INVITRO ANTILIPID PEROXIDATION AND FERRIC REDUCINGANTIOXIDANT POWER OF METHANOLIC STEM BARK *Lonchocarpus eriocalyx*

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DECLARATION

I declare that this research is my original work and has not been presented in any university for the award of degree. Any source of the information has duly been cited.

Signature…… Date ……

RUTH NJOGA

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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 to my dear Dad, Joseph Njoga, my sisters Esther, Phoebe and Ann Njoga for their unwavering support.

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ABSTRACT

Free radicals are species characterized by unpaired electrons and reactive in nature. These species mainly include two broad classes; reactive oxygen (ROS) species and reactive nitrogen species (RNS). Under normal condition they have beneficial role that involves cell signaling and beneficial oxidation that generates energy. However, under low levels of antioxidants or relapse in the antioxidant defense system free radicals are over produced and they surpass the antioxidant levels. This increase in the free radicals mainly ROS results into oxidation reactions that cause deleterious damages on the cell membrane and even may result into cell death. One of this oxidation reactions is the lipid peroxidation in which the lipids are oxidized by the attack of reactive oxygen species that interferes with the cell membrane functions. The free radicals have as well been linked to the genesis of various disorders and diseases. The control of these deleterious effects involves eradication of the free radicals. Antioxidants are used to scavenger these free radicals and the antioxidants of synthetic nature have been in use for many years. However, these gents have been associated with side effects and are expensive. Plants are alternative source of natural antioxidants that are safe and at the same time potent. In this study the anti-lipid peroxidation activity and the ferric reducing antioxidant power of the methanolic stem bark extract of *Lonchocarpus eriocalyx* was investigated. The anti-lipid peroxidation was assayed by determining the thiobarbituric reactive substances (TBARS) while the reducing power was determined by the ferric reducing antioxidant power method. In this study L-ascorbic acid was used as the standard antioxidant in both assays. The ferric reducing power results showed that the extract recorded a dose dependent reducing power which increased with increase in the concentration. At concentrations 3.125 mg/ml and 1.5625 mg/ml no significant difference in the percentage reducing power was noted ($p>0.05$). However, at the rest of the concentrations of the extract significant difference was noted in the percentage reducing power recorded ($p<0.05$). Similarly, for the L-ascorbic acid, significant difference in the percentage reducing power was noted at 6.25 mg/ml. However, at the other all concentration no significant difference was noted between them ($p>0.05$). At all concentration levels, L-ascorbic acid recorded significantly higher reducing power percentage as compared to the extract ($p<0.05$). The methanolic root extract of *Lonchocarpus eriocalyx* and L-ascorbic acid recorded EC_{50} values of 0.36937 mg/ml and 0.26188 mg/ml respectively. The results for lipid peroxidation inhibition showed that at concentrations 12.5 mg/ml and 6.25 mg/ml and 3.125 mg/ml and 1.5625mg/ml no significant difference in percentage inhibition was recorded for both the extract and the standard ($p>0.05$). However, significant difference was noted at concentrations 0.3906 mg/ml and 0.7813 mg/ml for both extract and the standard ($p<0.05$). The comparison of the peroxidation inhibition activity of the extract to that of standard showed that no significant difference was noted at concentration of 12.5 mg/ml ($p>0.05$). However, at all other concentrations the standard (L-Ascorbic acid) recorded significantly higher percentage inhibition of lipid peroxidation($p<0.05$). In conclusion the methanolic stem bark extract of *Lonchocarpus eriocalyx* it's a potential antioxidant agent with free radical scavenging properties.

