In Vitro ANTIOXIDANT ACTIVITIES OF THE ETHANOL EXTRACT OF *Anthocleista djalonensis*

A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY FACULTY OF LIFE SCIENCES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc.) (Hons) IN BIOCHEMISTRY

BY

ADEYANJU BENEDICTA ANI

LSC1705027

FACULTY OF LIFE SCIENCES

DEPARTMENT OF BIOCHEMISTRY

UNIVERSITY OF BENIN

BENIN CITY

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CERTIFICATION

We the undersigned hereby certify that this project work was carried out by ADEYANJU BENEDICTA ANI (LSC1705027) in the Department of Biochemistry, Faculty of Life Science, University of Benin, Benin City. In partial fulfilment of the requirements of the award of Bachelor of Science Degree (B.Sc.
Hons.) in Biochemistry.

PROF. C.C. OSUBOR
(Project Supervisor)

Date :

DR. S.I. OJEABURU
(Project coordinator)

Date:

PROF. K.E. IMAFIDON

(Head of Department)

Date:

DEDICATION

This piece of work is dedicated to God Almighty whose infinite love and grace never cease.

ACKNOWLEDGEMENTS

Words are not enough for me to appreciate God Almighty for His immense help, He has been the source of my strength, inspiration and He has been the provider for this research work.

My special gratitude also goes to my Parents, Mr and Mrs Leonard Ani for the special role they played in my education.

Special thanks to Prof. C.C. Osubor my project supervisor, Special gratitude to Dr. C.O Ugbeni who has truthfully being the backbone to the success of this work, whose patience, understanding, fatherly counsel, advice and truthful concern has propelled the idea of this work without which it wouldn't have been possible.

My thanks also goes to my friends and colleagues, roommates, siblings, family members, my WCCCF family and all my well-wishers. I must not forget to appreciate the good works of my lecturers.

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ABSTRACT

In vitro antioxidant activities were carried out on the ethanol extracts of the stem bark of *Anthocleista Djalonensis*, The methods used were standard procedures for assessment of its antioxidant properties using DPPH scavenging activity, hydrogen peroxide scavenging assay, thiobarbituricacid reactive assay, superoxide scavenging activity and ferric reducing-antioxidants power with their standard as Ascorbic acid. The stem washed clean of sand after which it was air-dried at room temperature and ground into fine powder. The powdered sample (168g) was extracted with 4.9L of Ethanol by maceration for 72 hrs with regular stirring, The mixture was filtered using muslin cloth and concentrated using rotary evaporator and subjected to freeze drying to obtain powdered form. The result of 1,1-diphenol-2picrylhydrazyl (DPPH) radical scavenging activity of ethanol extract shows that the scavenging activity of DPPH was significantly higher ($P>0.05$) than the standard used(Ascorbic acid),it possesses Antioxidant activities. In the Thiobarbituricacid reactive substance (TBARs) assay, ethanol extract of *A.djalonensis* observed to be at bar with the standard used(Ascorbic acid). Although it was highest in the standard used. Since the *A.djalonensis* has been suspected to have antioxidant activities which helps in relieving stress and reducing organic species that enhances damage to the systems of the body, hence the experiment. In conclusion, from the results gotten *A.djalonensis* possesses good antioxidant properties, since it can be found naturally, it's use should be encouraged to help scavenge radicals and manage stress.

CHAPTER ONE

INTRODUCTION

Anthocleista Djalonensis is a tree or shrublike plant which was previously classified under the *loganiaceae* family but is now classified under the *gentianaceae* family due to some morphological qualities. It belongs to the major group Angiosperm.(U.Brunken *et al.*, 2008; Anyanwu *et al*.,2015). *Anthocleista Djalonensis* shows preference for both normal low land dry forest and seasonally flooded environments (Edwin-Wosu *et al*., 2015).
The chemical compounds isolated from Anthocleista species fall into the class of phytochemicals such as secoiridoids, nor-secoiridoids, xanthones, phytosterols, triterpenes, alkaloids, and others of which majority of the

compounds were isolated from *A.djalonensis* (Anyanwu, *et al* 2015).
It has also been reported to have medicinal and health benefits including treatment and prevention of diabetes, hypertension and fertility enhancement (Lawal *et al*., 2010; Diallo *et al*.,2012; Gbolade, 2012).

This study sought to access the antioxidant potentials of the Ethanol extract of *A.djalonensis* stem bark with a view of studying the possibility of utilising the plant in the treatment and management of disorders.

ANTHOCLEISTA DJALONENSIS STEM BARK

LITERATURE REVIEW

Taxonomy and Nomenclature

Kingdom: *Plantae*

Clade: *Angiosperm*

Clade: *Eudicots*

Order: *Gentianales*

Family: *Gentianaceae*

Genus: *Anthocleista*

Species: *A.djalonensis*

DESCRIPTION

Anthocleista djalonensis is a tree growing up to 12 to 15 metres tall. The cylindrical bole is unbuttressed, it can be up to 40cm in diameter. The twigs sometimes have 2 erect spines or small cushions above the leaf axils
The tree is commonly harvested from the wild (Burkil, 2004).

MEDICINAL USE OF ANTHOCLEISTA DJALONENSIS

In African traditional medicine, the leaves, stems and roots of *A. djalonensis* are prepared as a decoction or macerated in water or alcohol, and the solution is given orally as a treatment for diabetes in Guinea, Nigeria (Madubunyi et al., 1994; Olowokudejo et al., 2008; Jiofack et al., 2010; Diallo et al., 2012; Gbolade, 2012; Soladoye et al., 2012; Tchacondo et al., 2012).

Some of the traditional uses of *A. djalonensis* include treatment of stomach aches, diabetes, malaria fever, constipation, typhoid fever, hypertension, haemorrids and syphilis. They are also used as a laxative, purgative and contraceptive (Anyanwu, *et al*.,2015).

OXIDATIVE STRESS AND HUMAN DISEASE

Oxidative damage to DNA, proteins, and other macromolecules has been implicated in the pathogenesis of a wide variety of diseases, most notably heart disease and cancer (Halliwell, 1994). A growing body of animal and epidemiological studies suggest that antioxidants may play a major role in preventing or slowing the progression of both heart disease and some forms of cancer (Hennekens and Gaziano, 1993; Block et al ., 1992).

Conditions Associated with Oxidative Damage

i. Atherosclerosis
ii. Cancer
iii. Pulmonary dysfunction
iv. Cataracts
v. Arthritis and inflammatory diseases
vi. Diabetes
vii. Shock, trauma, and ischemia
viii. Renal diseases
ix. Pancreatitis
x. Parkinson's and Alzheimer's disease
xi. Neonatal lipoprotein oxidation xii. Drug reactions
xiii. Skin lesions
xiv. Aging

i. Heart: Disease Heart disease is the leading cause of death in the United States. It is estimated that one in three Americans will eventually die from this disease (Hennekens and Gaziano, 1993). While several factors, such as high cholesterol levels, hypertension cigarette smoking, and diabetes, are believed to promote atherosclerosis, a growing body of evidence suggests a critical step in its development is the oxidation of low-density lipoprotein (LDL) within the arterial wall (Jialal and Fuller, 1993). This theory is supported by several epidemiological studies which link low intakes of dietary antioxidants to an increased frequency of heart disease (Hennekens and Gaziano, 1993). Additionally, an inverse relationship between heart disease and plasma antioxidant levels has been reported (Geyet al, 1987).

