ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF ACETONE LEAF EXTRACT OF *Senna singueana*

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BPHARM/ 53505/2016

A RESEARCH PROJECT SUBMITTED TO THE SCHOOL OF PHARMACY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF PHARMACY DEGREE OF MOUNT KENYA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF PHARMACY

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY

SEPTEMBER 2021

DECLARATION

I declare that this project is my original work and has not been submitted in any institution for award of degree or diploma.

Signature…………………………………… ... Date ……………………

MWANAMWINYI JUMA MWARASI

BPHARM/ 53505/2016

Supervisor' s approval

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 dedicate this work to my dear parents, especially my mother and siblings for their encouragement, support and guidance throughout my studies and my supervisor professor Bindu Madhavi for her continued guidance and moral support in the completion of my research project

ACKNOWLEDGEMENT

I would like to express my special thanks and gratitude to the Almighty God for the strength and good health he granted me during the entire five years and the research project period. His grace is sufficient.

I am in debt to my supervisor and mentor professor Bindu Madhavi for her timely and positive advices and guidance in the research project. Her advices kept me going throughout the time as I conducted may project. The laboratory technologist Mr. Elias Nelson is as well acknowledged for his guidance in various phases and ensured that the completion of the project was on time.

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LIST OF ABBREVIATIONS

DMSO	Dimethyl sulfoxide
DPPH	2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl
HART	Hydrogen atom transfer
MRSA	*Methicillin Resistant Staphylococcus aureus*
SET	Single electron transfer

LIST OF APPENDICES

ABSTRACT

Emergence of resistance among many commercially available antibiotics has dimmed all the efforts made in the antibacterial agent development. Many infections that results from bacteria exposure have as well resulted into development of other complications. The multidrug resistance has been ignited by misuse and improper use of the antibiotics. On the other hand the oxidation reaction in the body results into free radicals that have deleterious impact on the body. The increased concentration of these free radicals which are very reactive tend to attack the biomolecules resulting into damage and in some instances result into death of the cells or tissues. The increase free radicals results into a state of oxidative stress which is trigger in many conditions such as cancer, diabetes, neurodegenerative disorders and aging. The conventional medicines used in management of the bacterial infections and oxidative stress related conditions are expensive and in some cases have side effects and are less potent. Alternatives from plants have been identified as the savior to the current problems. Plants synthesized secondary metabolites that are pharmacologically active among many conditions. This has seen many plant being explored for their medicinal properties. *Senna singueana* has been widely used as an antimalarial agent in many countries including Kenya and Tanzania. This antimalarial property has been widely investigated by many researchers. However its antimicrobial and antioxidant activity is not well known. This study aimed at evaluating the antimicrobial and antioxidant activity of acetone leaves extract of *Senna singueana.* The antimicrobial activity was evaluated by the diffusion method employing the disc diffusion technique. The antimicrobial activity was investigated against four bacteria strains; three gram negative; *Escherichia coli*, *Acinetobactor baumanni* and *Salmonella enteritidis* and one gram positive *Methicillin Resistant Staphylococcus aureas.* The antioxidant property was investigated by monitoring its free radical scavenging property using the DPPH free radical. The zones of inhibition at 100 mg/ml concentration levels were 14.00±0.00 mm, 12.33±0.577 mm, 12.00±0.00 mm and 13.00±1.00 mm for *Acinetobactor baumanni*, *Salmonella enteritidis*, *Escherichia coli* and *Methicillin Resistant Staphylococcus aureas* respectively. Ciprofloxacin at 10 ug/ml recorded 30.00± 1.00 mm, 37.33±0.577 mm, 39.667±0.577 mm and 30.667±0.577 mm for *Acinetobactor baumanni*, *Salmonella enteritidis*, *Escherichia coli* and *Methicillin Resistant Staphylococcus aureas*. The antioxidant results showed that the acetone extract was significantly scavenged the DPPH free radicals at concentration levels 500 ug/ml, 250 ug/ml and 125 ug/ml. the activity at tis particular concentration levels was was comparaeble to L-ascorbic acid and no significant difference was noted between them ($p>0.05$). The antioxidant activity of the acetone extract at lower concentrations of 62.5 ug/ml, 41.25 ug/ml and 15.625 ug/ml was lower than the L-ascorbic acid. In conclusion Senna *singueana* is a potential antioxidant and antimicrobial agent and can be taken as an alternative to the conventional drugs.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Nature has been an adequate hub for many vital requirements that are essential for the survival of the human beings. Man has depended on nature for things such as medicines, shelters, food stuffs, flavors and fertilizers. Traditional medicines that are mainly from plants have been used by larger population of people all over the world. This has been evident in developing countries where herbal medicine has dominated the health care for a long time. The use of these medicinal plants has as well gained momentum in the developed countries (Refaz Ahmad Dar, Mohd Shahnawaz, 2017) in the recent days. This has seen medicinal plants used as important source of natural compounds in developing agents that are potent as anti-diabetic, anti-inflammatory, anticancer and antimicrobial as well (Mahomoodally, 2013).

Micro-organisms that comprises of bacteria, fungi and viruses are the main spreader of various human diseases. The attack and ability of the microbes to exceed the defense mechanism of the human beings has shown to negatively damage the host cells. Potent antimicrobial agents have been released in the past but the increasing rate of resistance by microbes to these antimicrobial agents has shuttered the advancements made in this area (Mutembei et al., 2018). Similarly, the resistance shown by the micro-organisms has as well resulted into the emergence of new and re-emergence of old eradicated infections (Waliullah et al., 2014).

