ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF METHANOL RHIZOME EXTRACT OF *Zingiber officinale*

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DECLARATION

I declare that this research project is my original work and has not been submitted by any other student to any institution for the award of degree or diploma. Any work gotten from other sources is dully cited.

Signature…… **Date …….……**

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BPHARM/53349/2016

Approval by the Supervisor

I confirm that this research project has been conducted and submitted with my approval as the student supervisor

Signature…… **Date …….……**

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DEDICATION

I would like to express my special thanks of gratitude my professor Bindhu Mandhav and School Of Pharmacy MKU Thika, for their able guidance and support in completing my project.

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I dedicate this work to my beloved parents for their endless sacrifice and encouragement I making sure I attain the best that education has to offer.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Antimicrobial drug resistance is one of the challenging problems that the world is facing currently. This situation is worsening each day due to the high cost of searching new antimicrobial agents. Additionally, the slow rate of new drug discovery is greatly contributing to the worsening of this state. The oxidation process in the body that results into free radicals has as well resulted into oxidative stress which is an etiology in many chronic conditions such as cancer. These chronic conditions are not easy to prevent with the conventional drugs hence need for alternatives. However, nature has shown to be the savior of this devastating global challenge by offering alternative agents with antimicrobial and antioxidant properties. These agents include the products from plants, herbs and shrubs with medicinal values, minerals and other products. This study aimed at evaluating the antibacterial and antioxidant activities of methanol rhizome extract of *Zingiber officinale.* Disc diffusion technique and DPPH free radical scavenging assay were used as methods for evaluating antibacterial and antioxidant. Ciprofloxacin and L-ascorbic acid were used as the standard antibiotic and antioxidant respectively. The antibacterial activity was tested aginst pure cultures of *staphylococcus aureus*, *pseudomonas aeruginosa* and *E.coli*. The results showed that the extract of *Zingiber officinale* was more active against psudomoan aeruginosa followed by *staphylococcus aureus*. The extract of *Zingiber officinale was less active against E.coli.* The antioxidant activity results showed that L-ascorbic acid recorded significantly higher percentage radical scavenging activity as compared to the extract of *Zingiber officinale* at all concentration levels ($p<0.05$). In conclusion it's evident that *Zingiber officinale* has antibacterial and antioxidant properties.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Natural products are the main genesis of many drugs and drug discovery. Their use has a long history that dates back many years. According to the WHO statistics, about 65 % of the world population relay on natural products as main source of primary healthcare (WHO, 2005). This percentage of the population is mainly from the developing countries while the remaining 35% comprise of the developed countries who directly use natural products as source of medicine (Santo Grace et al., 2017). Of the many natural products available globally, spices are as well important and have been used since ancient times to date. Their use has however, not been restricted to food flavoring but as food preservatives and colorants, and as well as prescribed in traditional medicine (Bellik, 2014).

The human health has been interfered by various chronic infections that have placed it at a stake. These infections have been ignited by various physiological processes such as metabolism. The metabolism process that utilizes oxygen results into production of free radicals as by- products (Shi et al., 2019). The inflammation process that is entirely the protective mechanism of the body against noxious stimuli, results into productive of free radicals too (Olela et al., 2020). These free radicals that comprises of both oxygen and nitrogen based reactive species, are unstable and very reactive hence attack biomolecules such as proteins, lipids and nucleic acids in the body resulting into oxidative damage. The oxidative stress the condition that is characterized by the imbalance between the free radicals and the antioxidants (G. A. Moriasi et al., 2020). This condition has been reported to be involved in the pathogenesis of chronic diseases such as neurodegenerative diseases, cancer, cardiovascular disorders, and inflammatory disorders and as well contributed to aging (Arika et al., 2019). Bacterial infections have greatly contributed to rise in infection

burden to many people globally. These infections have continued to contribute to the high mortality rate that is being observed among all ages of people (Ardiansah, 2018). Majority of the infections are caused by bacteria such as *Escherichia coli*, *Salmonella* species and *Staphylococcus aureus*.

Conventionally, the management of bacterial and fungal human disorders has been done by use of antibiotics. These antibiotics have been of great help in the past decades as they greatly reduced the case backload of microbial infections. However, with emergence of multidrug resistance among majority of the fungal and bacterial strains, the failure in efficacy of the antibiotics has been witnessed. Additionally, the use of antibiotics has as well been faced with the challenge of manifestation of side effects upon use. Similarly, some of the oxidative stress related disorders such as diabetes is managed by conventional drugs that have exhibited side effects upon use.

The use of natural remedies remains the better option in control of the chronic infections. These agents have been embraced due to the lower toxic effects and high potency associated with them (Arika et al., 2019). The World Health Organization statistics indicate that about 80 % of the population in some developing countries rely on them for the primary health care (World Health Organization (WHO), 2014). The natural products have served as the discovery point of many drugs that are in the market such as morphine. These natural, products can serve as promising alternatives antibacterial and antioxidant agents.

1.2 Problem statement and justification

Currently, all over the world, bacterial resistance to the commercial antibiotics is a serious problem (Chandra et al., 2017). This has been influenced by wide spread and easy availability of the antibiotics that has resulted in the indiscriminate use such as self-medication. Similarly, the absence of the appropriate program for antimicrobial use and sub doses of antimicrobial have as well played a role in the increased prevalence of drug-

resistant micro-organism resulting in the ineffectiveness of the antibiotics(Chandra et al., 2017).. The commercially available antibiotics have as well shown to have toxic effects and side effects that include hypersensitivity. The ever-increasing micro-organism resistance to the available antibiotics, toxicity and side effects of the synthetic antimicrobials and the growing consumer awareness towards use of the environmentally safe and health-friendly products has seen the embrace the use of natural products as alternatives to the synthetic drugs agents.

