and essential oil. Characterization of the isolated compound using 2D NMR and MS techniques is needed. Moreover, isolation of more bioactive compounds from the plant is recommended. Additional *in vitro* antibacterial studies are also recommended other resistant clinical isolate pathogenic bacteria such as methicillin-resistant *S. aureus, Enterococcus* and multidrug-resistant *Mycobacterium tuberculosis* and fungal test organisms. Future studies need to be done on a combination of the compounds isolated from the root extract as well as essential oils with standard first line drugs *in vitro* and *in vivo* to examine the potential of their antibacterial and antioxidant activity.

6. REFERENCES

Akinduti, P. A., B. Motayo, O. M. Idowu, P. O. Isibor, G. I. Olasehinde, Y. D. Obafemi, H. U. Ugboko, J. O. Oyewale, A. Oluwadun, and G. A. Adeyemi. 2019. Suitability of Spectrophotometric Assay for Determination of Honey Microbial Inhibition. P. 12131 in *Journal of Physics: Conference Series*. Vol. 1299. IOP Publishing.

Al-Amin, M., M. S. R. Khan, F. Begum, J. Hasan, and M. Asaduzzaman. 2009. "Isolation and Characterization of Bacterial Species from Enteric Fever Suspected Human Cases of Mymensingh Medical College Hospital." *Progressive Agriculture* 20(1–2):85–92.

Alben, JAMES O. 1996. "Fourier Transform Infrared Spectroscopy of Enzyme Systems." *Infrared Spectroscopy of Biomolecules* 19–38.

Alebiosu, C. O., and A. J. Yusuf. 2015. Phytochemical Screening, Thin-Layer Chromatographic Studies and UV Analysis of Extracts of *Citrullus lanatus*. *Journal of Pharmaceutical, Chemical and Biological Sciences* 3(2):214–20.

Alemu, Dawit, Chilot Yirga, and Adam Bekele. 2014. "Situation and Outlook of Maize in Ethiopia."

Al-Kobaisi, Muhannad F. 2007. "Jawetz, Melnick & Adelberg's Medical Microbiology." *Sultan Qaboos University Medical Journal* 7(3):273.

Altemimi, Ammar, Dennis G. Watson, Mary Kinsel, and David A. Lightfoot. 2015. Simultaneous Extraction, Optimization, and Analysis of Flavonoids and Polyphenols from Peach and Pumpkin Extracts Using a TLC-Densitometric Method. *Chemistry Central Journal* 9(1):1–15.

Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*, *48*(SUPPL. 1). https://doi.org/10.1093/jac/48.suppl_1.5

Aravind, G., Debjit Bhowmik, S. Duraivel, and G. Harish. 2013. Traditional and Medicinal Uses of *Carica papaya*. *Journal of Medicinal Plants Studies* 1(1):7–15.

ATEŞ, D. AYFER, and ÖZLEM TURGAY. 2003. Antimicrobial Activities of Various Medicinal and Commercial Plant Extracts. *Turkish Journal of Biology* 27(3):157–62.

Ayano, Ashenafi. 2015. “Significance of Vetivar Grass (Chrysopogon Zizanioides) in Coffee Based Farming System of Ethiopian (A Review).” *Significance* 5(21).

Aziz, Ramy K., Rita Kansal, Bruce J. Aronow, William L. Taylor, Sarah L. Rowe, Michael Kubal, Gursharan S. Chhatwal, Mark J. Walker, and Malak Kotb. 2010. “Microevolution of Group A Streptococci in Vivo: Capturing Regulatory Networks Engaged in Sociomicrobiology, Niche Adaptation, and Hypervirulence.” *PloS One* 5(4):e9798.

Bakkali, Fadil, Simone Averbeck, Dietrich Averbeck, and Mouhamed Idaomar. 2008. Biological Effects of Essential Oils–a Review. *Food and Chemical Toxicology* 46(2):446–75.

Balasankar, D., K. Vanilarasu, P. Selva Preetha, S. Rajeswari, M. Umadevi, and Debjit Bhowmik. 2013. Traditional and Medicinal Uses of Vetiver. *J Med Plants Stud* 1(3):191–200.

Barad, R., U. Atodariya, S. Bhatt, H. Patel, S. Upadhyay, and U. Upadhyay. 2013. Antibacterial and Preliminary Cytotoxic Activity of the Roots of Vetiveria zizanioides. *Int J Pharm Rev Res* 3(1):23–25.

Bar-On, Yinon M., Rob Phillips, and Ron Milo. 2018. “The Biomass Distribution on Earth.” *Proceedings of the National Academy of Sciences* 115(25):6506–11.

Baucells, Benjamin James, Maria Mercadal Hally, Airam Tenesor Álvarez Sánchez, and Josep Figueras Aloy. 2016. “Asociaciones de Probióticos Para La Prevención de La Enterocolitis Necrosante y La Reducción de La Sepsis Tardía y La Mortalidad Neonatal En Recién Nacidos Pretérmino de Menos de 1.500 g: Una Revisión Sistemática.” Pp. 247–55 in *Anales de pediatria*. Vol. 85. Elsevier.

Bérdy, János. 2012. “Thoughts and Facts about Antibiotics: Where We Are Now and Where We Are Heading.” *The Journal of Antibiotics* 65(8):385–95.

Bharathi Raja, M., K. Rajamani, J. Suresh, A. John Joel, D. Uma, Bharathi Raja, M. Bharathi, and John Joel. 2018. Chemical Composition of Vetiver Root Oil Obtained by Using GCMS Analysis. *~??? 1709 ~ Journal of Pharmacognosy and Phytochemistry* 7(6):1709–13.

Bhatia, Rajesh, and Jai P. Narain. 2010. The Growing Challenge of Antimicrobial Resistance in the South-East Asia Region-Are We Losing the Battle? *The Indian Journal of Medical Research* 132(5):482.

Bhuiyan, Md Nazrul Islam, Jasim Uddin Chowdhury, and Jaripa Begum. 2008. Essential Oil in Roots of Vetiveria Zizanioides (L.) Nash Ex Small from Bangladesh. *Bangladesh Journal of Botany* 37(2):213–15.

Bolan, Nanthi S., Jin Hee Park, Brett Robinson, Ravi Naidu, and Keun Young Huh. 2011. "Phytostabilization: A Green Approach to Contaminant Containment." *Advances in Agronomy* 112:145–204.

Boucher, Helen W., George H. Talbot, John S. Bradley, John E. Edwards, David Gilbert, Louis B. Rice, Michael Scheld, Brad Spellberg, and John Bartlett. 2009. Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. *Clinical Infectious Diseases* 48(1):1–12.

Bouhdid, S., Skali, S. N., Idaomar, M., Zhiri, A., Baudoux, D., Amensour, M., & Abrini, J. (2008). Antibacterial and antioxidant activities of Origanum compactum essential oil. *African Journal of Biotechnology*, *7*(10).

Brandenburg, KLAUS, and ULRICH Seydel. 1996. "Fourier Transform Infrared Spectroscopy of Cell Surface Polysaccharides." *Infrared Spectroscopy of Biomolecules* 203–38.

Chahal, Khushminder Kaur, Urvashi Bhardwaj, Sonia Kaushal, and Amanpal Kaur Sandhu. 2015. Chemical Composition and Biological Properties of *Chrysopogon zizanioides* (L.) Roberty Syn. *Vetiveria zizanioides* (L.) Nash-A Review.

Chanda, Sumitra, and Yogesh Baravalia. 2010. Screening of Some Plant Extracts against Some Skin Diseases Caused by Oxidative Stress and Microorganisms. *African Journal of Biotechnology* 9(21):3210–17.

