# CHAPTER ONE

## 1.0. INTRODUCTION

Plant-based natural products are the foundation for treating human ailments. The use of medicinal plants for the a treatment for illnesses has a long history in the context of human history; since humans have sought a tool in their environment to recover from a condition, plants have been their sole option. (Halberstein, 2005). Plants are used to produce pure compounds and create new products, and their chemical variety is unmatched. Novel medicinal agents for different diseases are natural products obtained from plants extracts (Faithazadet, 2012).

Over than a tenth of all plant species (more than 50 000 species) are utilized in pharmaceutical and cosmetic products, and medicinal plant distribution is not consistent over the world. (Huang & Rafieian, 2012).

Traditional medicinal herbs, according to the World Health Organization (WHO), are natural plant materials that are utilized for the treatment of diseases on a local or regional basis, either with or without industrial processing. (Tilburt & Kaptchuk,2008). They also calculated that 80 percent of the people in several Asian and African countries utilize herbal medicine to cure a variety of ailments.

*Alafia barteri* (Apocynaceae) is a high-climbing, scandent shrub with small, pure white or pink flowers (Irvine, 1961). Its common names include: Igbo (Obompa), ọta nza; Yoruba: agbárí ẹtù; Guinea fowl's crest (Millson) ìbò-agba (Yor. Dict.) (Burkill,1985).

It is used in ethnomedicine for the treatment of sickle cell anemia, rheumatism, eye infections, febrifuges, fever, as chew sticks and toothache through the application of twining stem, as binding materials for roots (Leeuwenberg, 1997).

## 1.1 Aim of the study

The aim of this study is to evaluate the effects of the aqueous and ethanol leaf and root extracts on oxidative stress parameters in the brain and heart tissue of formaldehyde induced arthritic mice.

## 1.2 Objectives of the study

- To evaluate the effect of *Alafia barteri* aqueous and ethanol leaf and root extracts on the body weight of formaldehyde induced Arthritic mice.
- To evaluate the effect of *Alafia barteri* aqueous and ethanol leaf and root extracts on the brain and heart oxidative stress parameters in formaldehyde induced Arthritic mice.

## 1.3 Scope of The Study

This study entails whether aqueous and ethanol extract of *Alafia barteri* will suppress the arthritis induced in the mice model or not. Also, the various parameters will be a factor in the determination of the study and a positive control for check.

## 1.4 Significance of The Study

There are so many medicinal plants in the market today; the extract of *Alafia barteri been* reported to improve human health and decrease inflammation (Sofidiya, Essien & Aigbe,2014).

Therefore, it is essential to investigate the effects of *Alafia barteri* extract on various parameters of Arthritis induced mice heart and brain parts

## **1.5** Definition of Terms

- Arthritis:  is any disorder that affects joints
- Oxidative stress:- is defined as any alteration in the equilibrium of antioxidants and pro-oxidants in favor of pro-oxidants, as a result of many variables such as age, inflammation, pharmacological action, and toxicity
- Reactive Oxygen Species (ROS):- could be free radicals or non-radical oxygen species
- Rheumatoid arthritis is an inflammatory disease that affects not just the joints but also other organ systems.
- Traditional medicinal herbs, according to the World Health Organization (WHO), are natural plant materials that are utilized for the treatment of diseases on a local or regional basis, either with or without industrial processing.

CHAPTER TWO

## 2.1.0. LITERATURE REVIEW

### 2.1.1 ARTHRITIS

Arthritis is any disorder that affects joints (NIAMS., 2014). The arthritis caused by bacteria, fungus, virus is called infectious arthritis which leads to the inflammation of the joint and is also known as Septic arthritis (Nayana, 2020). In the case of arthritis, the pain is mainly responsible for the faulty joints. Possible cartilage damage, a lack of synovial fluid, an autoimmune attack, and infections are all factors that can cause the disease. (Drake & Mitchell, 2009).