CHAPTER ONE: INTRODUCTION

1.1 Introduction

Oxidative stress is a well-known and established factor that has greatly affected the integrity of the biological systems and in the process has influenced the physiological state of the living organisms and chemical composition of the organic matrices as well. The damage due to oxidative stress can be triggered by various physiological processes such as the exposure to environmental factors such as heat and light or oxidizing chemical agents (Bussmann et al., 2006);(Bhat et al., 2015). The main inducer of the oxidative stress is the reactive oxygen species that are produced in the body via the various normal metabolic mechanisms. The oxidative stress condition usually arises due to the imbalance between these reactive oxygen species and the available antioxidant defense system. This is usually as a result of overproduction of these ROS or the decrease in the antioxidant defense system as compared to the available ROS (Hardman et al., 2016);(Sohal & Orr, 2012). The free reactive species that includes both the RNS and ROS attack the biomolecules (nucleic acids, proteins, carbohydrates and lipids) resulting into oxidative damage via the various chemical reactions such as peroxidation, carboxylation, nitration and nitrosylation (Newsholme et al., 2012);(Weidinger & Kozlov, 2015). This results in the decrease in the capacity of the endogenous antioxidant and injuries to the cellular system due to the oxidative stress. The decrease in the integrity of cellular system as a result of the action of the free reactive species (ROS and RNS) serves as the prequalification for the genesis for the various degenerative diseases and disorders that include obesity, aging, inflammation, cognitive impairment, cardiovascular diseases, cancer and Alzheimer's (Guidi et al., 2006).

Humans usually have a well-organized and equipped antioxidant defense system that is tasked with the role of neutralizing the effects of the free reactive species. This role of the antioxidant defense helps to maintain the equilibria between the reactive species and the

antioxidants hence the protecting both the cells and the organ systems from damages due to oxidative stress (Umar et al., 2012). The antioxidant defense system comprises of both the endogenous and the exogenous components that function together with the main aim of neutralizing and preventing the reaction mechanisms that results into generation of the free reactive species (Ishino et al., 2010). This antioxidant defense system comprises of both the non-enzymatic and the enzymatic antioxidants. The non-enzymatic components include the dietary antioxidants such as vitamins E, vitamin C, beta-carotene, glutathione and uric acid. The enzymatic components comprise of the superoxide dismutase (SOD), glutathione reductase, catalase and the glutathione peroxidase (Ishino et al., 2010).

The antioxidant defense system at time is overwhelmed and hence requires back up to avert the destructive effects that may arise to loss in the redox homeostasis. Due to this, various synthetic antioxidants have been used as food additives, preservatives and supplements that help to neutralize the free reactive species. These antioxidants components include the butylated hydroxytoluene (BHT), butylated hydroxyanisole, tertiary butyl hydroquinone, and propyl gallate to avert the lipid oxidation process. These are added as preservatives and food additives. However, various concerns have risen regarding the safety of the antioxidant of synthetic origin. These antioxidants have been proven to have adverse side effects such as hepatic damage, malignancies, allergy reactions and reduced potency in some of the animal models (Arika et al., 2019). This has as well raised concerns by the consumers on the status of the food and the cosmetic products that present in the market. In addition to the adverse side effects, the synthetic antioxidants are as well costly, short shelf life and limited in supply making and then un available to most of the people that are uneconomically stable. Due to this, researchers have increased the moment in search for potent alternative antioxidant that is less toxic, potent and at the same cost friendly from natural products and plants (Salazar et al., 2008).

Medicinal plants are a hub for many phytochemicals that have different pharmacological activities. These plants such *Launaea cornuta* have for the current days been used as the alternative to the many synthetic drugs used to manage various diseases such as cancer and inflammation (Onyancha et al., 2019). The various bioactive constituents have been screened in various scientific researches and in turn developed into the modern medicines(Fai-Chu Wong, 2018);(Arika et al., 2019). Many plants are available globally which are good source of antioxidants. these plants contain phytoconstituents that are effective free radical scavengers hence reducing the oxidation reactions as result of these free reactive species. In addition to the potency in eradication of the free reactive species, these plants render minimal cytotoxicity effects and are as well easily available and cost effective.

Traditionally various medicinal plants have been used in the African setup to treat various ailments that have affect man. *Lonchocarpus eriocalyx* is an example such plants that has been widely used in Kenya among the Embu and Mbeere communities. The *Lonchocarpus eriocalyx* exists as either a tree or shrub and belongs to the family Leguminosae. It has been used in the management of both diabetes mellites and high blood pressure as well as a cognitive enhancer (Moriasi et al., 2020). Through various studies, its ant plasmodial activity and analgesic activity have been validated. Despite the many uses of *Lonchocarpus eriocalyx*, its antioxidant activity has not been clearly elaborated. Hence this studied aimed at evaluating the anti-lipid peroxidation, metal chelating ant catalase activity of methanolic stem bark extract of *Lonchocarpus eriocalyx.*