Chen, Jiang Hong, and Chi-Tang Ho. 1997. Antioxidant Activities of Caffeic Acid and Its Related Hydroxycinnamic Acid Compounds. *Journal of Agricultural and Food Chemistry* 45(7):2374–78.

Christen, Yves. 2000. Oxidative Stress and Alzheimer Disease. *The American Journal of Clinical Nutrition* 71(2):621S-629S.

Chukwuemerie, Ogechukwu L., Samuel J. Bunu, Ezinne S. Iloh, Chika U. Ogujiuba, Josephat Obasi, Chukwuebuka C. Onwuzuluigbo, and Collins O. Okeke. 2022. Evaluation of the Anthelmintic Property of the Endophytic Extract of Senna alata in Mice Model. *Journal of Medicinal Plants* 10(3):52–54.

Chukwuka, K. S., Ikheloa, J. O., Okonko, I. O., Moody, J. O., & Mankinde, T. A. (2011). The antimicrobial activities of some medicinal plants on Escherichia coli as an agent of diarrhea in livestock. *Advances in Applied Science Research*, *2*(4).

Chun, Sung-Sook, Dhiraj A. Vattem, Yuan-Tong Lin, and Kalidas Shetty. 2005. Phenolic Antioxidants from Clonal Oregano (*Origanum vulgare*) with Antimicrobial Activity against *Helicobacter pylori*. *Process Biochemistry* 40(2):809–16.

Coates, Anthony, Yanmin Hu, Richard Bax, and Clive Page. 2002. The Future Challenges Facing the Development of New Antimicrobial Drugs. *Nature Reviews Drug Discovery* 1(11):895–910.

Council, National Research. 1993. *Vetiver Grass: A Thin Green Line against Erosion*. National Academies Press.

Danh, Luu Thai, Paul Truong, Raffaella Mammucari, Tam Tran, and Neil Foster. 2009. "Vetiver Grass, Vetiveria Zizanioides: A Choice Plant for Phytoremediation of Heavy Metals and Organic Wastes." *International Journal of Phytoremediation* 11(8):664–91.

Das, Padmini, Dibyendu Sarkar, Konstantinos C. Makris, and Rupali Datta. 2015. "Urea-Facilitated Uptake and Nitroreductase-Mediated Transformation of 2, 4, 6-Trinitrotoluene in Soil Using Vetiver Grass." *Journal of Environmental Chemical Engineering* 3(1):445–52.

Das, Padmini, Rupali Datta, Konstantinos C. Makris, and Dibyendu Sarkar. 2010. "Vetiver Grass Is Capable of Removing TNT from Soil in the Presence of Urea." *Environmental Pollution* 158(5):1980–83.

David, Andreea, Fang Wang, Xiaoming Sun, Hongna Li, Jieru Lin, Peilei Li, and Gang Deng. 2019. Chemical Composition, Antioxidant, and Antimicrobial Activities of *Vetiveria*

zizanioides (L.) Nash Essential Oil Extracted by Carbon Dioxide Expanded Ethanol. *Molecules* 24(10):1897-1906.

Davies, Julian, and Dorothy Davies. 2010. "Origins and Evolution of Antibiotic Resistance." *Microbiology and Molecular Biology Reviews* 74(3):417–33.

De Fátima, A., L. V Modolo, L. S. Conegero, R. A. Pilli, C. V Ferreira, L. K. Kohn, and J. E. De Carvalho. 2006. Styryl Lactones and Their Derivatives: Biological Activities, Mechanisms of Action and Potential Leads for Drug Design. *Current Medicinal Chemistry* 13(28):3371–84.

Deng, Gang, Jonathan D. Craft, Kelly Marie Steinberg, Pei Lei Li, Suraj Kumar Pokharel, and William N. Setzer. 2017. Influence of Different Isolation Methods on Chemical Composition and Bioactivities of the Fruit Peel Oil of *Citrus medica* L. Var. *sarcodactylis* (Noot.) Swingle. *Medicines* 4(1):1-23.

Devi, V. Subhadra, K. Asok Kumar, M. Uma Maheswari, A. T. Shanmugam, and R. Sankar Anand. 2010. In Vitro Antibacterial Activity of Ethanolic Extract of *Vetiveria zizanioides* Roots. *Int J Pharm Sci Res* 1:120–24.

Devprakash, D., P. Singh, K. K. Srinivasan, T. Subburaju, and S. K. Singh. 2011. Antifungal Activity of Alcoholic and Aqueous Extracts of *Vetiveria zizanioides*. *Journal of Pharmaceutical Research and Opinion* 1(3):85–88.

Di Donato, Mariangela, Rachel O. Cohen, Bruce A. Diner, Jacques Breton, Rienk Van Grondelle, and Marie Louise Groot. 2008. Primary Charge Separation in the Photosystem II Core from Synechocystis: A Comparison of Femtosecond Visible/Midinfrared Pump-Probe Spectra of Wild-Type and Two P680 Mutants. *Biophysical Journal* 94(12):4783–95.

Dikshit, A. 1984. Antifungal Action of Some Essential Oils against Animal Pathogens. *Fitoterapia* 55:171–76.

Djeussi, Doriane E., Jaurès A. K. Noumedem, Jackson A. Seukep, Aimé G. Fankam, Igor K. Voukeng, Simplice B. Tankeo, Antoine H. L. Nkuete, and Victor Kuete. 2013. Antibacterial Activities of Selected Edible Plants Extracts against Multidrug-Resistant Gram-Negative Bacteria. *BMC Complementary and Alternative Medicine* 13(1):1–8.

Donnenberg, Michael. 2013. *Escherichia Coli: Pathotypes and Principles of Pathogenesis*. Academic Press.

Dos Santos, D. S., Oberger, J. V., Niero, R., Wagner, T., Delle Monache, F., Cruz, A. B., Martin-Quintal, Z., & Filho, V. C. (2014). Seasonal phytochemical study and antimicrobial potential of Vetiveria zizanioides roots. *Acta Pharmaceutica*, *64*(4). https://doi.org/10.2478/acph-2014-0040

Dubey, Nidhi, C. S. Raghav, R. L. Gupta, and S. S. Chhonkar. 2010. Chemical Composition and Antifungal Activity of Vetiver Oil of North and South India against Rhizoctonia Solani. *Pesticide Research Journal* 22(1):63–67.

Duraipandiyan, Veeramuthu, Muniappan Ayyanar, and Savarimuthu Ignacimuthu. 2006. Antimicrobial Activity of Some Ethnomedicinal Plants Used by Paliyar Tribe from Tamil Nadu, India. *BMC Complementary and Alternative Medicine* 6(1):1–7.

Efe, Derya. 2019. The Evaluation of the Antibacterial Activity of *Vetiveria zizanioides* (L.) Nash Grown in Giresun, Turkey. *Alinteri J. Agr. Sci* 34:21–24.

Escobar-Páramo, Patricia, Catherine Giudicelli, Claude Parsot, and Erick Denamur. 2003. "The Evolutionary History of Shigella and Enteroinvasive Escherichia Coli Revised." *Journal of Molecular Evolution* 57:140–48.

Eshetu, Mulugeta, Negash Bedasso, Shure Seboka, Chala Chimdessa, Tilahun Chibsa, and Abduraman Hussen. 2017. "Multiplication and Distribution of Vetiver Grass (Vetiveria Zizanioides) for Soil and Water Conservation Measures in the Highland of Bale Zone, Southeastern Ethiopia." *Agric. & Environ. Sci* 17(6):514–18

Ezhilan, Bagavathi Perumal, and Ramasamy Neelamegam. 2012. GC-MS Analysis of Phytocomponents in the Ethanol Extract of *Polygonum chinense* L. *Pharmacognosy Research* 4(1):11.

Ferretti, Joseph J., Dennis L. Stevens, and Vincent A. Fischetti. 2016. "Streptococcus Pyogenes: Basic Biology to Clinical Manifestations [Internet]."