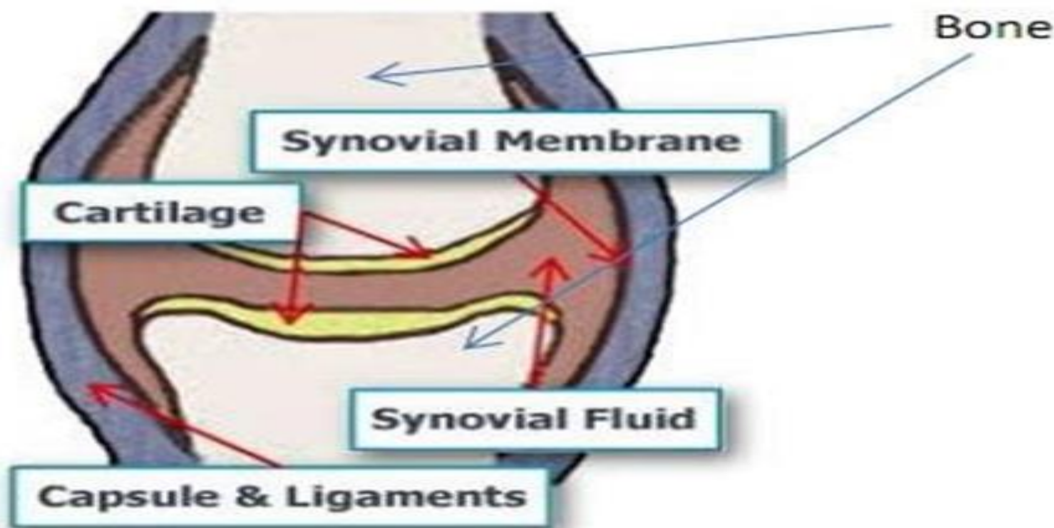


**Figure 2.1.** Typical picture of a bone joint (Knee) (Drake & Mitchell, 2009)

### 2.1.2 HISTORICAL PERSPECTIVE OF ARTHRITIS

Before the 1600's, the disease was rare. It then spread across the Atlantic during the Era of Discovery while in 1859 the disease acquired its current name. During the Age of Exploration, It expanded across the Atlantic, and the disease was given its current name in 1859. In 1800, a French physician published the earliest known description of rheumatoid arthritis. Dr. Augustin Jacob Landré-Beauvais (1772-1840) who was based in the famed Salpêtrière Hospital in Paris. The term "rheumatoid arthritis" was created in 1859 by British

rheumatologist Dr. Alfred Baring Garrod, a British rheumatologist, created the term "rheumatoid arthritis" in 1859. Rheumatoid arthritis is uncommon among Africans, and even more so among West Africans. The majority of the cases have been reported in Southern Africa. (Adelowo, 2010)

### 2.1.3 PREVALENCE OF ARTHRITIS IN AFRICA

The majority of the prevalence were from South Africa (44.4%, 12/27). In urban areas, rheumatoid arthritis prevalence ranged from 0.1 percent in Algeria to 0.6 percent in the DRC, with a prevalence rate of 2.5 % was found in a contextual in South Africa, while in rural areas, prevalence ranged from 0.07 percent in South Africa to 0.3 percent in Egypt to 0.4 percent in Lesotho. The far more frequent kind of arthritis was osteoarthritis, with 55.1 percent in urban areas in South Africa and 29.5 percent, 29.7%, and 82.7 percent in rural areas, all in South Africa.

Other findings include the highest prevalence of 33.1 % in rural South Africa for knee osteoarthritis, 0.1 percent in rural South Africa for ankylosing spondylitis, 4.4 % in urban South Africa for psoriatic arthritis, 0.7 percent in urban South Africa for gout, and 0.3 % in urban Egypt for juvenile idiopathic arthritis. A third of the studies (33.3 percent (9/27) had a low risk of bias, 40.8 percent (11/27) had a moderate risk, and 25.9% (7/27) had a high risk of bias.