Natural products from natural sources such as plants, minerals and animals has a great influence on the health of human beings. The use of these natural products as a remedy of many ailments has been positive. The therapeutic effects of the natural products is as a result of the secondary metabolites that plant synthesize. The bioactive compounds elicit different pharmacological properties such as antimicrobial, antioxidant and anti-inflammatory (G. Moriasi et al., 2020). Even though, the use of natural products from plants has been there for quite some time, much attention towards them has been witnessed in the recent decades. The attention has been intensified on their use in food preservation ad control of microbial origin diseases. This has seen very scanty information about the therapeutic properties of the natural products being available. Therefore this study was designed to evaluate the free radical scavenging activity and antibacterial activity of methanol rhizome extract of *Zingiber officinale* (ginger).

1.3 Objectives

1.3.1 General objective

To evaluate the antioxidant and antibacterial activity of methanol rhizome extract of *Zingiber officinale.*

1.3.2 Specific objectives

I. To evaluate the free radical scavenging activity of methanol rhizome extract of *Zingiber officinale*

II. To investigate the antibacterial activity of methanol rhizome extract of *Zingiber officinale*

1.4 Research questions

I. Does the methanol rhizome extract of *Zingiber officinale* have free radical scavenging properties?

II. Does the methanol rhizome extract of *Zingiber officinale* have antibacterial activity?

CHAPTER TWO

LITERATURE REVIEW

2.1 Antimicrobials

The rise in the human infections as a result of pathogenic bacteria has continued to affect man. This has seen many efforts put in place to compact this public problem. As a result many antimicrobials agents which are agents that either kill or inhibit the growth of micro-organisms have been developed. These agents exist in various forms such as antibiotics and can be products of micro-organisms or synthesized derivatives, antimicrobial proteins that are produced by complex organisms as well as some microbes, and medicinal plants.

2.2 Types and sources of antimicrobials

Antimicrobial agents exist in various forms such as antibiotics, antifungals, anti-protozoans, anti-parasitic, antiviral and many more. These types of antimicrobials are usually named after the microbe they act on or control. Antibiotics are the commonly used antimicrobial agents as they control bacterial infections which are the most common. The source of antibiotics can be either natural or synthetic in nature. The antibiotics whose origin is from natural sources include phenyl propanoids (chloramphenicol), polyketides (tetracycline), aminoglycosides (streptomycin, gentamycin), macrolides (vancomycin) and second-generation β-lactams (cephalosporins). The antibiotics obtained from synthetic sources include sulphonamoides, quinolones and oxazolidiones.

2.3 Bacterial resistance

2.3.1 Natural resistance

Many micro-organisms are naturally resistance to variety of antimicrobial agents. This natural resistance can either be intrinsic where it's always expressed in the microbe species or can as well be induced. The induction of the resistance usually happens where the genes responsible for resistance are always present but are only expressed upon exposure to the antibiotic (Giedraitiene et al., 2011). Intrinsic resistance is regarded as the trait that is

universally shared between the species of a particular bacteria and is independent of the history of antibiotic exposure. Additionally, this mode of resistance is not related to horizontal gene transfer. Most common resistance mechanisms that employ intrinsic natural resistance include increased impermeability of the outer membrane and efflux pumps. On the other hand the induced resistance is employed by resistance mechanisms such as muilt-drug efflux pumps (Chandra et al., 2017).

2.3.2 Acquired resistance

Micro-organisms mainly bacteria can acquire resistance to particular antibiotics through all the possible means of bacteria resistance. This is mainly through acquiring the genes responsible for bacterial resistance through routes such as transformation, transportation and conjugation. Similarly, the bacteria may experience mutation to its own chromosomal DNA. The acquisition of this genetic material responsible for antibiotic resistance by bacterial species may be permanent or temporary (Chandra et al., 2017).

The transmission of the resistance genes is achieved via various routes where the outside genetic is inserted into the bacteria. The plasmid-mediated transmission is the most common whereas the bacteriophage-borne transmission is mainly rare. However, other bacteria species including *Acinetobactor species* are naturally skilled and are able to acquire the genes from the environment directly. Internally, the genetic material are moved around by insertion sequences and integrins while stress induction factors such as starvation, UV radiation, chemicals and many more on bacteria are the most initiators of genetic mutations such as deletion and substitutions. Generally bacteria are characterized by mutation rate of one per every 106 to 109 cell divisions and this pose a deleterious effect to the cell. Mutation responsible or eliciting antimicrobial resistance only happens in fewer genes. These include genes that are responsible for encoding drug targets, drug transporters, encoding regulators of drug transporters and the antibiotic modifying enzymes (Sirijan Santajit & Nitaya

Indrawattana, 2016). Mutation does not only cause the changes in the genetic make up for acquiring resistance to the antimicrobial agents but also cause deleterious effects on the bacteria. For instance the mutation that result into *Staphylococcus aureus* acquiring resistance against methicillin as well caused significant decrease in the growth rate of this bacteria.