Filippi, J. J., Belhassen, E., Baldovini, N., Brevard, H., & Meierhenrich, U. J. (2013). Qualitative and quantitative analysis of vetiver essential oils by comprehensive two-dimensional gas chromatography and comprehensive two-dimensional gas

chromatography/mass spectrometry. *Journal of Chromatography A, 1288*. https://doi.org/10.1016/j.chroma.2013.03.002

Filippi, Jean-Jacques, Emilie Belhassen, Nicolas Baldovini, Hugues Brevard, and Uwe J. Meierhenrich. 2013. Qualitative and Quantitative Analysis of Vetiver Essential Oils by Comprehensive Two-Dimensional Gas Chromatography and Comprehensive Two-Dimensional Gas Chromatography/Mass Spectrometry. *Journal of Chromatography*

GERwERT, KLAUS, Georg Souvignier, and Benno Hess. 1990. "Simultaneous Monitoring of Light-Induced Changes in Protein Side-Group Protonation, Chromophore Isomerization, and Backbone Motion of Bacteriorhodopsin by Time-Resolved Fourier-Transform Infrared Spectroscopy." *Proceedings of the National Academy of Sciences* 87(24):9774–78.

Gerwert, Klaus. 1993. "Molecular Reaction Mechanisms of Proteins as Monitored by Time-Resolved FTIR Spectroscopy." *Current Opinion in Structural Biology* 3(5):769–73.

Giamarellou, H. (2010). Multidrug-resistant Gram-negative bacteria: How to treat and for how long. *International Journal of Antimicrobial Agents*, *36*(SUPPL. 2). https://doi.org/10.1016/j.ijantimicag.2010.11.014

Giamarellou, Helen. 2010. Multidrug-Resistant Gram-Negative Bacteria: How to Treat and for How Long. *International Journal of Antimicrobial Agents* 36:S50–54.

Gow, Neil A. R., and Bhawna Yadav. 2017. "Microbe Profile: Candida Albicans: A Shape-Changing, Opportunistic Pathogenic Fungus of Humans." *Microbiology* 163(8):1145–47.

Hall, Robert D. 2006. "Plant Metabolomics: From Holistic Hope, to Hype, to Hot Topic." *New Phytologist* 169(3):453–68.

Hatano, Tsutomu, Hiroshi Uebayashi, Hideyuki Ito, Sumiko Shiota, Tomofusa Tsuchiya, and Takashi Yoshida. 1999. Phenolic Constituents of Cassia Seeds and Antibacterial Effect of Some Naphthalenes and Anthraquinones on Methicillin-Resistant *Staphylococcus aureus*. *Chemical and Pharmaceutical Bulletin* 47(8):1121–27.

Hickman, Meleah A., Guisheng Zeng, Anja Forche, Matthew P. Hirakawa, Darren Abbey, Benjamin D. Harrison, Yan-Ming Wang, Ching-hua Su, Richard J. Bennett, and Yue

Wang. 2013. “The ‘Obligate Diploid’Candida Albicans Forms Mating-Competent Haploids.” *Nature* 494(7435):55–59.

Hudzicki, J. (2012). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol Author Information. *American Society For Microbiology*, *December 2009*.

Iwu, Maurice W., Angela R. Duncan, and Chris O. Okunji. 1999. New Antimicrobials of Plant Origin. *Perspectives on New Crops and New Uses. ASHS Press, Alexandria, VA* 457:462.

Jain, S. K. (1994). Ethnobotany and research on medicinal plants in India. In *Ciba Foundation symposium* (Vol. 185). https://doi.org/10.1002/9780470514634.ch11

Joshi, A., M. Bhobe, and A. Sattarkar. 2013. Physicochemical and Phytochemical Investigation of the Roots of *Grewia microcos* Linn. *American J Pharm Health Res* 1(4):54–65.

Kabir, M. Anaul, Mohammad Asif Hussain, and Zulfiqar Ahmad. 2012. “Candida Albicans: A Model Organism for Studying Fungal Pathogens.” *International Scholarly Research Notices* 2012.

Kadosh, David. 2019. “Regulatory Mechanisms Controlling Morphology and Pathogenesis in Candida Albicans.” *Current Opinion in Microbiology* 52:27–34.

Kannan, P., S. R. Ramadevi, and Waheeta Hopper. 2009. Antibacterial Activity of *Terminalia chebula* Fruit Extract. *African Journal of Microbiology Research* 3(4):180–84.

Katz, Leonard, and Richard H. Baltz. 2016. “Natural Product Discovery: Past, Present, and Future.” *Journal of Industrial Microbiology and Biotechnology* 43(2–3):155–76.

Kaushik, J. C., Arya Sanjay, N. N. Tripathi, and S. Arya. 2002. Antifungal Properties of Some Plant Extracts against the Damping-off Fungi of Forest Nurseries. *Indian Journal of Forestry* 25(3–4):359–61.

Kede, Maria Luiza F. M., Daniel Vidal Pérez, Josino Costa Moreira, and Marcia Marques. 2017. “Effect of Phosphates on the Bioavailability and Phytotoxicity of Pb and Cd in Contaminated Soil and Phytoextraction by Vetiver Grass.” *Journal of Environmental Engineering* 143(3):5016008.

Kemp, William. 1991. Energy and the Electromagnetic Spectrum. Pp. 1–17 in *Organic spectroscopy*. Springer.

Kim, Hye Kyong, Young Hae Choi, and Robert Verpoorte. 2010. "NMR-Based Metabolomic Analysis of Plants." *Nature Protocols* 5(3):536–49.

Koeth, Laura M., Jeanna M. DiFranco-Fisher, and Sandra McCurdy. 2015. A Reference Broth Microdilution Method for Dalbavancin *in vitro* Susceptibility Testing of Bacteria That Grow Aerobically. *JoVE (Journal of Visualized Experiments)* (103):e53028.

Kumaran, A. 2006. "Antioxidant and Free Radical Scavenging Activity of an Aqueous Extract of *Coleus aromaticus*." *Food Chemistry* 97(1):109–14.

Kusuma, Heri Septya, A. Altway, and Mahfud Mahfud. 2017. Alternative to Conventional Extraction of Vetiver Oil: Microwave Hydrodistillation of Essential Oil from Vetiver Roots (*Vetiveria zizanioides*). P. 12015 in *IOP Conference Series: Earth and Environmental Science*. Vol. 101. IOP Publishing.

Lal, C., & Verma, L. R. (2006). Use of certain bio-products for insect-pest control. In *Indian Journal of Traditional Knowledge* (Vol. 5, Issue 1).

Lambert, John, Jitendra Srivastava, and Noel Vietmeyer. 1997. *Medicinal Plants: Rescuing a Global Heritage*. Vol. 355. World Bank Publications.

Lavania, U. C. 2004. "MEETING REPORT: Vetiver System Ecotechnology for Water Quality Improvement and Environmental Enhancement." *Current Science* 86(1):11–14.

Lenart-Boroń, Anna, Katarzyna Wolny-Koładka, Katarzyna Juraszek, and Andrzej Kasprowicz. 2017. "Phenotypic and Molecular Assessment of Antimicrobial Resistance Profile of Airborne Staphylococcus Spp. Isolated from Flats in Kraków." *Aerobiologia* 33(3):435–44.

Levin, I. W., & Bhargava, R. (2005). Fourier transform infrared vibrational spectroscopic imaging: Integrating microscopy and molecular recognition. *Annual Review of Physical Chemistry, 56*. https://doi.org/10.1146/annurev.physchem.56.092503.141205

Lewis, R. N. 1996. "Fourier Transform Infrared Spectroscopy in the Study of Hydrated Lipids and Lipid Bilayer Membranes." *Infrared Spectroscopy of Biomolecules* 159–202.