Antioxidants have been shown to prevent LDL oxidation in vitro and retard the progression of atherosclerosis in animal models. Several human studies found supplemental vitamin E increased vitamin E levels in LDL, increased the

resistance of LDL oxidation, and decreased the rate of LDL oxidation (Hennekens and Gaziano, 1993). In a recent retrospective study,(Stampfer, et al) found that health workers who consumed higher amounts of vitamin E on a regular basis had a 41% lower incidence of heart disease than health workers who consumed the lowest level of vitamin E from their diet and supplements (Stampfer et al ., 1993).
It has been estimated that dietary increases in antioxidant vitamins may reduce the risk of heart disease by 20-30% (Hennekens and Gaziano, 1993).

ii. Cancer: Cancer is the second leading cause of death in the United States. It is estimated that diet may account for as much as 35% of all human cancers (Hennekens, 1994). Epidemiological evidences consistently relates low antioxidant intake or low blood levels of antioxidants with increased cancer risk (Block et al ., 1992). In fact, low dietary intake of fruits and vegetables doubles the risk of most types of cancers (Ames, 1994).
Oxidants are capable of stimulating cell division, which is a critical factor in mutagenesis. When a cell with a damaged DNA strand divides, cell metabolism and duplication becomes deranged. Thus, a mutation can arise which in turn is an important factor in carcinogenesis. It is believed that antioxidants exert their protective effect by decreasing oxidative damage to DNA and by decreasing abnormal increases in cell division. Both cigarette smoking and chronic inflammation, two of the major causes of cancer have strong free radical components in their mechanisms of action. Some research has indicated that people who smoke tend to have lower antioxidant levels than nonsmokers and are at an increased risk for both cancer and cardiovascular disease. Well over 100 studies have reported that reduction in cancer risk is associated with a diet high in vitamin C.
As mentioned earlier, the amount of fruits and vegetables included in the diet appears to have a significant impact on cancer risk. Although antioxidant activity is believed to be responsible for much of the protection against tumour genesis, additional anticancer activities have been observed from several plant-derived substances (Milner, 1994). Sulphur- containing phytochemicals, such as the allyl sulphides found in the allium family (garlic, onions, and leeks), and isothyocyanates and sulphoraphane (cabbage, broccoli, and cauliflower) have been shown to inhibit various steps in tumour development in animal and in vitro studies (Milner, 1994). Indoles, also found in cruciferous vegetables, and terpenes, natural constituents of citrus oils, may also be protective.

REACTIVE OXYGEN SPECIES (ROS)

Reactive oxygen species (ROS) is a term which encompasses all highly reactive oxygen-containing molecules including free radicals. Types of ROS include; the hydroxyl radical, the superoxide anion radical hydrogen peroxide singlet oxygen, nitric oxide and various lipid peroxides. All are capable of reacting with membrane lipids nucleic acids proteins and enzymes and other small molecules resulting in cellular damage.

ROS are generated by a number of pathways. Most of the oxidants produced by cells occur as:

i. A consequence of normal aerobic metabolism: approximately 90% of the oxygen utilized by the cell is consumed by the mitochondrial electron transport system.

ii. Oxidative burst from phagocytes (white blood cells) as part of the mechanism by which bacteria and viruses are killed and by which foreign proteins (antigens) are denatured.

iii. Xenobiotic metabolism i.e. detoxification of toxic substances.

Things like vigorous exercise, which accelerates cellular metabolism, exposure to allergens (which may trigger allergic reactions), Drugs or toxins such as cigarette smoke, pollution, pesticides, and insecticides may all contribute to an increase in the body's oxidant load.

ANTIOXIDANT PROTECTION

Oxidative stress and free radicals are generally known to be detrimental to human health, so to protect the cells and system of the body against Reactive Oxygen Species, Cells deploy an antioxidant defensive mechanism which involves the breaking down and removal of free radicals (Jacob, 1995). These components include:

i. Nutrient-derived antioxidants like ascorbic acid (vitamin C), tocopherols and tocotrienols (vitamin E), carotenoids.

ii. Antioxidant enzymes, e.g,., Catalase(CAT) superoxide dismutase(SOD),

glutathione peroxidase(GPx), and glutathione reductase, which catalyse free radical extinguishing reactions.

iii. Metal binding proteins, such as ferritin, lactoferrin, albumin, and ceruloplasmin that seize free iron and copper ions that are capable of catalysing oxidative reactions.

ANTIOXIDANTS

Antioxidants have been known to play a positive protective role in human body against dangerous effects of reactive free radicals and it has been defined as any substance that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1990). They are chemical compounds that neutralize free radicals by giving up some of their own electrons. In making this sacrifice, they act as a natural off switch for the free radicals. These effects include oxidative damage to membranes and enhanced susceptibility to lipid peroxidation or enzyme inactivation (Farombi and Fakoya, 2005; Sathishsckar and Subramanian, 2005).

Free radicals are formed from molecules via the breakage of a chemical bond such that each fragment keeps one electron, by cleavage of a radical to give another radical and also via redox reactions (Halliwell and Gutteridge, 2008; Bashorun *et, al* , 2006). Free radicals include hydroxyl (OH), superoxide (0!), nitric oxide (NO), nitrogen dioxide (NO), peroxyl (ROO!) and lipid peroxyl (LOO). Also, hydrogen peroxide (H2O2), ozone (03), singlet oxygen (0), hypochlorous acid (HOCI), nitrous acid (HNO), peroxynitrite (ONOO), dinitrogen trioxide (N20), lipid peroxide (LOOH), are not free radicals but generally, they are called oxidants, although, they can easily lead to free radical reactions in living organisms (Genestra, 2007., V.Lobo *et,al* 2010).

Biological free radicals are thus highly unstable molecules that have electrons available to react with various organic substrates such as lipids, proteins, DNA and eventually progress to oxidative stress. Antioxidant containing foods like fruits and vegetables could have strong protective effect against the risk of major diseases such as cancer and cardiovascular diseases (Kaur and Kapoor, 2001; Arnie *et,al* 2003).

EXAMPLES OF ANTIOXIDANT

GLUTATHIONE
Glutathione is one antioxidant that can “donate” an electron and a hydrogen atom to oxidized molecules. This allows it to stop oxidizers in their tracks by “feeding” their need for an electron – and to repair molecules that have been damaged by oxidation, by returning the electron they lost.

