A CASE STUDY OF ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF METHANOL RHIZOME EXTRACT OF *Beta vulgaris* (BEET ROOTS)

By

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DEPARTMENT OF PHARMACEUTICS

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DECLARATION

This research study is my original work and has not been presented to any other Institution. No part of this research should be reproduced without the authors' consent or that of Mount Kenya University.

Sign _____ Date _____

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Declaration by the supervisor(s)

This research has been submitted with my approval as The Mount Kenya University Supervisor(s).

Sign _____ Date _____

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DEDICATION

To the Almighty God for His strength and guidance throughout my studies.

My mother, my siblings and friends for their support, encouragement and prayers.

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I would like to acknowledge my supervisor, Prof. Bindu Madhavi, for her guidance, understanding and supervision. Mr. Nelson Elias for guiding me through the laboratory work of the project. My mother and siblings for their financial support and prayers.

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ABSTRACT

The huge cost of medication and treatment of many chronic conditions that are caused by bacteria and the oxidative stress as a result of imbalance between the antioxidant and excess free radicals continue to be a major hindrance to better health for many people. Addition to the cost, the increased side effects and toxicity of modern medicine and reduced efficacy that has seen many bacteria strains gain resistance are some of demerits of the conventional drugs. The increased use of medicinal plants has been witnessed recently. The aim of this has been to be able to get alternatives that are potent and safer at the same time. Recent studies have weighed in and shown that vegetables and fruits play a great role in disease prevention. The health benefits of these products has been nailed at the many biologically active compounds synthesized by these plants. Beta vulgaris is a common vegetable in many countries and many pharmacological activities have been reported. Based on its beneficial claims from various sources, the baseline of this study aims to investigate the antioxidant and antimicrobial activity of methanol rhizome extracts of *Beta vulgaris*. Standard methods for antioxidant and antimicrobial assays were used and the extract was evaluated at different concentration. Antioxidant activity was evaluated by DPPH radical scavenging method while the antimicrobial activity was evaluated by the disc diffusion method. L-ascorbic acid and Ciprofloxacin was used as the standard antioxidant and antibiotic respectively. Three bacteria strains; *Staphylococcus aureus, E. coli* and *Pseudomonas aeruginosa* were used. The methanol rhizome extract showed antimicrobial activity against both the gram negative and gram positive bacteria. The extract inhibited more the positive bacteria; *Staphylococcus aureus* with zone of 14.5±0.5 mm followed by the gram negative bacteria *Pseudomonas aeruginosa* and *E. coli* with zones of 12.0±1.0 and 11.0±0.00 mm respectively. Ciprofloxacin recorded the highest inhibition against *E. coli* with zone of 38.5±0.5 mm, followed by *Pseudomonas aeruginosa* with zone of 31.5±0.5 mm and *Staphylococcus aureus* had the least inhibition with zone of 30.0±0.0 mm. The antioxidant activity of the methanol rhizome extract recorded percentage radical scavenging activity of 88.983±0.141, 72.310±3.299, 20.007±4.366, 6.040± .317, 4.713±1.431 and 3.269±0.385 %. The antioxidant activity was high at 100 ug/ml and 200 ug/ml concentration level. The antioxidant activity of the Beta vulgaris extract was however, lower than that of the standard at all concentration levels. In conclusion the present study revealed that Beta vulgaris is a potent antioxidant and antimicrobial agent.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

In the recent years an urge to have a healthy people in the world has been on the rise. This has been put forward by advocating for proper health diet among many people globally. This healthy diet has mainly concentrated on having the so called functional foods. Similarly, the consumers have as well turned their efforts in acquiring foods that are safer for their general health as well as the living standards. The well documented health benefits of having a diet consisting of fruits and vegetables has greatly influenced the rising need for such type of diet (Clifford et al., 2015). These health benefits of both fruits and vegetable are more than enough. These include ensuring the cardiovascular health, inhibiting the reactions of free radicals, protection against many forms of cancer, constipation, diabetes, obesity (Mikołajczyk-Bator & Pawlak, 2016) and micro-organism infections. This health benefits on consuming fruits and vegetables have been confirmed through various previous studies. In these studies it has been illustrated that consumption of fruits and vegetables has greatly helped protect the body against numerous chronic diseases that are linked to aging such as cancer, cardiovascular and hepatic diseases, brain and immune dysfunction.