Liquier, Jean. 1996. “Infrared Spectroscopy of Nucleic Acids.” *Infrared Spectroscopy of Biomolecules*.

Luqman, S., Srivastava, S., Darokar, M. P., & Khanuja, S. P. S. (2005). Detection of antibacterial activity in spent roots of two genotypes of aromatic grass Vetiveria zizanioides. *Pharmaceutical Biology*, *43*(8). https://doi.org/10.1080/13880200500387471

Mackie, T. J., and J. E. McCartney. 1989. “Microbial Infections.” *Medical Microbiology. 13th Edition Longman Group Limited, London* 236–45.

MacNee, W. (2005). Pulmonary and systemic oxidant/antioxidant imbalance in chronic obstructive pulmonary disease. *Proceedings of the American Thoracic Society*, *2*(1). https://doi.org/10.1513/pats.200411-056SF

Maffei, Massimo. 2002. *Vetiveria: The Genus Vetiveria*. CRC Press.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. In *American Journal of Clinical Nutrition* (Vol. 79, Issue 5). https://doi.org/10.1093/ajcn/79.5.727

Marasini, B. P., Baral, P., Aryal, P., Ghimire, K. R., Neupane, S., Dahal, N., Singh, A., Ghimire, L., & Shrestha, K. (2015). Evaluation of antibacterial activity of some traditionally used medicinal plants against human pathogenic bacteria. *BioMed Research International*, *2015*. https://doi.org/10.1155/2015/265425

Marston, A. (2007). Role of advances in chromatographic techniques in phytochemistry. In *Phytochemistry* (Vol. 68, Issues 22–24). https://doi.org/10.1016/j.phytochem.2007.08.004

Matsuo, Y., Maeda, S., Ohba, C., Fukaya, H., & Mimaki, Y. (2016). Vetiverianines A, B, and C: Sesquiterpenoids from Vetiveria zizanioides Roots. *Journal of Natural Products*, *79*(9). https://doi.org/10.1021/acs.jnatprod.6b00140

Medina, A. L., Lucero, M. E., Holguin, F. O., Estell, R. E., Posakony, J. J., Simon, J., & O’Connell, M. A. (2005). Composition and antimicrobial activity of Anemopsis californica leaf oil. *Journal of Agricultural and Food Chemistry*, *53*(22). https://doi.org/10.1021/jf0511244

Mehta, S. R., Yusuf, S., Peters, R. J. G., Bertrand, M. E., Lewis, B. S., Natarajan, M. K., Malmberg, K., Rupprecht, H. J., Zhao, F., Chrolavicius, S., Copland, I., & Fox, K. A. A. (2001). Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: The PCI-CURE study. *Lancet*, *358*(9281). https://doi.org/10.1016/S0140-6736(01)05701-4

Mekonnen, Mulatie, Saskia D. Keesstra, Leo Stroosnijder, Jantiene E. M. Baartman, and Jerry Maroulis. 2015. "Soil Conservation through Sediment Trapping: A Review." *Land Degradation & Development* 26(6):544–56.

Meyer, Edenilson, Diana Marcela Morales Londoño, Rafael Dutra de Armas, Admir José Giachini, Márcio José Rossi, Shantau Camargo Gomes Stoffel, and Cláudio Roberto Fonsêca Sousa Soares. 2017. "Arbuscular Mycorrhizal Fungi in the Growth and Extraction of Trace Elements by Chrysopogon Zizanioides (Vetiver) in a Substrate Containing Coal Mine Wastes." *International Journal of Phytoremediation* 19(2):113–20.

Milne, A., & Beamish, T. (1999). Inhalational and local anesthetics reduce-tactile and thermal responses in mimosa pudica. *Canadian Journal of Anaesthesia*, *46*(3). https://doi.org/10.1007/BF03012612

Mukhtar, M. D., Orah, S. A., & Mohammed, Y. (2017). Antimicrobial activity of moringa on ear, nose and throat associated fungi, and vancomycin resistant cocci isolated at Aminu Kano Teaching Hospital, Kano, Nigeria. *African Journal of Clinical and Experimental Microbiology*, *18*(3). https://doi.org/10.4314/ajcem.v18i3.5

Mulinacci, N., Prucher, D., Peruzzi, M., Romani, A., Pinelli, P., Giaccherini, C., & Vincieri, F. F. (2004). Commercial and laboratory extracts from artichoke leaves: Estimation of caffeoyl esters and flavonoidic compounds content. *Journal of Pharmaceutical and Biomedical Analysis*, *34*(2). https://doi.org/10.1016/S0731-7085(03)00552-1

Muthamilarasan, Mehanathan, and Manoj Prasad. 2013. "Plant Innate Immunity: An Updated Insight into Defense Mechanism." *Journal of Biosciences* 38:433–49.

Muthukrishnan, S., & Manogaran, P. (2018). Phytochemical analysis and free radical scavenging potential activity of Vetiveria zizanioides Linn. *Journal of Pharmacognosy and Phytochemistry*, *7*(2).

Nabedryk, E. 1996. “Light-Induced Fourier Transform Infrared Difference Spectroscopy of the Primary Electron Donor in Photosynthetic Reaction Centers.” *Infrared Spectroscopy of Biomolecules* 39–81.

Nabedryk, Eliane, Winfried Leibl, and Jacques Breton. 1996. “FTIR Spectroscopy of Primary Donor Photooxidation in Photosystem I, Heliobacillus Mobilis, and Chlorobium Limicola. Comparison with Purple Bacteria.” *Photosynthesis Research* 48:301–8.

Nash, John H. E., Andre Villegas, Andrew M. Kropinski, Renan Aguilar-Valenzuela, Paulina Konczy, Mariola Mascarenhas, Kim Ziebell, Alfredo G. Torres, Mohamed A. Karmali, and Brian K. Coombes. 2010. “Genome Sequence of Adherent-Invasive Escherichia Coli and Comparative Genomic Analysis with Other E. Coli Pathotypes.” *BMC Genomics* 11(1):1–15.

Natarajan, D., Britto, S. J., Srinivasan, K., Nagamurugan, N., Mohanasundari, C., & Perumal, G. (2005). Anti-bacterial activity of Euphorbia fusiformis - A rare medicinal herb. *Journal of Ethnopharmacology*, *102*(1). https://doi.org/10.1016/j.jep.2005.04.023

Passos, Marieta L. C., and M. Lúcia M. F. S. Saraiva. 2019. “Detection in UV-Visible Spectrophotometry: Detectors, Detection Systems, and Detection Strategies.” *Measurement* 135:896–904.

Pate, Smit, Aniket Raulji, Diya Patel, and Diya Panchal. 2022. “Prof. Mitali Dalwadi, Dr. Umesh Upadhyay, A Review on ‘Uv Visible Spectroscopy.’” *International Journal of Pharmaceutical Research and Applications* 7(5):1144–51.

Phusantisampan, Theerawut, Weeradej Meeinkuirt, Patompong Saengwilai, John Pichtel, and Rattanawat Chaiyarat. 2016. “Phytostabilization Potential of Two Ecotypes of Vetiveria Zizanioides in Cadmium-Contaminated Soils: Greenhouse and Field Experiments.” *Environmental Science and Pollution Research* 23:20027–38.

Pripdeevech, P., Wongpornchai, S., & Promsiri, A. (2006). Highly volatile constituents of Vetiveria zizanioides roots grown under different cultivation conditions. *Molecules*, *11*(10). https://doi.org/10.3390/11100817

Radha, Puri, S., & Kumar, V. (2019). Phytochemical screening of medicinal plants used by tribal migratory shepherds in Western Himalaya. *Annals of Biology*, *35*(1).