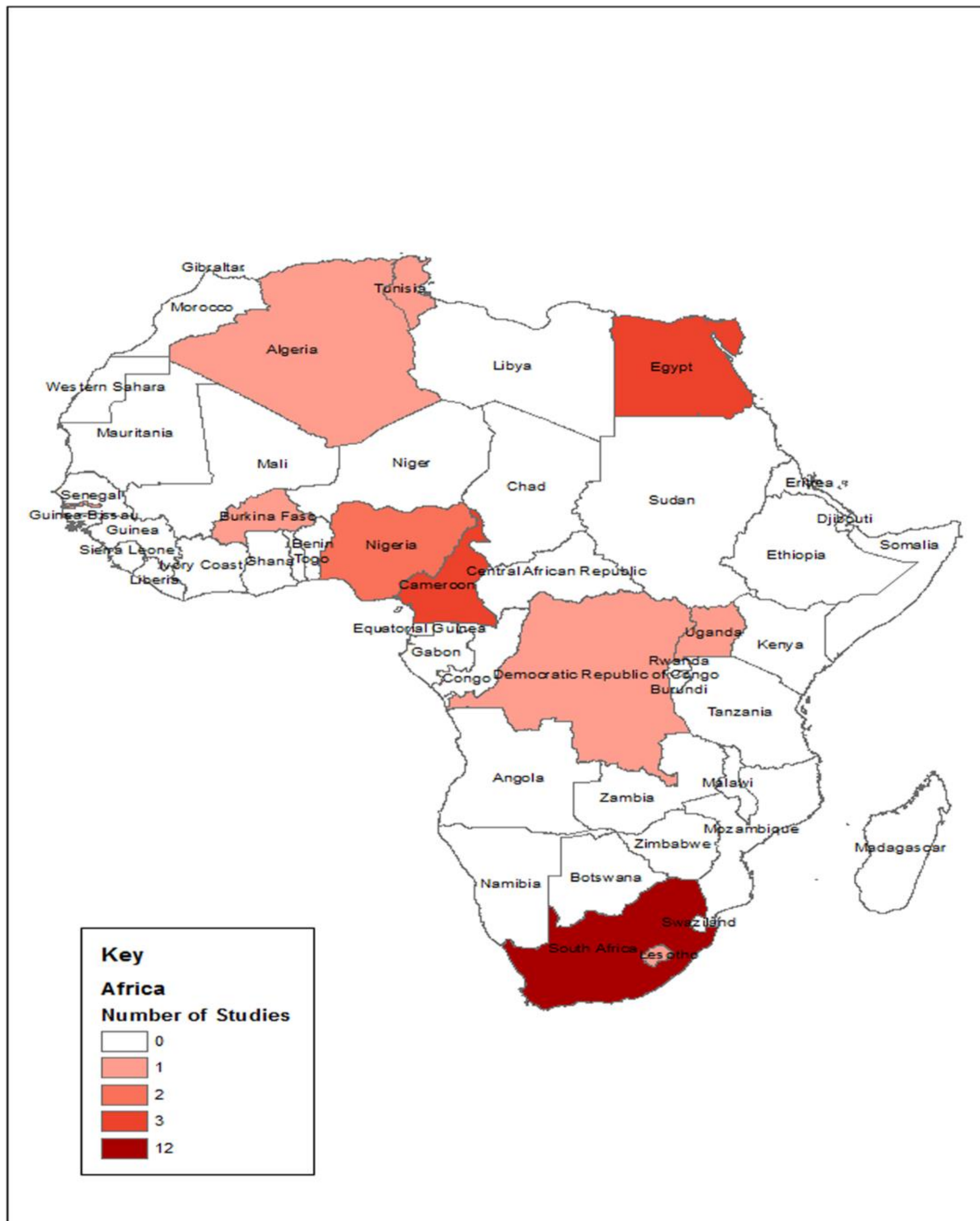


**Figure 2.2**: Map of Africa showing the distribution of the number of arthritis prevalence studies from each country published from 1975 to 2014.

(https://doi.org/10.1371/journal.pone.0133858.g002)

### 2.1.4 SIGNS AND SYMPTOMS OF ARTHRITIS

Pain is a common symptom in virtually all types of arthritis. Other symptoms include:

1. Inability to use the hand or walk
2. Stiffness in one or more joints
3. Rash or itch
4. Malaise and fatigue
5. Weight loss
6. Poor sleep
7. Muscle aches and pains
8. Tenderness
9. Difficulty moving the joint (Nancy Garrick, 2017)

### 2.1.5 TYPES OF ARTHRITIS

There are over 100 types of arthritis. The few common ones are:

**1**. **Osteoarthritis (OA):** Here, the cartilage undergoes a slow damage due to stiffness developed by losing the elasticity**.** Therefore, it no longer can act as a proper shock absorber is the common types of arthritis which affects humans and animals e.g. dogs, cats, horses. Osteoarthritis affects both larger and smaller joints in human body such as hands wrists, feet back, hip and knee (VanItallie, 2010). More than 30 percent of women have some osteoarthritis severity by age 65. Other risk factors for osteoarthritis include prior joint trauma, obesity, and a sedentary lifestyle (Zhang & Jordan, 2010).

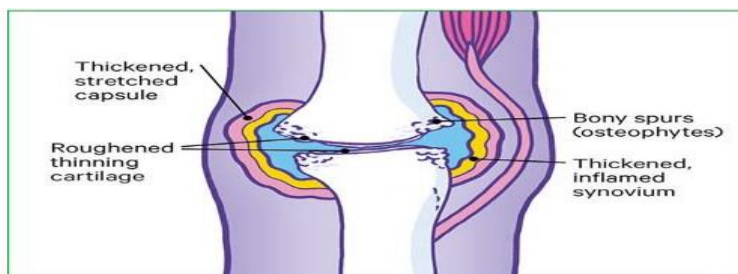


**Figure 2.3**: A joint affected by osteoarthritis (versusarthritis.org)

**2. Rheumatoid Arthritis (RA):** is a disorder in which the body's own immune system starts to attack body tissues(www.cdc.gov.). Women are three times more likely than males to develop RA between the ages of 40 and 60, while infants are rarely affected. **(**Hamermann, 1997**).** Because the joints are swollen from inflammation, they feel stiff, especially when first waking up in the morning. If it is

touched, patients feel tenderness showing red or puffy colors at the affected areas. Patients often feel tired and experience weight losses.

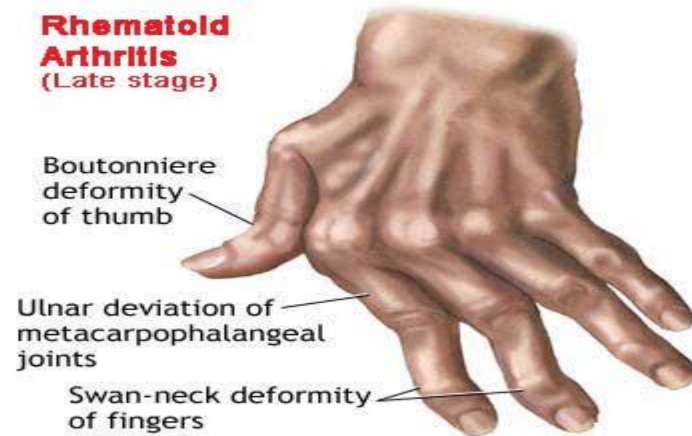


**Figure 2.4:** Rheumatoid arthritis of the hand (Sadura & Kasprzak 2016)

**3. Gout:** Gout is a painful and debilitating disease characterized by uric acid / urate crystal deposition in joints such as the toes, fingers, and ankles, resulting in an inflammatory gouty condition. The primary causes that raise the rise of serum uric acid level resulting in its deposit while inflicting significant pain and sufferings inducing inflammation are incorrect purine metabolism creating Hyperuricemia or mal-excretion of uric acid / urate due to inadequate renal filtration. (Mitra, 2012). In the early stages, the gouty arthritis usually occurs in one joint, but with time, it can occur in many joints and be quite crippling. In gout, the joints become inflamed and lose function. Gout is a type of arthritis that has an effect on the bone joints, especially those in the extreme reaches of the body, such as the toes. The condition is more common in men, but when women reach menopause, the rate equalizes, implying that estrogen plays a significant role in preventing it. (Becker & Michael, 2005).