Oxidative stress is characterized by disturbance in the normal redox balance that exist between the reducing (antioxidant) and oxidizing (pro-oxidant) agents in the body (G. Moriasi et al., 2020). The latter is usually more as compared to the reducing agents and this state has been associated with many chronic disorders (G. Moriasi et al., 2021). Conditions such as cancer, diabetes and neurodegenerative and cardiovascular diseases have been linked to oxidative stress as a result of continual and excess production of reactive oxygen and nitrogen species that induce long term cellular disruption (Lobo et al., 2010). Antioxidants constitutes compounds that are able to eliminate the excess free radicals and reinstate the redox balance between the free radicals and the antioxidants (Pradedova et al., 2011). These compounds do not only scavenge the free radicals but also do the detoxification of the system to eliminate the damaged tissues as a result of uncontrolled oxidation process (Pradedova et al., 2011). The antioxidants are either of endogenous origin naturally produced in the body or exogenous origin which are introduced in the body to back up the activity of endogenous antioxidants(Aiyelaagbe et al., 2010). The secondary metabolites of plants known as phytochemicals are also good source of alternative antioxidants that are less toxic and potent at the same time.

The reduced efficacy of modern medicines that are used to treat and manage the infections caused by bacteria and chronic disorders as a result of the oxidative stress has created a great challenge in many health sectors (G. A. Moriasi et al., 2021). This has seen many researches engage themselves on finding alternatives that are potent and whose efficacy is on the high note as well. Similarly the toxicity of the agents used as drugs has been an area of concern and this has as well not been left out as the search of the alternative agents to the modern medicine whose efficacy has reduced continues (Nyaboke et al., 2017). Plants which have been in use as the remedies for many communities in different countries and continents has been the only remaining alternative. This has seen an increase in the researches that try to evaluate the various composition and the pharmacological properties of the plants. The fact that plants used as medicine contain many compounds that are biologically active and possess diverse pharmacological properties has greatly influenced their choice as medicines or source of agents in drug development. Plants are as well less toxic and readily available hence contributing to their choice as the alternative source of medicine for the many ailments affecting man and his animals (Onyancha et al., 2019).

Many of these plants are found to be indigenous in various countries and their therapeutic effects have been reported in various studies. An example of such plant is *Senna singueana* that belongs to the genus *senna* and family Caesalpinioideae. This plant exist as a shrub or a small tree that grows to a height of about 400-1500 cm and characterized by reddish stem-bark which turns to grey-brown and become rough as the plant ages. This plant has wide uses in the east African countries as it's traditionally used to treat malaria in Tanzania. In this country the plant is squeezed and the sap from the plant taken orally. In Kenya and Burkina Faso, the leaves are dried and grounded prior to boiling and the resultant decoction taken orally to treat malaria and fever (Sobeh et al., 2017). The reported pharmacological activity of this plant include the antiplasmodial activity. The methanol extract of the leaves has shown great antiplasmodial effect against *plasmodium berghei*. Also many compounds have been isolated from this plants such as Lupeol (Sobeh et al., 2017). This is atriterpene that is attributed to the antimalarial property shown by the plant. However even though the plant has wide uses, its antioxidant and antimicrobial activity has not been well evaluated. Therefore this study aimed at evaluating the antioxidant and antimicrobial property of acetone leaves extract of *Senna singueana.*

1.2 Problem statement

Oxidative stress that arises due to disturbance in the redox balance between the antioxidants and the reactive free radicals in the body (Arulselvan et al., 2016). These reactive free radicals and molecules have deleterious effects on the biomolecules when produced in larger populations. The free radicals attack the biomolecules; lipids, proteins and nucleic acid causing oxidative damage and to some instance result into cell deaths (Kurutas, 2016). The attack on the on lipids interferes with the structure of the lipids which resulting food damage and condition such as dementia in human beings. The oxidative stress has as well be linked to development of many chronic conditions such as cardiovascular, hearth diseases, aging, cancer and inflammatory disorders (Dai & Mumper, 2010). The emergence of resistance as well has limited the advancements being made in the development of antibiotics. Many bacteria are gaining resistance to the available antibiotics and this tread is a threat to the human health (Waliullah et al., 2014). The available conventional medicines used as antioxidant and antibacterial agents are becoming less potent hence in effective increasing the cost of health care and time taken in the health facilities (Parham et al., 2020). The side effects that are associated with these drugs and high cost of obtaining them has as well limited their use. This reason has seen many efforts put in place to find alternative remedy is potent as antioxidant and antibacterial agent.

1.3 Justification

Plants are the major sources of natural remedies that are potent against many chronic conditions. Their uses in the management of diseases dates back many years before the introduction of the modern medicine. The presence of the bioactive compounds in these plants has significantly played a vital role in the reported pharmacological properties (Parham et al., 2020). The lower toxicity effects and high efficacy has as well contributed to the increased demand for the herbal remedies. The high body compatibility and multidisciplinary uses of the herbal medicine has played a major role in preventing many diseases (Olela et al., 2020). Even though many plants that are used as traditional medicine in the African set up mostly in developing countries have been claimed to heal many diseases, these claims have not been validated. A few plants that have been studied only few properties that including phytochemical screening and in vitro studies have been done. To bridge this gap more studies on the pharmacological properties of plants need to be done. Therefore this study is designed to evaluate the antioxidant and antibacterial activity of the acetone leaves extract of *Senna singueana*.