Remy, André, and Klaus Gerwert. 2003. “Coupling of Light-Induced Electron Transfer to Proton Uptake in Photosynthesis.” *Nature Structural & Molecular Biology* 10(8):637–44.

Rivero-Cruz, J. F., Granados-Pineda, J., Pedraza-Chaverri, J., Pérez-Rojas, J. M., Kumar-Passari, A., Diaz-Ruiz, G., & Rivero-Cruz, B. E. (2020). Phytochemical constituents, antioxidant, cytotoxic, and antimicrobial activities of the ethanolic extract of mexican brown propolis. *Antioxidants*, *9*(1). https://doi.org/10.3390/antiox9010070

Rochfort, Simone. 2005. “Metabolomics Reviewed: A New ‘Omics’ Platform Technology for Systems Biology and Implications for Natural Products Research.” *Journal of Natural Products* 68(12):1813–20.

Romero, C. D., Chopin, S. F., Buck, G., Martinez, E., Garcia, M., & Bixby, L. (2005). Antibacterial properties of common herbal remedies of the southwest. *Journal of Ethnopharmacology*, *99*(2). https://doi.org/10.1016/j.jep.2005.02.028

Rubió, L., Motilva, M. J., & Romero, M. P. (2013). Recent Advances in Biologically Active Compounds in Herbs and Spices: A Review of the Most Effective Antioxidant and Anti-Inflammatory Active Principles. *Critical Reviews in Food Science and Nutrition*, *53*(9). https://doi.org/10.1080/10408398.2011.574802

Ryan, Kenneth James, C. George Ray, and John C. Sherris. 2004. “Sherris Medical Microbiology: An Introduction to Infectious Diseases.” *(No Title)*.

Samaan, M., Ebid, M., & Thabet, M. (2022). GC-MS Analysis, Antioxidant Capacity and Antimicrobic Action of Vetiveria zizanioides. Essential Oil Cultivated in North Egypt. *Journal of Plant Production*, *13*(9). https://doi.org/10.21608/jpp.2022.147290.1138

Sangeetha, D., & Stella, D. (2012). Screening of Antimicrobial Activity of Vetiver Extracts against Certain Pathogenic Microorganisms. In *International Journal of Pharmaceutical & Biological Archives* (Vol. 3, Issue 1).

Sarajlija, H., Čukelj, N., Novotni, D., Mrčič, G., Brnčič, M., & Čurič, D. (2012). Preparation of flaxseed for lignan determination by gas chromatography-mass spectrometry method. *Czech Journal of Food Sciences*, *30*(1). https://doi.org/10.17221/107/2010-cjfs

Sarmah, Ajit K., Michael T. Meyer, and Alistair B. A. Boxall. 2006. “A Global Perspective on the Use, Sales, Exposure Pathways, Occurrence, Fate and Effects of Veterinary Antibiotics (VAs) in the Environment.” *Chemosphere* 65(5):725–59.

Seger, Christoph, and Sonja Sturm. 2007. “Analytical Aspects of Plant Metabolite Profiling Platforms: Current Standings and Future Aims.” *Journal of Proteome Research* 6(2):480–97.

Shankar, V., Venkatesh, A., Hofacker, C., & Naik, P. (2010). Mobile marketing in the retailing environment: Current insights and future research avenues. *Journal of Interactive Marketing*, *24*(2). https://doi.org/10.1016/j.intmar.2010.02.006

Siebert, F., & Hildebrandt, P. (2008). Vibrational Spectroscopy in Life Science. In *Vibrational Spectroscopy in Life Science*. https://doi.org/10.1002/9783527621347

Snigdha, M., Kumar, S. S., Sharmistha, M., & Deepa, C. (2013). An overview on Vetiveria zizanioides. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *4*(3).

Solanki, S. L., Modi, C. M., Patel, H. B., Patel, U. D., & Bhadarka, D. H. (2019). Phytochemical screening and thin-layer chromatography of six medicinal plants from the surroundings of Junagadh , Gujarat , India. *JPP*, *8*(4).

Soni, A., & Dahiya, P. (2015). Screening of phytochemicals and antimicrobial potential of extracts of vetiver zizanoides and Phragmites karka against clinical isolates. *International Journal of Applied Pharmaceutics*, *7*(1).

Spencer, J. P. E. (2008). Flavonoids: Modulators of brain function? In *British Journal of Nutrition* (Vol. 99, Issue E-SUPPL. 1). https://doi.org/10.1017/S0007114508965776

Sumner, Lloyd W., Pedro Mendes, and Richard A. Dixon. 2003. “Plant Metabolomics: Large-Scale Phytochemistry in the Functional Genomics Era.” *Phytochemistry* 62(6):817–36.

Surewicz, Witold K., Henry H. Mantsch, and Dennis Chapman. 1993. “Determination of Protein Secondary Structure by Fourier Transform Infrared Spectroscopy: A Critical Assessment.” *Biochemistry* 32(2):389–94.

Takaidza, S., Mtunzi, F., & Pillay, M. (2018). Analysis of the phytochemical contents and antioxidant activities of crude extracts from Tulbaghia species. *Journal of Traditional Chinese Medicine*, *38*(2). https://doi.org/10.1016/j.jtcm.2018.04.005

Tenaillon, Olivier, David Skurnik, Bertrand Picard, and Erick Denamur. 2010. "The Population Genetics of Commensal Escherichia Coli." *Nature Reviews Microbiology* 8(3):207–17.

Upstone, Stephen L. 2000. "Ultraviolet/Visible Light Absorption Spectrophotometry in Clinical Chemistry." *Encyclopedia of Analytical Chemistry* 1699–1714.

Urbano, M., Luque De Castro, M. D., Pérez, P. M., García-Olmo, J., & Gómez-Nieto, M. A. (2006). Ultraviolet-visible spectroscopy and pattern recognition methods for differentiation and classification of wines. *Food Chemistry*, *97*(1). https://doi.org/10.1016/j.foodchem.2005.05.001

Verma, A. (2020). Vetiveria zizanioides (L.) Nash: A review of magic grass. *~ 58 ~ Journal of Medicinal Plants Studies*, *8*(1).

Verpoorte, R., Y. H. Choi, and H. K. Kim. 2007. "NMR-Based Metabolomics at Work in Phytochemistry." *Phytochemistry Reviews* 6:3–14.

Wahlberg, I., and C. R. Enzell. 1987. "Tobacco Isoprenoids." *Natural Product Reports* 4:237–76.

Wang, H. H., Manuzon, M., Lehman, M., Wan, K., Luo, H., Wittum, T. E., Yousef, A., & Bakaletz, L. O. (2006). Food commensal microbes as a potentially important avenue in transmitting antibiotic resistance genes. *FEMS Microbiology Letters*, *255*(2). https://doi.org/10.1111/j.1574-6968.2006.00138.x

Wang, H., J. C. McEntire, L. Zhang, X. Li, and M. Doyle. 2012. "The Transfer of Antibiotic Resistance from Food to Humans: Facts, Implications and Future Directions." *Revue Scientifique et Technique (International Office of Epizootics)* 31(1):249–60.

Wang, W., Wu, N., Zu, Y. G., & Fu, Y. J. (2008). Antioxidative activity of Rosmarinus officinalis L. essential oil compared to its main components. *Food Chemistry*, *108*(3). https://doi.org/10.1016/j.foodchem.2007.11.046

Ward, Jane L., John M. Baker, and Michael H. Beale. 2007. “Recent Applications of NMR Spectroscopy in Plant Metabolomics.” *The FEBS Journal* 274(5):1126–31.