The health benefit of fruits and vegetables in entirely protecting the body and ensuring food health of the entire body is attributed to specific components contained in the fruits and vegetables. These include fibers, minerals, vitamins, carotenoids, betalains, anthocyanins, polyphenols and other vital phytochemicals (Koubaier et al., 2014). Many vegetables have been sought for in the recent days. These include, the beta vulgaris L. popularly known as red beet root whose biological activity has been under investigation by many researchers. Its unending potential utility as a healthy promoting and diseases preventing plant hence its recognition as a functional food (Guldiken et al., 2016).

The red beetroot scientifically known as Beta vulgaris L. is a traditional and common vegetable found all over the world. This plant is popular in Eastern and Central Europe where it’s the main ingredient in various preparations such as salads

(Jasmitha et al., 2018). Nowadays beet root is consumed on a regular basis as part of the normal diet. It's taken both when fresh or upon processing or fermentation. In the food industries it's used as a food coloring agent identified as E162 (Sawicki & Wiczkowski, 2018). Beet root contain vital phytochemicals such as betalains, carotenoids, phenols, B-vitamins (B1, B2, B3, B6 and B12), folate minerals and fibers (Sawate AR, 2018) and inorganic nitrates (Clifford et al., 2015). The entire beet root plant has medicinal properties that include antioxidant, anti-depressant, antimicrobial and anti-inflammatory (Liliana & Oana-Viorela, 2020). Even though the consumption of beet root in many countries by most people is on the high note, most of the consumers are not aware of its health benefits and the importance of consuming it (Liliana & Oana-Viorela, 2020). Therefore the current study was designed to evaluate the antimicrobial activity and antioxidant activity of the red beet root rhizomes found locally in the market.

1.2 Problem statement and Justification

Poor nutrition is the major contributing factor to genesis of many chronic conditions that are threat to the human health (Medical Research Council, 2017). Practices such as smoking have immensely contributed to the generation of free radicals whose reactions contribute to ageing and oxidative stress related disorders. Similarly smoking has been regarded as an influence in the genesis of cancer and about 30-35% cancer cases are linked to poor diet (Medical Research Council, 2017). Nutrition, physical activity levels and health go hand in hand hence change in one has an impact on the other. The increase in poor nutrition among many people globally has seen increase in cases of physical inactivity which is the fourth leading cause of mortality worldwide. Additionally, physical inactivity has as well been linked to pathogenesis of chronic conditions such as cancer, heart diseases and diabetes. The management of these nutrition related conditions has seen use of synthetic products being introduced in the daily diets. Even though they have played a great role in preventing some conditions, side effects have been observed. Some are as well very expensive to be obtained by the less fortunate in the communities and some are toxic. This has seen the change of heart into the use of natural fruits and vegetables as the alternatives.

Fruits and vegetables have been used since ancient times as they formed part of the diet for human beings and animals as well. Their high concentration of vitamins, more so vitamin C and E, minerals and phytochemicals mostly those related to antioxidant properties has seen their usage increase drastically (Slavin & Lloyd Beate, 2012). In addition vegetables and fruits are good source of fibers hence it's advised that they do not miss in the diet (Slavin & Lloyd Beate, 2012). The inclusion of vegetables and fruits in the daily diet has been reported to result in reduction of many chronic diseases such as cancer (Wang et al., 2014), cardiovascular diseases, high blood pressure and diabetes as well (Muraki et al., 2013). However, very few vegetables and fruits have been evaluated for their therapeutic properties and this leaves a huge consignment of un-evaluated fruits and vegetables that are still used. This therefore, formed the pin point of this study which aimed at evaluating the antimicrobial and antioxidant activity of *Beta vulgaries* commonly known as Beetroot that is a common vegetable worldwide.

1.3 Objectives

1.3.1 General objective

To evaluate the antimicrobial and antioxidant activity of methanol extract of *Beta vulgaris L*.

1.3.2 Specific objectives

I. To evaluate the antimicrobial activity of methanol extract of *Beta vulgaris L.*

II. To investigate the free radical scavenging activity of methanol extract of *Beta vulgaris L*.

1.4 Research questions

I. Does the methanol extract of Beta vulgaris have antimicrobial activity?

II. What is the free radical scavenging capabilities of methanol extract of *Beta vulgaris L*.?

CHAPTER TWO: LITERATURE REVIEW

2.1 Oxidative stress

Oxidative stress is a condition whose genesis is as result of lose in the balance between the reactive oxygen species and the antioxidants. This condition has been reported to play a very big role in the genesis of various diseases. The reactive free radicals that comprises of the reactive oxygen species and the reactive nitrogen species attacks the biomolecules (lipids, proteins and DNA) in the body resulting into their damage. These free radicals include hydrogen peroxide, organic hydrogen peroxide, nitric oxide, superoxide and hydroxyl radicals (T. Rahman et al., 2012). They are usually produced during the normal physiological process in the body that are crucial to human being survival (Nathan & Nathan, 2003).