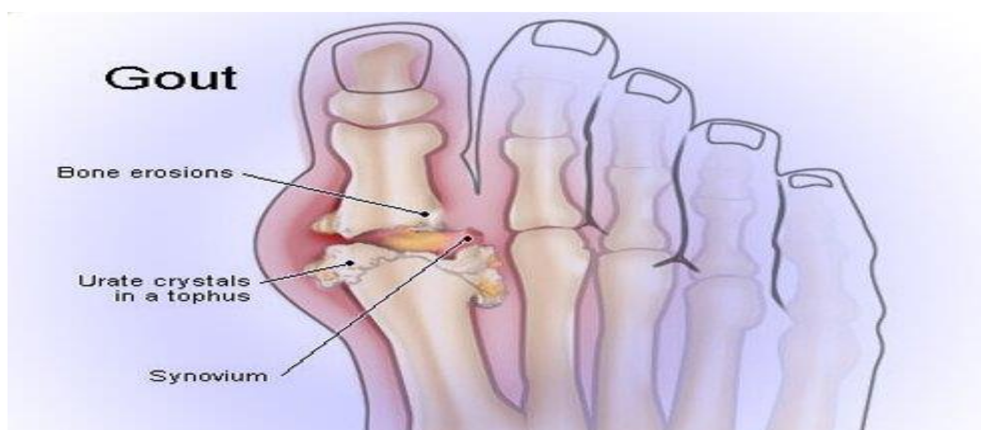


**Figure 2.5:** Gout arthritis of the toe (William, 2021)

**4. Ankylosing Spondylitis (AS):** This is an inflammatory autoimmune disease of the spinal joints or between spine and pelvis (Prakash, Mehra & Bhargava 1984) The exact cause remains unidentified but suspicion points it to be genetic. The disease affects more males than the females and often starts at the ages of 20 – 40 (Herzberg & Laiho, 2006).

**5. Lupus Arthritis, (LA):** Lupus is a common collagen vascular disorder that can be presented with severe arthritis. Other features of lupus include a skin rash, extreme photosensitivity, hair loss, kidney problems, lung fibrosis and constant joint pain (Fessel, 1974). Lupus arthritis is a systemic autoimmune disorder affecting nearly 1.5 million people in the US alone, 90% of the lupus patients suffer from joint and muscle pain and about 35% of them bear LA (Grossman, 2009).

**Figure 2.6**: Lupus facial rash (mayo, 2021).

**6. Infectious Arthritis (IA):** Another severe kind of arthritis is infectious arthritis.
Chills, fever, and joint discomfort appear all of a sudden. Bacteria from other parts of the body are to blame for the illness. Inflammation can occur when a bacterium, fungus, or virus penetrates the joint. Salmonella and Shigella are examples of these organisms, as are medical disorders including Hepatitis C, Chlamydia, and gonorrhoea. In order to avoid irreparable joint damage, infectious arthritis must be detected and treated quickly.

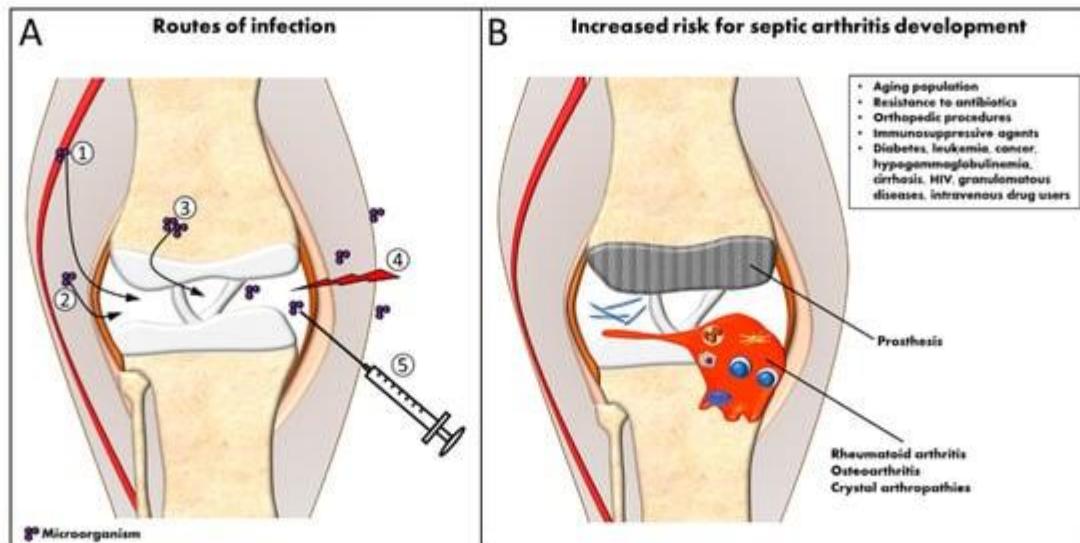


**Figure 2.7:** Routes of bacterial infection and risk factors for septic arthritis development (Mathews & Weston, 2010).