1.4 Objectives

1.4.1 General objective

To evaluate the antioxidant and antibacterial activity of acetone leaves extract of *Senna singueana*.

1.4.2 Specific objectives

I. To investigate the free radical scavenging activity of acetone leaves extract of *Senna singueana.*

II. To evaluate the antibacterial activity of acetone leaves extract of *Senna singueana.*

1.5 Research questions

I. Does the acetone leaves extract of Senna singueana have free radical scavenging properties?

II. Does the acetone leaves extract of Senna singueana have antibacterial activity?

1.6 Significance of the study

The surge in the number of antibiotics upon which bacteria are resistance to needs an urgent solution. Similarly, the problem of oxidative stress which is an initiator to many conditions such as cancer and diabetes need critical attention. Plants with potential antibacterial phytochemicals are the recent are of investigation. Therefore the need to evaluate the antioxidant and antimicrobial activity of plant extracts such as *Senna singueana* is the main drive for the present study.

CHAPTER TWO: LITERATURE REVIEW

2.1 Reactive free radicals

Oxidation process is key as it is responsible for formation of the free radicals in the animal and human body systems, foods and drugs as well. The free radicals that comprises of the two major groups reactive oxygen and nitrogen species are under constant production during the vital aerobic metabolic process in the body (Alfadda & Sallam, 2012). During these processes the free radicals are produced as by-products. The characteristic structure of the free radicals is having unpaired electrons that gives them their high reactive nature due to instability. The generation of the free radicals in the body is through cascade of many contributing factors such as environmental pollutants, radiations, toxins, smoking, and chemicals (Alfadda & Sallam, 2012). Physical factors such as stress has as well be implicated in the generation of the free radicals as they result into exhaustion of the antioxidants in the body. Many free radicals and reactive molecules are produced in the body systems and include superoxide radical ($O^{2-\bullet}$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2) and singlet oxygen (O_2). Theses free radicals and reactive molecules are produced contribute to the oxidative stress which has been implicated in the many disorders in humans such as atherosclerosis, diabetes, ischemia, cancer, inflammatory disorders and aging (Sghaier et al., 2016);(Youssef et al., 2017).

2.2 Antioxidants

Antioxidant is a vital substance in the body system or any other system found in either foods, drugs or humans being and is tasked with the role of either delaying, inhibiting or breaking the chain reactions of the free radicals and reactive molecules. Through this process the oxidizable substrates in the body for instance lipids, proteins and nucleic acids are protected as these species are neutralized.

Living organisms such as human beings are equipped with the protective antioxidant system. This defense system is characterized by high regenerative power and keeps in check the free radicals and the reactive molecules limiting their deleterious effects (Sobeh et al., 2017). This defense system employs enzymatic mechanisms that use enzymatic antioxidants such as superoxide dismutase, catalase and glutathione-peroxidase-glutathione system. Similarly, non-enzymatic antioxidants are involved in the protective role of the body against free radical and these includes ascorbic acid, glutathione, uric acid, tocopheral, carotenoids, polyphenols and some hormones such as melatonin (Mamta et al., 2014).

2.2.1 Classification of antioxidants.

Antioxidants are classified based on various criteria that include function and origin criteria. Based on the function criteria two type of antioxidants are identified; primary and secondary antioxidants. The primary antioxidants also known as the chain breaker, reduce the reactivity of the free radicals by inhibiting the first reaction step of the free radical chain reactions (initiation step) (Brewer, 2011). This is done by the antioxidant reacting with the lipid radical. The primary antioxidant may as well inhibit the propagation step of the free radical chain reactions by reacting with peroxyl or alkoxyl radicals. The secondary antioxidant are also known as the preventative antioxidants and they slow down the rate of oxidation. Based on the origin of the antioxidants, two categories are recognized; natural and synthetic antioxidants (Mamta et al.,(2014).

2.2.2 Mechanism of action of antioxidants

The mechanism of action of antioxidant is dependent on the type of the free radical being acted on. For this reason different antioxidants have different mechanism on different free radicals or oxidants sources as a result of the different characteristics (Sehwag & Das, 2013). However, one antioxidant may act on the free radicals through different set of mechanisms. Antioxidants are able to deactivate free radicals and other oxidants through two main mechanisms. These are hydrogen atom transfer (HAT) and single electron transfer mechanisms (SET). The antioxidants that act through the HAT mechanism are able to donate a hydrogen atom to the oxidant of free radical. On the other hand the antioxidants that act through the SET mechanism they donate one electron to the radical hence reducing the compound or molecule such as metals, carbonyls and radicals (Sehwag & Das, 2013).

2.3 Antimicrobial agents

The antimicrobial agents are molecules or substances that either inhibit or totally kill the growth of the micro-organisms. The antimicrobial agents are found in various forms such as the antibacterial, antifungal and antiviral agents. The different form of antimicrobial agents are named as per the micro-organism that they act on. The antibacterial agent act on bacteria, antifungals act on the fungi and antiviral act on the viruses. The antimicrobial exist as either synthetic or natural(Cowan, 1999). The antimicrobial agents can be obtained from different sources such as plants and micro-organisms as well (Jenssen et al., 2006).