The regulation of the oxidative stress is usually via maintaining the hemeostasis between the free radicals and the antioxidants. Antioxidant is any substance present at low concentrations in the body as compared to oxidizable substrate and it remarkably prevents or delay the oxidation of that particular substrate (K. Rahman, 2007). An ideal antioxidant is characterized by being readily absorbed by the body, being able to quench or prevent free radical formation or chelate redox metals at physiologically acceptable levels. It should as well be vase tile in its function that is it should be able to work in aqueous and /or membrane environs and effect gene expression in a positive manner (K. Rahman, 2007). The endogenous antioxidant defense system helps in reverting the oxidative stress condition by maintaining the cellular redox homeostasis. This defense system comprises of enzymes such Superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT). These enzymes regulate the levels of the ROS to maintain the homeostasis (T. Rahman et al., 2012). The defense system is usually complex and helps in minimizing the levels of reactive oxygen species while at the same time allowing the ROS to elicit their beneficial roles of cell signaling and redox regulation (Halliwell, 2011). The increased or prolonged action of the free radicals at same instance overtakes the action of the endogenous antioxidant defense system. At these instances the exogenous antioxidants are supplement to offer support to the

overwhelmed defense system. The exogeneous antioxidants includes of vitamins A, C and E, glutathione, polyphenols, resveratrol and Nacetylcysteine.

2.2 Regulation of ROS

To prevent the deleterious effects of the reactive oxygen species on both cells, tissues and organs of the body, humans' body have evolved a mechanism that involves highly sophisticated and complex antioxidant protection system. This mechanism involves various components, both endogenous and exogenous in origin, that function synergistically to avert the action of the free radicals by neutralizing them. The endogenous antioxidant components comprises of the enzymes such as superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT) that regulates overly the levels of ROS to maintain homeostasis (T. Rahman et al., 2012).

SODs (metal containing proteins) catalyzes the removal of superoxide by generating hydrogen peroxide as the final product.

$$2\ O_2^{*-} + 2\ H^+ \longrightarrow H_2O_2 + O_2$$

Intracellular GSH is converted to GSSG by selenium containing GSH peroxidase in the process catalyzing the reduction of H_2O_2, is coupled with oxidation of glucose-6-phosphate and 6-phosphogluconate which provides NADPH for the reduction of GSSG by GSSG reductase as indicated below.

$$H2O2 + GSH \longrightarrow H_2O + GSSH$$

$$GSSH + NAD\ (P)H \longrightarrow GSH + NAD\ (p)^+$$

Thus, this reaction prevents peroxidation of membrane lipids by free radicals, thus inhibiting the formation of malondialdehyde (Raghuvanshi *et al.,* 2007).

The non-enzymatic endogenous antioxidant components include the glutathione, histidine-peptides, the iron binding proteins transferrin and ferritin, dihydrolipoic acid, melatonin, urate and plasma protein thiols (Sharma and Aggarwal, 2004). Although several small molecular weight substances such; ascorbate, alpha-tocopherol, cysteine, thioredoxin and vitamins also acts as antioxidants. Failure of

these systems causes oxidative stress which in turn cause or exacerbate disease state (Duganath *et al.,* 2010; Bellance *et al*., 2009).

2.3 Antibacterial agents

2.3.1 Mechanism of action of antibacterial agents

2.3.1.1 Bacterial protein biosynthesis

Protein synthesis involves many molecular steps. These steps include initiation, elongation and termination of the protein assembly by the ribosomes of the bacteria. When the synthesis of the protein in the bacteria is altered by either interfering with the ribosomal subunits the functions of the bacteria are also affected and the bacteria won't survive any more. In the process the infection of the bacteria are reduced completely. Some of the antibiotics with such mode of actions include the macrolides, tetracycline, aminoglycosides and axazolidinones (Walsh, 2003).

2.3.1.2 Inhibiting nucleic acid synthesis

DNA is the vital component of any living organism as it's the origin of the genetic material of that organism. In bacteria all the procedures of the supercoiling and uncoiling of the DNA is conducted by the DNA gyrase enzyme. Therefore, this enzyme is very important as the matter of DNA synthesis, replication, repair and transcription is considered. The interference of the functions of this enzyme results into the alteration of all the procedures as the matter of bacterial DNA is regarded. The antibacterial agents and antibiotics that act through this mechanism of targeting the DNA gyrase enzyme include fluoroquinolones such as ciprofloxacin and nalidixic acid (Maxwell, 1997).