7. **Psoriatic arthritis (PA):** Psoriatic arthritis can arise from psoriasis. Most people with psoriatic arthritis get the skin issue first, then the arthritis. Consistent joint aches, stiffness, and edema are common symptoms. Although the condition does return after periods of remission, there is no treatment for it. A tiny number of people acquire a painful and destructive form of arthritis that damages the small joints in the hands, resulting in lifelong impairment and loss of hand function (Psoriatic Arthritis Archived, 2010).

### 2.1.6 THE DIFFERENCE BETWEEN ARTHRITIS TYPES

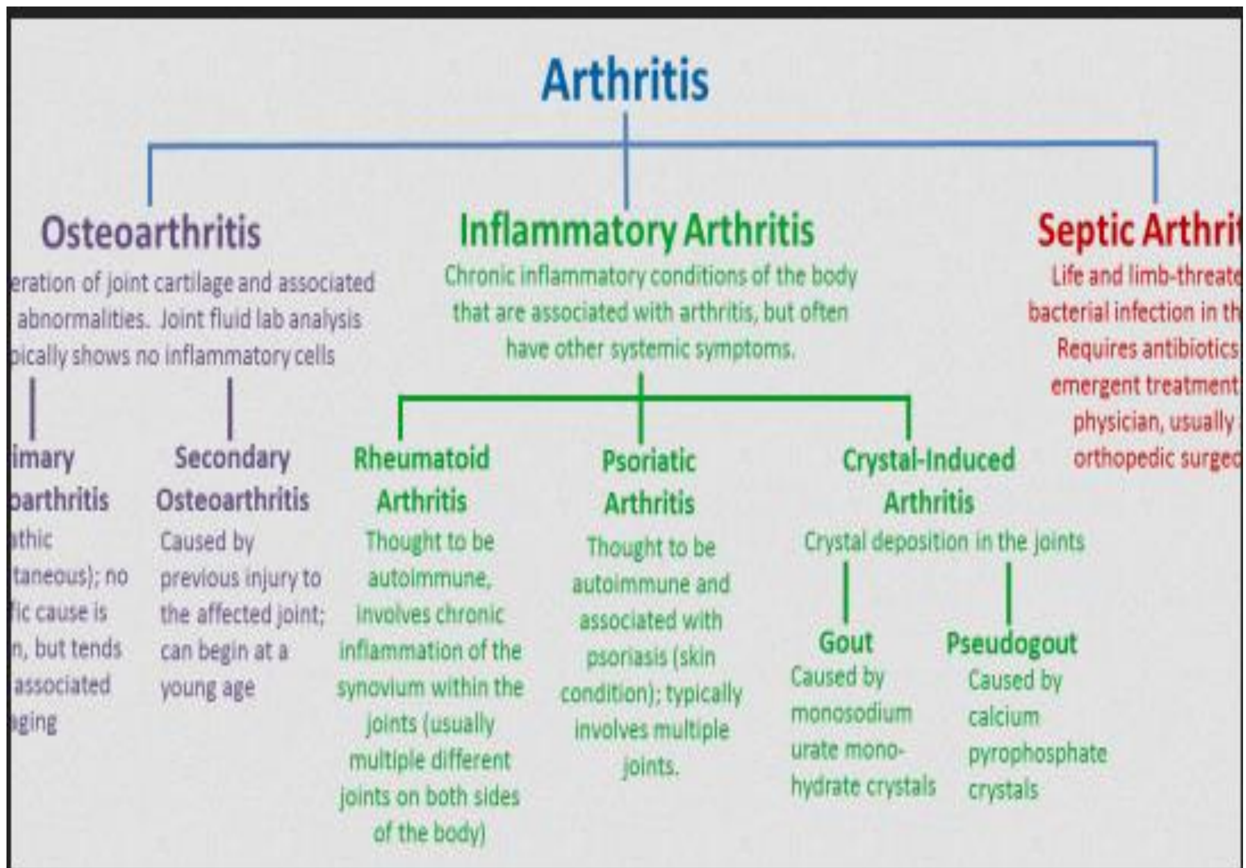


**Figure 2.8**: Arthritis Differences (Medicals, 2017)

### 2.1.7 TREATMENT OF ARTHRITIS

Controlling pain, reducing joint deterioration, and improving or maintaining function and all of these aims revolve around improving one's quality of life in arthritis treatment. Variety of available treatments include:

1. **MEDICATIONS**: Non-inflammatory kinds of arthritis, such as osteoarthritis, are frequently treated with pain-reducing medicines, physical exercise, weight loss and self-management education. Anti-inflammatory medicines such as corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac sodium, disease-modifying anti-rheumatic drugs (DMARDs), and a relatively new class of pharmaceuticals known as biologics are also

used to treat inflammatory forms of arthritis, such as Rheumatoid Arthritis. Medications will depend on the type of arthritis. Commonly used drugs include:

I. **Analgesics**: These reduce pain, but have no effect on inflammation. Acetaminophen (Tylenol), tramadol (Ultram), and opioids including oxycodone (Percocet, Oxycontin) or hydrocodone (Hycodone) are examples (Vicodin, Lortab). Tylenol can be ordered via the internet.
II. **NSAIDs (Nonsteroidal Anti-Inflammatory Medicines):** These pharmaceuticals are used to treat both pain and inflammation. Ibuprofen (Advil, Motrin IB) and naproxen sodium are two NSAIDs that can be purchased over-the-counter or online (Aleve). Some NSAIDs come in creams, gels, or patches that can be applied directly to specific joints.
III. **Counter-irritants**: Some creams and ointments containing menthol or capsaicin, the hot element in hot peppers, are among them. Rubbing them on the skin over a sore joint can reduce pain by modulating pain signals from the joint.
IV. **Disease-modifying antirheumatic drugs (DMARDs)**: are used to treat Rheumatoid Arthritis (RA).They slow or inhibit the immune system from attacking the joints. Examples include methotrexate (Trexall) and hydroxychloroquine (Plaquenil).
V. **Biologics**: They are often used with DMARDs. Biologics are frequently utilized in conjunction with DMARDs. These pharmacologic response modifiers are medications that have been genetically designed to target specific protein components involved in the immune response. Two examples are the drugs etanercept (Enbrel) and infliximab (Remicade).
VI. **Corticosteroids**: Examples are prednisone and cortisone which reduce inflammation and suppress the immune system.

2. **NON-PHARMACOLOGIC THERAPIES:** This section covers natural therapies such as eating a healthy, balanced diet and getting enough exercise, not smoking, and not drinking too much alcohol. Although no specific diet has been discovered to cure arthritis, certain foods may help reduce inflammation.
3. **PHYSICAL OR OCCUPATIONAL THERAPY:** Physical therapy is also used as it has been discovered to overcome some of the challenges of arthritis and to reduce limitations on mobility. Forms of physical therapy that may be recommended include:

- **Warm water therapy**: Exercises in a warm-water pool are known as warm-water therapy.

The weight is supported by the water, which relieves pressure on the muscles and joints.

- **Physical therapy**: consists of exercises that are adapted to the patient's condition and demands, and is sometimes paired with pain-relieving therapies like ice or heat packs, as well as massage.

  - **Occupational therapy**: Occupational therapy provides practical assistance on completing daily chores, selecting specific aids and equipment, preventing additional joint damage, and coping with weariness.

4. **SURGERY, INCLUDING JOINT REPLACEMENT:** Joint surgery can offer several benefits: The most important advantage of joint surgery is pain management. Many patients with arthritis are in excruciating pain all of the time. Rest, heat and cold treatments, exercise, splints, and prescription medicine can help alleviate some of the pain. If these treatments fail to alleviate the pain, surgery may be considered. Joint surgery can also help you move more freely and use your joint more effectively. Joints, tendons, and ligaments can be injured or dragged out of place as a result of chronic inflammation and the wear and tear of bone and cartilage. The loss of use of a joint, such as the hip, knee, hand, elbow, or shoulder, can significantly limit a person's activities. When this happens, surgery to replace or stabilize the joint may be suggested.

### 2.1.8 SYSTEMIC EFFECTS OF ARTHRITIS

RA systemic symptoms and consequences, such as pulmonary, cardiovascular, neurological, and musculoskeletal involvements; glucocorticoid (GC)-induced osteoporosis (GIOP); and infection, occur in around 40% of patients and have a substantial impact on disease outcomes. (Myasoedova *et al.,* 2011)

HEART – inflammation of the tissue around the heart can lead to pericarditis, which causes chest pain. Rheumatoid arthritis patients are susceptible to cardiovascular diseases (CVD). CVD is a broad term that refers to a variety of heart and blood vessel disorders, including life-threatening issues like heart attack and stroke. It's unclear why patients with rheumatoid arthritis have a higher risk of these complication.

Different CVDs associated with Rheumatoid arthritis include:

1. Heart attack: People with RA are twice as likely to have a heart attack as those without the disease. A heart attack occurs when a piece of plaque (a fatty deposition in your arteries) breaks off, a clot forms, and blood supply to the heart is interrupted.

2. Stroke: Also known as a brain attack, a stroke occurs when the brain's blood supply is cut off, most commonly due to a blood clot. People with RA are 60 to 100 percent more likely to have a stroke than people without the disease.

3. A pulmonary embolism: is a potentially fatal blood clot in the lungs. A pulmonary embolism is substantially more common in people with RA than in the general population, but those with high levels of rheumatoid arthritis disease activity are at the greatest risk.

4. Coronary artery disease: The most common type of heart disease, coronary artery disease occurs when the arteries leading to your heart become clogged with too much plaque (made of cholesterol and other substances). A clot can form if a piece of plaque breaks off, blocking blood flow to the heart and causing a heart attack. In comparison to the general population, patients with RA had a 1.5 to twice greater risk of coronary artery disease, according to studies.

5. Heart failure: Heart failure is a chronic disorder in which the heart is unable to pump effectively, causing you to feel weak. Everyday tasks can become difficult to do. You're twice as likely to get heart failure if you have RA.