1.2 Problem statement and justification

Oxidative stress, the condition due to loss of homeostasis between the reactive oxygen species and the already present antioxidant defense system, plays a very big pathophysiological role in the manifestation of the various diseases such as the liver disease, cancer, aging, autoimmune disorders and cardiovascular as well as the neurodegenerative

diseases (Bhat et al., 2015). The increased production of the reactive oxygen species such as the hydroxyl radicals, hydrogen peroxide and the nitric oxide radical results into the oxidative degradation of the lipids (Stark, 2005). This is a result of their act on the polyunsaturated fatty acids that are present in the plasma membranes. Additionally, the oxidation of the lipids triggers cellular and tissue damage via the covalent binds resulting into lipid peroxidation, damage to the DNA, inflammation and eventually cell aptosis (Chang & Kim, 2018). In the food industries the oxidation of the lipids present in the foods and the related food products results in the reduced quality, bad odor and synthesis of toxic compounds. In this manner the shelve-life of the food and food products, feeds and cosmetic products is interfered with resulting into loses.

To avert all this, synthetic antioxidants are added in foods as preservatives and food additives mainly to present the production of the excess ROS. These synthetic antioxidants scavenge the free radicals that are produced or prevent the production of the free radicals. This results into inhibition of the lipid peroxidation. The used synthetic antioxidants include the BHT. However, the use of these synthetic antioxidants both in the food industries and health systems is declining. This is as a result of the negative effects that are associated with these products on both the human health and the environment (Weidinger & Kozlov, 2015). The negative effects have resulted into the need for the natural antioxidants by the manufacturers of foods that are prone to lipid peroxidation. This has been ignited by the growing market for the healthy food. Therefore, there is need for the alternatives to these synthetic antioxidants that are safer and as well cost effective. Plants of medicinal values have been for the better part of man's history the source of relive for the ailments that has affected man. Medicinal plants are reported to contain various phytochemicals that elicit different biological activities such as antioxidant, antidiabetic, antimicrobial and anti-inflammation. Even though plants are the after sought as the reservoirs for the natural antioxidants, little information is available

about their activities. The various plants have not been tested for and even their mode of action in preventing the mechanisms that results in the production of the ROS and even mode of action in inhibiting the oxidation of lipids. In this study the anti-lipid peroxidation and the metal chelating activity of the *Lonchocarpus eriocalyx* was evaluated by adopting the various antioxidant models.

1.3 Objective

1.3.1 General objective

To evaluated the anti-lipid peroxidation and ferric reducing antioxidant power of methanolic bark extract of *Lonchocarpus eriocalyx.*

1.3.2 Specific objectives

I. To evaluate the lipid peroxidation inhibition activity of methanolic stem bark extract of *Lonchocarpus eriocalyx.*

II. To determine the ferric reducing power of methanolic stem bark extract of *Lonchocarpus eriocalyx*

1.4 Research questions

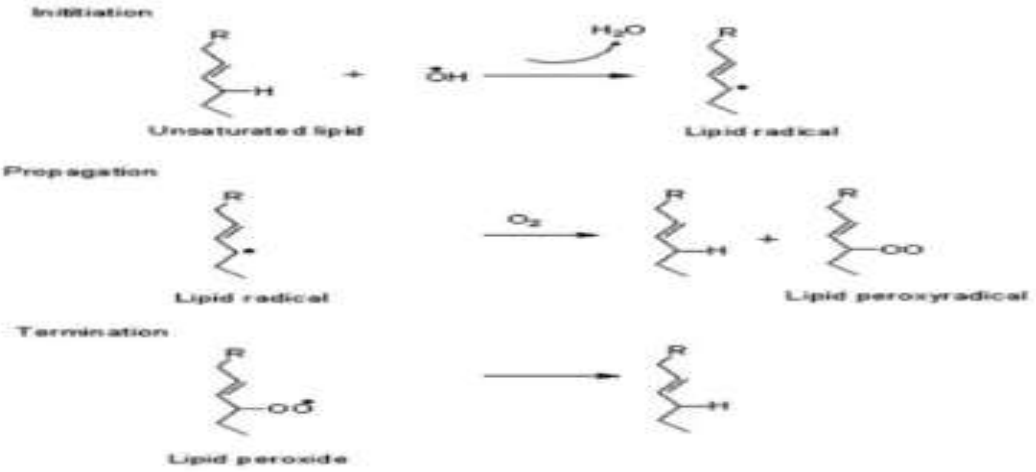
I. Do the methanolic stem bark extract of *Lonchocarpus eriocalyx* have the anti-lipid peroxidation ability?