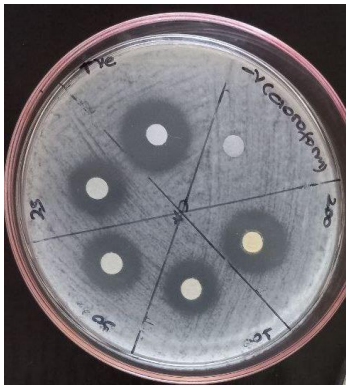
WHO. (2002). The World Health Organization Report 2002: reducing risks, promoting healthy life. *WHO Library Cataloguing-in Publication Data*.

Xu, R., Zhao, W., Xu, J., Shao, B., & Qin, G. (1996). Studies on bioactive saponins from Chinese medicinal plants. *Advances in Experimental Medicine and Biology*, *404*. https://doi.org/10.1007/978-1-4899-1367-8_30

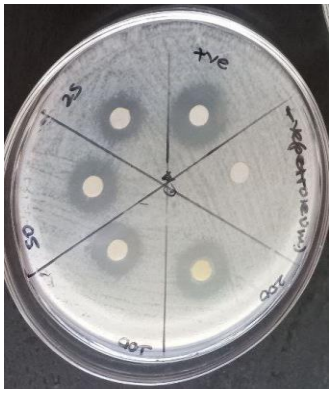
Zahoor, S., Shahid, S., & Fatima, U. (2018). Review of Pharmacological Activities of Vetiveria zizanoide (Linn) Nash. *Journal of Basic & Applied Sciences*, *14*.

7. APPENDICES

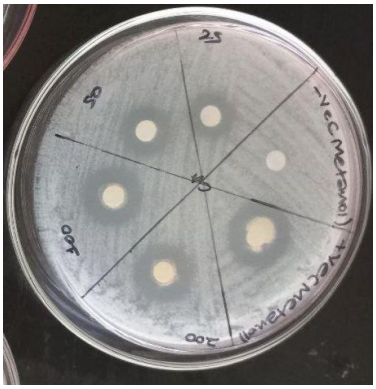
Appendix 1: Inhibition zone of *C. zizanioides* root crude extracts against *C. albicans*.



Chloroform extract

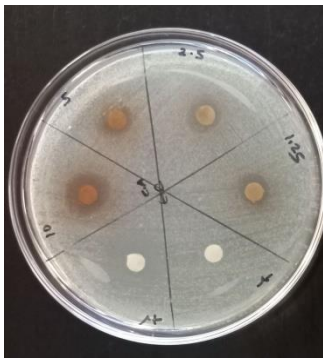


Petroleum ether extract

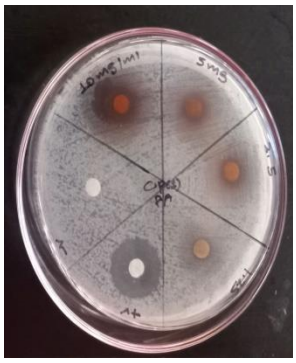


Methanol extract

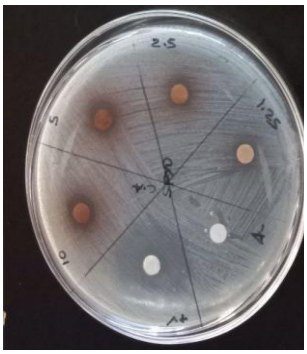
Appendix 2: Inhibition zones of isolated compounds of *C. zizanioides* root on tested bacterial strains in different concentration.