CAROTENOIDS (Vitamin A)
Carotenoids are yellow and orange pigments found in plants, including vegetables such as carrots and sweet potatoes. Scientists have also genetically engineered an orange-hued species of “golden rice,” which helps people in nutrient-poor environments to avoid severe carotenoid deficiencies.

Carotenoids are thought to break the “chain reaction” of oxidation by donating electrons to oxidized species. Although the carotenoid is oxidized itself, it is stable in its oxidized form, so it does not go on to damage any other molecules. The “chain reaction” of oxidation then stops there, instead of being passed on to other molecules which might continue to pass the damage along.

VITAMIN C (Ascorbic Acid)
Vitamin C can act as an antioxidant in two ways. It can interact directly with reactive oxygen species to neutralize them; or it can donate an electron to regenerate Vitamin E, another important antioxidant species.
Like so many other antioxidants, Vitamin C serves more than one purpose in the body. In addition to acting as an antioxidant, it is essential for the formation of collagen – the protein that gives your skin, bones, and muscles its elastic strength.

FUNCTIONS OF ANTIOXIDANT

Antioxidants act as a shield to protect vital molecules, such as DNA, from oxidizing molecules that can appear inside of cells. They can accomplish this in several ways:

Binding to oxidizers:- Some antioxidants bind to oxidizing molecules, preventing them from interacting with other, vulnerable molecules. Some of these can even carry oxidizers such as heavy metal out of the body through the bloodstream and kidneys.

Shielding vulnerable molecules:- Some antioxidants attach to the most important molecules – such as DNA – and serve as buffers, preventing oxidizing molecules from reaching the DNA.

Repair:- Some antioxidants actually repair oxidative damage: they may carry an extra electron or hydrogen atom, which can be donated to molecules that have lost theirs to oxidation reactions.

Damage control:- Some antioxidants also serve as messengers promoting self induced death in cell through apoptosis. While this might not sound very protective, cells that have been severely damaged by oxidation can become cancerous. In this way, these damage controllers protect the whole organism (Chang *et, al* 2017).

BENEFITS OF ANTIOXIDANT

There are some controversies as to whether eating antioxidants makes people healthier.
It has been known for a long time that people who eat diets high in antioxidants are healthier than those who don't. However, foods that are high in antioxidants – such as berries, nuts, vegetables, whole grains, and fish – are healthy for the body in many ways.
These foods are low in sugar and saturated fat, which are major contributors to many common and serious diseases. They are high in fibre, vitamins, minerals, proteins, and unsaturated fats – all of which work to promote health throughout the body, and are deficient in most modern diets (Magee, 2017).
Results so far have shown clearly that taking an antioxidant pill or supplement cannot replace the good health effects of eating a healthy diet that is high in antioxidants.
Scientists caution against the dangers of people opting for antibiotic supplements instead of healthy diets. People who take vitamin A, C, and E supplements alone have not been found to be healthier than those who don't; and 0ne study actually found that taking large doses of vitamin A may actually have been more helpful to cancer cells than to healthy cells (Crane, 2001).

AIM AND OBJECTIVE OF THE STUDY

The aim of this study is to evaluate the in vitro Antioxidant Property of Ethanol extract of *Anthocleista Djalonensis.*
The Specific objectives of this study is to:

I. To determine the invitro antioxidant activity of the Extract of *Anthocleista Djalonensis*.

i. Diphenyl - 2 picryl- hydrazyl (DPPH) radical scavenging activity.

ii. Hydroxyl radical scavenging activity.

iii. Ferric Reducing Antioxidant Power.

iv. Super oxide radical scavenging activity.

v. Thiobarbituric acid.

CHAPTER TWO

MATERIALS AND METHOD

EQUIPMENT/APPARATUS

Beakers

Conical Flasks

Test tubes

Test tube racks

Spatula

Plastic cans

Hand gloves

Spectrophotometer

Micropipette

Weighing Balance

Filter paper

Nose Mask

pH meter

Rotary evaporator

Water bath

Centrifuge

REAGENTS USED

Distilled water

Ethanol

20% Trichloroacetate acid

0.67% Thiobarbituric acid

Tris-Hcl buffer(PH 8.0)

Nitroblue tetrazolium (NBT)

Phenazine methosulphate(PMS)

2Deoxyribose

Sodium hydroxide(NaoH)

FeCl3

Hydrogen peroxide (H202)

Ascorbic acid

DPPH solution

Sodium Acetate

Hydrochloric acid

Acetate buffer

Glacial acetic acid

2, 3, 5- triphenyl-1, 3, 4-triaza-2-azoniacyclopenta-1, 4-dienechloride (TPTZ).

COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

The stem of *A. djalonensis* were collected from Uselu market, Benin City, Nigeria in January 2022. The entire plant was authenticated by Dr. H.A Akinnibosun, a Botanist at the Herbarium of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, where a voucher specimen (UBH-A594) has been deposited.

PREPARATION OF PLANT EXTRACT.

The stem washed clean of sand after which it was air-dried at room temperature and ground into fine powder. The powdered sample (168g) was extracted with 4.9L of Ethanol by maceration for 72 hrs with regular stirring, The mixture was filtered using muslin cloth and concentrated using rotary evaporator and subjected to freeze drying to obtain powdered form.

METHODOLOGY

Diphenyl2-picryl-hydrazyl (DPPH) Radical Scavenging Activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay is the most commonly used antioxidant assay for plant extract(Alam, *et al*.,2012).

PRINCIPLE: DPPH radical is scavenged by antioxidants by donating proton forming the reduced DPPH. The colour changes from purple to yellow after

reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant by their hydrogen donating ability. The electrons become paired off and solution loses colour stochiometrically depending on the number of electrons taken up (Sannigrahi, et al ., (2009).

PROTOCOL: The free radical scavenging capacity of the ethanol extracts of *A.djalonensis* against 1,1-diphenol-2-picrylhydrazyl (DPPH) radical was determined by a slightly modified procedure of William et al, 1995).

0.5ml of 0.3Mm DPPH solution in methanol was added to 2ml of various concentrations of the extracts. The reaction tubes were shaken and incubated for 15min at room temperature in a dark room. Absorbance was read at 517nm and this process was carried out in triplicate. Ascorbic acid was used as the standard control with similar concentrations as the test tube prepared. A blank solution containing 0.5ml of 0.3mM DPPH and 2ml methanol was prepared and treated as the test tubes (Alam, *et al*.,2012).