One major problem of the antimicrobial agents is the resultant in resistance upon use. This resistance may develop even after using lower or higher concentrations of the antimicrobials. The antimicrobials agent may lead to selection of high–level resistance in successive bacterial generations

2.4 Antimicrobial resistance mechanism

Micro-organisms shown resistance to the antimicrobial agents various mechanisms. Many of the resistance mechanisms shown by micro-organisms exist with the major four being; limiting drug uptake, modification of the drug targets, inactivation of drugs and use of efflux pumps (figure 2.1). These mechanisms falls under the two broad resistance means that are intrinsic and acquired resistance. The intrinsic resistance encompasses mechanisms such as, limiting uptakes, drug inactivation and drug efflux whereas the acquired resistance makes use of drug target sit modification, drug inactivation and drug efflux as well.

Based on the structure of the different bacteria, there is varied type of mechanism used by either gram-negative or gram-positive. For instance the gram-negative bacteria use both the four resistance mechanism while gram-positive bacteria rarely use the limiting uptake of drugs and other drug efflux mechanisms.

2.4.1 Drug inactivation

The drug is much effective when is completely metabolized, however, bacteria have developed mechanism that inhibit this. They have been able to inactivate the drug and this is achieved by either degrading the drug or changing the structure of the drug by transferring

the chemical group to the drug. The drugs that bear the β-lactam group in their chemical structure have been degraded by hydrolyzation that is aided by the β-lactamase enzymes. Similarly, other drugs such as tetracycline are inactivated by hydrolyzation via the *tetX* gene. Deactivation as a result of the transfer of a chemical group mainly involves transfer of groups such as acetyl, phosphoryl and adenyl through respective mechanisms. This transfer mechanisms acylation, phosphorylation and adenylation are achieved by the transferase enzymes. Of all the transfer mechanisms, acetylation has been frequently used and it's the main cause of resistance to aminoglycosides, chloramphenicol, streptogramins and fluroquinolones. However, phosphorylation and adenylation are primarily responsible for resistance to aminoglycosides (Blair et al., 2015)..

2.4.2 Drug target modifications

The delivery of the drugs to the target site is vital for the drug to elicit intended action. The modification of these drug target site renders the drugs un-useful. The structure of the bacteria has various components that may act as target sites for drugs. However, many of these components have been modified resulting in acquisition of the resistance to these antimicrobial agents. The penicillin-binding proteins that are the target points for the β-lactam drugs which are mostly used against gram positive bacteria have been altered both in structure and number resulting into resistance to these drugs. The change in number is witnessed either an increase in the PBPs which have reduced drug binding properties or a reduction in number of PBPs reducing the drug pointing point. Similarly, the change in the structure of the penicillin binding proteins results into reduced ability of the drug to bind or even result into total inhibition of drug binding (Sirijan Santajit & Nitaya Indrawattana, 2016).

2.5 Antioxidants

Oxidation reaction in the human and animal body is very vital to life. As this reaction plays a critical supportive role in physiological process, it's as well destructive. Oxidation reaction which is the process of changing a chemical substance, releases free radicals as by products. These free radicals undergo various chain reactions that results into cell damage (Arika et al., 2019). Environmental factors such as increased radiation cause an increase in the reactive oxygen species that damages the cells and tissues resulting into diseases such as heart disease, cancer, neurodegenerative disorders and aging.

Antioxidants counteract the effects of free radicals by maintaining the oxidative balance between the free radicals and the pro-oxidants. This reduces the deleterious effects of the free radicals that cause to oxidative stress related disorders. Antioxidants quench or neutralize the free radicals ensuring the homeostasis balance that results into normal cellular functions (G. Moriasi et al., 2021).

A larger pool of antioxidants are available both natural and synthetic. Both are categorized under either endogenous or exogenous. The endogenous antioxidants comprises of both enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants consist of the enzymes such as catalase, glutathione peroxidase and superoxide dismutase. The non-enzymatic antioxidants include, uric acid, lipoic acid, glutathione, bilirubin and metatonin.

2.6 Zingiber officinale

2.6.1 Botanical description

Zingiber officinale popularly known as ginger is a perennial herb that is slender and grows to about 2 m tall. It's characterized by greenish yellow flowers and fleshy rhizome. The rhizome is horizontal, branched and has a characteristic yellowish or whitish to brown colour. Additionally, the rhizome is thick and lobed as well. Leaves are long with sheathing base, 2-3 cm broadness and blades that are gradually tapering to point. Flowers are usually

rare, rather small, carlyx superior, gamosepolous, three toothed, open by splitting on one side, corolla of three sub equal oblong to lanceolate connate greenish segments. The ginger herb produces a characteristic test and odour that is as a result of the volatile oils (Santo Grace et al., 2017).

2.6.2 Biological activities of ginger

The medicinal history of ginger dates backs to almost 2500 years in countries such as china and India. Ginger has been reported to be therapeutically important as many pharmacological activities have been reported. These activities have been as a result of the compounds found in ginger. Ginger compounds such as 6-shogaol is proven to have anti-inflammatory, antipyretic, anti-tussive, analgesic and hypotensive effects (3,4). Similarly, ginger is widely accepted as the herbal remedy for management of inflammatory diseases.

2.6.3 Antimicrobial activity

The ginger rhizome has been reported in various studies to have both antibacterial and antifungal effects against varied micro-organisms. The antimicrobial activity has been reported against various pathogenic micro-organisms such as *Escherichia coli*, *Salmonella typhinirium*, *Bacillus subtilis* and *Candida albicans*.

