TOTAL FLAVONOID CONTENT, TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF *Uveriodendron anisatum* ROOT AND FRUIT METHANOL EXTRACTS

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DECLARATION

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

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Signature …… Date ……

Supervisor's approval

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

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DEDICATION

I dedicate this work to my parents Mr Mhamed abdi and Mrs asha Mohamed abdi jilal and my uncle abdi Mohamed abdi jilaal for their support throughout my entire education. Their affection, unconditional love, encouragement and dedication for the uplifment in my life is incredible, I owe them a lot for their selfless support towards my education.

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ABSTRACT

Medicinal plants have gained favor among many researchers globally. This has been attributed to their therapeutic properties that has been key in management of many diseases including microbial infections, inflammation and neurodegenerative diseases. This pharmacological properties are as result of the bioactive compounds such as phenolics that are widely distributed in many plants. These compounds play a great role as antioxidants that aid in quenching free radicals preventing the development of oxidative stress and its related disorders. The evaluation of these bioactive compounds and their pharmacological properties is still insufficient. This study aimed at evaluating the total phenolic, total flavonoid and radical scavenging activity of methanol fruit and root extract of *Uvariodendron anisatum.* Folin-Ciocalteu's method and calorimetric aluminum chloride method were used to evaluate total phenolic content and total flavonoid content respectively. The 2.2-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging assay was used to evaluate the antioxidant. L-ascorbic acid, gallic acid and catechin were used as the standards for antioxidant, total phenolic content and total flavonoid content respectively. The total phenolic content and total flavonoid were calculated from the regression equation of the gallic standard and catechin standard curve and presented as mg GAE/ g of dry weight and CE /g of dry weight respectively. The methanol root and fruit extract recorded total phenolic content of 14.627±0.078 mg GAE /g and 15.013±0.047 mg GAE /g and total flavonoid content of 7.894±0.002 mg CE /g and 8.057±0.009 mg CE /g respectively. The total phenolic content of fruit extract was significantly higher from that of root extract ($P<0.05$). However, the total flavonoid content in both the two extracts were not significantly different from each other ($P>0.05$). The 2.2-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging activity results showed a dose depended activity in both the fruit and root methanol extract of *Uvariodendron anisatum* and L-ascorbic acid. The methanol root extract at 0.01 µg/ml,0.1 µg/ml and 1 µg/ml recorded significantly higher percentage radical scavenging activity as than both methanol fruit extract and L-ascorbic acid ($p<0.05$). The standard, L-ascorbic acid at 10 µg/ml, 100 µg/ml and 1000 µg/ml significantly recorded high percentage radical scavenging activity as than both methanol root and fruit extracts ($p<0.05$). Methanol fruit and root extract recorded IC_{50} of 4.680 µg/ml and 5.080 µg/ml for fruit and root respectively while L-ascorbic acid record the IC_{50} of 3.420 µg/ml. The methanol fruit extract showed higher antioxidant activity that correlated to its higher phenolic compound. In conclusion, this plant is a potential antioxidant agent with the ability to scavenge the free radicals.

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LIST OF ABBREVIATIONS

%RSA	Percentage radical scavenging activity
BHA	Butylatedhydroxyl anisole
BHT	Butylatedhydroxyl toluene
CE	Catechin
DNA	Deoxy nucleic acid
DPPH	2,2 diphenyl-1-picrylhydrazyl
EGCG	Epigallocatechin-3- gallate
mgGAE/ g DW	Milligram of gallic acid per gram dry weight
TPC	Total phenolic content

CHAPTER ONE: INTRODUCTION

1.1 Background Information

The herbal medicine arena is gaining popularity each time as the human population that continues to grow (Arika et al., 2019). The use of herbs in treating a range of ailments continues to grow globally and this is evident in the world health organization report (WHO, 2013). In this report about 65%-85% of the global population that mostly reside in the rural setups in developing countries relies entirely on medicinal remedies from plants as the source of primary health care (Kigen et al., 2014). This has been the case since the rural are characterized by lower numbers of medical personnel (Arika et al., 2019). Additionally, many of the modern medicines are believed to be from the medicinal plants' products and are isolated. These compounds have been used as active ingredients in the allopathic medicines. Many of these bioactive plant-derived constituents including phenolic compounds, flavonoids, alkaloids, tannins and many more are reported to confer different pharmacological activities such as antioxidant, antimicrobial and anti-inflammatory (G. Moriasi et al., 2020).

The oxidation reaction is an important physiological process in the human body. This process involves the production of the reactive free radicals that are important in cell signaling as by products. However, the overproduction of these free reactive species of either oxygen or nitrogen origin results into the imbalance between the prooxidants and the antioxidants (Durairaj et al., 2014). Free radicals contribute greatly on genesis of oxidative stress which is characterized by increased population of the free radicals in the body as compared to the antioxidants and reduced quenching of these free radicals. Free radicals attack biomolecules; proteins, lipids and nucleic acid (DNA) causing oxidative damage to them or even death through apoptosis or necrosis (Kurutas, 2016). The oxidative damage to the biomolecules has been associated with the development of many chronic conditions such cancer, hypertension, depression, coronary artery diseases, diabetes and degenerative disorders such as Alzheimer's

disease (Aryal et al., 2019; Losada-Barreiro & Bravo-Díaz, 2017). Human and animal bodies eradicate excess free radicals by using endogenous antioxidant defense system that comprises of both the antioxidant enzymes and non-enzymatic antioxidants (Kurutas, 2016). These antioxidants are synthesized by the body and act as the first line of defense against deleterious effects of free radicals (Arika et al., 2019). The body also gets antioxidants from foods such as fruits, vegetables, seeds, nuts, meats and oil (G. Moriasi et al., 2021).

The human body constantly removes the free radicals in the body as the normal routine cheek up. However, with time the internal antioxidant defense system is overwhelmed (Aryal et al., 2019) calling for the need to incorporation other antioxidant agents (Leverve, 2009). Many synthetic antioxidant agents such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) are used in this case. They are mostly added as food preservatives to prevent the various oxidation reactions such as lipid peroxidation which destroy food and cosmetic products and even resulting into carcinogenesis (G. A. Moriasi et al., 2020a). Many pharmaceutical products are available and are used to manage many oxidative associated conditions. For example, donepezil is used for treating the dementia condition (G. A. Moriasi et al., 2020b). However, the synthetic antioxidants and the other pharmaceutical drugs have been reported to result into side effects (Arika et al., 2019). Additionally, they are expensive and with reduced efficacy as many of the products end up only reducing the symptoms alone (Olela et al., 2020). This has shown the need for the research in the natural product field with the aim of finding an alternative natural agent with potent antioxidant properties.