6. Atrial fibrillation: Atrial fibrillation is a type of irregular and usually rapid heartbeat that puts you at risk for a stroke. RA patients are 60% more prone than the general population to get the disease.

Reasons Rheumatoid Arthritis Raises the Risk of Heart Problems include:

1. Inflammation which is dangerous to the heart and vessels
2. Lack of function of HDL (“good”) cholesterol
3. Magnification of heart disease risk factors (Barbara Brody, 2021).

### 2.1.9 ARTHRITIS EFFECT IN THE BRAIN TISSUE

Rheumatoid arthritis is an inflammatory disease that affects not just the joints but also other organ systems. Brain fog is one of the condition's lesser-known symptoms. Rheumatoid arthritis is an inflammatory disease that affects not just the joints but also other organ systems. Brain fog is one of the condition's lesser-known symptoms. These symptoms, known as brain fog, are widespread in people with chronic inflammatory conditions (Nancy Carteron, 2018).

The effects of chronic inflammation on the brain were investigated in a 2018 study published in Nature Communications Trusted Source. They took scans of the brains of 54 persons with RA using MRI scanners. The findings revealed a correlation between RA inflammation and changes in brain connectivity patterns. It also revealed a decrease in gray matter volume in the inferior parietal lobe, which is located in the back of the brain (Nancy Carteron, 2018). According to the research, these brain changes are linked to weariness, pain, and a reduced ability to think.

Researchers believe these alterations to brain tissues may have a role in converting inflammation signals to the rest of the central nervous system.

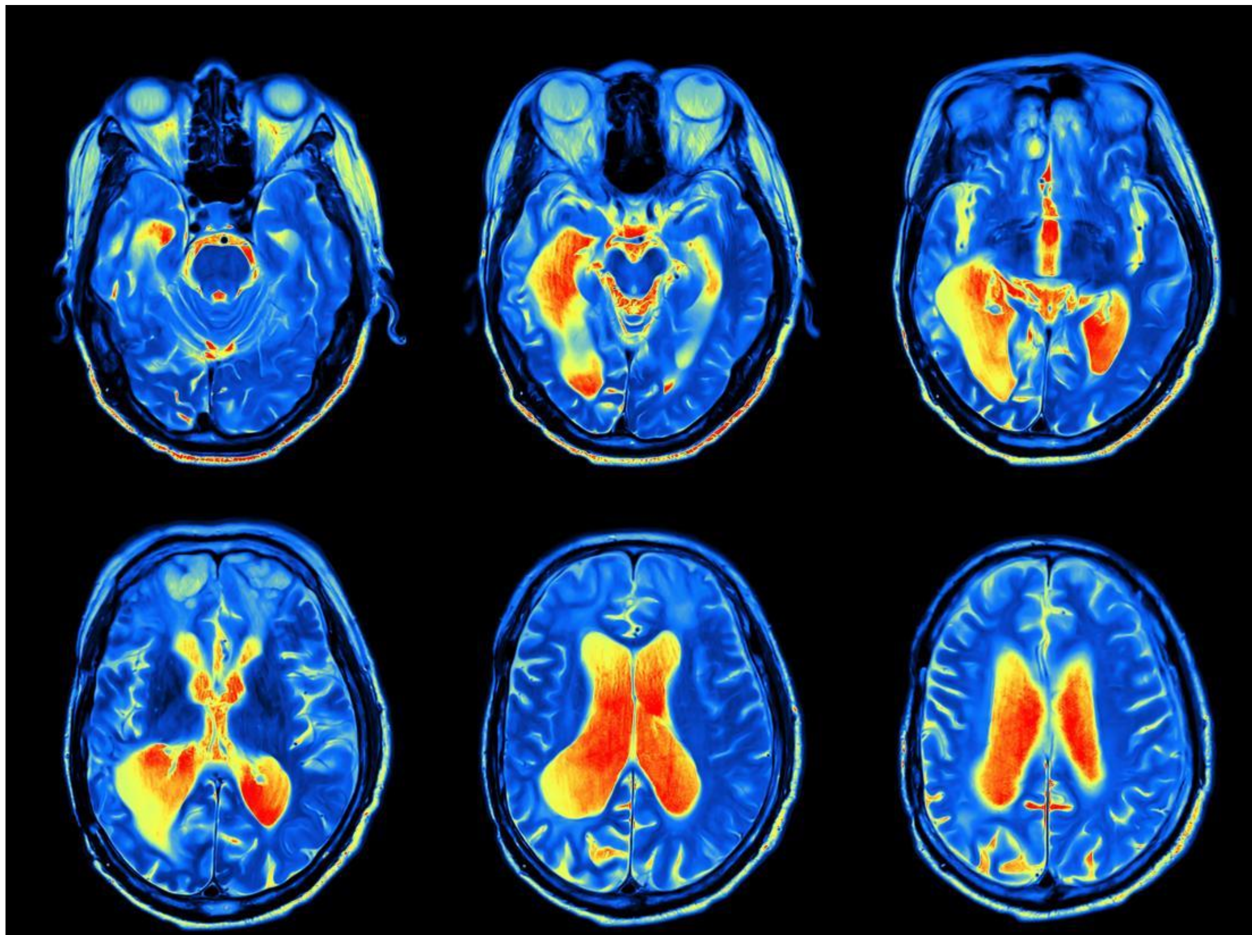

**Figure 2.9:** Using MRI how rheumatoid arthritis inflammation changes the brain (Ana Sandoiu, 2018).

An MRI scan creates a detailed cross-sectional image of interior organs and structures using a powerful magnet, radio waves, and a computer. The scanner is usually shaped like a long tube with a table in the middle into which the patient can slide. An MRI scan differs from CT scans and X-rays, as it does not use potentially harmful ionizing radiation (Judith Marcin, 2018).