II. Do methanolic stem bark extract of *Lonchocarpus eriocalyx* have reducing power properties?

CHAPTER TWO: LITERATURE REVIEW

2.1 Lipid peroxidation

Lipid peroxidation involves the oxidative damage of the lipids which is generated as a result of attack by reactive oxygen species resulting into formation of the peroxides. The peroxidation of the lipids results into the disruption of the lipid bilayer present in the cell membrane. This interrupts the physiological roles that includes the selective permeability of the cell membranes. When there is rearrangement in the lipid bilayers the inactivation of the receptors and the enzymes that bind on the membrane may and in the process resulting into increased permeability(Marisa Repetto, 2012). Peroxides which ae the products of lipid peroxidation are compounds with generalized structural formula 'R-O-O-R' where by the 'O' are the oxygen molecules with oxidative state -1. The peroxides include the isoprostanes and thiobarbituric acid reactive species such as MDA. These products are used the markers of oxidative stress. Lipids are prone to peroxidation due the methylene groups present adjacent to the double bonds in the structure of the lipids. The mainly attacked lipids includes the PUFAs, that are either in the form of triacyl glycerides (TAGs) or free fatty acids (FFAs). The polar lipids that contain the PUFAs such as the glycolipids, phospholipids and sphingolipids and cholesterol.



Mechanism of lipid peroxidation in unsaturated fatty acid molecule(s) (World Wide Web. http://en.wikipedia.org/wiki/Lipid peroxidation. Accessed on October 30, 2013.)

2.2 Mechanism of lipid peroxidation

The lipid peroxidation is a stepwise process that entirely consist of three steps; initiation, propagation and termination (Yin et al., 2011). Initiation, the first step involves removal of hydrogen from the lipid by the ROS such as hydroxyl radical resulting into formation of the lipid radical. In the second step, propagation, the resultant lipid radical from the first step reacts with oxygen to form lipid peroxy radical. The lipid peroxy radical formed removes hydrogen from anew lipid molecule to form another lipid radical and lipid hydroperoxide (LOOH). The lipid radical from step two continues the chain reaction. The last step is termination and it involves donation of the hydrogen to the lipid peroxy radical by the antioxidants such as vitamin E. the resultant corresponding vitamin E, eventually reacts with another lipid forming a product that is not a radical.

2.2.1 Initiators of lipid peroxidation

Lipid peroxidation is initiated mainly by exposure of the lipid molecule to the oxygen and the reactive oxygen species that are generated via the various chemical mechanisms, light or even

high temperatures (Yin et al., 2011). The reactive oxygen species are generated by various means,

2.2.1.1Reactive oxygen species

These comprises of the reactive species that are of molecular oxygen origin and are the by-products of the normal cellular metabolism. They are grouped into two main categories, the free radicals and the nonradicals. The free radicals are those that have either one or more unpaired electrons hence having the reactivity status while the nonradicals are those that results after the combination of two free radicals and in the process sharing the unpaired electrons. The free radical ROS include the superoxide anion (O^{-2}) and the hydroxyl radical ($^{\cdot}$OH) while the nonradical include the hydrogen peroxide(H_20_2). The reactive oxygen species are both of endogenous and exogenous origin. The endogenous source of reactive oxygen species are the mitochondria, plasma membrane, endoplasmic reticulum and the peroxisomes (Chang & Kim, 2018). The ROS from these sources are generated via a variety mechanism such as enzymatic reactions. The in vivo sources of ROS are through the exogenous stimuli such as ionizing radiation, agricultural chemicals, tobacco smoke, infections due to pathogens and ultraviolet radiations (Halliwell, 2012).

2.2.1.1.2The endogenous origin

In the food and cosmetic industries, the oxidation of the lipids has greatly impacted this sector negatively. The food and cosmetic products that are prone to lipid oxidation by the reactive oxygen species have been affected as lipid oxidation is the major cause of the chemical deterioration of these products. The alteration of the lipid oxidation of these products negatively affects the appearance, test and smell off the products. These industries have for many years employed the use of synthetic antioxidants such as the butylated hydroxytoluene (BHT), butylated hydroxyanisole, tertiary butyl hydroquinone, and propyl gallate to avert the lipid oxidation process (Fai-Chu Wong, 2018). These are added as

preservatives and food additives. However, various concerns have risen regarding the safety of the antioxidant of synthetic nature. These antioxidants have been proven to have adverse side effects such as. This has resulted in the concerns by the consumers on the status of the food and the cosmetic products that present in the market.