Nature has been the main source of the natural remedies that man has been using for a very long time in treating various diseases (Nyaboke et al., 2017). The use of these plants was entirely depended on the folktales which were different among different cultures. The therapeutic properties that have been claimed to be due to the various plants have been as result of the active compounds known as phytochemicals. These compounds are synthesized by

plants and vary from one region to the other. Phytochemicals that exhibit antioxidant activities include phenolics, flavonoids, tannins and alkaloids that show various biological activities such as antioxidant, antidiabetic and anticancer. Flavonoids and phenolic are phytochemical compounds characterized by an aromatic ring bearing at least one hydroxyl group. These compounds naturally occur in plants and about 8000 phenolic compounds have been reported. Most of these compounds have been reported to be potent antioxidants, anticancer, antibacterial, cardioprotective agents, immune-modulatory agents and skin protecting agents. These biological properties have presented them as the best suitable candidates for both pharmaceutical and medical applications (Tungmunnithum et al., 2018);(Chen et al., 2015). The various beneficial effects of the flavonoids and other phenolic compounds to human health have greatly facilitated their increased attention by many researchers. Many plants that are used as antioxidants exist all over the world including Kenya. *Hypericum perforatum* for example is a potent antioxidant that is used as an antidepressant. *Uvariodendron anisatum* is a shrub or small tree that grows to about 9 m tall. It's endemic in the central and southern parts of Kenya and locally identified as "Mutonga" and "Mutunga" by the kikuyu and Meru communities. This plant is traditionally use as aremedy for stomach ache and anaplasmosis among the Meru communities (Mutembei et al., 2018a)

1.2 Problem Statement and Justification

The adverse effects associated with oxidative stress in human being body has become public health problem globally. Oxidative stress is the imbalance of the free radicals and the counter reactors antioxidants with the free radicals being the main etiology of this condition. Free radicals consisting of the reactive oxygen and nitrogen species attack the biomolecules (lipid, protein and DNA) causing oxidative damage and in some severe cases resulting in cell death. Oxidative stress is also associated with many chronic disorders such as cancer, diabetes, degenerative diseases such as aging, cardiovascular diseases and neurodegenerative disorders

such as Alzheimer's disease (Ahmed et al., 2016; Ilahi et al., 2013). The treatment of the oxidative related disorders mainly requires quenching of the free radicals to their required levels that does not result into deleterious effects. This has seen use of the synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) that are able to eliminate the free radicals. However, these antioxidants have in the recent days received backlash due to the side effects observed upon use(Ahmed et al., 2016). For instance in the study conducted it relieved that these synthetic antioxidants resulted into liver damages and carcinogenesis in laboratory animals(Kumar G et al., 2010). In other circumstances some synthetic drugs are used to manage the oxidative related diseases. These drugs are very expensive and in most of the times they only relieve signs and symptoms. Other therapies react with the body for example the chemotherapies done to the cancer patients. This has seen many consumers turn away from them and look for alternatives from natural sources such as plants. In the same spirit, the manufacturers have appreciated the fact that the search for an alternative antioxidant that are safe and potent is inevitable.

Antioxidants from plants have gained much attention from all the researchers due to their important value both in nutrition and therapeutic. Antioxidant property of many plant and plant-derived compounds and extract remains the most important pharmacological activity. This has been the case as the antioxidants show varied biochemical activities that are beneficial to the body. Antioxidants act as inhibitors of the reactive oxygen species generation, scavengers of the free radicals and altering the intracellular redox potential. Additionally, antioxidants have been shown to have anticancer or anticarcinogenic properties. For instance, Epigallocatechin-3- gallate (EGCG) present in green tea was reported through a study to be able to scavenge free radicals and inhibit carcinogen-induced in both skin, lung, fore stomach and colon of rodents. All this has presented undeniable evidence that plants are source of natural antioxidants. These antioxidants are not only safe but cheap and easily accessible as

well. The studies of the bioactive compounds from plants is therefore important as plants are the links to safer therapy of many diseases affecting many. The increasing reliance of these natural antioxidant plants needs an increased exploitation of the plants with the various important pharmacological properties. Hence the need for further exploitation and study of the various bioactive compounds such as phenolics and flavonoids and the antioxidant properties of plants is inevitable. Therefore, this study aimed at evaluating the antioxidant activity and determining the total phenolic and flavonoid contents of methanolic root and fruit extracts of *Uvaridendron anisatum*.

1.3 Objectives

1.3.1 General objective

Evaluation of the total phenolic content, total flavonoid content and antioxidant activity of the methanol root and fruit extract of *Uvaridendron anisatum*

1.3.2 Specific objectives

I. To determine the total phenolic content of methanol root and fruit extracts of *Uvaridendron anisatum*

II. To determine the total flavanoid content of methanol root and fruit extracts of *Uvaridendron anisatum*

III. To evaluate the DPPH free radical scavenging activity of methanol root and fruit extract of *Uvaridendron anisatum*

1.4 Research questions

I. What is the quantity of the total phenolic content in methanol root and fruit extract of *Uvaridendron anisatum*?

II. What is the quantity of total flavonoid content in methanol root and fruit extract of *Uvaridendron anisatum*?

III. Does the methanol root and fruit extract of *Uvaridendron anisatum* have free radical scavenging properties?

CHAPTER TWO: LITERATURE REVIEW

2.1 Natural Phenolic compounds

Phenolics are compounds characterized by either one or more aromatic rings. The rings either possess one or more hydroxyl groups. These compounds are widely distributed in the plant kingdom and are the most abundant secondary metabolites of plants. To date more than 8000 phenolic structures are known and these range from simple molecules including phenolic acids to more polymerized molecules such as tannins. Plant phenolic are very vital in both plants and when used by human beings. They have been stated to offer a defensive mechanism against, Uv- radiations or deleterious effects of pathogens and parasite in human beings (Platzer et al., 2021). In plants, phenolic compounds help in keeping away predators and as well contribute majorly to the colour of plants. Phenolics form part of many plant food including fruits, vegetables, cereals and legumes and as well as beverages such as tea, coffee, beer and wine. Phenolic compounds have been partly involved in the overall organoleptic properties of plant foods (Dai & Mumper, 2010). For instances, phenolics plays a role bitterness and stringency of fruit and fruit juices that occurs as a result of the interaction the phenolics mostly procyanidin and glycoprotein in saliva. The orange, red, blue and purple colours found in many fruits and vegetables such as apples, berries, beets and anion as a result of the anthocyanins which is one of the six members of the flavonoids (Dai & Mumper, 2010).