CALCULATIONS: The ability to scavenge DPPH was calculated by the following equation:

DPPH radical scavenging % = (Abr-Aar) × 100
Where Abr = absorbance before reaction
Aar= absorbance after reaction

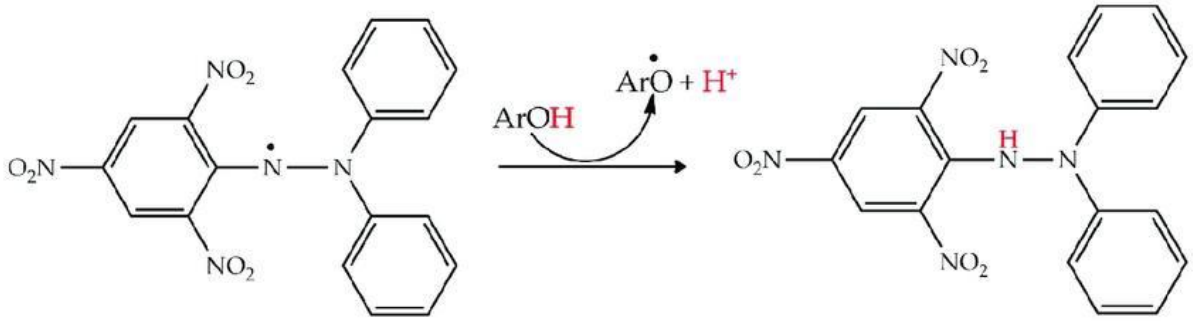
Chemical reactions:

$$DPPH^{\bullet} + ArOH \rightarrow DPPH\text{-}H + ArO^{\bullet} \quad \text{(HAT mechanism)}$$

$$DPPH^{\bullet} + ArOH \rightarrow DPPH - [ArOH]^{\bullet+} \quad \text{(SET mechanism)}$$

where ArOH: phenolic AO

Mechanism of reaction: HAT



Deep purple
2, 2-diphenyl-1-picrylhydrazyl
(DPPH)

Pale yellow
2, 2-diphenyl-1-picrylhydrazine
(DPPH-H)

$\lambda = 517$ nm

Deep purple **Pale yellow**

THIOBARBITURIC ACID REACTIVE SPECIES ASSAY

PRINCIPLE: Among free radicals OH- is the most harmful ROS which can damage cell membranes and destroy sugar groups and DNA base sequences and even cause apoptosis and mutation (Taihua M. 2017).

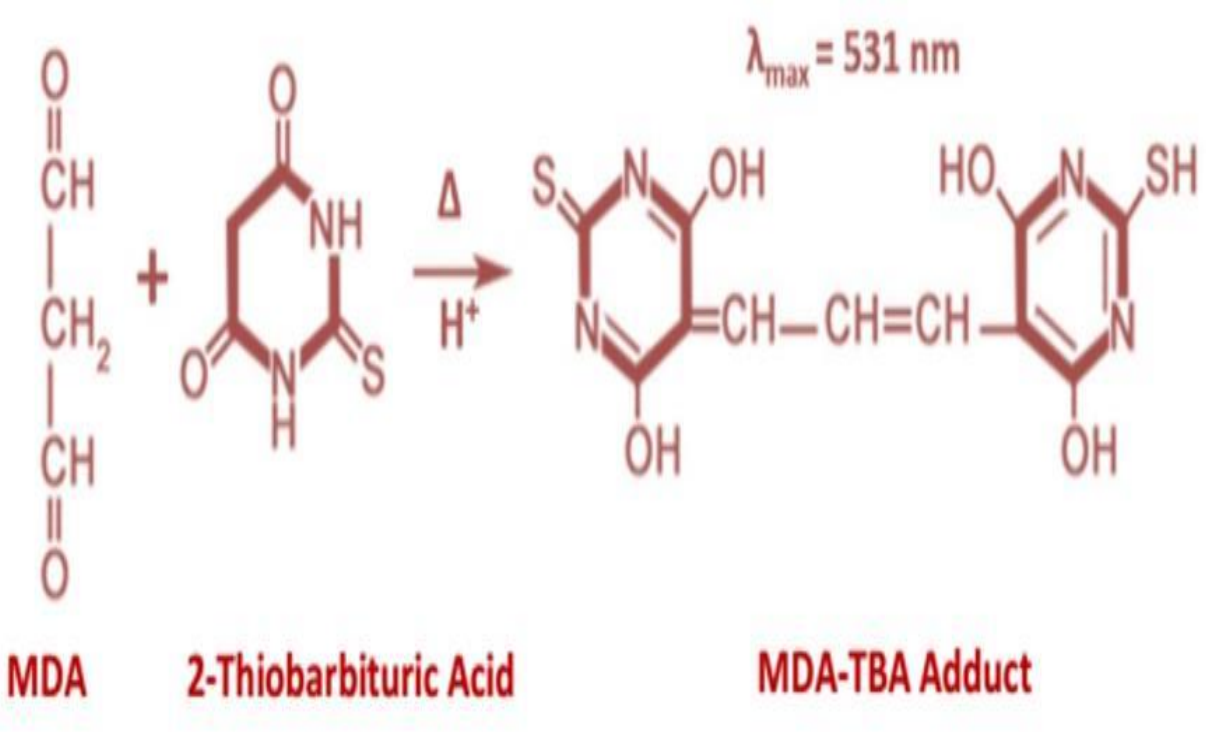
Thiobarbituric acid reactive species assay is a way of measuring lipid peroxidation in cells and tissues (Kohn H.I and Liversedge .M. 1944). The reaction was initially used to determine the rate of reaction between HO- and molecule having therapeutic importance. The same methodology is still used to evaluate radical activity between HO and antioxidants with slight modification. The assay is started by adding EDTA to Fe^{2+} which then reacts with H_2O_2 to generate the HO- radical following a fenton reaction (Grotto D and Maria L.S et al 2009). The incubation temperature for the generation of the radical is 37 degree census for a duration of about 12hrs.the generated OH- radical then attacks the deoxyribose sugar in the presence of amino acid to form a mixture product.

PROTOCOL: To prevent the formation of MDA in this assay, inhibition of deoxyribose degradation is needed. The scavenging activity towards the OH- radical is measured based on the inhibition of deoxyribose degradation. The purpose of adding amino acid is to increase the rate of deoxyribose degradation by the radical, In the absence of the HO- radical the deoxyribose sugar does not undergo any degradation, thus, hindering the formation of MDA and MDA-TBA adduct. In the absence of MDA-TBA chromogen, the colouration of the solution remains pale yellow, indicating good antioxidant activity.

CALCULATION: Inhibiting of lipid peroxidation (%) was calculated with the formula.

$(C-E)/C \times 100\%$

C= absorbance value of the fully oxidized control.