2.3.1 Classification of Antimicrobial agents

Antimicrobial are grouped in various categories. These classification is done based on various aspects; activity spectrum, mode of action and the chemical makeup. The antimicrobial agent is regarded as either broad spectrum or narrow spectrum (Parham et al., 2020). The ability of the antimicrobial agent to act on a wide range of bacteria both gram negative and gram positive is classified as broad spectrum while the antimicrobial with the ability to act on only one set of bacteria that is either gram negative or gram positive is classified as narrow spectrum (Awouafack et al., 2013).

The mode of action of the antimicrobial agent as aspect of classification recognizes that antimicrobial agents act following one major mechanism (Reygaert, 2016). However many other mechanism of action are known and include that inhibition of protein synthesis in the bacteria, inhibition of cell wall synthesis, inhibition of biosynthesis of the nucleic acids, inhibition of functions of the cell membrane and those that inhibit the other metabolic processes in the bacteria (Reygaert, 2016).

2.3.2 Use of complementary medicine in bacterial infection management

The wide spread of resistance shown by many bacteria against the available antibiotic need an urgent solution. Complementary medicine that involve use of herbal and plant based products as antimicrobial agent many pathogenic bacteria strains (Iwu et al., 1999). The characteristic of herbal medicines; safety, less toxicity, high potency and easy availability has contributed to the immense use of plants as medicines (Safary et al., 2009).

Many herbs, vegetables, fruits and plants are used as remedies for many bacterial infections. The availability of bioactive compounds known as phytochemicals are responsible for this activity. one such plant is *Glycyrrhiza glabra* which has been reported to be effective in the management of respiratory diseases that include coughs, sore throat and bronchitis (Nitalikar et al., 2010). *Mahonia aquifolium* is used as a remedy for the skin infections (Rackova et al., 2007). The *Achillea millefolium* and Arctostaphylos uva-ursi have been reported to be effective in management of the urinary tract infections(Yakhkeshi et al., 2012).

2.4 Study plant

2.4.1 *Senna singueana*

2.4.1.1 Botanical description and distribution

Senna singueana exist as a shrub or a small tree and is deciduous in nature. This plant is characterized by alight and open crown. It grows to a height of about 4-15 meters tall. This plant is found in the genus *Senna singueana* that is represented by about 350 species. The bark is usually reddish that turns to grey-brown and becomes grey upon aging (Sobeh et al., 2017).

The *Senna singueana* plant is well distributed in the Africa continent. In east Africa it's found both in Kenya and Tanzania. In Tanzania is found in Morogoro, Tanga and Arusha and Dodoma region.

2.4.1.2 Traditional uses of *Senna singueana*

Senna singueana has a wide traditional uses across the African countries. As an antimalarial agent it's used both in Kenya, Burkina Faso and Tanzania. In Tanzania the sap resulting from the squeezing of the leaves is taken orally as remedy for malaria. In Kenya and Burkina Faso, leaves are powdered, boiled and the resultant decoction is taken while hot as remedy for both fever and malaria (Sobeh et al., 2017).

2.4.1.3 Reported pharmacological properties of *Senna singueana*

Many biological properties have been reported from previous works. The methanol root bark extract has been reported to have antiplasmodial activity against the plasmodium berghei. Similarly, the root bark has been reported to contain Lupeol which is atriterpene that has wide spectrum of pharmacological activities including antimalarial. As an antimalarial agent it's potent against the chloroquine (CQ)-resistant plasmodium falciparum (Sobeh et al., 2017).

2.4.1.4 Isolated compounds senna singueana

A wide range of bioactive compounds that have medicinal properties have been identified in this plant. In the root four compounds; tetrahydroanthracene, singueanol-I and II, torosachrysone and germichrysone have been isolated. Other compounds such as pentacyclic triterpene, lupeol and steroids have as well been identified (Sobeh et al., 2017).

2.4.1.5 Other uses of *Senna singueana*

The various parts of *Senna singueana* have other uses other than medical uses. These include as dyes or as tanning agents. In Zambia and Ethiopia the stem bark is useful as a dye for textile while in the larger parts of East Africa the stem bark is used for tanning hides and skins. The fruits in Sudan is used for tanning skins as well. The dyeing property in the leaves is attributed to the flavonoid leucopelargonidin. The leaves are as well used as a ripening agent for the bananas while the stems are used in building huts, small furniture, tool handles and carvings (Sobeh et al., 2017).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Extraction of the *Senna singuenna* leaves

The *Senna singuenna* leaves were extracted with acetone. The extraction followed the standard extraction procedures. Cold maceration extraction method was used and this briefly involved soaking 200 g of the coarse grounded leaves powder in 400 ml of analytical grade acetone. The materials remained soaked for a period of 72 hours and each day the extraction vessel was agitated to ensure complete solvent powder interaction. After the 72 extraction hours the filtrate was separated from the residue by filtering under vacuum with the aid of the filtration apparatus. The resultant filtrate was concentrated using a rotatory evaporator and complete drying done in a hot air oven, completely sealed and stored in the fridge at 6 ^{0} C.

3.2 Chemicals and solvents

The chemicals used in this study included 2,2-Diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH)(sigma Aldrich AR), dimethyl salfoxide (DMSO)(Loba Chemie), L-ascorbic acid (Loba chemie AR), nutrient agar (Hi-Media laboratories), 0.5 McFarland standard (Hi-Media laboratories) and ciprofloxacin (Sigma Aldrich AR).