2.6.4 Antioxidant Activity

Oxidation of biological molecules induces many pathological diseases such as cancer, neurodegenerative diseases as well accelerate aging. These damages are as a result of the presence of free radicals. The inhibition of these free radicals is done by either inhibiting or terminating the chain reactions involving t6he free radicals. This is usually done by antioxidants that are either produced by the body or those introduced through diet. Ginger extract through previous studies it has shown to be a potent antioxidant. Studies has shown that the extract was able to inhibit DPPH radical recoding an IC_{50} of 4.25 mg/ml.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant sample collection and preparation

Fresh ginger rhizomes were obtained from the mukereti market located in Thika sub-county, Kiambu County. These rhizomes were then taken to the pharmacognosy laboratory in Mount Kenya University. Here they were washed to remove any dust and soil from them followed by slicing into smaller pieces. These pieces were then well spread on the laboratory benches and allowed to shade dry for seven days. The well dried ginger rhizomes were grounded into fine powder using plant grinding machine and the powder stored in the khaki envelopes.

3.2 Methanol extraction

The fine ginger powder was extracted using methanol as the solvent by adopting the method of .exactly 200 g of the powder was weighed using analytical balance and emptied in a clean 1000 ml conical flask. Pure analytical grade methanol, 400 ml, was added in the powder and allowed to cold macerate for 48 h under occasional shaking. On the third day the sample was filtered and the residue soaked for the second and third time. The total filtrated was then concentrated under vacuum using a rotary evaporator and water bath set at 40 ^{0}C. The concentrated sample was put in a clean glass bottle that was already weighed and left in the hot air oven (40 ^{0}C) to completely dry.

3.3 Preparation of the plant sample dilutions and reagents

The plant sample for antioxidant activity was prepared by exactly weighing our 10 mg of the crude extract on a foil paper using analytical balance. This extract was then dissolved in 10 ml of analytical grade resulting into a stock solution of 1 mg/ml. From the stock solution serial dilution with a factor of 5 was done to obtain other five concentrations of 200, 40, 8, 1.6 and 0.32 ug/ml.

For the antibacterial activity, exactly 100 mg/ml was weighed and dissolved in 1 ml of 5 % of DMSO. This stock solution was serially diluted with a factor of two to obtain the different concentrations of 50, 25, 12.5, 6.25 and 3.125 mg/ml.

3.4 Antioxidant activity of methanol rhizome extract of Ginger

The free radical scavenging activity of methanol extract of ginger was evaluated using DPPH assay as a measure of antioxidant activity. The method described by with minor modifications was used. This was conducted as follows, 1.4 ml of the various sample/standard concentration was added in into clean test tubes with respective labels. Into this 2.6 ml of 0.1mM, methanolic DPPH solution was added. Into the control only 1.4 ml of methanol and 2.6 ml of DPPH solution was added. The content of all test tubes were mixed by swirling and incubated in the dark for exactly 15 minutes. The absorbance of the sample/standard were then measured at 517 nm using UV-vis spectrophotometer (labetech double beam). The percentage radical scavenging activity of both sample and standard were calculated using the formulae

$$Percentage\ Radical\ Scavenging\ Activity = \frac{Abs\ C - Abs\ T}{Abs\ C} \times 100$$

3.5 Antibacterial activity of methanol extract of ginger.

3.5.1 Media preparation

Nutrient agar (Hi-media) was used in this stud and was prepared as directed by the manufacturer. The manufacturer instructs that 28 g of the powder be suspected in 1000 ml of distilled water. In this study, 6.72 of nutrient agar powder was weighed and suspended in 240 ml distilled water. The media was then heated to boiling to completely dissolve and then sterilized by autoclaving at 121 ^{0} C for 15 minutes. The sterile media was then cooled to around 50 ^{0} C and aseptically poured into sterile petri-dishes.

3.5.1 Test Microorganisms and preparation of inoculants

Three bacteria strains *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E.coli* were used. These bacteria strains were obtained from the micro-biology laboratory of Mount Kenya University. The sub-culturing of the bacteria was done on the nutrient agar a day before the study to check the viability and as well obtain fresh colonies.

The inoculants of the respective bacteria strains was prepared in sterile normal saline. Using hot flame sterilized loop wire, well isolated colonies of the respective bacteria strain were scoped and suspended in normal saline. The turbidity of the respective bacteria suspension was compared to that of 0.5 McFarland standard.

3.5.2 Antibacterial activity of methanol extract of ginger.

The antibacterial activity of methanol rhizome extract of ginger was conducted against the three bacterial strains. The disc diffusion test was used in this study to check the sensitivity of the methanol extract of ginger against the bacteria strains as highlighted by Mama et al. (2019) with minor modifications. The inoculants of each bacteria strain was inoculated on the sterile media uniformly using sterile swab to cover the entire surface. On to this inoculated media sterile filter paper disc were laid using hot flame sterilized forceps. Only five paper discs were put on one plate to ensure no overlap of the zones. The methanol extract of ginger at different concentration levels was top-loaded onto the discs. Using micropipette, exactly 15 µl of the extract at different concentration level was directly loaded on the filter paper and then allowed half an hour to dry prior to incubating. On the discs at the center of each Petridish 10ul of 0.0005g/ml of ciprofloxacin was loaded as the standard antibiotic. While on the negative control disc 0.1% DMSO was loaded. All the plates were then incubated at 37 ^{o}C for 18 -24 hours. Antimicrobial activity was then evaluated by measuring the zones of inhibition around the discs in millimeters.