2.3 Herbal management of oxidative stress

Herbal medicine is regarded as the oldest form of healthcare to humanity as its use dates back to many centuries (Petrovska, 2012). The therapeutic value of the herbal medicine and its ability to treat majority of the emerging diseases has been boosted by support from the WHO (Olela et al., 2020). Due to its affordability, efficacy and safety it has been regarded as the most sought for means of achieving total health when compared to the conventional medicine (S. Antwi-Baffour, 2014). The medicinal plants are widely used with about 80 % of the global population relying on them as the primary source of healthcare (WHO 2015). In most of the African countries herbal medicine is part of their culture hence they are more accepted than the modern medicine(Obakiro et al., 2020).

Through various studies, medicinal plants have shown to be potential source of medicine for management of various ailments. This has been linked to the many phytoconstituents that plants contain (Obakiro et al., 2020). These bioactive compounds that are present in the various plant are thought to be the leads to medicine in use currently such as the anticancer and analgesics (Misonge et al., 2015). Various medicinal plants that are potent antioxidant are available and used in compacting oxidative stress and its related disorders. These include *Hypericum keniense, Piliostigma thonningii* and many more. The potential antioxidant activities of these plants have been shown through the various studies (Cai et al., 2004). The pure compounds or crude extracts from these plants have shown to be effective antioxidants as compared to even vitamin E and BHT via the invitro antioxidant assays (Gu & Weng, 2001). Additionally, the medicinal plants have shown to exhibit much higher antioxidant

activity that is directly proportional to the higher levels of phenolic compound as compared to some of the common vegetables and fruits (Cai et al., 2004).

2.4 *Lonchocarpus eriocalyx*

2.4.1 Botanical description

Lonchocarpus eriocalyx is a small deciduous plant or shrub. Its slender and grows to heights of between 3-12 m tall. The tops usually form round crowns and the barks are pale greyish in colour (Jong et al., 2012).

2.4.2 Ethnomedicinal uses

The infusion of the bark is used as a remedy for fever, headache, diarrhea and as an insecticide as well (Ceres et al., 1981; Kokwaro, 2009; Adem et al., 2018). The barks have also been used in the management of blood pressure and reduce the sugar levels among the Embu and Mbeere communities in Kenya (Kareru et al. 2006).

2.4.3 Phytochemistry

The various phytochemical studies on the *Lonchocarpus eriocalyx* revealed the presences lupeol triterpene. This compound has been reported to possess anti-plasmodial activity against plasmodium ovale (Ochung et al., 2020). The phytochemical screening of the chloroform and methanolic leaves extracts resulted into isolation of eight compounds. The study conducted by Moriasi et al.(2020) on the aqueous and methanolic stem bark extract revealed the presence of cardenolide glycosides, coumarins, phenols, steroids, saponins, and flavonoids in both the extracts. However, alkaloids and tannins were only present in the aqueous extract.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Collection and preparation of the plant material

The bark stem plant samples of the *Lonchocarpus eriocalyx* were gotten from the healthy *Lonchocarpus eriocalyx* trees by using a knife. The plants were identified as by the local herbalist and further identified at the museum of Kenya where it was given the voucher number MMK/BOT/CTX/2/2. Two voucher specimens were prepared and one deposited at the herbarium of national museum of Kenya and the other in the Mount Kenya herbarium in the school of pharmacy. These barks were transported to pharmacognosy laboratory in Mount Kenya university. They were then sorted and unwanted material separated then air dried for two weeks by spreading on the laboratory benches. The dried samples were then powdered by help of the plant mill and then packed in bags awaiting extraction.

3.2 Extraction

The stem bark samples of *Lonchocarpus eriocalyx* were extracted with methanol following the procedures of (Moriasi et al., 2020a). About 250 g of the powdered plant materials were soaked into 500ml of analytical grade methanol in one-liter conical flask and kept under occasional agitation for 48 hours. On the third day the sample was filtered and the filtrate concentrated under reduce pressure in a rotary evaporator. The concentrated extract was the transferred into the already weighed sample bottle, completely dried in a hot air oven at 35 ^{0}c for one week and then weighed again to demine the yield. The sample bottle was then completely capped and sealed with parafilm and then kept in a fridge at 6 ^{0}c awaiting the analysis.