2.2 Groups of plant phenolics

The plant phenolic compounds include common compounds such as, phenolic acids, flavonoids, tannins and less common compounds such as stilbenes and lignans.

Of all the phenolic compounds, flavonoids are the most common and most abundant in the human diet. These class of polyphenols are characterized by a basic structure containing flavan nucleus made up of 15 carbon atoms that are arranged in three rings (C6-C3-C6) labeled as A, B and C. Flavonoids are further divided in six subgroups; flavones, flavanols, flavanones, isoflavones, and anthocyanins as per the oxidation state of the central carbon ring. The variation

among the subgroups structurally is partially as a result of the degree and pattern of hydroxylation, methoxylation, prenylation, or glycosylation. Examples of the flavonoid polyphenol compounds include; quercetin, found in more quantities in onion, broccoli, and apple; catechin, found in tea and several fruits; naringenin, found in grapefruit; cyanidin-glycoside, present in berry fruits (black currant, raspberry, blackberry, etc.); and daidzein, genistein and glycitein, found in in soybean (Dai & Mumper, 2010).

The phenolic acids polyphenol compounds are further divided into two groups; that's benzoic acid derivatives that include gallic acid, and cinnamic derivatives such as coumaric, caffeic and ferulic acid. Of all these phenolic acids, caffeic acid is the most common and abundant in many fruits and vegetables. In most cases, caffeic acid undergoes esterification wi bth quinic acid as in chlorogenic acid mainly present in coffee. Additionally, the second abundant phenolic acid compound is the ferulic acid. This polyphenolic compound mostly found in cereals undergoes esterification to form hemicellulose in the cell wall (Dai & Mumper, 2010).

Tannins phenolic compounds as well constitutes a larger part of our diet and are mainly divided into two groups; hydrolysable tannins and condensed tannins. Hydrolysable tannins, a subclass of the larger tannin polyphenols also identified as gallotannins are compounds that contain the central core of glucose or another polyol that has undergone esterification with Gallic acid.

2.3 Antioxidant properties of phenolic compounds

Antioxidants are compounds with the ability to either delay, inhibit or prevent the oxidation reaction of the free radicals. This prevents the oxidizable compounds such as protein, DNA and lipids from being attacked by the free radicals as they try the avenue to gain stability. The prevention of the oxidation reaction is usually via the scavenging of these free radicals and in the process inhibition the genesis of the oxidative stress. Oxidative stress which is state of an imbalance between the free radicals and the antioxidants with the free radicals being in higher concentration. Oxidative stress has being implicated in the development of chronic

degenerative diseases including cancer and aging (Schieber & Chandel, 2014). In the current days, phenolic compounds have been considered powerful antioxidants via the in vitro methods. These compounds have been proved to be even more potent as compared to vitamin C and E and carotenoids (Azad et al., 2019; Panche et al., 2016) Additionally, studies have shown an inverse relationship between the developments of the oxidative stress related diseases such as cardiovascular diseases and cancer to the intake of vegetables and fruits. This inverse relationship has been partly attributed to the phenolic that are found in both fruits and vegetables (Zhan et al., 2016). Various studies have proposed that the antioxidant mechanism of the phenolic compounds to be through method such as scavenging of the free radical species, suppressing free radical formation by inhibiting certain enzymes or chelating trace metals that are vital in free radical generation and up regulating or protecting antioxidant defense (Platzer et al., 2021).

2.3.1 Phenolic compound as free radical scavengers and metal chelators

Phenolic compounds act as both as free radical scavengers and similarly as chain breakers. This is done by interfering with the oxidation reactions of lipids and other biomolecules by donating a hydrogen atom to the free radical (Dai & Mumper, 2010).

$$R + POH \rightarrow RH + PO^{\cdot}$$

The resultant phenoxy radical intermediates ($PO^{\cdot}$) are usually stable and this limits the initiation process of other free radical chain reactions. Additionally, the phenoxy radical intermediate acts as a terminator of propagation of other free radicals reaction by reacting with these free radicals (Dai & Mumper, 2010).

$$PO^{\cdot} + R^{\cdot} \rightarrow POR$$

Phenolic compounds have an ideal structure that aids in its free radical scavenging activity. This is attributed to many factors that include the hydroxyl groups that form part of the

phenolics and have the ability to donate a hydrogen or electron to the free radicals. Similarly, the phenolic compounds are able to extend conjugated aromatic system to delocalize an unpaired electron (Dai & Mumper, 2010).

2.3.2 Pro-oxidant activity of phenolic compounds

Phenolics have the ability to initiate the autoxidation process and act as pro-oxidants (Kumbhare et al., 2012) under certain conditions. The phenoxy radical intermediates ($PO^{\cdot}$) as a pro-oxidant do not react with the second free radical to terminate the chain reaction, but instead react with the oxygen producing quinones (P = O) and superoxide anion ($O2^{\cdot -}$) (Panche et al., 2016).

$$PO^{\cdot} + O2 \rightarrow P{=}O + O2^{\cdot}$$

2.4 Uvariodendron anisatum Verdec

2.4.1 General description of *Uvariodendron anisatum* Verdec

Uvariodendron anisatum Verdec is a shrub or small tree found in the family *Annonaceae*. It is called Mutonga (Kikuyu) and Mutongu (Meru) (Beentje, 1994; Bernard and Verdcout, 1971). It is rare, indigenous and endemic to Central and Eastern parts of Kenya (Beentje, 1994).