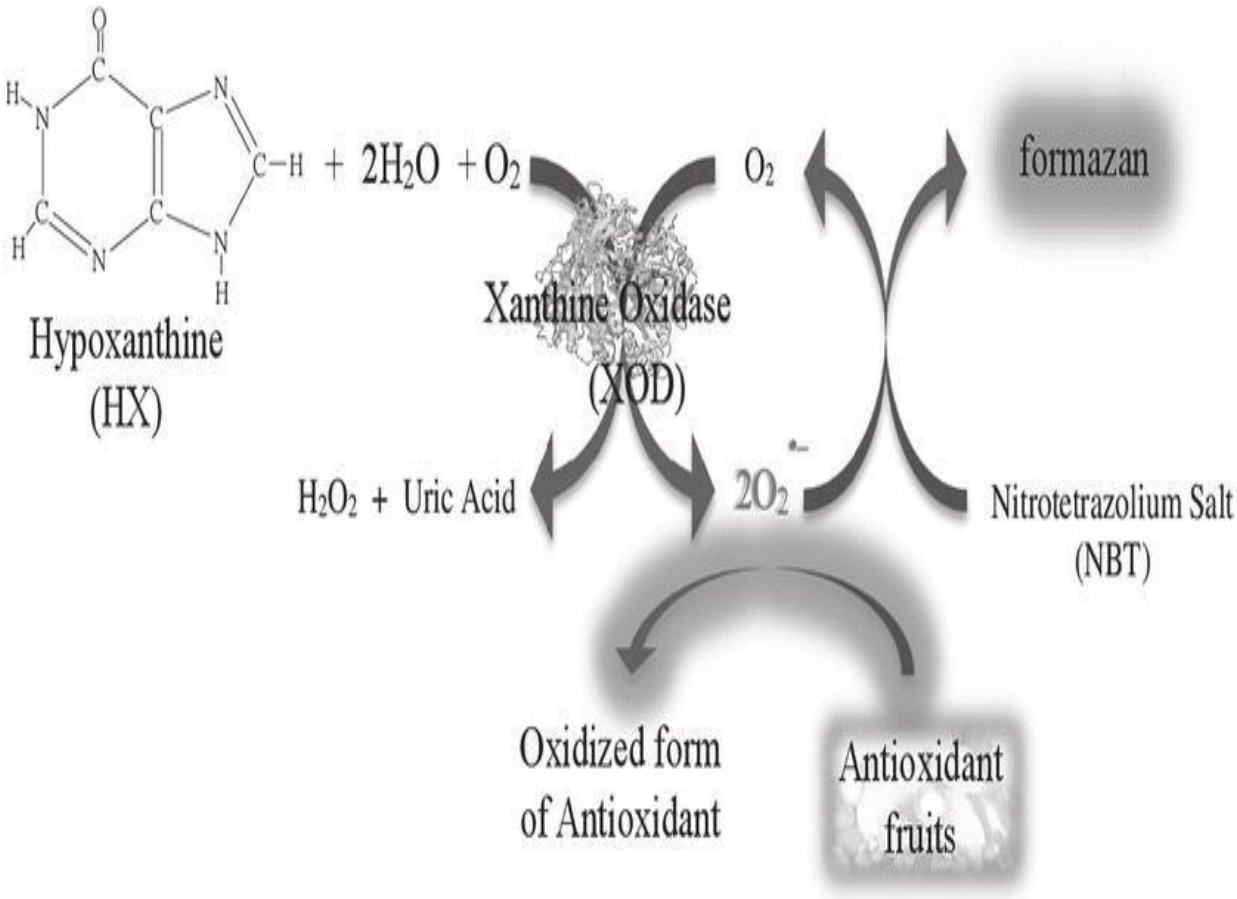
Superoxide Radical Scavenging Assay

Superoxide ultimately produces powerful and dangerous hydroxyl radicals as well as singlet oxygen even though it's a weak oxidant, both of which contribute to oxidative stress (Meyer and Isaken, 1995). During a normal respiration process superoxide (O2-) radicals are formed, which reduces 1-3% of the oxygen that we breathe, Under normal environmental condition in the mitochondria, reduction of the molecular oxygen takes place (Boveris A and Cadenas E. 2013).

The Antioxidant enzyme that is responsible for quenching O2- radical is called superoxide dismutase (SOD) (Koppenol W.H 1998).SOD converts O2- into H2O2, which is further converted into O2 and water by glutathione peroxidase

and catalase (Nimse S.B and Pal .D 2015). This activity was measured by the reduction of NBT according to a previously reported method. The non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce nitro blue tetrazolium (NBT) to a purple formazan.

The scavenging activity of AOS towards (O2-) is assessed in term of their ability to prevent (O2-) generation. Prior to the reduction process caused by O2-. NBT is a pale- yellow soluble salt. However, upon reduction occurring at a pH of 7.4, the tetrazole ring is disrupted leading to dismutation which subsequently result in an intense blue insoluble diformazan product (Nimse S. and Pal. D 2015).



Ferric reducing-antioxidants Power(FRAP) assay

PRINCIPLE: The FRAP assay is based on the ability of PH to reduce Fe^{3+} to Fe^{2+}. The FRAP reaction is conducted at acidic pH 3.6 to maintain iron solubility, so the reaction at low pH decreases the ionization potential that drives hydrogen atom transfer and increases the redox potential, which is the dominant reaction mechanism. FRAP assay measures the ability of antioxidants to reduce ferric iron (Alam, *et al*.,2012) ,This method was based on the reduction of Ferric tripyridyltriazine (Fe(III)(TPTZ))3+ giving an intense blue coloured ferrous complex [Fe(II)(TPTZ)2+ under acidic condition of pH 3.6. intense blue colouration is developed from this assay.

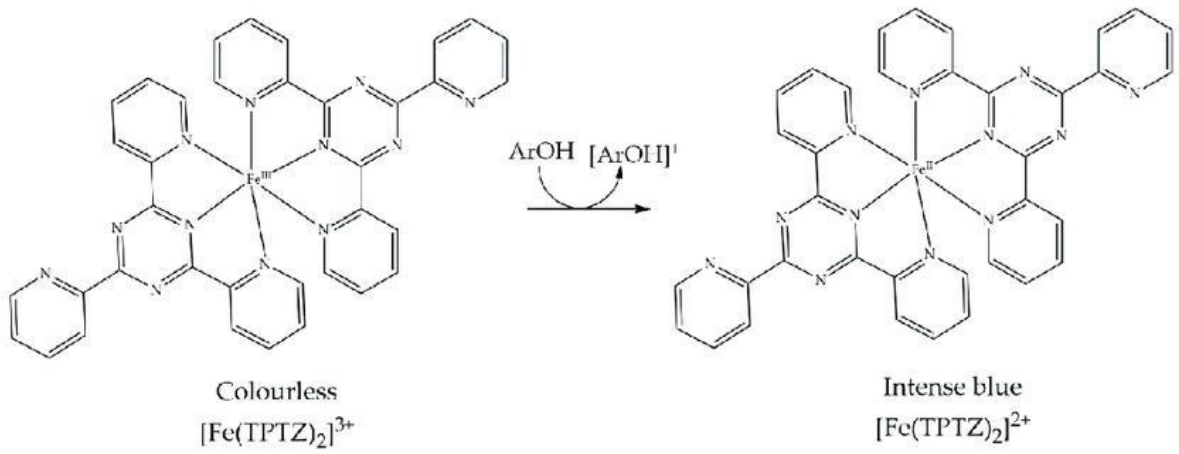
PROTOCOL: The absorbance is read at 593nm. The redox potential of Fe(II) is approximately 0.70V which is comparable to the redox potential of ABTS+ (0.68V) according to the review compiled by Haung et al (2005).