3.6 Data management and statistical analysis

The antibacterial activity data that consisted of the duplicate zones of inhibition and the antioxidant activity data that included the percentage radical scavenging activity were first noted in the laboratory note book. These values were then tabulated in the excel spread sheet. This was then transferred into the GraphPad prism statistical software. Descriptive statistics was performed and zones of inhibition together with the percentage radical scavenging activity presented as Mean ± SEM. To determine the level of significance between the mean zones of inhibition between the different concentrations and standard one-way anova was used. While the level of significance between methanol extract of ginger and L-ascorbic acid was done by the student t-test. The results were then presented in form of tables and graph.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Antibacterial activity of methanol rhizome extract of ginger

The results for the antibacterial activity of methanol ginger extract against *Staphylococcus aureus, Pseudomonas aeruginosa* and *E.coli* are summarized in table 4.1 and figure 4.1. Methanol extract of ginger rhizome recorded zones of 17.50 mm, 15 mm and 12.5 mm against *Staphylococcus aureus, Pseudomonas aeruginosa* and *E.coli* at 100 mg/ml concentration. At concentration level of 50 mg/ml zones of inhibition of 14.50.5, 12.5 0.5 and 11.000.00 were recorded for *Staphylococuous aureus, Pseudomonas aeruginosa* and *E.coli* respectively. At concentration level of 25 mg/ml the zones 13.000 0.00, 11.50 0.500 and 10.00 0.00 for *Staphylococuous aureus Pseudomonas aeruginosa* and *E.coli*. Standard antibiotic ciprofloxacin at concentration level of 0.1mg/ml was used as the standard and recorded zones of inhibition 30.000.00, 35.5000.50 and 39.50 0.500 for *Staphylococuous aureus, pseudomonas aeruginosa* and *E.coli* respectively. The comparison of the different mean zones of inhibition recorded by the three bacteria at each concentration level reveled that as the concentration reduced the zones of inhibition reduced as well (Fig 4.1). From the graph as indicated, *E.coli* recorded the least activity that was shown by the significantly smaller zones of inhibition recorded at all concentration levels against all the studied micro-organisms ($P<0.05$; fig 4.1). However, ciprofloxacin (0.1 mg/ml) recorded significantly larger zone of inhibition against E. coli followed by pseudomonas aeruginosa and the least zone of inhibition was recorded against staphylococcus aureus ($p<0.05$; fig 4.1).

The methanol ginger rhizome extract recorded significantly larger zone of inhibition against *pseudomonas aeruginosa* as compared to both *Staphylococuous aureus* and *E.coli*. At concentration levels of 100mg/ml, 25 mg/ml and 12.5 mg/ml ($p<0.05$; fig 4.1). however at concentration levels of 50 mg/ml,6.25 mg/ml and 3.125 mg/ml there was no significant

difference in the zones of inhibition recorded between *Staphylococuous aureus and Pseudomonas aeruginosa* ($p>0.05$; table 1 and figure 1).

Table 1: Antibacterial activity of methanol rhizome extract of ginger

Concentration mg/ml	**ZONE OF INHIBITION (MEAN ± SEM)**		
	Staphylococcus aureus	Pseudomonas aeruginosa	E. coli
100	23.00 ± 0.577	26.00± 0.577	7.333±0.333
50	20.00 ± 0.000	20.333± 0.333	6.000±0.00
25	18.333 ± 0.333	19.667± 0.333	6.000±0.00
12.5	17.333 ± 6.884	17.667± 0.667	6.000 ± 0.00
6.25	8.820 ± 3.087	15.667± 0.333	6.000±0.00
3.125	2.735 ± 1.325	13.667± 0.333	6.00±0.00
Ciprofloxacin (0.1 mg/ml)	30.333 0.333	36.000± 0.000	40.00±0.00