2.4.2 Biological activity of *Uvariodendron anisatum* Verdec

Only limited bioassay reports of *Uvariodendron anisatum* extracts are available. The aqueous root extract of *U. anisatum* was reported by Misonge *et al*., (2014) to possess oxytocic like effects on isolated rat uterus. Antimicrobial activity against gram positive bacteria such as *Staphylococcus aureus* and gram negative bacteria such as *Escherichia coli* have also been studied (Mutembei et al., 2018b). However, this antimicrobial activity was only against bacteria at this plant was inactive against fungal species; *Candida albicans*.

2.4.3 Phytochemistry of *Uvariodendron anisatum* Verdec

Bergenin was reported by Onyancha et al. (2019). In addition, phytochemical groups including alkaloids, saponins, glycosides, terpenoids, volatile oils, steroids and phenols have been

detected in the leaf and root powder (Misonge *et al*., 2014). However, in a study conducted by Mutembei *et al*.(2018) in both aqueous and methanol extracts, steroids, terpenoids, cardiac glycoosides, anthroquinones, tannins and reducing sugars were present. Saponins and alkaloids were absecent in the two extracts (Mutembei et al., 2018b).

2.4.4 Ethnobotanical uses of *Uvariodendron anisatum* Verdec

The root decoction of *Uvariodendron anisatum* is used to easy labour or remove after birth if it is late or retained while the root infusion is used to manage impotence in men (Gachathi, 2007). The wood is used as a walking sticks and axe handles (Beentje, 1994).

2.5 Annonaceae family and antioxidant activities

The *Annonaceae* family consist of many plants that are categorized in about 120 genera with more than 2000 species. The species of this family are source of edible fruits and oils making the family to be of economic importance. For instance the seed oils of some *Annonaceae* family plants are used in soap production and as edible oils while flowers are used as perfumes in cosmetics. The plants in this family have been are traditionally used as remedies for treating various types of tumors and cancer (Biba et al., 2014).

Antioxidant activities have been reported in various species of the family *Annonaceae.* The aqueous and ethanol root extracts of *Uvaria chamae* were assessed for their antioxidant activity. 1, 1 diphenyl-2picrylhydrazyl (DPPH) radical scavenging, ferric reducing antioxidant power (FRAP) and lipid peroxidation inhibition were evaluated (Monon et al., 2015). In the study it was noted that the root extract had antioxidant activity with minimum concentration required to scavenge or reduce 50 % of the free radicals being 4.02±0.50 µg/ml and 14.35±4.86 µg/ml for ethanol and aqueous for 1,1 diphenyl-2picrylhydrazyl (DPPH) radical scavenging. For the reducing power, 9.00±0.66 µg/ml and 14.35±4.86 µg/ml for ethanol and aqueous (Monon et al., 2015).

2.6 *Annonaceae* and phenolic

Phenolic which constitutes the majority of the plant secondary metabolites have been identified in the plant species of the family *Annonaceae*. The phenolic compounds such as flavonoids, phenols and tannins have been determined in some plant species of the family *Annonaceae*. The evaluation of the total phenolic, total flavonoid and total tannins of the ethanol and aqueous root extracts of *Uvaria chamae*. The results showed presence of considerable quantity of these phenolic compounds in both ethanol and aqueous extracts (Monon et al., 2015). The polyphenols formed the larger percentage followed by tannins and flavonoids. Total phenols were 21.14±0.51 mg GAE/ g and 8.917±0.92 mg GAE/ g, tannins were 9.519±0.96 mg TAE/ g and 6.253±1.10 mg TAE/ g and flavonoids were 12.13±1.68 mg QE/g and 6.833±0.01 mg QE/g for ethanol and aqueous respectively (Monon et al., 2015).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Source of the extract

The methanolic and aqueous root and fruit extract of *Uvariodendron anisatum* was used in this study. These extracts were provided by my project supervisor Dr. Jared Onyancha of Mount Kenya university school of pharmacy from his previous work. The extract which was kept under 6-8 ^{0} C conditions was diluted in methanol to obtain a stock concentration of 1000ug/ml which was serially diluted by a factor of 10 to obtain the working concentrations for the 2.2-diphenyl-2-picryl-hydrazil (DPPH) free radical scavenging assay.

3.2 Chemicals and reagents

The chemicals and reagents used in this study were all analytical grade obtained from reputable sources. They included methanol, ethanol, Gallic acid (Loba chemie), catechin (Loba chemie), sodium carbonate (Loba chemie), 2.2-diphenyl-2-picryl-hydrazil (DPPH) (Sigma Aldrich), L-Ascorbic acid (Loba chemie), and Folin- ciocalteu's All these chemicals and reagents were kept under the conditions instructed by the manufacturer prior to and after the study day. 2.2-diphenyl-2-picryl-hydrazil (DPPH) was kept in the fridge at 6-8 ^{0} C and the other reagents were kept under room temperature.

3.3 Total phenolic content of the methanolic root and fruit extract of *Uvariodendron anisatum*

The method of Sousa et al. (2016) and modified by Al-Rifai et al.(2017) was used to evaluate the total phenolics content of the methanolic root and fruit extract of *Uvariodendron anisatum* using the Folin - Ciocalteu assay. Briefly this involved mixing 0.3 ml of the extract at concentration of 1 mg/ml/ Gallic acid (standard) at concentrations (150 ppm, 75 ppm, 37.5 ppm, 18.75 ppm, 9.375 ppm and 4.687 ppm) with 1.5 ml of 10 % Folin-Ciocalteu reagent in clean test tubes. The content of the tubes was mixed by carefully swirling to avoid spillage for five minutes. To the reaction mixture 1.5 ml of 7.5 % aqueous sodium carbonate (Na_2CO_3) (7.5 g in 100 ml of distilled water) solution, was added and mixed by swirling prior to incubation

in the dark for two hours under room temperature. Absorbance of the reaction mixture was read and wavelength 760 nm using reaction mixtures without the sample/standard as the blank. Using the absorbance readings of the standard (Gallic acid) and its various concentration, standard calibration curve was plotted and from its regression equation the concentration of total phenolics in the extracts was calculated. The total phenolic content in plant extract was then calculated using the formula below and expressed as Gallic acid equivalent per gram of dry weight (GAE, mg/g dw).

$$concentration\ \left(\text{GAE}, \frac{\text{mg}}{\text{g}}\text{dw}\right) = \frac{c \times v}{m}$$

Where c is the concentration from the standard Gallc acid curve, v is the volume of the sample and m is the mass of the extract weighed.