3.3 Reagents and chemicals

The reagents and chemicals used in this study were all pure and of analytical grade. These included, acetic acid, dichromate, potassium ferrocyanide, sodium dihydrogen phosphate and

disodium hydrogen phosphate, ferric chloride, thiobarbituric acid (TBA), trichloroacetic acid and ferrous sulphate.

3.4 Preparation of the egg yolk homogenate

The hen egg was broken and then the yolk separated. It was then homogenized and from the homogenate 10% v/v working solution was prepared with phosphate buffer.

3.4 The reducing power of the methanolic stem bark extract of *Lonchocarpus eriocalyx*

The reducing power of the methanolic stem bark extract was determined by adopting the protocols of (Moriasi et al., 2020b) with minor modifications. Briefly 1 ml of the different concentrations of the extract were added into the respective labeled test tubes and then 2.5 ml of phosphate buffer added into each test-tube followed by 2.5 ml of1%v/v potassium ferricyanide. The reaction mixtures in the test-tube were then mixed by swirling and then incubated in water at 50 ^{0} C for 20 minutes. The mixtures were cooled to room temperature and then 2.5 ml of TCA was added, completely mixed and then centrifuged at 5000rpm for 10 minutes. The supernatant was then separated and 2.5 ml of it mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% v/v $FeCl_3$. The absorbance of the mixture was then measured at wavelength 700nm using the UV-Vis double beam spectrophotometer.

3.5 Invitro inhibition of lipid peroxidation

The lipid peroxidation inhibition ability of the methanolic extract of *Lonchocarpus eriocalyx* was determined by the modified TBARS method as outlined by (Yoshino & Higashi, 2005). Briefly, 0.5 ml of the egg yolk homogenate and 0.1 ml of the different concentrations of the methanolic extract/ascorbic acid were added into different labeled tubes. Into these tubes 0.5 ml of 24mM of ferrous sulphate was added followed by 0.5ml of phosphate buffered saline buffer. 1 ml of the 0.8% TBA was added and the reaction mixtures incubated in aware bath

at for minutes. The samples were then cooled to room temperature and the reaction stopped by adding 0.5 ml of 20 % TCA

3.6 Statistical analysis and data management

The lipid peroxidation and ferric reducing power data was tabulated in the excel spread sheet and then imported in the mini-tab software for descriptive statistics. The data was then presented as mean ± standard error of mean. The data was then analyzed by the one-way anova followed by Fisher's LSD test to determine the level of significance. The results were then presented in form of tables.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Ferric reducing antioxidant power of the methanolic stem bark extract of *Lonchocarpus eriocalyx*

The results for the ferric reducing antioxidant power of the *Lonchocarpus eriocalyx* are presented in table 4.2. the results showed that the ability of the methanolic stem bark extract of *Lonchocarpus eriocalyx* to reduce the ferric ions at concentrations 12.5, 6.25, 0.7813 and 0.390625 mg/ml was significantly different ($p<0.05$). However, at concentration 3.125 and 1.5625 mg/ml no significant difference in the percentage radical scavenging activity was recorded ($p>0.05$). L- Ascorbic acid at concentration 6.25 mg/ml recorded significant difference in ferric reducing power ($p<0.05$). however, at concentrations 12.5 and 3.125 mg/ml there was no significant difference in the ferric reducing power recorded ($p>0.05$). likewise, no significant difference was recorded at concentrations 1.5625, 0.7813 and 0.390625 mg/ml in the ferric reducing power ($p>0.05$). the comparison of the methanolic stem bark extract of *Lonchocarpus eriocalyx* with the L-Ascorbic acid revealed there was significant difference in the percentage radical scavenging activity at all the concentrations ($p<0.05$). the L-ascorbic acid recorded significantly higher ferric reducing power as compared to the methanolic stem bark extract of LE at all concentrations. The a methanolic stem bark extract of *Lonchocarpus eriocalyx* and L-ascorbic acid recorded IC 50 of 0.36937 and 0.26188 respectively. This showed that the L-ascorbic acid generally performed better than the plant extract in reducing iron (iii) to iron (ii).