3.3 Determination of the antioxidant activity of acetone extract of *Senna singuenna*

The antioxidant activity of the *Senna singuenna* acetone extract was done using the DPPH radical scavenging method (Arika et al., 2019). Extract solutions of concentration range 200 ug/ml to 500 ug/ml were prepared in analytical grade methanol. DPPH solution at concentration of 0.3Mm was freshly prepared in methanol as well and stored in the dark prior to use. L-ascorbic acid was also prepared in methanol in the concentration range of 200-500 as that of the extract and used as the standard. Exactly 1 ml of the extract/ standard at different concentration was mixed with 1.4 ml of the DPPH solution. In the control 1.6 ml of methanol was mixed with 1.4 ml of DPPH solution. Fifteen minutes later upon incubation in the dark, the absorbance of the reaction mixed was measured at 517 nm. The ability of the extract to scavenge the DPPH free radical was calculated as the percentage radical scavenging activity using the equation;

$$\% \, RSA = \frac{Abs\ Control - Abs\ Test}{Abs\ Control} \times 100$$

3.4 Antibacterial activity

3.4.1 Preparation of the extract solutions at different concentrations

The acetone extract of *Senna singuenna* was at concentration of 100 mg/ml was prepared by dissolving 100 mg of the dried extract in 1000 ul of 5% DMSO. In order to ensure total dissolution of the extract the tube containing the extract was put in ultrasonic sonicator for a period of 10 minutes. The 100 mg/ml stock solution of the extract was diluted in a serial twofold dilution method to obtain concentrations 50, 25, 12.5, 6.25 and 3.125mg/ml. Ciprofloxacin was taken as the standard antibiotic to compare with the antibacterial activity of *Senna singueana* extract. The ciprofloxacin solution was prepared by dissolving in 5% DMSO to obtain the 10 ug/ml concentration.

3.4.2 Bacteria strains used

The antibacterial activity of *Senna singueana* was tested upon four bacteria strains. Three gram negative bacteria strains; *Escherichia coli, Acinetobactor baumanni* and *Salmonella enteritidis*, and one gram positive bacteria *Methicillin Resistant Staphylococcus aureas*. All these bacteria strains were pure cultures obtain from Kenya medical research institute.

3.4.3 Preparation of the bacteria inoculums

The four bacteria strains were first sub cultured on the nutrient agar by streaking a loop wire full of the microbe on sterile media and incubation for 18 hours. From the fresh colonies well isolated colonies were scoped and suspended in sterile normal saline. The inoculum of each specific bacteria was adjusted to the McFarland standard (0.5).

3.4.4 Preparation of the culture media

Nutrient media was used as the culture media in this study. The media was prepared according to the manufacturer instructions; 28g/I l. the nutrient agar powder was dissolved in distilled water and the resultant mixture heated to boiling on a hot plate set at 100 ^{0}C. The boiled and well dissolved media was then covered with foil paper and sterilized by autoclaving at 121 0C and 15 bar pressure for 15 minutes. Sterile media was then cooled to around 50 ^{0} C and then plated in sterile petri-dishes and left for some time to solidify.

3.4.5 Sensitivity test

The sensitivity of the *Senna singueana* extract against the selected gram negative and gram positive bacteria was evaluated by the disc diffusion technique (Monon et al., 2015). On to the respective bacteria labeled sterile media the inoculum of the specific bacteria was inoculated using sterile cotton swab which was dipped into the inoculant and the excess bacteria removed by squeezing on the wall of the tube. Five sterile paper discs of diameter 6 mm were then laid onto each plate using a sterile forceps. The dis s were well spread across the plant allowing adequate space between the discs. The disc at the center was then loaded with 10 ul of the ciprofloxacin solution while the other three discs were loaded with 290 ul of the extract at different concentrations. The fourth disc on the same plate was loaded with 10 ul of the 5% DMSO solution as the negative control. The different solutions were ten allowed to complete dry and the plates covered then incubated while inverted for a period of 18- 24 hours at 37 ^{0} C. After the elapse of the incubation time zones of inhibitions around the discs were measured using a ruler.

3.5 Data analysis

The absorbance of both the extract and L-ascorbic acid were tabulated in the laboratory research book. Suing the relevant formula the percentage radical scavenging activity of both the extract and L-ascorbic was calculated. All the triplicate values of the percentage radical scavenging activity and zones of inhibition were then manually entered in the cells of the graph pad prism. Descriptive statistics to obtain the mean values and standard error of the mean was done on both data. The different resultant means of extract and L-ascorbic acid were compared to each at different concentration in the antioxidant assay using the one-way analysis of variance. Similarly, the means of the extracts were compared to the mean of ciprofloxacin in and antibacterial activity using the one-way analysis of variance. The comparison was done using the tukeys post hoc test where difference in the level of significance between the different means was identified at $p<0.05$.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Antibacterial activity