2.3.1.3 Destruction of bacterial membrane

The bacterial membrane is very important structure that protects the internal components of the bacteria. The membrane is made up of the lipopolysaccharides. Some classes of the antibacterial agents and antibiotics showcase their bacterial activity by destroying these membranes. For example antibiotics such as polymyxins may attach themselves on to one part of the component that make up the lipopolysaccharide identified as the lipid A. this alters the entire structure of

the bacterial cell membrane by means of phospholipid exchange that may result into an osmotic imbalance resulting into bacterial death later (Tenover, 2006). This mechanism of antibacterial agent activity has been witnessed for quite some time even with other chemicals constituents such as the anesthetics or disinfectants (McBain et al., 2003) The external membrane when is disrupted may rapture resulting into destruction of the cytoplasmic membrane which are the energy metabolism cells. This later result into loss of permeability, the intracellular constituents leaking to the outside environment and in some cases the cytoplasm may coagulate.

2.3.2 Antibacterial activity screening methods

2.3.2.1 Diffusion methods

2.3.2.1.1 Agar disc diffusion test

This is the official and most frequently used method in the clinical microbiology laboratories for the antimicrobial susceptibility tests. It was first developed in the year 1940 (Heatley & Wiuiam, 1943). In this test, the agar plates are usually inoculated with 0.5 McFarland standardized inoculum of the test micro-organism under aseptic conditions. Disc of diameter either 6 mm or 5 mm made from a filter paper and then impregnated with the compound of desired and known concentration are aseptically transferred to the gar surface. The petri dishes are then incubated upside down under suitable conditions with the incubation temperature and time depending on the particular test micro-organism. This test follows the principle that the test antimicrobial agent diffuses into the agar and either inhibits the growth of the microorganism. The diameter of the inhibition zones around the discs is measured (Hudzicki, 2016).

2.3.2.1.2 Agar well diffusion method

This method has widely been used in the assessment of the antimicrobial activities of plants or microbial extracts (Magaldi et al., 2004; Valgas et al., 2007). Its procedures are similar to those of the agar disc diffusion only that in place of the disc a well is made in the agar. The standardized inoculum of the test microbe is

plated completely to cover the entire surface of the agar. A well of diameter 6 mm is then made into the already microbe plate agar using sterile straw or cork borer. The antimicrobial compound or the extract solution (20-100ul) at the desired and known concentration is then loaded directly in the wells and then allowed to dry before incubation under the suitable conditions. The diameter of the inhibition zones around the well is measured.

2.4 Beta vulgaris

Beta vulgaris is a biennial or annual plant that taxonomically belongs to the family *chenopodiaceae* (Babarykin et al., 2019; Jasmitha et al., 2018) The members of this family including Beta vulgaris are all dicotyledonous. This plant is characterized by bright crimson color and thick flashy roots. It's usually erected and characterized by tuberous root stocks. Beta vulgaris is identified by common names such as beet, chard, spinach beet, beetroot or table beet (Babarykin et al., 2019). This plant is ranked in the group of the best ten vegetables that are potent as antioxidant agents. It has as well been used in the dietary supplement that is not only limited to the richness in minerals, nutrients and vitamins but as well as other vital phytochemicals that possess important pharmacological properties (Jasmitha et al., 2018)..

2.4.1 Bioactive compounds and nutrients of Beta vulgaris

Many phytochemicals that are of biological or pharmacological importance are found in the various parts of the beta vulgaris that include the most valuable part the tuberous root. These include betalains, flavonoids, polyphenols and saponins (Koubaier et al., 2014). The betalains are the most abundant phytoconstituents of beta vulgaris and include betacyanins and betaxanthins. Similarly inorganic molecules such as nitrate is also found in this plant. Additionally, beta vulgaris is also a rich source of a wide range of minerals that include potassium, sodium, phosphorous, calcium, magnesium, copper, iron, zinc and manganese that have health importance on the body.

Beta vulgaris is prepared in different forms such as juice, powder, bread, gel, boiled, oven-dried, pickled, pureed or jam-processed as per different cultures.

2.4.2 Pharmacological activity of Beta vulgaris

The different parts of beta vulgaris contain different compounds that are of medical importance. These compounds are responsible for different therapeutic properties that are shown by the beet root. These include antioxidant, antimicrobial, anti-inflammatory, and ant-diabetic and hepato-protective activities (Babarykin et al., 2019).