Table 4. 1 Ferric reducing power of the methanolic stem bark extract of *Lonchocarpus eriocalyx*

Concentration (mg/ml)	Percentage FRAP	
	Methanolic stem bark extract of *L. eriocalyx*	L- Ascorbic acid
0.390625	$52.81\pm1.87^{D}_{b}$	$74.173\pm0.573^{B}_{a}$
0.7813	$58.20\pm 1.26^{C}_{b}$	$72.797\pm0.882^{B}_{a}$
1.5625	$64.91\pm1.43^{B}_{b}$	$73.917\pm0.846^{B}_{a}$
3.125	$67.210\pm0.794^{B}_{b}$	$80.217\pm0.401^{A}_{a}$
6.25	$68.33\pm0.838^{AB}_{b}$	$77.00\pm3.64^{AB}_{a}$
12.5	$71.86\pm1.14^{A}_{b}$	$80.88\pm1.10^{A}_{a}$
EC_{50}(mg/ml)	0.36937	0.26188

Values are expressed as mean±SEM; means with similar superscript letters within the same column are not significantly different (One-Way ANOVA with Fisher's LSD test; P>0.05), whereas, means with dissimilar subscript letters across the rows are not significantly different (Unpaired student t-test statistic; p>0.05)

4.2 lipid peroxidation activity of the methanolic stem bark extract of *Lonchocarpus eriocalyx*

The results for the anti-lipid peroxidation activity of the methanolic stem bark extract are outlined in table 4.2. the results showed there was no significant difference in the percentage inhibition of lipid peroxidation by the methanolic extract at concentration 12.5 and 6.25 mg/ml and at concentration 3.125 and 1.5625 respectively ($p>0.05$). however, there was significant difference in the percentage inhibition of lipid peroxidation recorded at concentrations 0.7813 and 0.3906 mg/ml ($p<0.05$). L-Ascorbic acid the positive control showed no significant difference in the percentage inhibition of lipid peroxidation at concentrations 12.5 and 6.25 mg/ml and at 3.125 and 1.5625 mg/ml ($p>0.05$). however, at concentration 0.7813 and 0.3906 mg/ml significant difference in the percentage inhibition lipid peroxidation was noted ($p<0.05$). at concentration 12.5 mg/ml for both the methanolic extract and L-Ascorbic acd no significant difference was noted in the percentage inhibition of lipid peroxidation ($p>0.05$). however, there was no significant difference in the percentage inhibition of lipid peroxidation for both the L-ascorbic acid and the extract at concentrations 6.25 mg/ml, 3.125 mg/ml,1.5625 mg/ml, 0.7813 mg/ml and 0.3906 mg/ml.

Table 4. 2 lipid peroxidation activity of the methanolic stem bark extract of *Lonchocarpus eriocalyx*

Concentration (mg/ml)	**Percentage inhibition of Lipid peroxidation *in vitro***	
	Methanolic stem bark extract of *L. eriocalyx*	**L-Ascorbic acid**
12.5	$64.880\pm3.450^{a}_{a}$	$69.140\pm3.080^{a}_{a}$
6.25	$61.452\pm0.709^{a}_{b}$	$66.125\pm0.677^{a}_{a}$
3.125	$53.533\pm0.048^{b}_{b}$	$59.168\pm0.023^{b}_{a}$
1.5625	$48.360\pm2.520^{b}_{b}$	$54.620\pm2.290^{b}_{a}$
0.7813	$28.692\pm0.060^{c}_{b}$	$38.203\pm0.817^{c}_{a}$
0.3906	$10.381\pm0.548^{d}_{b}$	$22.890\pm1.280^{d}_{a}$
0.0000	$0.0000\pm0.000^{e}_{a}$	$0.0000\pm0.000^{e}_{a}$
IC_{50} (mg/ml)		

Values are presented as $\bar{x} \pm SEM$; Means sharing a superscript letter within the same column are not significantly different by One-Way ANOVA followed by Fisher's LSD test ($p>0.05$), whereas means that share a subscript letter within the same concentration (across the rows) are not significantly different by unpaired student t-test statistic ($p>0.05$).