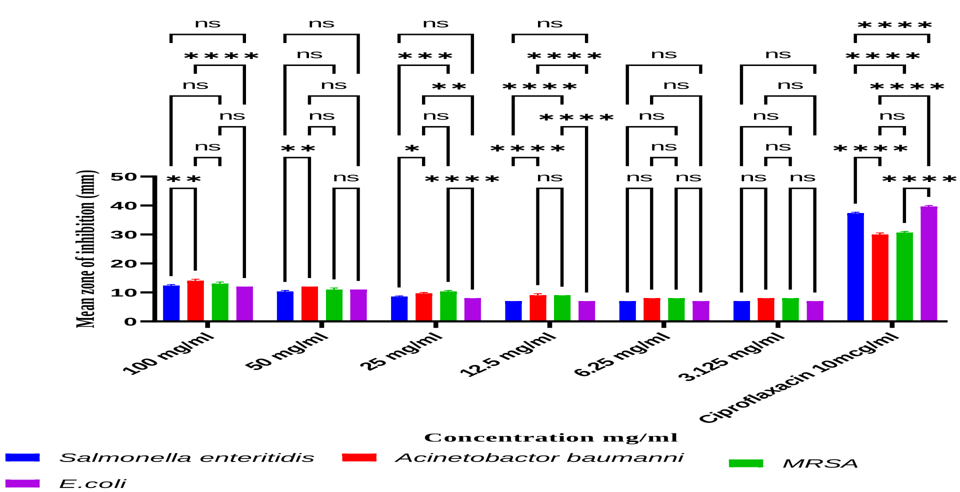
The screening of the potential of *Senna singueana* extract as antibacterial agent was done by the disc diffusion technique. The disc diffusion method is the initial method of determining the antibacterial activity of natural products against various micro-organism. In this study antibacterial activity was tested against *Acinetobactar bauamanni*, *Salmoneall enteritidis*, *E.coli* and *MRSA*. *MRSA* is a gram positive bacteria while the rest three are gram negative. At the stock solution of 100 mg/ml the extract was able to record zone of inhibition indicating that the extract was active against the four bacteria strains. The extract at this concentration level was more active against *Acinetobactar baumanii* with zone of inhibition of 14.00±0.00 mm this was followed by *MRSA* with inhibition zone of 13.00±0.00. At the same concentration level inhibition zones of 12.33±0.577 mm and 12.00±0.00 for *Salmonella enteritidis* and *E.coli* respectively were recorded (table 4.1). Statistically, a significantly larger zone of inhibition was recorded against *Acinetobactar bauamnni* and this zone was significantly larger than that recorded against *Salmonella enteritidis* and *E.coli* respectively (Figure 4.1). However, this zone of inhibition was not significantly different from the zone of inhibition recorded against *MRSA* (figure 4.1).

Table 4. 1 Antibacterial activity of acetone extract of *Senna singueana* gram positive and negative against bacteria

Concentration	Zone of inhibition			
	Acinetobacter baumannii	*Salmonella enteritidis*	*E.coli*	*MRSA*
100 mg/ml	14.00±0.00	12.33±0.577	12.00±0.00	13.00±1.00
50 mg/ml	12.00±0.00	10.333±0.577	11.00±0.00	11.00±1.00
25 mg/ml	9.657±1.00	8.5 0±0.500	8.00±0.00	10.333±0.577
12.5 mg/ml	8.00 ±0.00	7.0±0.00	7.00±0.00	9.000±0.000
6.25 mg/ml	8.00±0.00	7.00±0.00	7.00±0.00	8.00±0.00
3.125 mg/ml	8.00± 0.00	7.00±0.00	7.00±0.00	8.00± 0.00
Ciprofloxacin(10 ug/ml)	30.00± 1.00	37.33±0.577	39.667±0.577	30.667±0.577

Results presented as Mean ± SEM of the three replicates values (SEM-Standard error of mean)

Figure 4. 1 Antibacterial activity of acetone extract of *Senna singueana* gram positive and negative against bacteria



***** = Significant difference (p<0.05), ns = no significant difference (p>0.05)**

4.2 antioxidant activity of *Senna singueana* acetone leaves extract.

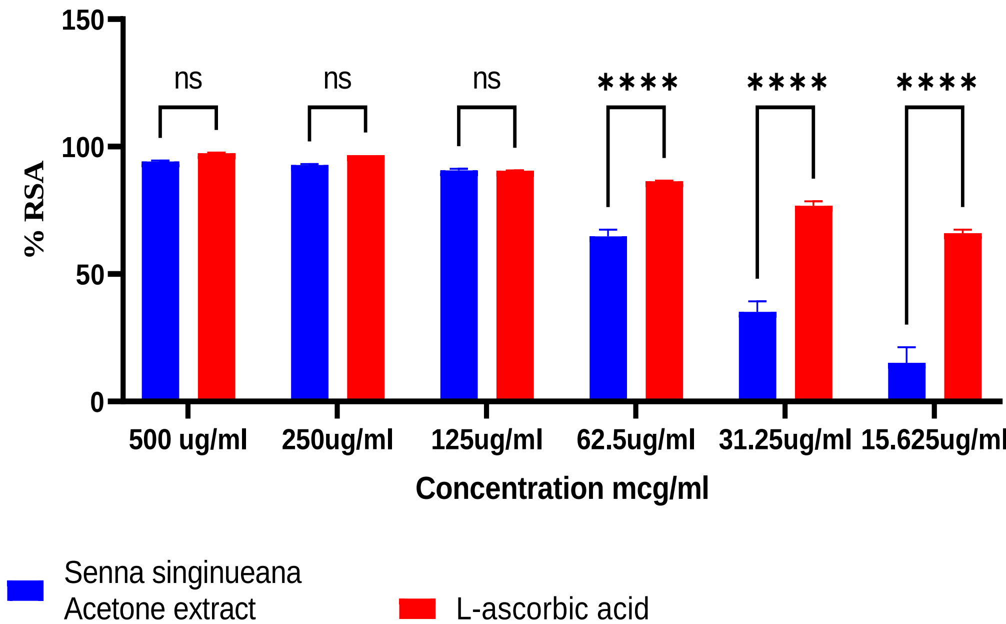
From the antioxidant results it can be observed that the DPPH radical scavenging activity of both acetone leaves extract of *Senna singueana* and L-ascorbic acid the standard antioxidant positively correlates with the concentration. This has been shown by an increase in the percentage radical scavenging activity as the concentration of the extract is increased (table 4.2). The results showed that the between concentrations levels 500 ug/ml, 250 ug/ml and 125 ug/ml no significant difference was noted in the percentage radical scavenging activity recorded by both L-ascorbic acid and acetone leaves extract of *Senna singueana* ($p>0.05$;figure 4.2). However from concentration levels 62.5 ug/ml, 41.25 ug/ml and 15.625 ug/ml, L-ascorbic acid recorded significantly higher percentage radical scavenging activity as compared to the percentage radical scavenging activities recorded by acetone leaves extract of *Senna singueana* ($p<0.05$;figure 4.2).