CHAPTER THREE: MATERIAL AND METHODS

3.1 study design

This study was a laboratory based controlled experiment and it was conducted in both microbiology and biochemistry laboratories in Mount Kenya university.

3.2 plant collection and preparation

Fresh *Beta vulgaris* (beet root) rhizomes were bought from Mukereti market located in Thika town. These rhizomes were then brought to the Pharmacognosy laboratory from where they cleaned by first removing the excess mud and soil using a sharp spatula and then washed with clean running water. The cleaned rhizomes were then chopped in smaller pieces using a clean and sharp knife and then spread on the laboratory benches to dry under the shade. For a period of two weeks, the small pieces of the rhizome were turned each day to preventing rotting and fermentation that might reduce the medicinal properties of this plant. Well dried rhizomes of beet roots were grinded into fine powder by help of plant grinder and stored into khaki bugs.

3.2.1 Extraction

Beta vulgaris were extracted with methanol as solvent. The cold maceration process as described by was used. Approximately 200 grams of the beet root rhizome powder was weighed in a clean 500 ml conical flask and labeled. Into this 250 ml of the pure analytical grade methanol was added to completely soak the powder. The methanol was allowed to wet the powder completely and then an extra 50 ml of methanol was added to completely immerse the powder. The extract process was conducted for a period of 72 hours with occasionally shaking to aid in complete extraction. After the third day, the entire content of the conical flask was filtered by aid of the vacuum pump and the filtrate was transferred in a different flask while the residue was returned in the extraction conical flask. The filtrate was concentrated using a rotary evaporator to recover the extraction solvent. The concentrated extract was transferred in pre-weighed glass bottle and completely

dried in a hot air oven overnight. The well dried extract was then weighed and the percentage yield calculated.

3.3 Antibacterial activity of the rhizome methanol extract of *Beta vulgaris*.

The ability of the *Beta vulgaris* methanol extract to inhibit the growth of the bacteria was evaluated using the disc diffusion technique. The method outlined by with minor modifications was followed. The inoculants of the respective bacteria strains were prepared in normal saline and the turbidity adjusted to the 0.5 McFarland standard. The nutrient agar culture media was prepared as per the instruction of the manufacturer and sterilized by autoclaving at 121 ^{0} C and 15 bars pressure. The sterile media was then plated by carefully pouring approximately 20 ml of the media into the labeled culture plates and allowed to solidify. Using sterile cotton wool swab that had been dipped in the bacteria inoculant, the bacteria strain was uniformly inoculated on the media. Clean forceps made sterile by first dipping in ethanol and heating on a hot ethanol flame, was used to place the sterile discs on the bacteria inoculated media. The beet root extract serially diluted using 0.5 % dimethyl salfoxide to obtain (100, 50, 25, 12.5, 6.25 and 3.125 mg/ml) concentrations was top loaded onto the respective discs using micropipette. Onto each disc exactly 15 microliter of the extract was added and allowed to dry prior to incubation. Ciprofloxacin at 0.1 mg/ml was used as the standard antibiotic and was added onto the center disc in all the plats while 0.5 % dimethyl salfoxide solution was used as the negative control. All the plates were then incubated at 37 ^{0} C for 18-24 hours.

3.4 Radical scavenging activity of methanol rhizome extract of beet root.

The free radical scavenging activity of the methanol rhizome extract of *Beta vulgaris* was evaluated by DPPH assay. The protocol of with minor modifications were used. The plant extract and L-ascorbic acid were prepared by dissolving 10 mg in 10 ml of analytical grade methanol. This formed the stock solutions which were serially diluted by a dilution factor of 5 to obtain the working concentrations 1000, 200, 40, 8, 0.6, and 0.32. Exactly 1.4 ml of methanol extract/ L-ascorbic acid at respective concentrations was mixed with 1.4 ml of 0.1 Mm methanol DPPH

solution. The control was prepared by mixing 1.4 ml of methanol with 1.4 ml of 0.1 Mm methanol DPPH solution. The reaction mixture was then incubated in the dark for 15 minutes and the absorbance measured at 517 nm against methanol as the blank and percentage radical scavenging activity calculated following the formulae.

$$\% RSA = \frac{AbC - AbT}{AbC} \times 100$$

3.5 Data management and analysis

The triplicate zones of inhibition measured with ruler were recorded in the laboratory note book. The percentage radical scavenging activity calculated from the absorbance of the tests and control and noted in the note book as well. Both data (antimicrobial and antioxidant activity) was then entered manually in the graph pad prism statistical software. First descriptive statistics was done and the data presented as Mean ± SEM (standard error of the mean). Further, respective means were then compared to each another by one-way anova to determine the level of significance. The tukeys post hoc test with levels of significance being observed at p-value of $p<0.05$

3.6 Ethical review

This study did not require any ethical review since it did not include use of animals or human beings.