3.4 Total flavanoid content of the methanolic root and fruit extract of *Uvariodendron anisatum*

The aluminum chloride calorimetric method as described by Al-Rifai et al.(2017) was used to evaluate the total flavonoid content of the methanolic root and fruit extract of *Uvariodendron anisatum.* The methanolic extracts were diluted in methanol to obtain the concentration of 1 mg/ml. This involved accurately weighing out exactly 10 mg of the dry powder and dissolving in 10 ml of methanol to form the stock solution. Catechin at concentration range of 400 mg/ml – 12.5 mg/ml in methanol was used as the standard flavonoid. The reaction mixture was reconstituted by mixing 0.125 ml of the sample/ catechin with 0.1 ml of sodium nitrate and then incubated at room temperature for 6 minutes. To this reaction mixture 0.075 ml of 4 % sodium hydroxide was added followed by 0.750 ml of aluminum chloride to give final volume of 1 ml. The volume was made to 2.5 ml using distilled water and the absorbance measured at 510 nm using UV-Vis spectrophotometer double beam (Labtech). From the catechin celebration curve an equation of the straight line was obtained and using this the concentration

of total flavonoid was calculated. The concentration of total flavonoid in the dry weight sample was further calculated using the equation below and presented as (CE, mg/g dw).

$$Concentration\ \left(\text{CE}, \frac{\text{mg}}{\text{g}}\text{dw}\right) = \frac{c \times v}{m}$$

Where c is the concentration from the standard catechin curve, v is the volume of the sample and m is the mass of the extract weighed.

3.5 The 2, 2-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of the methanolic root and fruit extract of *Uvariodendron anisatum*

The ability of methanolic root and fruit extract of *Uvariodendron anisatum* to scavenge the free radicals was evaluated by 2, 2-diphenyl-2-picrylhydrazyl (DPPH) assay. The protocol described by Al-Rifai et al.(2017)with minor modifications was adopted in this study. The 4 ml reaction mixture was reconstituted by adding 1.6 ml of the extract/ascorbic acid (standard antioxidant) at study concentrations of 1000 ug/ml, 100 ug/ml, 10 ug/ml, 1 ug/ml, 0.1 ug/ml and 0.01 ug/ml. This was followed by addition of 2.4 ml of methanolic solution of 0.3 mM DPPH and then mixed by swirling prior to incubation in the dark for quarter an hour. The negative control was prepared by adding 1.6 ml of methanol and 2.4 ml of methanolic DPPH solution. The absorbances of the sample and standard were read at wavelength 517 nm against methanol as the blank using double beam lab tech UV-Vis spectrophotometer. The percentage radical scavenging activity was then calculated following the formula.

$$\%\ RSA = \frac{Abs.C - Abs.T}{Abs.C} \times 100$$

Where Abs.C is the absorbance of the control (methanol + DPPH), Abs.T is the absorbance of the test (sample/L-ascorbic + DPPH).

3.6 Data management and statistical analysis

The total phenolic content and the total flavonoid content concentration were obtained from the respective standard calibration curves. The antioxidant data was first calculated to find the percentage radical scavenging activity and the concentration of total phenolic and flavonoids. All this data was then tabulated in different excel spread sheet prior to importation in the prism GraphPad prism software version 9.1.0. Descriptive statistic was conducted on both the percentage free radical scavenging activity, total phenolic and total flavonoid data for descriptive statistics. The data was then expressed as Mean±SEM. After the descriptive statistic, two- way analysis of variance followed by the turkeys post hoc test was performed to determine the level of significance between the different means. The results were then presented in form of tables and graphs.

CHAPTER FOUR RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Total phenolic content and total flavonoid content of methanol fruit and root extract of *Uvariodentron anisatum.*

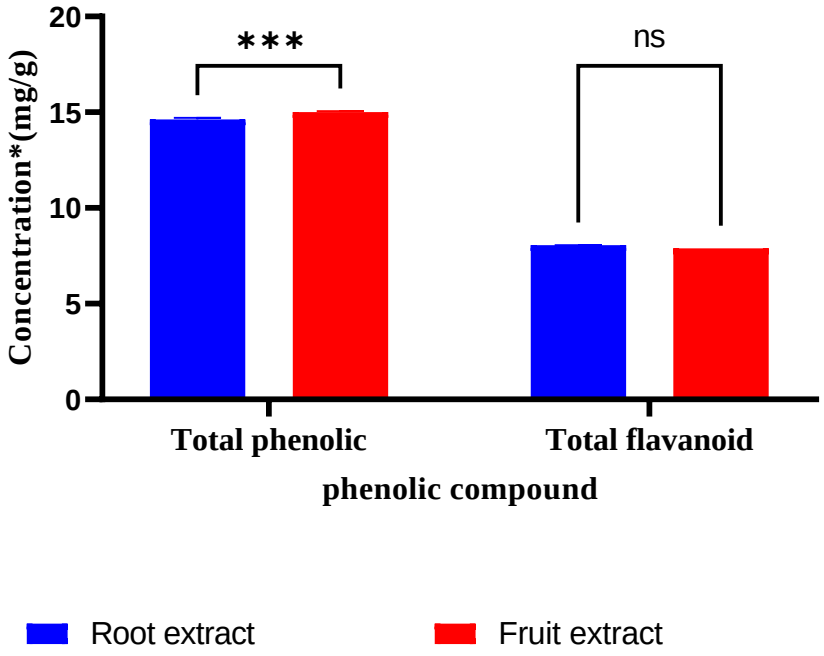
The different concentrations of total phenolic and flavonoid content in methanol fruit and root extract of *Uvariodentron anisatum* are in table 4.1 and fig 4.1. The concentration of these phenolic compounds were calculated from the standard curve of Gallic (Y= 115.26x -16.097; R^2= 0.9962) acid and catechin (Y= 42.338x - 3.308; R^2= 0.8959) for total phenolic and total flavonoid respectively. The total phenolic content in methanol root and fruit extracts was 14.627±0.078 mg GAE /g and 15.013±0.047 mg GAE /g respectively. The content of flavonoid in methanol root and fruit extracts was 7.894±0.002 mg CE /g and 8.057±0.009 mg CE /g. Methanol fruit extract recorded significantly higher total phenolic content as compared to the methanol root extract ($p<0.05$; fig 4.1). For both root and fruit methanol extract of *Uvariodentron anisatum*, there was no significant difference in the total phenolic content recorded ($p>0.05$; fig 4.1.)