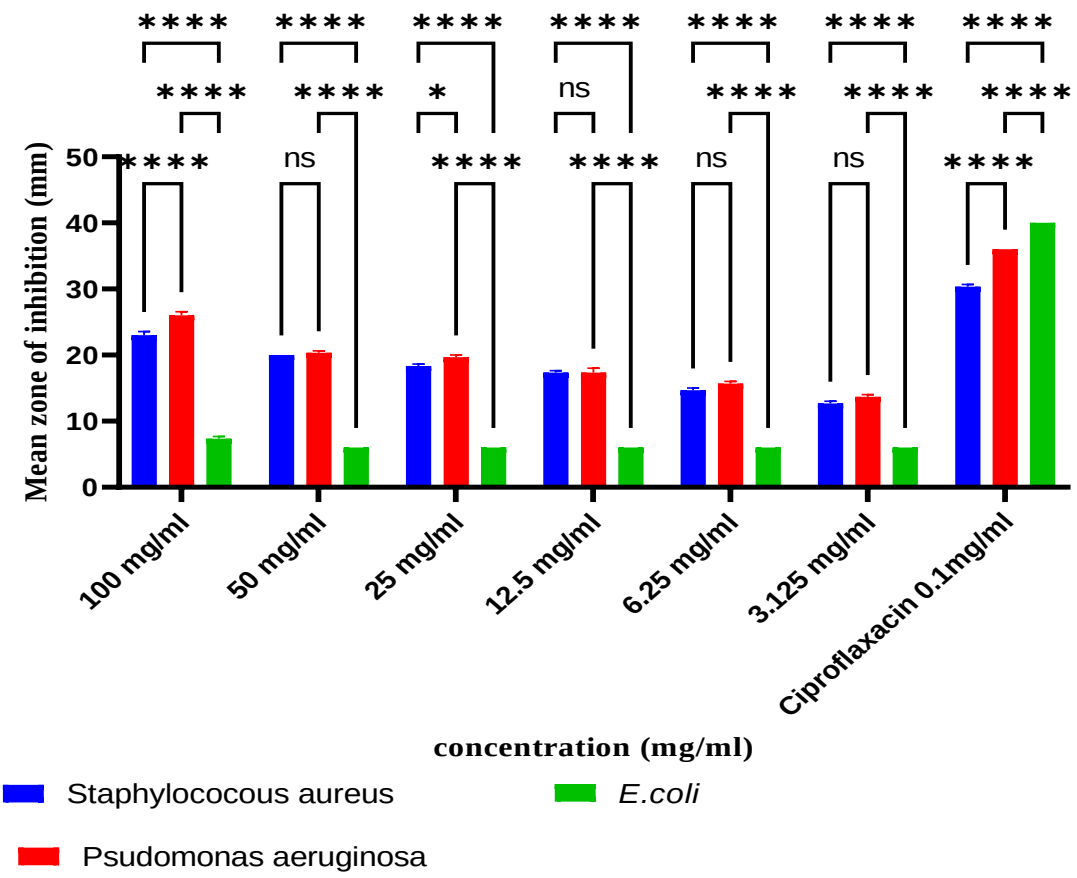

Figure 1: Antibacterial activity off methanol rhizome extract of ginger

4.2 DPPH free radical scavenging activity of methanol rhizome extract of ginger.

The ability of the ginger extract to elicit its antioxidant activity was investigated by evaluating its radical scavenging ability. The results as presented in table 2 and figure 2 and 3, showed an activity that entirely depended on the dose (fig 4.2). The free radical scavenging activity of ginger and *L*-ascorbic acid as the standard was investigated at concentration levels 1000, 200, 40, 8, 1.6 and 0.32. Ginger extract at concentration levels of 0.32 and 1000 recorded significantly lower and higher percentage radical scavenging activity respectively ($p<0.05$;fig 2). The percentage radical scavenging activity recorded between contractions 1000 and 200 and between 1.6 and 0.32 showed no significant different from each other ($p>0.05$; fig 2). the percentage radical scavenging activity recorded

at the rest of the concentration levels was significantly different from each other ($p<0.05$;fig 4.2). the percentage radical scavenging activity recorded by L-ascorbic acid between concentration levels 1000 and 200, 1000 and 40, 200 and 40 and 1.6 and 0.32 respectively was not significantly different from each other ($p>0.05$;fig 4.2).

The comparison of the percentage radical scavenging activity of methanol rhizome extract of ginger to the standard antioxidant agent; L-ascorbic acid, revel that at concentration level no significant was noted ($p>0.05$; fig 4.3).however, from concentration level 200 to 0.32, L-ascorbic acid significantly recorded higher percentage radical scavenging activity as compared to methanol rhizome extract of ginger ($p<0.05$; figure 2).