CHAPTER FOUR: RESULTS AND DISCUSSIONS

4.1 Antibacterial activity of methanol extract of *Beta vulgaris*

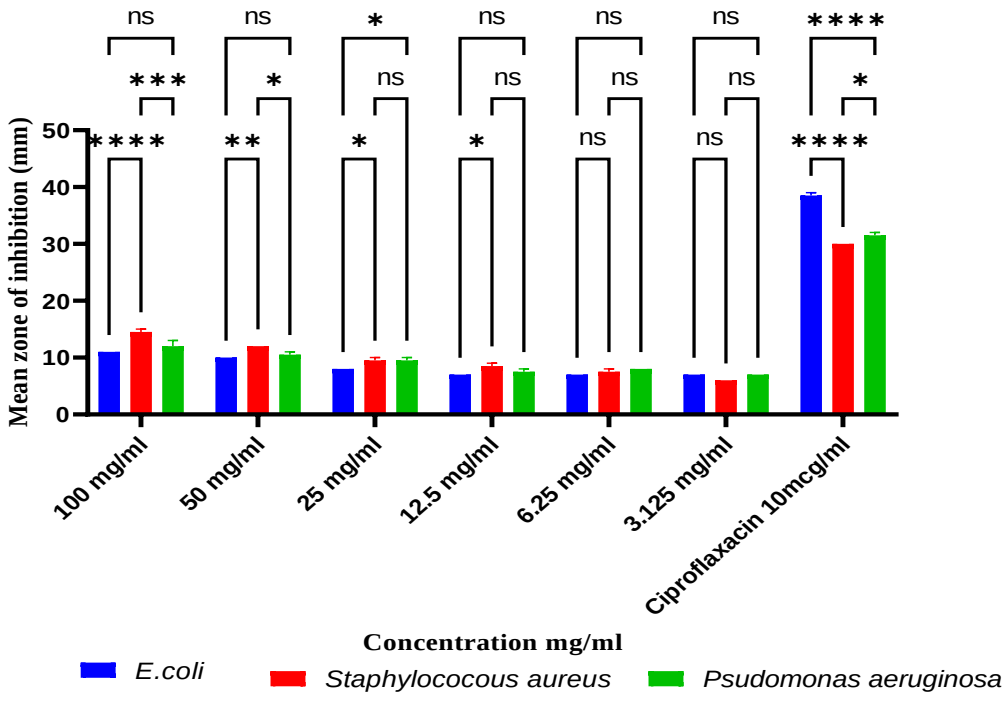
The antimicrobial activity of methanol extract of beet root was evaluated against three bacteria strains that are reported to be prone to resistance. The study showed that the *Beta vulgaris* methanol extract was active against all the bacteria strains as zone of inhibition was recorded in each case (table 4.1 and figure 4.1). Ciprofloxacin at dose level of 10 mcg/ml recorded the best antibacterial activity as seen from the larger zones of inhibition than those recorded by the beet root methanol extract. The antibacterial activity of the extract was decreasing as the dose of the extract reduced. The higher dose of 100 mg/ml recorded the highest antibacterial activity while the least activity was observed in the lower investigation concentration of 3.125 mg/ml against all the three bacteria strains. The extract was more effective against *S.aureus* with zone of inhibition of 14.5± 0.5 mm followed by *P. aeruginosa* and *E. coil* with zones of inhibition of 12.0±1.0 mm and 11.0±0.0 mm respectively (table 4.1). Statistically, the antibacterial activity recorded by methanol *Beta vulgaris* extract was significantly higher than that recorded against both *P. aeruginosa* and *E. coil* ($p<0.05$; table 4.1) at both concentration levels of 100 mg/ml and 50 mg/ml. However, at similar concentration levels the antibacterial activity methanol beet root rhizomes against *P. aeruginosa* and *E. coil* was no significantly different from each other ($p>0.05$; table 4.1). At 25 mg/ml the antibacterial activity of methanol extract of beet root against *S.aureus* and *P. aeruginosa* showed no any significant difference between them ($p>0.05$;table 4.1) while against *E.coli* the activity was significantly lower as compared to both the two bacteria strains ($p<0.05$;table 4.1). At both concentration levels 6.25 mg/ml and 3.125 mg/ml no any significant difference was noted in the between the three bacteria strains ($p>0.05$; table 4.1).