It is important to add reagents in a specific order when preparing FRAP reagent e.g. acetate buffer is added first, followed by FeCl3, while TPTZ is added last, this order is important to prevent the reduction of FeCl3 by TPTZ and to ensure the result isn't altered or disrupted.

Chemical reaction:

$$[Fe^{III}(TPTZ)_2]^{3+} + ArOH \rightarrow [Fe^{II}(TPTZ)]^{2+} + ArO^{\bullet} + H^{+}$$ Scheme 12

Mechanism of reaction:



$\lambda = 593$ nm

Colourless **Intense blue**

Hydrogen Peroxide Scavenging Activity

PRINCIPLES: Hydrogen peroxide is a chemical compound, it's one of the potent reactive oxygen species of the biological system that reacts with polysaturated fat acid parts of cell membrane phospholipids and causes damage to cell. In it's sure form, it is a pale blue, clear liquid

PROTOCOL: The ability of the extract in scavenging H2O2 was estimated according to the method of (Ruch *et,al* 1989). A solution of H2O2 (40mM) is prepared in Phosphate buffer 50Mm pH7.4) The concentration of hydrogen peroxide is determined by absorption at 532nm using a spectrophotometer.

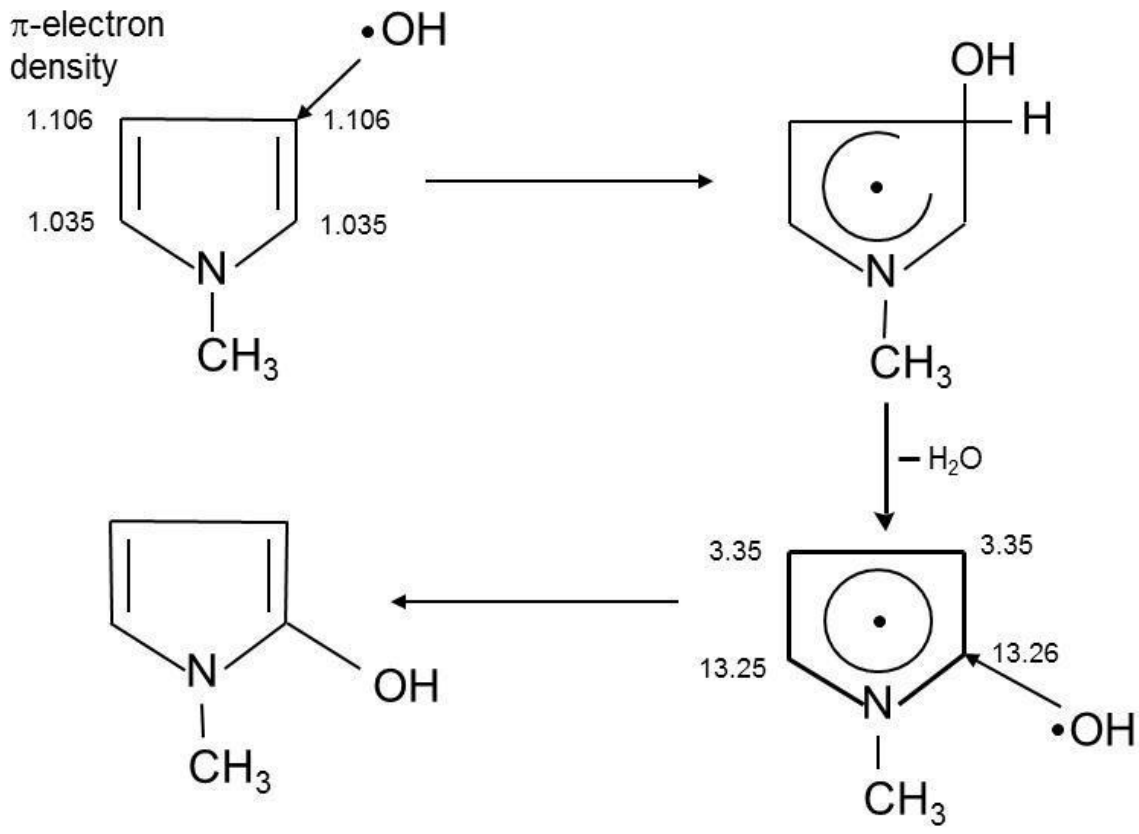
Extract (20-60ug/ml) in distilled water is added to H2O2and absorbance at 532nm was determined after 20min against a blank solution containing phosphate buffer without H2O2.

CALCULATION: The % of H2O2scavenging

% Scavenged (H2O2) =[(A1- At)]?100

Ar. Absorbance of control

At = absorbance of test


π-electron density
•OH
1.106
1.106
1.035
1.035
N
CH3
OH
H
N
CH3
– H2O
3.35
3.35
13.25
13.26
N
CH3
•OH
N
CH3
OH

CHAPTER THREE

RESULTS

The in-vitro Antioxidant activities of the ethanol extract of *Anthocleista djalonensis* was investigated in this study. The results obtained from this study are represented graphically below.

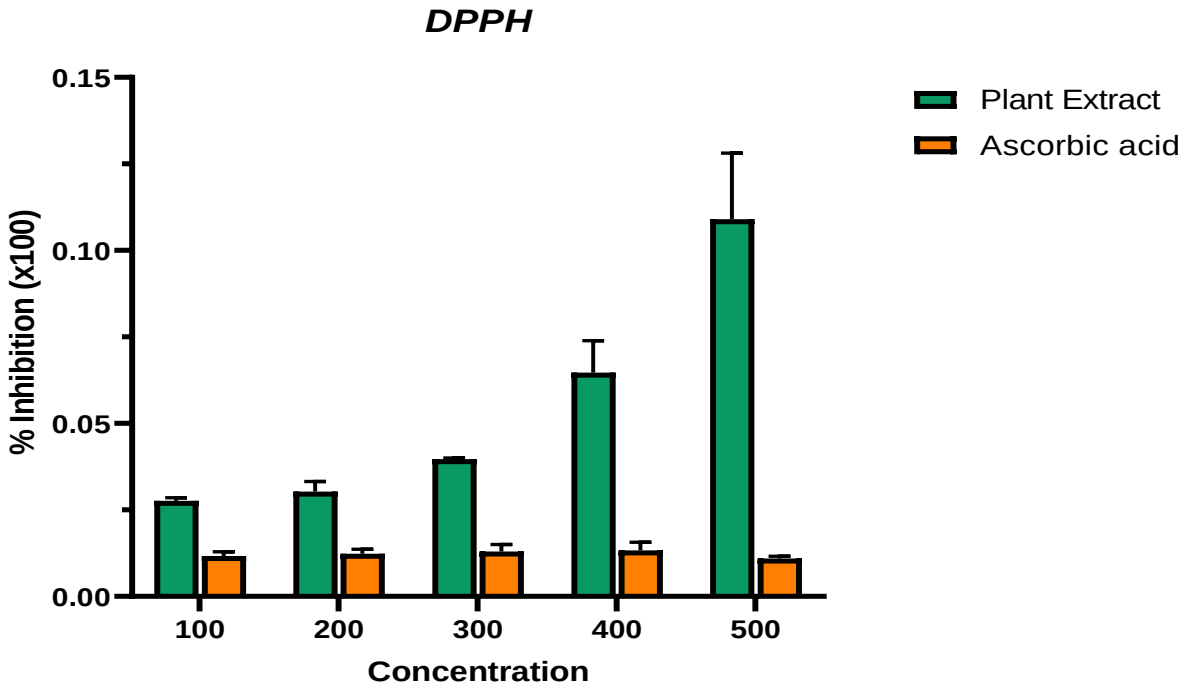
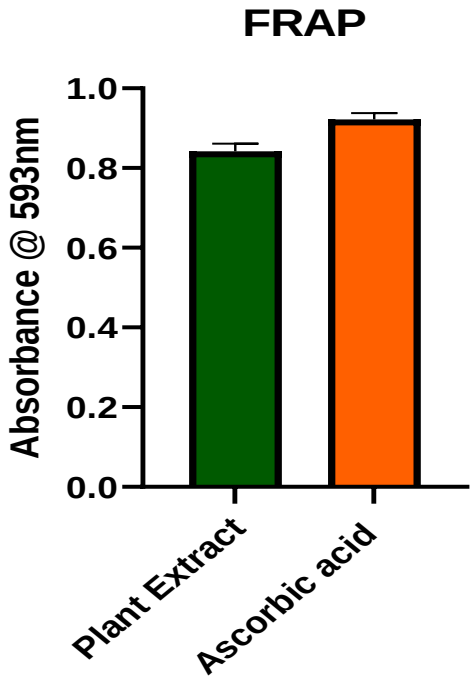