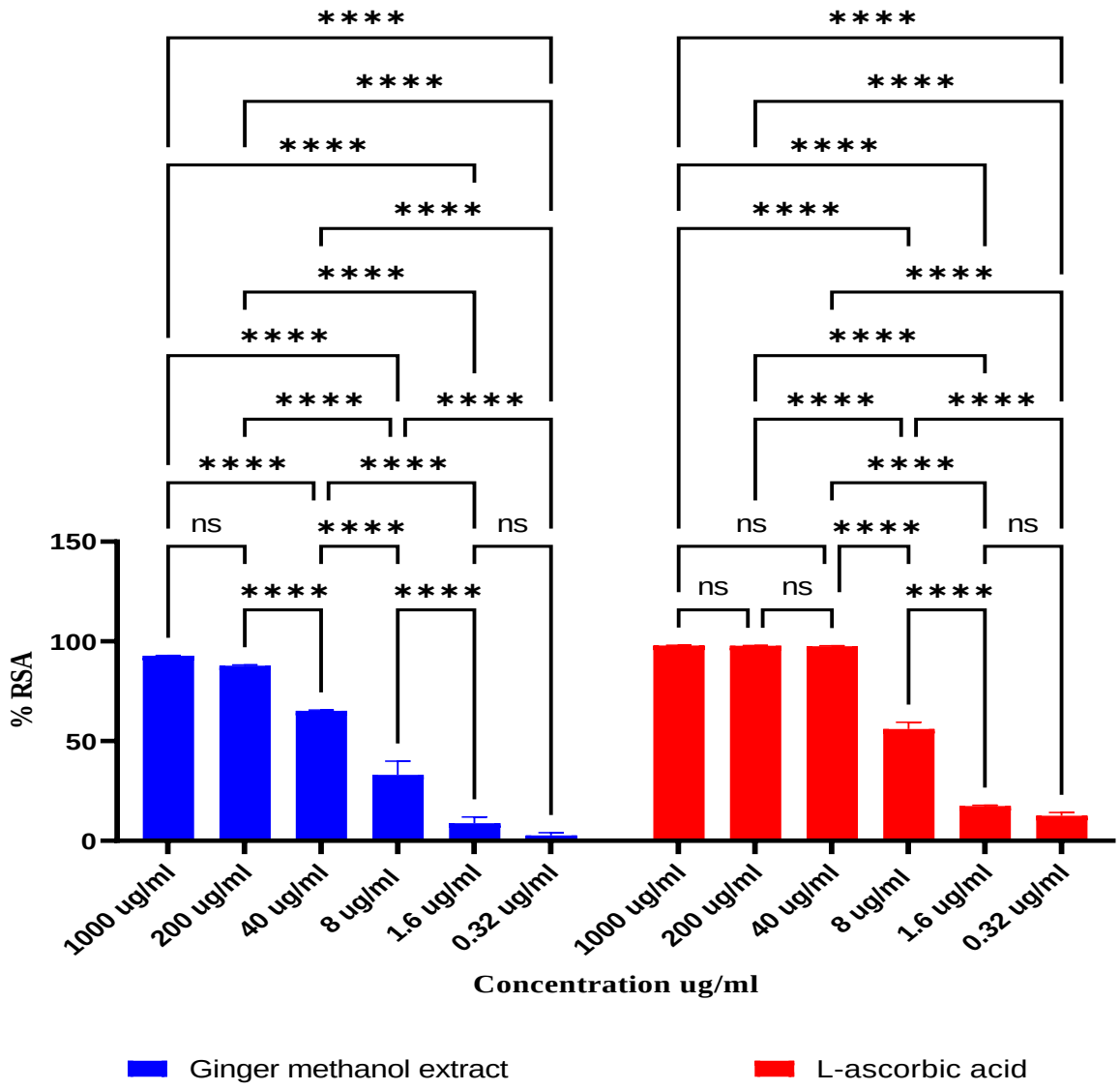

Figure 2: DPPH free radical scavenging activity methanol of ginger

Table 2: DPPH free radical scavenging activity methanol of ginger

Concentration	**% RSA (MEAN±SEM)**	
Mcg/ml	Methanol rhizome extract of Zingiber officinale	L-Ascorbic acid
1000	92.867 ± 0.098	97.997 ± 0.048
200	87.787 ± 0.368	97. 839 ± 0.134
40	65.247 ± 0.373	97.523 ± 0.260
8	33.060 ± 6.884	56.040 ± 3.383
1.6	8.820 ± 3.087	17.450 ± 0.315
0.32	2.735 ± 1.325	12.554 ± 1.609
IC_{50}	37.72	54.53

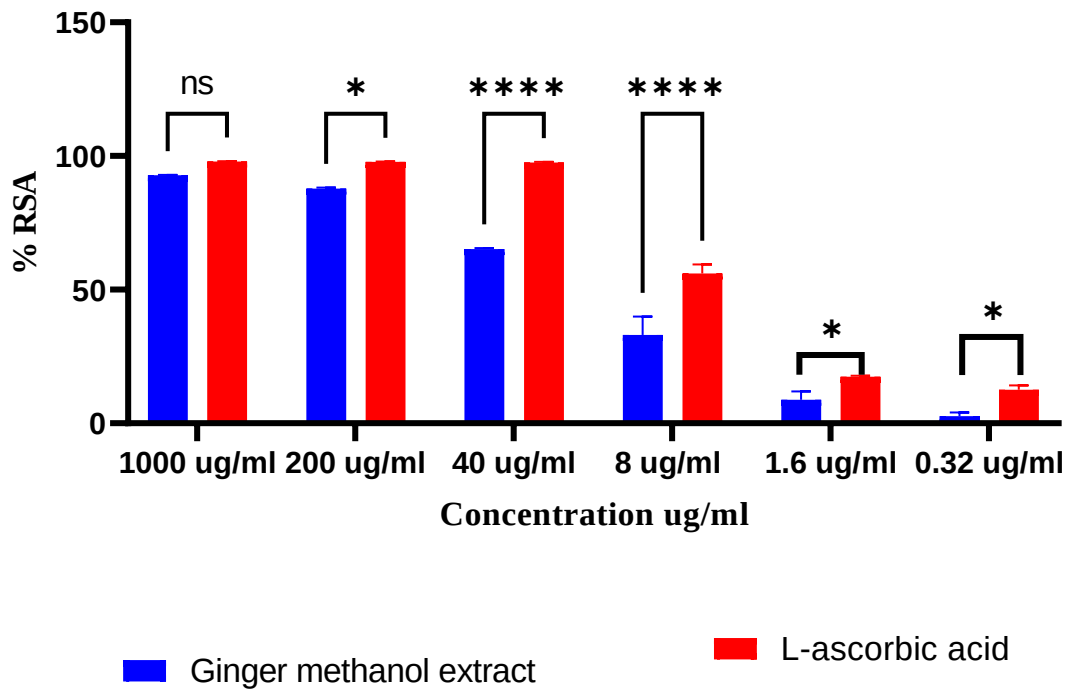

Figure 3: DPPH free radical scavenging activity of methanol rhizome extract of ginger

4.2 Discussion

Herbal medicines have played great role in complementing the conventional medicine which has been faced with many challenges. The reduced efficacy of modern medicine as well as side effects that accompanies the medicine upon use has greatly influenced the reduced demand of these medicine by majority of many people globally. Additionally, the high cost of maintaining the use of conventional medicine has as well contributed to their low demand. Herbal medicine are characterized by low cost of obtaining and at the same time are safer. The wide scale presence of many bioactive compounds in the plants have been reported to be the main contributors to the medicinal properties observed in these plants. Medicinal plants may contain majority of the phytochemicals making it to have varied therapeutic activities such antioxidant, antimicrobial, anti-inflammatory and analgesic.