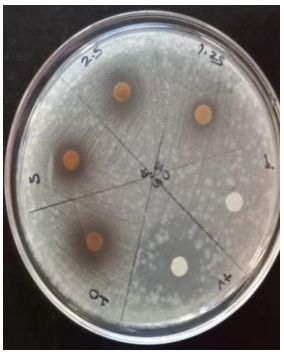
E. coli



P. aeruginosa

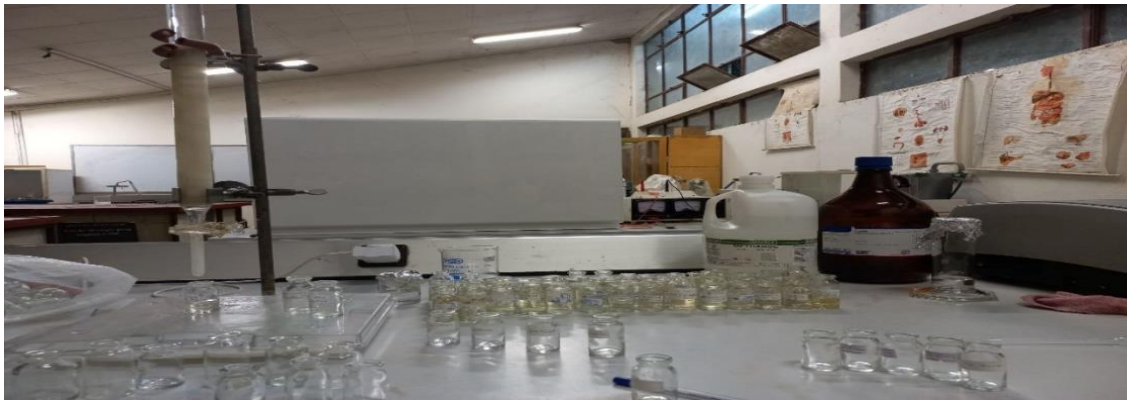


S. pyogenes

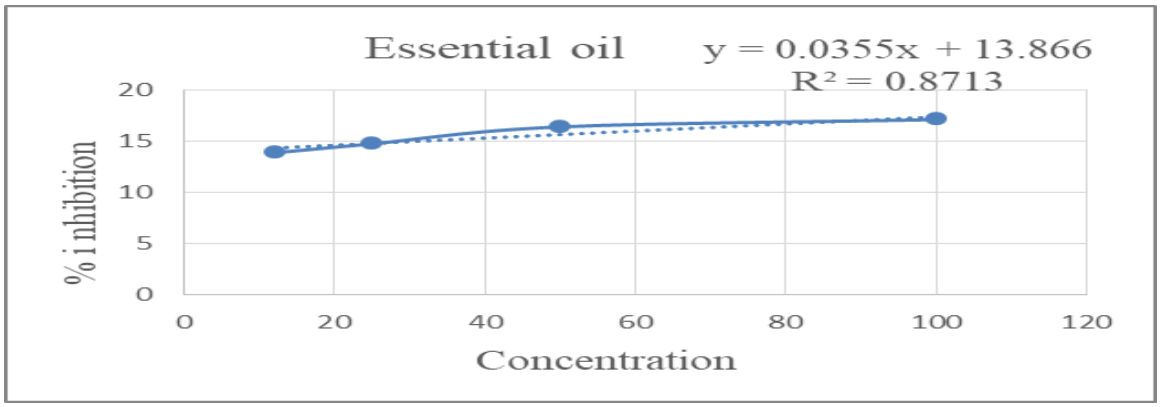


S. aureus

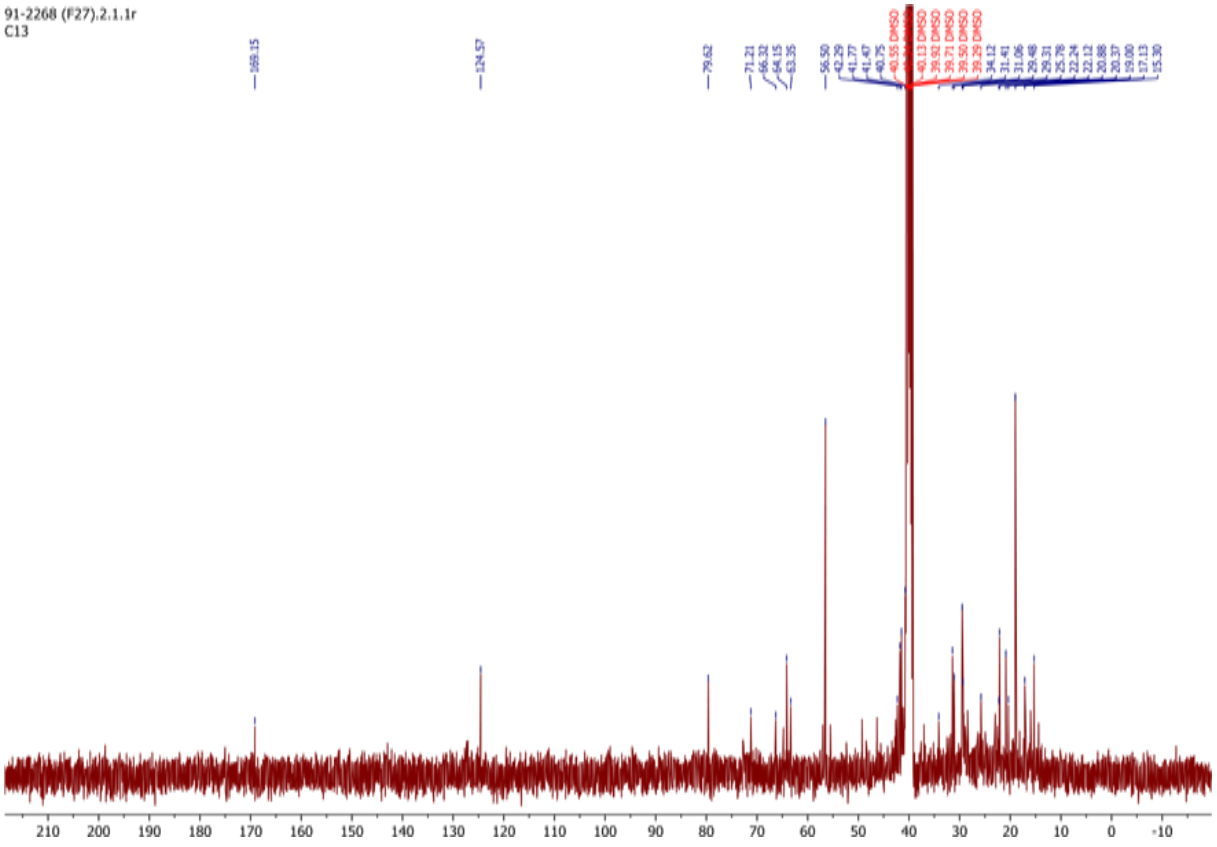
Appendix 3: Compound isolation using column chromatography.



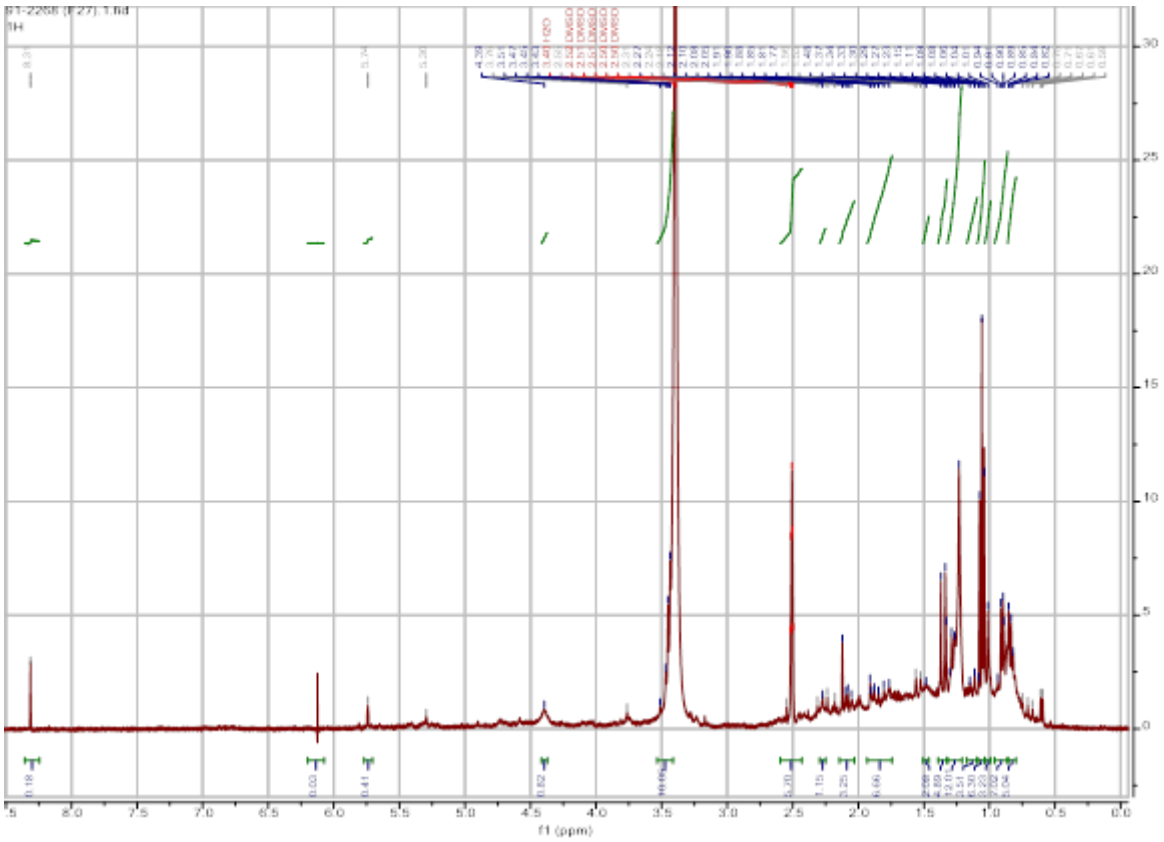
Appendix 4: DPPH radical scavenging activity (%) of *C. zizanioides* essential oil.



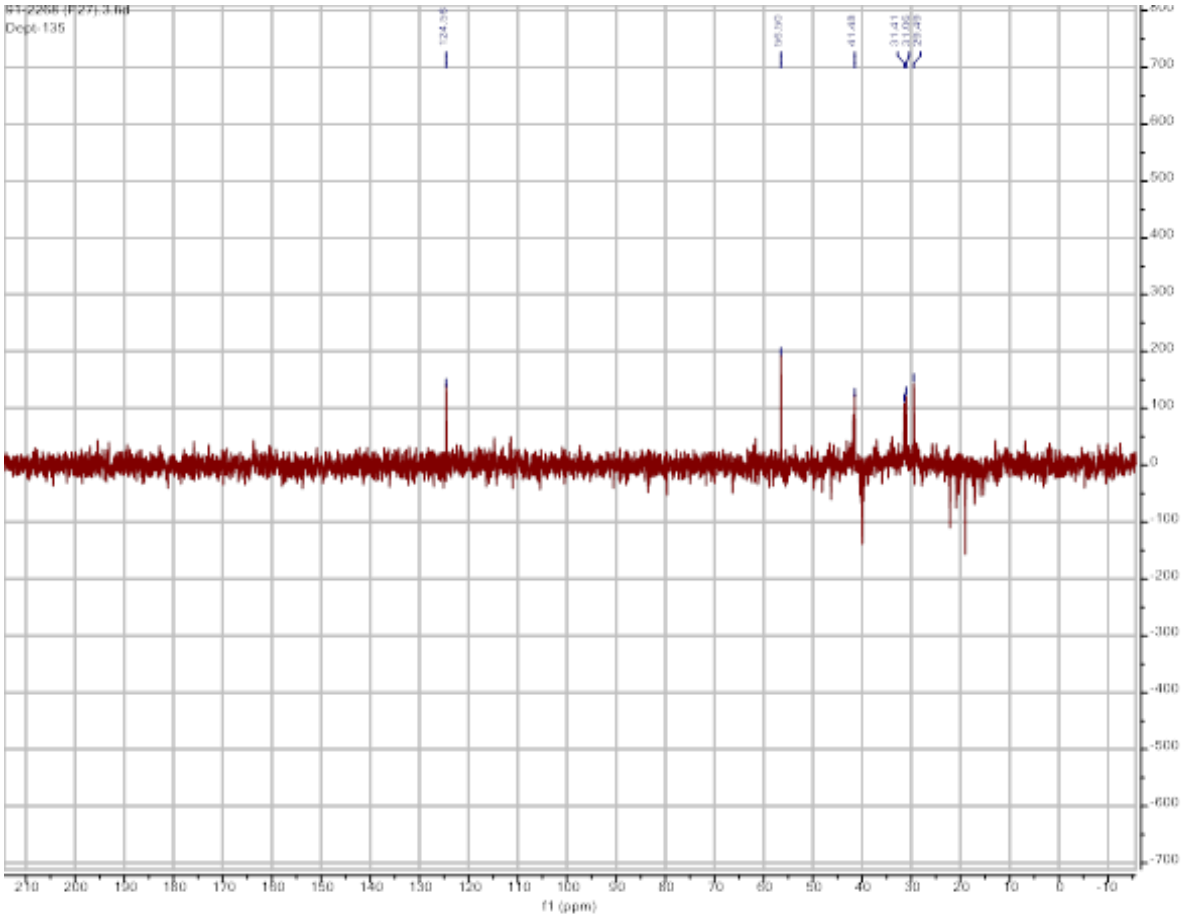
Appendix 5: ^{13}C-NMR spectrum of compound 1.