Figure 1: *DPPH* Scavenging Activity of Ethanol Extract

Figure 2: Ferric Reducing Antioxidant Power of Ethanol Extract



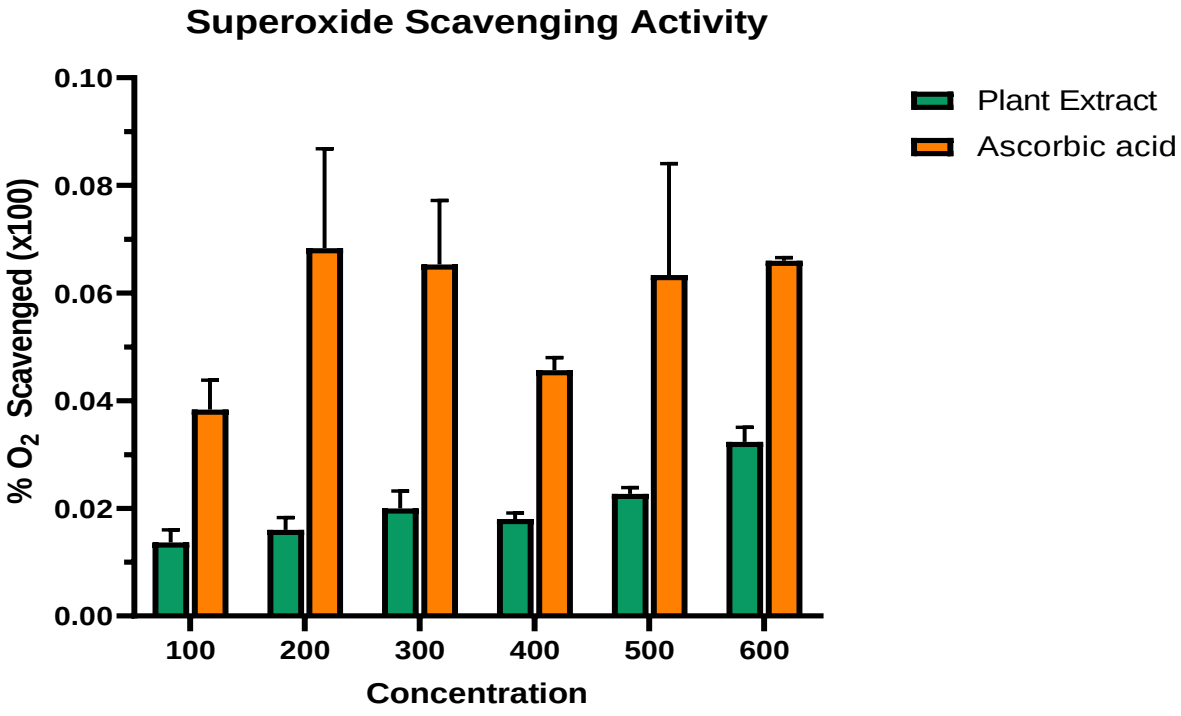


Figure 3: Super oxide (O_2^-) Scavenging Activity of Ethanol Extract

Figure 4: Hydroxyl (OH^-) Scavenging Activity of Ethanol Extract

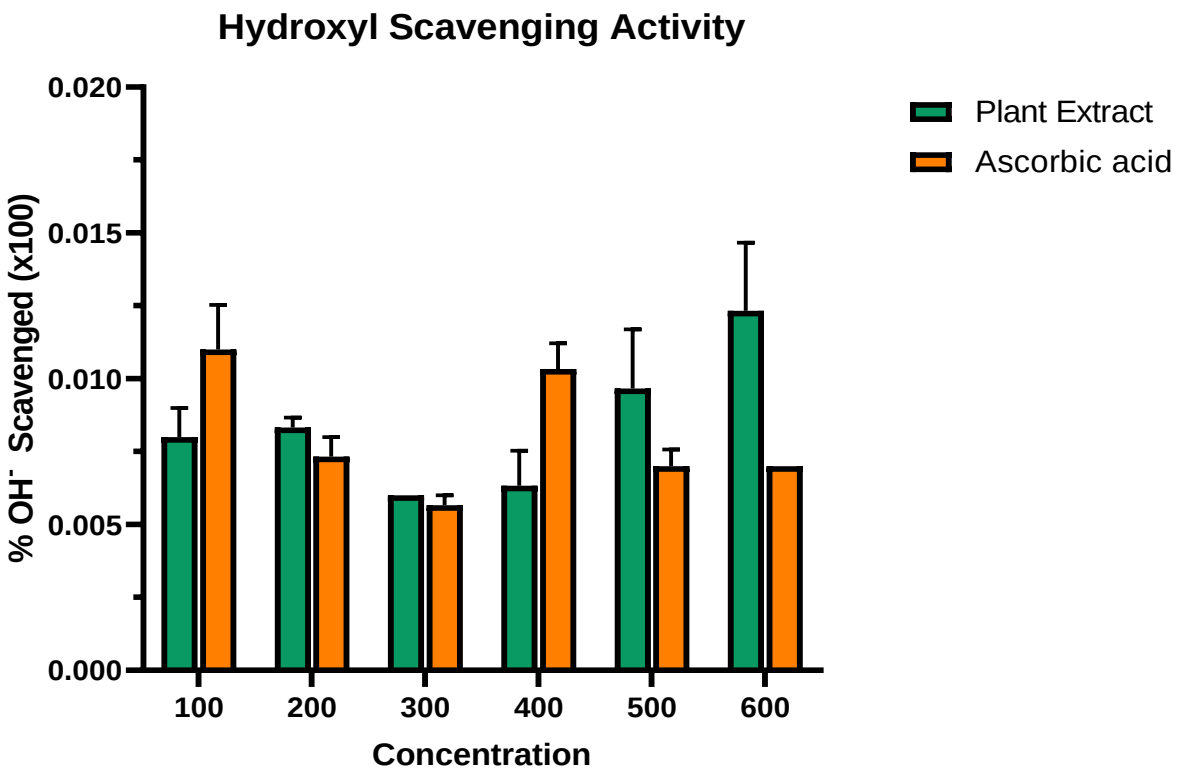
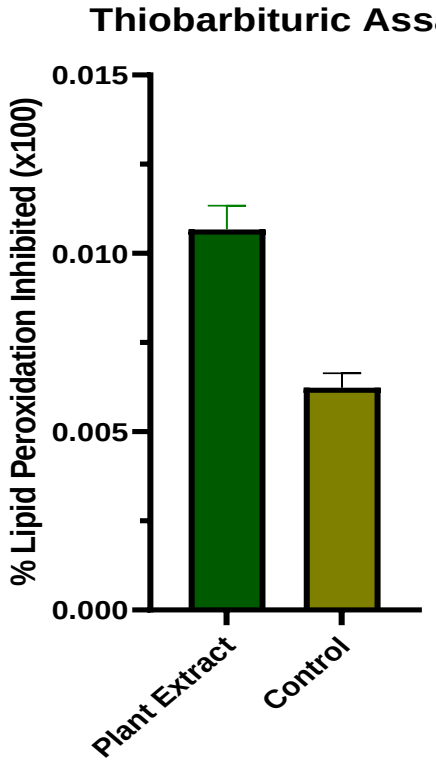