In the present study, the antibacterial activity and antioxidant activity of the ginger rhizome extracted with methanol was evaluated. The antibacterial activity was evaluated by the diffusion technique in which the discs diffusion was adopted. The extract was evaluated at six different concentrations; 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml while ciprofloxacin was used as the standard antibiotic at concentration of 0.1 mg/ml. The results were then evaluated by the antibacterial evaluation scale of De Almeida Alves et al., (2000), where the diameter of zone of inhibition was used to give the strength of the extract as an antibacterial agent. Zones of less than 9 mm were interpreted as inactive; 9-12 mm, partially active; 13-18 mm, active; and more than 18 mm, very active. In this study methanol rhizome extract of ginger significantly inhibited the growth of *pseudomonas aeruginosa* followed by *Staphylococcus aureus*. The extract was however not more sensitive against *E.coli.*

The antioxidant activity was evaluated by determining the free radical scavenging ability. DPPH a free radical that upon gaining an electron changes from purple to black with wavelength of maximum absorbance at 517 nm was used. From the results it was noted that both L-ascorbic acid and Methanol extract significantly scavenged the free radicals at higher concentrations. The ability of the extract to scavenge the free radical was decreasing with a decrease in the concentration. The L-ascorbic acid however, significantly scavenged the free radicals more at all concentration levels as compared to the methanol rhizome extract of ginger.

Chapter five: conclusion and recommendation

5.1 Conclusion

Based on the results its evident that ginger rhizome has antibacterial and antioxidant activity. the antioxidant activity may be due to the free radical scavenging mechanisms. The reported activities may be due to the presence of the phytochemicals such as phenolic compounds.

5.2 Recommendation

The following recommendation were made based on the findings of the study

I. Antibacterial activity be conducted against the resistant bacteria strains

II. The minimum inhibitory concentration and minimum bactericidal concentration be determined.

III. Antioxidant activity be evaluated by other antioxidant evaluation assays.

IV. *In vivo* antioxidant activity of ginger rhizome be evaluated.

REFERENCE

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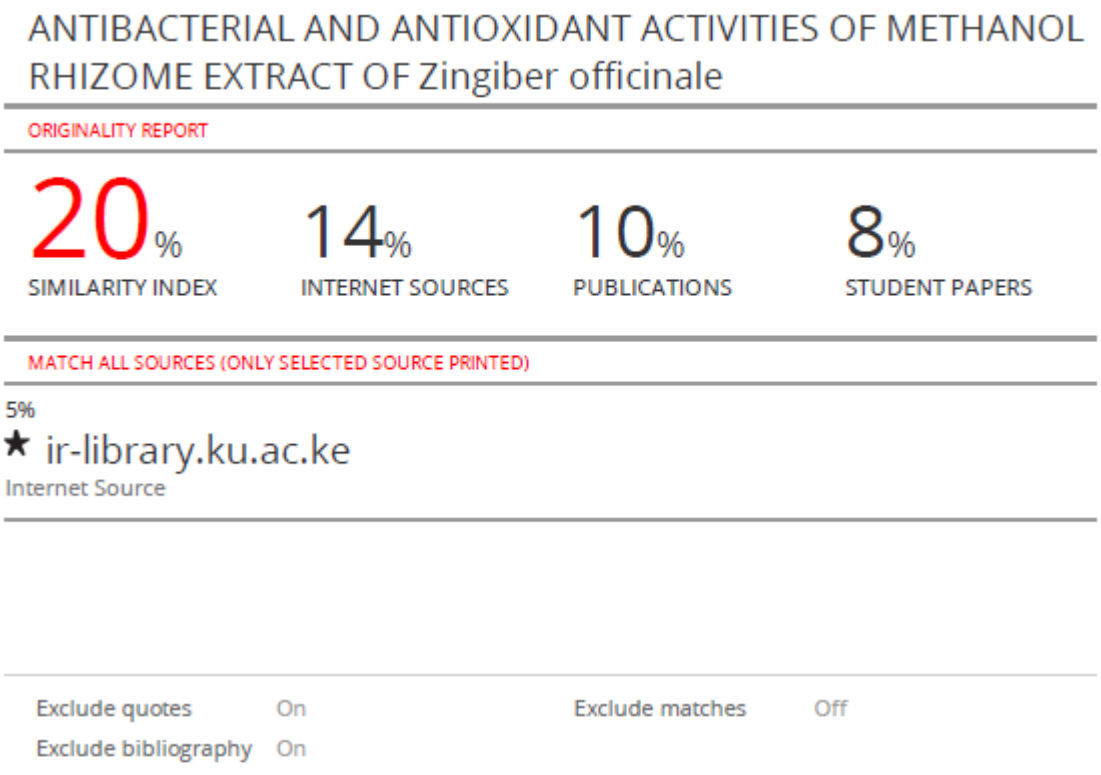
APPENDIXES

Appendix I: Similarity Index



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