Table 4. 1 Total phenolic content and total flavonoid content of methanol fruit and root extract of *Uvariodentron anisatum.*

Plant extract	Concentration (mg/g)	
	Total phenolic content	Total flavonoid content
Root extract	14.627±0.078	7.894±0.002
Fruit extract	15.013±0.047	8.057±0.009

Values are presented as mean of the triplicate values ± Standard error of mean

Figure 4. 1Total phenolic content and total flavonoid content of methanol fruit and root extract of *Uvariodentron anisatum.*



4.1.2 DPPH radical scavenging activity of methanol fruit and root extract of *Uvariodentron anisatum*.

The antioxidant activity results for the methanol root and fruit extract of *Uvariodentron anisatum* are summarized in in table 4.1 and figures 4.1 and 4.2. The results showed significant increases in the percentage radical scavenging activity of both methanol extracts (root and fruit) and L-ascorbic acid. This increase was in a dose dependent manner with the lower dose recording the least percentage radical scavenging activity while the high concentration recording higher percentage radical scavenging activity (table 4.1 and figure 4.2).

The methanol root extract at concentration level 0.01 ug/ml and 1000 ug/ml recorded significantly lower and high percentage radical scavenging activity respectively ($p<0.05$;figure 4.1). The percentage free radical scavenging activity recorded at concentration level 0.1 ug/ml was significantly different high as compared to 0.01 ug/ml. However, this activity was not significantly different from the percentage radical scavenging activity recorded at from that 1

ug/ml. similarly between concentrations 10 ug/ml and 100 ug/ml no significant difference was noted in the percentage radical scavenging activity ($p>0.05$; fig 4.1). However, the percentage radical scavenging activity at 100 ug/ml was significantly higher as compared to all the other lower concentrations 0.01, 0.1, 1 and 10 ug/ml and significantly lower as compared to concentration level 1000 ug/ml ($p<0.05$; fig 4.1).

Methanol root extract at concentration level 0.01,0.1 and 1 ug/ml recorded significantly higher percentage radical scavenging activity as compared to both methanol fruit extract and L-ascorbic acid ($p<0.05$; fig 4.2).similarly, L-ascorbic acid at concentration 0.1 and 1 ug/ml significantly recorded higher percentage radical scavenging activity as compared to the methanol fruit extract ($p<0.05$; fig 4.2). However, at concentration 0.01 ug/ml L-ascorbic acid and methanol fruit extract did not show any significant different in the percentage radical scavenging activity between them ($p>0.05$; fig 4.2).

L-ascorbic acid at concentrations 10 ug/ml, 100 ug/ml and 1000 ug/ml significantly recorded high percentage radical scavenging activity as compared to both methanol root and fruit extracts ($p<0.05$; fig 4.2). Similarly, at concentration 100 ug/ml and 1000 ug/ml, methanol fruit extract recorded significantly high percentage radical scavenging activity as compared to methanol root extract ($p<0.05$; fig 4.2). However, at 10 ug/ml, the methanol root extract significantly recorded higher percentage radical scavenging activity as compared to methanol fruit extract($p>0.05$; fig 4.2).

L-ascorbic acid recorded the lowest IC_{50} value of 3.420 ug/ml, methanol root extract recorded IC_{50} value of 5.0800 ug/ml and methanol fruit extract 4.6800 ug/ml (fig 4.3).

Table 4. 2 The DPPH free radical scavenging activity of methanol root and fruit extract of *Uvariodendron anisatum*.

Concentration (µg/ml)	% Radical Scavenging Activity		
	Methanol Root Extract	Methanol Fruit Extract	L-ascorbic acid
0.01	30.72±0.778	4.0±0.855	5.77±0.254
0.1	36.10±0.541	5.4±0.230	9.15±1.053
1	38.18±0.121	8.87±1.472	17.96±2.198
10	43.33±0.439	15.00±1.331	88.33±0.866
100	46.51±0.228	64.06±1.673	96.46±0.033
1000	89.23±0.728	92.76±0.176	97.10±0.099

Values are presented as mean of the triplicate values ± Standard error of mean

Figure 4. 2 The DPPH free radical scavenging activity of L-ascorbic acid and methanol root and fruit extract of *Uvariodendron anisatum*