Table 4. 2 Percentage free radical scavenging activity of acetone leaves extract of *Senna singueana*

Concentration ug/ml	Acetone leaves extract	L-ascorbic acid
500	94.230±0.283	97.424±0.239
250	92.846±0.335	96.715±0.030
125	90.721±0.611	90.502±0.174
62.5	64.805±2.688	86.400±0.342
31.25	35.212± 4.098	76.763±1.818
15.625	15.202± 6.062	66.043±1.429
IC_{50} (ug/ml)	2.420	0.500

Results presented as Mean ± SEM of the three replicates values (SEM-Standard error of mean)

Figure 4. 2DPPH free radical scavenging activity of acetone leaves extract of *Senna singueana*



***** = Significant difference (p<0.05), ns = no significant difference (p>0.05)**

4.3 Discussion

More efforts in the recent days have been put forward in finding the valuable and more potent remedies to the many chronic diseases and infections that are threatening the health of human beings. This is gaining more consideration due to the reduced efficacy and potency of the conventional medicines that have been the main pillar in management of diseases and infections for most of the time. The conventional antimicrobial agents that include the antibiotics that managed the bacterial infections have been greatly affected with resistance. Many factors have contributed to the resistance of the bacteria strains to multiple antibiotics. The resistance has posed many challenges that including difficulties in caring out other procedures such as surgeries and management of newborns in the newborn units. Similarly many environment factors that include exposure to radiation and other health practices such smoking have as well contributed high population of the free radicals in noticed in the body. These radicals have a tendency to attack the biomolecules (proteins, lipids and nucleic acids) in the efforts to gain stability into damages as well as death of the tissues and cells. The synthetic antioxidants that are used to quench the free radicals and reduce their effects have as well resulted into genesis of other conditions such as cancer. The management of the oxidative stress disorders such as cancer is as well expensive and as well results into both social effects such as depression.

The alternatives which are from plants have been the most preferred as the remedies to these life threating diseases and infections. The safety and high efficacy of plants has greatly influenced the high usage of these plants as therapeutic agents. The varied pharmacological properties that are witnessed upon use of these plants has been linked to many secondary metabolites that are synthesized by the plants in different parts as a result of stress. These secondary metabolites known as phytochemicals are responsible for various activities such as antioxidant and antimicrobial which need to be evaluated.

The current study evaluated the antioxidant and antimicrobial activities of the leaves of *Senna singueana.* The leaves were extracted using acetone and evaluated for antioxidant activity at different concentrations that ranged from 500-15.625 ug/ml for and antibacterial activity the concentration ranged between 100 mg/ml - 3.125 mg/ml for antimicrobial activity. The extract significantly showed good antioxidant and antimicrobial activities. The extract was able to scavenge the DPPH free radicals and this activity was comparable to the ability of L-ascorbic acid the standard antioxidant at concentration levels 500 ug/ml, 250 ug/ml and 125 ug/ml. however at lower concentrations the activity of the extract was lower than that of L-ascorbic acid.

The extract was also able to act as antibacterial agent against both the gram negative bacteria and gram positive bacteria. This was an indicator the extract of *Senna singueana* has broad spectrum activity. The antibacterial activity shown by the extract was moderate according to the evaluation scale of De Almeida Alves et al., (2000).

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Antioxidant and antimicrobial activities of the leaves of *Senna singueana* were performed in this study. The antioxidant activity was investigated using the DPPH radical scavenging methods and the antimicrobial activity was evaluated using the disc diffusion method. The antioxidant efficacy of the acetone leaves extract of *Senna singueana* was higher and not significantly different from that of L-ascorbic acid at concentration levels 500 ug/ml, 250 ug/ml and 125 ug/ml. The antioxidant activity of acetone leaves extract of *Senna singueana* at concentration levels 62.5 ug/ml, 41.25 ug/ml and 15.625 ug/ml was lower than that of the L-ascorbic acid. Therefore, the acetone extract of *Senna singueana* at lower concentrations has less antioxidant activity. The antibacterial property of the acetone extract of *Senna singueana* was moderate although lower than the standard antibiotic (ciproflaxacin). The higher antibacterial activity was observed between *Acinetobactor baumanni* and *MRSA* while the least activity was against *Salmonella enteritidis* and *E.coli*. The activity was high at the higher concentration of 100 mg/ml. From this studied it can be concluded that the extract of *Senna singueana* has both antimicrobial and antioxidant properties even though the two pharmacological activities are lower than the standards. This activities could be as a result phytochemicals such as alkaloids, phenols and flavonoids that are present in the plant. For these reasons the plant *Senna singueana* could be used as an alternative source of the antimicrobial agent against many bacteria strains reported to have developed resistance and source of antioxidant to prevent the development of many oxidative related disorders.