Table 4. 1 Antibacterial activity of methanol rhizome extract of *Beta vulgaris*

Concentration in mg/ml	Mean zone of inhibition		
	E.coli	*S. aureus*	*p. aeruginosa*
100	11.0±0.0	14.5± 0.5	12.0±1.0
50	10.0±0.0	12.0±0.0	10.5 ±0.5
25	8.0±0.0	9.5± 0.5	9.5± 0.5
12.5	7.0±0.0	8.5± 0.5	8.0±0.0
6.25	7.0±0.0	7.5± 0.5	7.5 ±0.5
3.125	7.0±0.0	6.0±0.0	7.0±0.0
Ciprofloxacin(10 mcg/ml)	38.5±0.5	30.0±0.0	31.5± 0.5

Results are presented as Mean ± SEM (SEM-Standard Error of the Mean) of the three replicate values

Figure 4. 1 Antibacterial activity of methanol rhizome extract of *Beta vulgaris*



4.2 Antioxidant activity of methanol *Beta vulgaris* (beet root) extract.

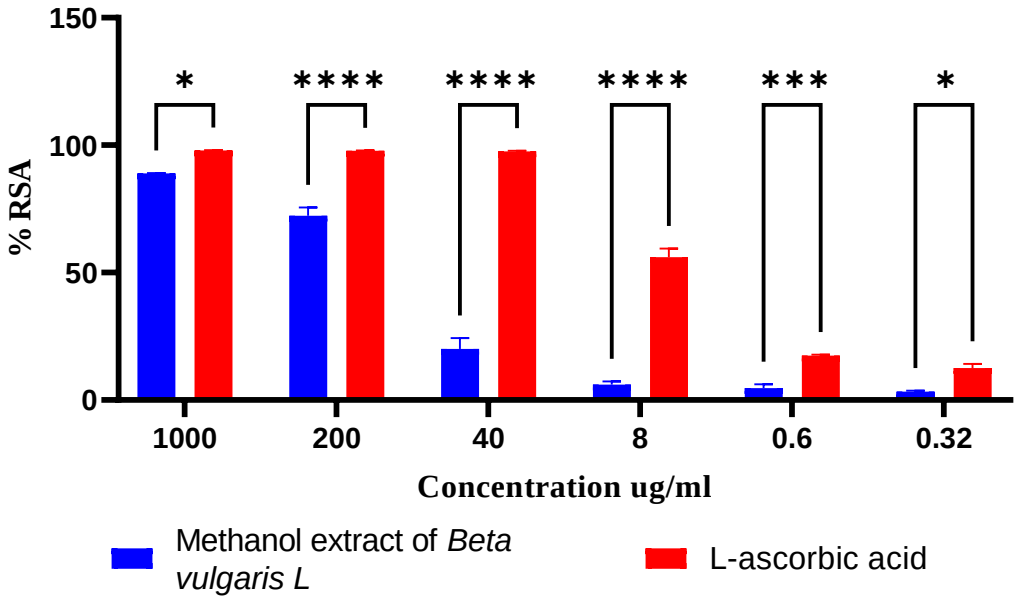
The free radical scavenging ability of the beet root was investigated using the DPPH antioxidant assay. The standard antioxidant used in this study was L-ascorbic acid. The extract and L-ascorbic acid both scavenged the DPPH radical in a dose depended manner. The methanol extract of *Beta vulgaris* recorded percentage radical scavenging activity (%RSA) of 88.983±0.141, 72.310±3.299, 20.007±4.366, 6.040± .317, 4.713±1.431 and 3.269±0.385% while L-ascorbic acid recorded percentage radical scavenging activity (%RSA) of 97.997±0.048, 97.839±0.138, 97.523±0.260, 56.040±03.383, 17.450±0.315 and 12.554±1.609 %. At all study concentrations, L-ascorbic acid recorded significantly higher percentage radical scavenging activity as compared to the methanol extract of *Beta vulgaris L* ($p<0.05$; figure 4.2).