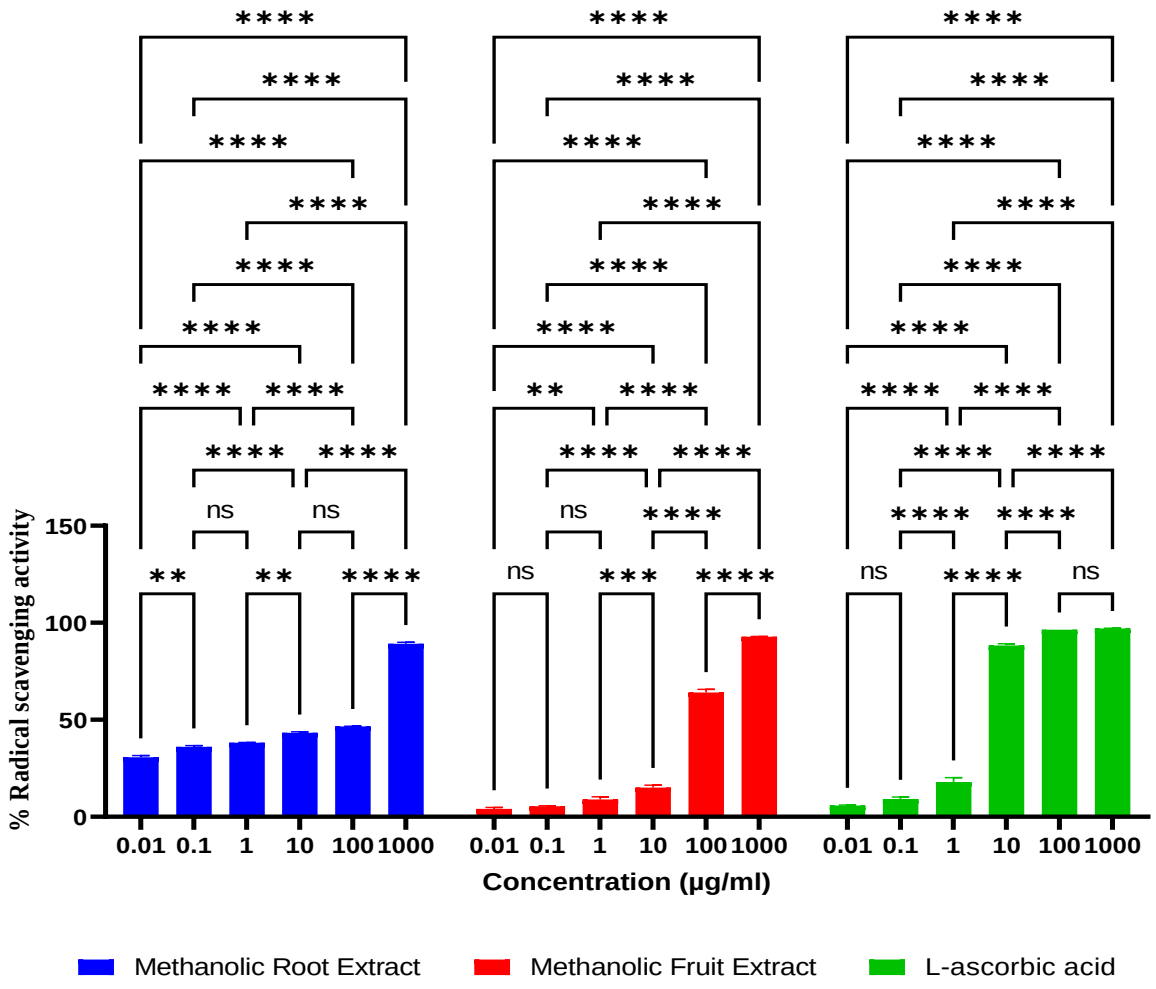


Figure 4. 3 The DPPH free radical scavenging activity of L-ascorbic acid and methanol root and fruit extract of *Uvariodendron anisatum*

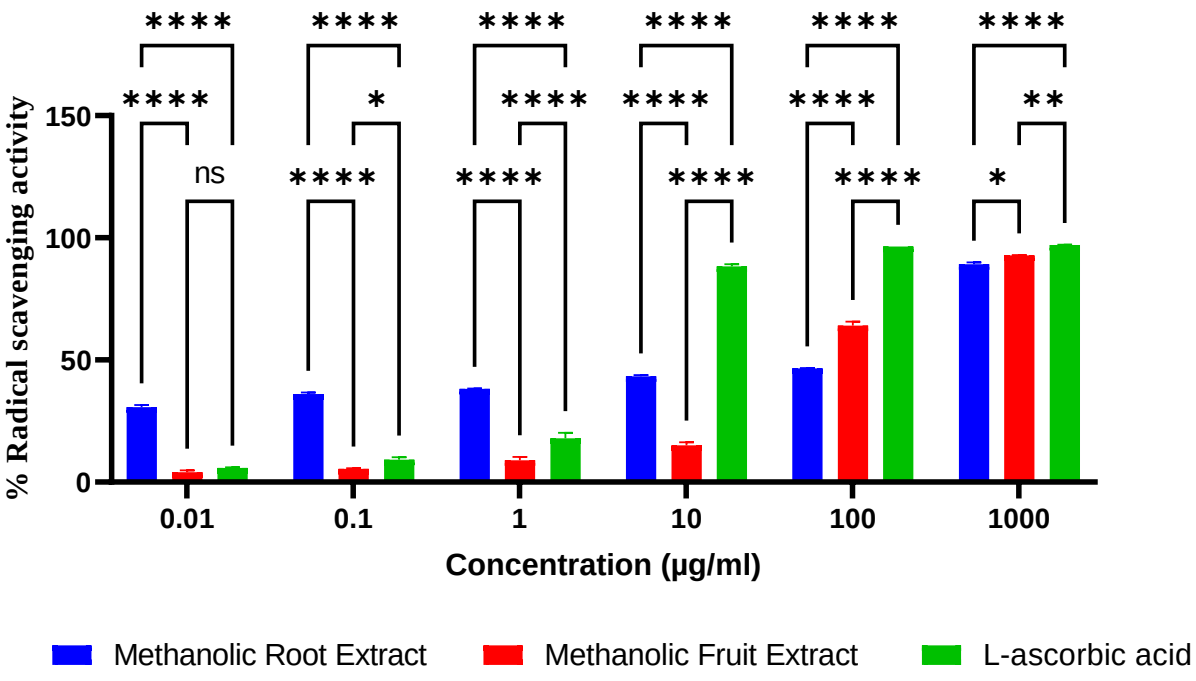
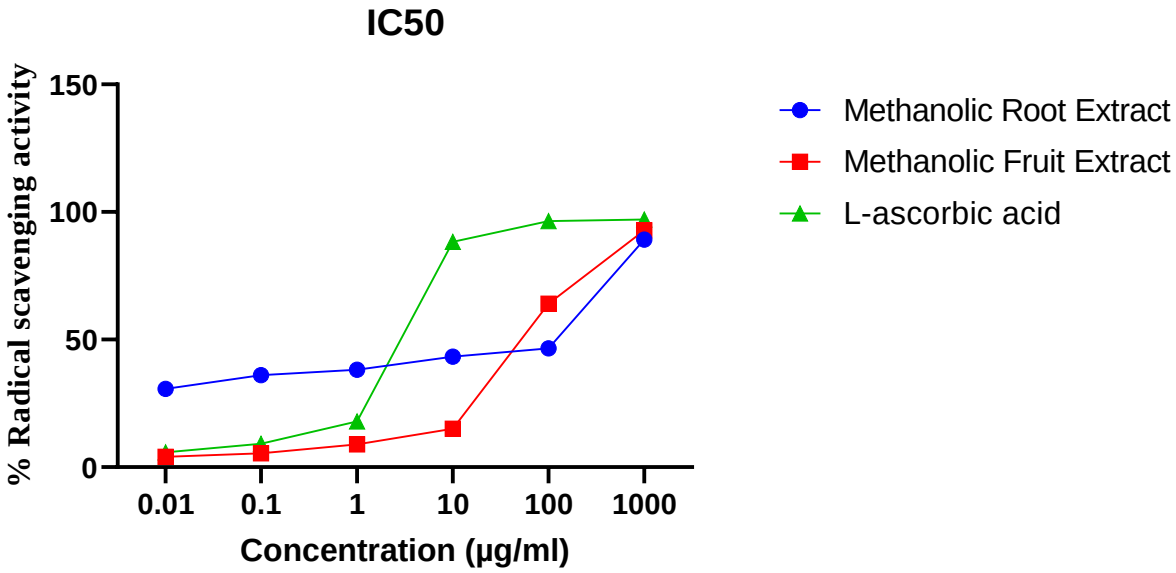