5.2 Recommendation

Recommendation made from this study are

I. Investigation of the quantity of the specific phytochemicals be done
II. Antioxidant property made evaluated using other methods
III. Isolation of the compounds responsible for antioxidant and antimicrobial activity be done

IV. The minimum inhibitory and bactericidal concentration be determined.

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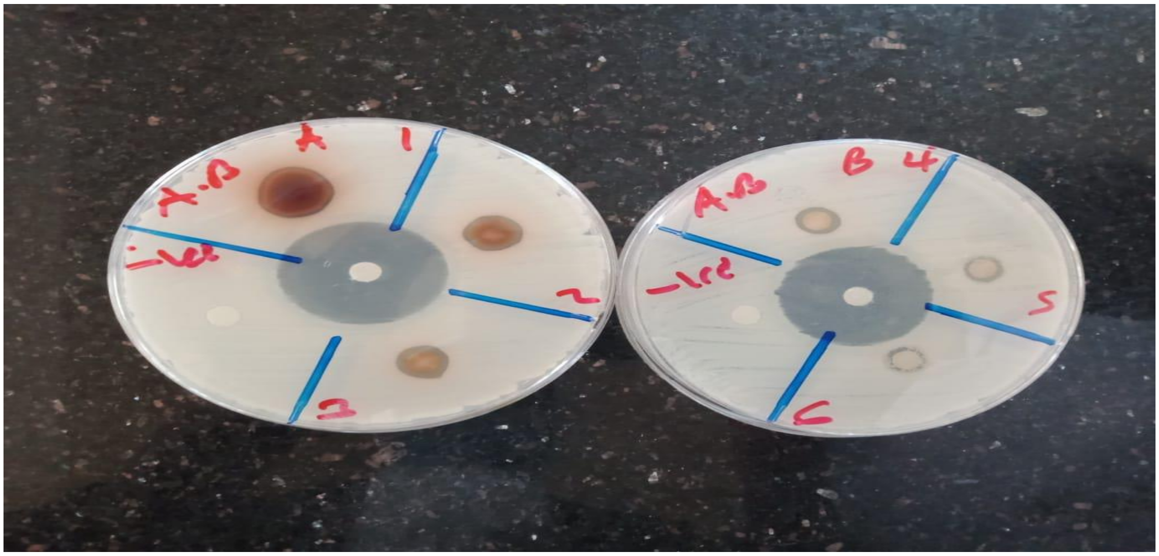
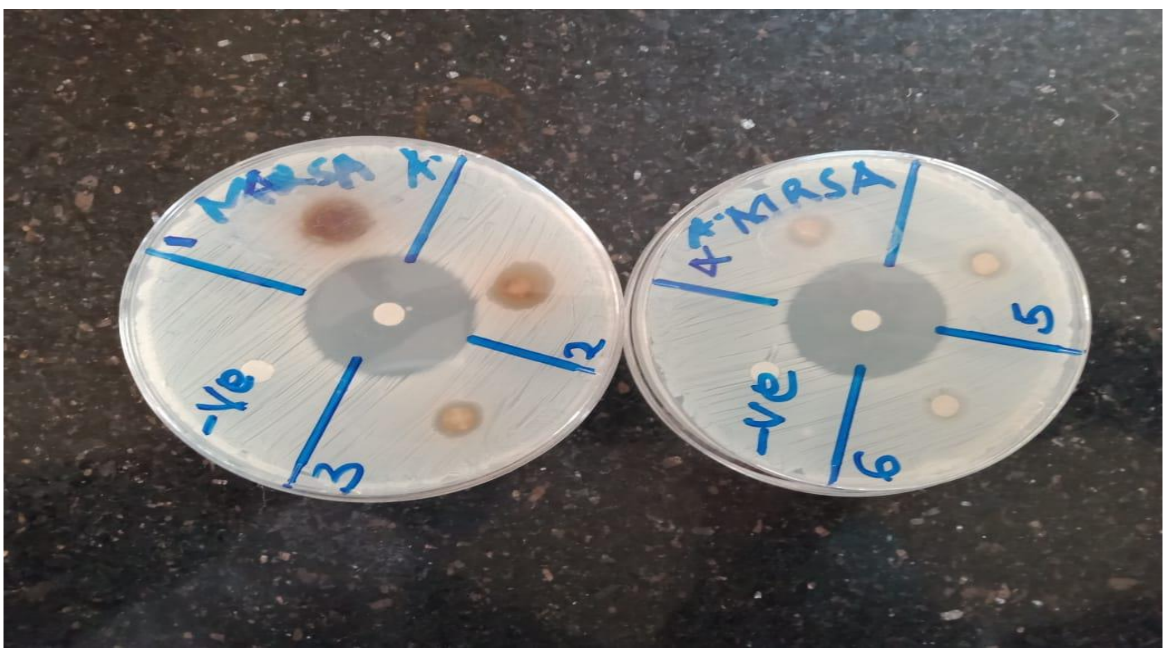
APPENDICES

Appendix 1. 1 Zones of inhibition in the petri dishes





Appendix 1. 2 Antioxidant work in progress