Table 4. 2 DPPH free radical scavenging activity of methanol rhizome extract of *Beta vulgaris*

Concentration mcg/ml	% Radical scavenging activity	
	Methanol *Beta vulgaris* extract	L-ascorbic acid
1000	88.983±0.141	97.997±0.048
200	72.310±3.299	97.839±0.138
40	20.007±4.366	97.523±0.260
8	6.040± .317	56.040±03.383
0.6	4.713±1.431	17.450±0.315
0.32	3.269±0.385	12.554±1.609

Results are presented as Mean ± SEM (SEM-Standard Error of the Mean) of the three replicate values

Figure 4. 2DPPH free radical scavenging activity of methanol rhizome extract of *Beta vulgaris*



4.3 Discussion

The presence of many valuable phytochemicals in vegetables and fruits in addition to the medicinal plants has resulted into a change of heart towards consumption of vegetables and fruits. This has been drive by the need of a healthy life style among many consumers that has seen mass exodus from consumption of the synthetically produced substances. Many chronic diseases such as cancer, brain related disorders such as alzemers disease, cardiovascular disorders and diabetes have been reported to decrease in the individuals whose vegetables and fruits form the larger percentage of their diets (Karagöz et al., 2015).. This is due to their antioxidant activity in which the vegetables and fruits has compounds which has a deactivating power of the free radicals and reactive molecules. The stabilization or putting in check of the excess free radicals in the body system play a greater role in prevention and management of the disorders related to them. The free radicals contribute to the loss in balance between them and the antioxidant system hence a condition known as oxidative stress. The oxidative stress is directly and indirectly linked to

genesis of many disorders such as diabetes, atherosclerosis, aging, immunosuppression and neuro-degenerative diseases which are life threatening. The use of natural source of antioxidant in management of these disorders is therefore in evitable. The lower side effects and easy availability of the vegetables, fruits, herbs and plants rich in compounds responsible for antioxidant and antimicrobial properties has contributed to the use of complementary medicine in diseases management.

The present study investigated the antimicrobial and antioxidant activity of the *Beta vulgaris*. The rhizomes of this plant were extracted with methanol and thereafter subjected to the antioxidant and antimicrobial activity. The free radical scavenging assay that involved scavenging of the DPPH radical by the extract was used as a measure of the antioxidant property. The antimicrobial activity was done by the diffusion method with disc diffusion technique being used. The antimicrobial activity was evaluated against three bacteria strains one gram positive; *Staphylococcus aureus* and two gram negative; *E. coli* and *Pseudomonas aeruginosa*. The antioxidant results showed the extract of Beta vulgaris has high radical scavenging abilities. This was due to its ability to be able significantly scavenger the DPPH free radical at all the studied concentrations. This antiradical activity was in a dose dependent manner. L-ascorbic acid was used as the standard against which the free radical scavenging power of the extract was compared to. The standard however had a higher scavenging power than the extract. This high antioxidant activity of the extract can be attributed to the phytochemicals such as phenols and flavonoids that forms majority of the compound composition in the Beta vulgaris.

The antimicrobial activity of Beta vulgaris was more in the gram positive bacteria as compared to the gram negative bacteria. This activity was similarly higher at the higher concentration of 100 mg/ml and reduced with reduction in the concentration of the extract. Ciprofloxacin was used as the standard and it significantly inhibited the growth of all the bacteria more than the extract of *Beta vulgaris*. The activity of ciprofloxacin against gram negative bacteria was more than gram positive bacteria.

E.coli was inhibited more followed by *Pseudomonas aeruginosa* and the least inhibited was *Staphylococcus aureus*.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Medicinal plants have continued to take a higher position in the health care system since time in memorial. They are currently being use as the alternatives in the place of modern medicine and in some instances are being used alongside with modern medicine. Their potency in curing and preventing many chronic without any signs and symptoms of toxicity being noticed is incredible. The presence of many phytochemicals in the plants that mainly involves vegetables and fruits has significantly contributed to the larger population turning towards them. These compounds play a vital role in the therapeutic properties such as ant inflammation, antioxidant and antimicrobial. The present studies evaluated the antioxidant and antimicrobial activity of Beta vulgaris a common vegetable that has a purple colored rhizome. The plant had higher antioxidant activity seen through the higher percentage radical scavenging activity of the DPPH free radical. However the antioxidant activity was significantly lower as compared to the L-ascorbic acid the standard at all studied concentrations. The antimicrobial activity of Beta vulgaris showed that it was sensitive to both the gram positive and gram negative bacteria. The antimicrobial activity against gram positive bacteria; *Staphylococcus aureus* was higher than their gram negative counterparts. In conclusion the consumption of Beta vulgaris can significantly result into better health status of the human and animal body. It's a potential antioxidant and antimicrobial agents towards the disorders such as cancer and the bacterial infections.

5.2 Recommendation

From this study the following recommendations were made;

I. The antioxidant activity be investigated in other specific antioxidant assays
II. Quantification of the common antioxidant and antimicrobial phytochemicals be done
III. Toxicity profile of the plant to be conducted

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