Appendix 6:^{1}H NMR spectrum of compound 1.



Appendix 7 : DEPT-135 spectrum of compound 1.



Appendix 8 : Significance of chloroform extract against Some Human Pathogenic strains.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) concentration	(J) concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
(ATCC 25923)	200	100	2.667*	.760	.036	.17	5.17
		50	3.333*	.760	.009	.83	5.83
		25	4.333*	.760	.001	1.83	6.83
		ciprofloxacin	-19.667*	.760	.000	-22.17	-17.17
	100	200	-2.667*	.760	.036	-5.17	-.17
		50	.667	.760	.899	-1.83	3.17
		25	1.667	.760	.257	-.83	4.17
		ciprofloxacin	-22.333*	.760	.000	-24.83	-19.83
	50	200	-3.333*	.760	.009	-5.83	-.83
		100	-.667	.760	.899	-3.17	1.83
		25	1.000	.760	.689	-1.50	3.50
		ciprofloxacin	-23.000*	.760	.000	-25.50	-20.50
	25	200	-4.333*	.760	.001	-6.83	-1.83
		100	-1.667	.760	.257	-4.17	.83
		50	-1.000	.760	.689	-3.50	1.50
		ciprofloxacin	-24.000*	.760	.000	-26.50	-21.50
	ciprofloxacin	200	19.667*	.760	.000	17.17	22.17
		100	22.333*	.760	.000	19.83	24.83
		50	23.000*	.760	.000	20.50	25.50
		25	24.000*	.760	.000	21.50	26.50
(ATCC 27823)	200	100	1.00000	1.11555	.892	-2.6714	4.6714
		50	3.00000	1.11555	.126	-.6714	6.6714
		25	6.00000*	1.11555	.002	2.3286	9.6714
		ciprofloxacin	-14.33333*	1.11555	.000	-18.0047	-10.6620
	100	200	-1.00000	1.11555	.892	-4.6714	2.6714
		50	2.00000	1.11555	.427	-1.6714	5.6714
		25	5.00000*	1.11555	.008	1.3286	8.6714
		ciprofloxacin	-15.33333*	1.11555	.000	-19.0047	-11.6620
	50	200	-3.00000	1.11555	.126	-6.6714	.6714
		100	-2.00000	1.11555	.427	-5.6714	1.6714
		25	3.00000	1.11555	.126	-.6714	6.6714

		ciprofloxacin	-17.33333*	1.11555	.000	-21.0047	-13.6620
	25	200	-6.00000*	1.11555	.002	-9.6714	-2.3286
		100	-5.00000*	1.11555	.008	-8.6714	-1.3286
		50	-3.00000	1.11555	.126	-6.6714	.6714
		ciprofloxacin	-20.33333*	1.11555	.000	-24.0047	-16.6620
	ciprofloxacin	200	14.33333*	1.11555	.000	10.6620	18.0047
		100	15.33333*	1.11555	.000	11.6620	19.0047
		50	17.33333*	1.11555	.000	13.6620	21.0047
		25	20.33333*	1.11555	.000	16.6620	24.0047
(ATCC 25923)	200	100	2.66667	2.19089	.743	-4.5437	9.8771
		50	2.66667	2.19089	.743	-4.5437	9.8771
		25	7.33333*	2.19089	.046	.1229	14.5437
		ciprofloxacin	-12.00000*	2.19089	.002	-19.2104	-4.7896
	100	200	-2.66667	2.19089	.743	-9.8771	4.5437
		50	.00000	2.19089	1.000	-7.2104	7.2104
		25	4.66667	2.19089	.280	-2.5437	11.8771
		ciprofloxacin	-14.66667*	2.19089	.000	-21.8771	-7.4563
	50	200	-2.66667	2.19089	.743	-9.8771	4.5437
		100	.00000	2.19089	1.000	-7.2104	7.2104
		25	4.66667	2.19089	.280	-2.5437	11.8771
		ciprofloxacin	-14.66667*	2.19089	.000	-21.8771	-7.4563
	25	200	-7.33333*	2.19089	.046	-14.5437	-.1229
		100	-4.66667	2.19089	.280	-11.8771	2.5437
		50	-4.66667	2.19089	.280	-11.8771	2.5437
		ciprofloxacin	-19.33333*	2.19089	.000	-26.5437	-12.1229
	ciprofloxacin	200	12.00000*	2.19089	.002	4.7896	19.2104
		100	14.66667*	2.19089	.000	7.4563	21.8771
		50	14.66667*	2.19089	.000	7.4563	21.8771
		25	19.33333*	2.19089	.000	12.1229	26.5437
(ATCC 11778)	200	100	3.00000	.98883	.074	-.2543	6.2543
		50	5.66667*	.98883	.001	2.4124	8.9210
		25	7.00000*	.98883	.000	3.7457	10.2543
		ciprofloxacin	-16.66667*	.98883	.000	-19.9210	-13.4124
	100	200	-3.00000	.98883	.074	-6.2543	.2543
		50	2.66667	.98883	.124	-.5876	5.9210
		25	4.00000*	.98883	.016	.7457	7.2543
		ciprofloxacin	-19.66667*	.98883	.000	-22.9210	-16.4124
	50	200	-5.66667*	.98883	.001	-8.9210	-2.4124
		100	-2.66667	.98883	.124	-5.9210	.5876
		25	1.33333	.98883	.670	-1.9210	4.5876

		ciprofloxacin	-22.33333*	.98883	.000	-25.5876	-19.0790
	25	200	-7.00000*	.98883	.000	-10.2543	-3.7457
		100	-4.00000*	.98883	.016	-7.2543	-.7457
		50	-1.33333	.98883	.670	-4.5876	1.9210
		ciprofloxacin	-23.66667*	.98883	.000	-26.9210	-20.4124
	ciprofloxacin	200	16.66667*	.98883	.000	13.4124	19.9210
		100	19.66667*	.98883	.000	16.4124	22.9210
		50	22.33333*	.98883	.000	19.0790	25.5876
		25	23.66667*	.98883	.000	20.4124	26.9210

*. The mean difference is significant at the 0.05 level.

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