Figure 5: Thiobarbituric Acid Reactive Substances (TBARS) of Ethanol Extract



CHAPTER FOUR

DISCUSSION AND CONCLUSION

DISCUSSION

The results of *in vitro* antioxidant activities showed that ethanol extract exhibited effective and excellent scavenging potential against free radicals.

SCAVENGING ACTIVITY OF DIPHENYL-2-PICRYL-HYDRAZYL (DPPH) RADICALS ON *A.DJALONENSIS*

In Figure 1, Ethanol extract showed a significantly ($p < 0.05$) higher ability to scavenge DPPH radicals which was dose-dependent when compared with the standard ascorbic acid. DPPH stable free radical method is an easy, rapid and sensitive method employed to analyse the antioxidant potential of a specific compound or plants(Ethanol extract of *Anthocleista djalonensis*) (Koleva *et al*., 2002). The result gotten from this study shows that the ethanol extract of *A.djalonensis* is a very strong scavenger of free radicals and can serve as an intervention or preventive method for diseases and any oxidative damage done to the systems of the body (Gyamfi *et al*., 1999).

SCAVENGING ACTIVITY OF FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ON *A.DJALONENSIS*

In Figure 2, Ferric reducing antioxidant power (FRAP)ability of the ethanol extract Ethanol extract revealed a significant value for FRAP though not as much as the standard.
The extracts had significantly lower ($p<0.05$) ability to reduce ferric ions to the ferrous form when compared with the ascorbic acid standard. Although the extracts possessed significantly lower ($p<0.05$) reducing potential than the ascorbic acid standard, This result indicates that maximum activity is shown at this dose by the extract (Benzie *et al*.,1996, Halvorsen *et al*.,2002).
This result showing that the standard has a more potent antioxidant activity than the extract might be as a result of the limitations that comes with the FRAP assay(Camila *et al*.,2016, Pinchuk 2012).

SUPEROXIDE SCAVENGING ACTIVITY ON *A.DJALONENSIS*

In Figure 3, The result shows that the Super oxide (O2-) scavenging ability of the ethanol extract is not as effective as the standard ($p < 0.05$). This study is meant to assess if the *A.djalonensis* is a good primary antioxidant using the Superoxide scavenging ability compared to the standard used(Shahidi, 2015), The ability to reduce NBT by PMS-NADH coupling can measure the superoxide radicals generated from dissolved oxygen(Gangwar et al.,2014), but the result shows that the standard(Ascorbic acid) with a percentage less than 0.05 is more effective as an antioxidant than the ethanol extract of the plant used, which suggests that the standard is a more potent scavenger of superoxide radical than the ethanol extract *A.djalonensis* (Gangwar *et al*.,2014).

HYDROXYL SCAVENGING ACTIVITY ON *A.DJALONENSIS*

In Figure 4, The ethanol extract of *A.djalonensis* showed a high percentage compared to the standard (Ascorbic acid).
The results shows that the ethanol extract of *A.djalonensis* had higher values compared to the Ascorbic acid although with a difference of ($p > 0.05$). As seen across the chart, the ethanol extract and the standard both have the ability to inhibit the activity of hydroxyl radicals, Hydrogen peroxide itself isn't very reactive, but it can be toxic because of it's tendency of giving rise to hydroxyl radical in the cell(Halliwell, 1991). Hence, the removal of hydrogen peroxide is important in defending the cells in the body.

SCAVENGING ACTIVITY OF THIOBARBITURIC ACID (TBA) ON *A.DJALONENSIS*

In Figure 5, the ethanol extract of A.djalonensis showed significantly increased values in Thiobarbituric reactive substances ($p > 0.05$) compared to the standard (Ascorbic acid).

Oxidative stress in cells and tissues can be best monitored by its lipid peroxidation assay(Gangwar *et al*., 2014).

The result reveals that the ethanol extract of *A.djalonensis* has an excellent potential of inhibiting lipids more than the standard, this shows that the plant *A.djalonensis* possesses antioxidant properties that prevents damage or deterioration of the body systems which is caused by reactive oxygen species(ROS) (Badmus, 1994., Olubomehin, 2014). It also shows that this plant is a readily available and natural source of in managing oxidative stress(Muanya,Odukoya 2008).

CONCLUSION

All antioxidant methods showed that the ethanol extract of *Anthocleista Djalonensis* possess high antioxidant properties although with slight differences. However the solvent type plays an important role in detecting antioxidant factors, the *A.djalonensis* extract has an effect that inhibits lipid peroxidation which

means it prevents damage or deterioration of the body systems which is caused by reactive oxygen species(ROS) (Badmus, 1994., Olubomehin, 2014). The extract showed a high hydroxyl scavenging activity,

This explains the wide acceptability and uniqueness of *A.djalonensis* in folklore medicine to prevent or slowdown the progress of various oxidative stress-related diseases. In addition to that, the systematic and continuous use of naturally occurring antioxidants should be encouraged (Rong, 2015).

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