The imbalance between the free radicals and the antioxidants as a result of the increased production of the reactive oxygen species results into oxidative stress as they form the catalyst for the oxidation of the biomolecules (Hazra et al., 2008). The antioxidants are tasked with the role of reacting, neutralizing with the reactive oxygen species or even competing for the substrates with the free radicals (Arika et al., 2019). Originally the body has the antioxidant defense system that is usually helps in eradicating the excess reactive oxygen species. The defense system consists of both the enzymatic and non-enzymatic antioxidants. In the situation the antioxidant defense system is overwhelmed, supplementary antioxidants are provided. These consists of the synthetic antioxidants that are commercially available. However, they are reported to have side effects that includes high toxicity and even being carcinogenic. These emerging side effects of the synthetic antioxidants have offered the natural antioxidants whose origin is from plants a chance to be the better alternative that helps in the reduce the oxidative damages that arises as a result of the free radicals (Wojdyło et al., 2007). Herbal plants with medicinal value have been reported to be useful therapeutic

agents that help in the prevention and management of the disorders that include the degenerative diseases that are related to the oxidative stress (Oyedemi et al., 2010). In this study the methanolic stem bark extract of *Lonchocarpus eriocalyx* was investigated for its anti-lipid peroxidation and the ferric reducing antioxidant power.

Iron (ii) has been regarded as the catalyst for process of generating hydroxyl radicals that are generally highly reactive in nature and results into damages on the cells and even tissues (Ott et al., 2007);(Stark, 2005). These radicals cause oxidative damages by attacking the biomolecules (Protein, lipids and DNA) (Balu et al., 2005). The hydroxyl radicals react with the polyunsaturated fatty acids present in the phospholipids bilayers of the cell membranes resulting into lipid peroxidation (Valko et al., 2007). They as well cause damages to the DNA by breaking the linkages that holds together the nucleotides and this alters the structure of the DNA bases structure and this contributes to cytotoxicity, mutagenicity and carcinogenicity (Balu et al., 2005). The evaluation of the ferric reducing activity is therefore an important marker for antioxidant activity elicited by natural antioxidants (Brewer, 2011). In this study, the ferric reducing power of the methanolic stem bark extract of LE was investigated. The reducing ability of the study compounds is usually based on the reductive capacity in the iron (iii)- iron (ii) system (Gülçin, 2005). The natural bioactive compounds that have the ferric reducing power are regarded as electron donors and they are capable of reducing the intermediates prone to oxidation such as those involved in the process of lipid peroxidation (Wojdyło et al., 2007). The results obtained from the ferric reducing activity of the methanolic stem bark extract of *Lonchocarpus eriocalyx* at all studied concentrations followed the bear lamberts law at 700nm (Bajpai et al., 2014). That is the reducing activity increased with increase in the absorbances recorded. The reduction of the iron (iii) to iron (ii) indicates the electron donating ability (Farhan et al., 2012).

The anti-lipid peroxidation activity of the methanolic stem bark extract of *Lonchocarpus eriocalyx* was as well evaluated in this study. This was through determining the thiobabuturic acid reacting substances such MDA which are used as the markers of oxidative stress. The MDA is produced as by product of lipid peroxidation and it has been associated with the pathogenesis of various diseases and disorders such as inflammation and inflammatory diseases, cancer, atherosclerosis, diabetes mellitus and Alzheimer's disease (Arika et al., 2019). The results for the anti-lipid peroxidation activity of the methanolic stem bark extract of *Lonchocarpus eriocalyx* that at concentrations 12.5 mg/ml and 6.25 mg/ml and concentrations 3.125 mg/ml and 1.5625 mg/ml showed similar anti-lipid peroxidation activity respectively. At concentrations 0.7813mg/ml and 0.3906 mg/ml significant difference in the ability of the methanolic stem bark extract of *Lonchocarpus eriocalyx* ta to inhibit lipid peroxidation.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion basing on the findings from this study its evident that the methanolic stem bark of *Lonchocarpus eriocalyx* is a potential antioxidant. It has the ability to donate electrons to the reactive oxygen species in the body preventing them from attacking the biomolecules. This prevents the oxidation reactions such as lipid peroxidation that has been linked with pathogenesis of various disorders and diseases. The ferric reducing activity and the anti-lipid peroxidation activity of the methanolic stem bark extract of *Lonchocarpus eriocalyx* may be attributed to the various bioactive compounds that are present in this plant.

5.2 Recommendation

From this study the following recommendations can be made:

I. The toxicity test of this extract to be conducted to evaluate its safety.
II. *Invivo* antioxidant activities to be conducted
III. A guided isolation of the active compounds by column chromatography should be done

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