Figure 4.3 IC $_{50}$ values of methanol root and fruit extracts of *Uvariodentron anisatum*



4.2 DISCUSSION

Plants hub many phytochemicals that are characterized by diverse biological activities such as anti-inflammatory, antibacterial and antioxidant. Of these bioactive compounds, include phenolic, steroids alkaloids and saponins. Phenolic compounds form a diverse group of

compounds such as simple phenolic acids, anthacynanins, hydroxycinnamic acid derivatives and flavonoids. Phenolic compounds have the after sought for due to their diverse biological functions such as free radical scavenging, anticarcinogenic and antiinflammatiory activity.

The ability of plant extracts to act as antioxidants has been linked to the presences of the phenolic compounds. This has been due to their ability to scavenge the free radicals which has been investigated in the laboratory via various methods (Al-Rifai et al., 2017). Commonly used method is the DPPH free radical scavenging assay. The DPPH being a free radical is able to accept an electron from the antioxidant compounds present in plant extract to form DPPH-H molecule that has a maximum absorbance at 517 nm (Bhanja Dey et al., 2016). The DPPH free radical is usually purple in color and upon accepting a hydrogen molecule it changes to yellow product (DPPH-H). Many standard antioxidants including; Vitamin C, butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are used as the reference antioxidant molecules in this method. However, vitamin C commonly known as ascorbic acid is the widely used standard due to its strong DPPH scavenging property (Sagar et al., 2018). In this study, the DPPH free radical scavenging ability of methanol fruit and root extract of *Uvariodentron anisatum* was evaluated with *L*-ascorbic acid as the standard. The methanol fruit and root extracts were evaluated at concentration levels 0.01, 0.1, 1, 10, 100 and 1000 mcg/ml and both the extracts recorded a dose dependent antioxidant activity. This was evident with increasing percentage radical scavenging activity for both the extracts and standard. The methanol root extract significantly scavenged the DPPH free radicals more as compared to both fruit and L-ascorbic acid at concentration range 0.01 mcg/ml to 10 mcg/ml and 0.01 mcg/ml to 1 mcg/ml respectively. At higher concentration levels 100 mcg/ml and 100 mcg/ml methanol fruit extract significantly quenched the DPPH free radicals more as compared to root extract. However, L-ascorbic acid showed higher radical free scavenging ability at concentration levels 10 mcg/ml to 1000 mcg/ml as compared to both methanol fruit and root extract. The lower, the value of

the IC_{50} the more active is the natural product as an antioxidant. In this study, L-ascorbic acid recorded significantly lower concentration of 3.420 mcg/ml, followed by methanol fruit extract with 4.680 mcg/ml and lastly methanol root extract with 5.080 mcg/ml that was required to scavenge 50 % of the free radicals. Generally, L-ascorbic acid had a higher antioxidant activity followed by methanol fruit extract and methanol root extract had the least antioxidant activity.

Phenolic compounds constitutes a class of phytochemicals that have aromatic ring attached with one or more hydroxyl groups. These compounds have been named to be radical scavengers due to the presence of the hydroxyl group in their structure (Soobrattee et al., 2005). The quantity of two phenolic compounds, total phenolic content and total flavonoid content concentration in the methanol fruit and root extracts were evaluated. Foilin-Ciocalteu's method and calorimetric aluminum chloride method were used to evaluate total phenolic content and total flavonoid content respectively. Phenolic are usually reduced in the presence of molybdenum in Foilin-Ciocalteu's reagent in the alkaline medium to generate a blue color solution with maximum absorbance at 760 nm (Hudz et al., 2019). The methanol fruit extract recorded a significantly higher total phenolic content of 15.013±0.047 mg/g GAE as compared to the total phenolic content in root extract of 14.627±0.078 mg GAE / g. The flavonoid content recorded in both the two extracts was not significantly different from each other. The methanol root and fruit extracts recorded 7.894±0.002 mg/g CE and 8.057±0.009 mg/g CE respectively.

The amount of the total phenolic content in the sample is directly proportional to the antioxidant activity. In this study the investigation of total phenolic content showed that the methanol fruit extract had higher total phenolic content as compared to the root extract. These values are in agreement with the antioxidant activity shown by the two respective extracts.

CHAPTER FIVE: CONCLUSION AND DISCUSSION

5.1 Conclusion

The consumption of natural antioxidants mainly from fruits, vegetables and medicinal plants is on the rise nowadays. This has been attributed to the fact that these materials contain phenolic compounds that are able to act as antioxidants. Phenolic compounds are able to act as free radical scavengers, terminators of the chain reaction of the free radicals and as pro-oxidants. This antioxidant activity has been directly linked to the structure of the phenolic compounds that include polyphenols and flavonoids. The current study has revealed that plants contain these phenolic compounds in various proportion and the quantity depend on the solvent of extraction. In this study it was noted that *Uvariodentron anisatum* has antioxidant properties. However, this antioxidant property was more in the fruits as compared to the roots. This excellent antioxidant activity could be as a result of the phenolic compounds that were present in considerable amount more so in the fruit than roots. The antioxidant activity of the extracts could be as result of the free radical scavenging mechanism in which the phenolics of the extract are able to donate a hydrogen or an electron free to the free radical. In conclusion, this plant can be an important agent with ability to either prevent or manage the oxidative stress related disorders such as the cancer and neurodegenerative diseases.

5.2 Recommendation

Based on the finding of this study, the following recommendation were made:

I. Other antioxidant assays that that target specific reactive oxygen species such as hydroxyl radicals be used.
II. The antioxidant activity through the in vivo models to be conducted.
III. Guided isolation of the specific compounds with the antioxidants activity be done using column chromatography.

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