### 2.2.0 *Alafia barteri* plant

Plant *Alafia barteri* The infusion of the leaves and twining stem, which are used for the treatment of inflammation and fever, is regarded in the traditional medicine system in Nigeria and other African countries as an anti-inflammatory and fever remedy (Sofidiya, Essien & Aigbe,2014). (Sofidiya, Essien & Aigbe,2014). In South-Western Nigeria (Lagos), *Alafia barteri* has been used for the treatment of malaria. Alafia barteri decoctions, together with the stem and root decoctions, are used to cure rheumatic pains, toothaches, eye infections, and

sickle-cell anemia. (Olowokudejo & Kadiri,2008). The inhabitants of South-Western Nigeria (Lagos) name *Alafia barteri* agbari-etu, which means "quick fever treatment." In Nigeria and other African countries, Alafia barteri leaf infusions and root decoctions are used to treat malaria (Dalziel, 1937). (Dalziel, 1937). Alafia barteri stem and root decoctions are utilized in Nigerian traditional medicine. for treating rheumatic pains, toothache, eye infection and sickle-cell anemia (Olowokudejo, Kadiri & Travil, 2008). Polyphenols, flavonoids and alkaloids have been reported for wide varieties of pharmacological activities, including ant plasmodial activity (Makkar & Sidhuraju, 2007). High levels of polyphenols and flavonoids reported in the roots and leaves fractions of *Alafia barteri* could be responsible for its ant plasmodial activity (Lasisi, Olayiwola & Balogun, 2016)

**Taxonomy**

Kingdom:Plantae

Clade: Tracheophytes

Clade: Angiosperms

Order: Gentianales

Family:Apocynaceae

Genus: Alafia

Species:A. barteri

**Binomial name:** *Alafia barteri* Oliv. (Royal Botanic Gardens, 2020)

### 2.3.0 OXIDATIVE STRESS

Oxygen, though the very gas that support life, could also lead to necrosis and cell death when it generates reactive species due to oxidation. Oxygen is capable of producing reactive oxygen species (ROS) such as hydroxyl radical ($OH^-$), superoxide radical ($SO_2^-$), and hydrogen peroxide ($H_2O_2$) which are exogenous ROS. Cellular ROS include NADPH oxidase and hydroperoxyl (Somogyi *et al.,*2007).

Oxidative stress is defined as any alteration in the equilibrium of antioxidants and pro-oxidants in favor of pro-oxidants, as a result of many variables such as age, inflammation, pharmacological action, and toxicity, is referred to as oxidative stress. (Asmat *et al.* 2015). Oxidative stress results in the inefficiency of the functions of some body cells and also a damage in the structure of cells resulting into different diseased conditions, examples of such structural impairment and diseased conditions caused by oxidative stress are listed in table 1.

**Table 2.1:** Organs affected and diseases caused by oxidative stress (Asmat *et al.* 2015)

| Organs | Diseases |
|---|---|
| Lungs | Asthma, chronic bronchitis |
| Kidneys | Glomerulonephritis, chronic renal failure |
| Joints | Arthritis, rheumatism |
| Brain | Alzheimer's disease Parkinson's disease, memory loss, and stroke |
| Eyes | Cataract, retinal diseases |
| Fetus | Preeclampsia, IU growth restriction |
| Heart vessels | Arteriosclerosis, hypertension, ischemia, cardiomyopathy, heart failure |
| Multiorgan | Cancer, diabetes, inflammation infection, aging |
| | |

Oxidative stress occurs via two mechanisms. To begin with, this is related to a reduction in antioxidant levels (which may be as a result of mutated antioxidant enzymes, toxins, or low intake of nutritional antioxidants). Second, oxidative stress is caused by an increase in the amount of free radicals such as reactive oxygen species (ROS) as a result of chronic inflammation. (Somogyi *et al.,* 2007).

Oxygen possesses a property of free radicals as a result of its two unpaired electrons that have a parallel spin in separate antibonding orbitals that supports its stability and paramagnetic property, this however can be altered by a single electron exchange which converts oxygen to an oxidizing agent and electron transfer to oxygen is catalyzed by the enzyme oxidase to produce energy or substrate oxidation. (Chikezie *et al.,* 2015).

Reactive Oxygen Species (ROS) could be free radicals or non-radical oxygen species. Reactive oxygen species can be produced either by stimulation of NADPH oxidase or by the mitochondrial respiratory chain. In the later ROS become unwanted byproducts of the metabolism, which is as a result of the oxidative stress, this then leads to overproduction of ROS that can cause alteration of proteins, and DNA also causing lipid peroxidation (Somogyi *et al.,* 2007), and this consequently result in cell damage or cell injury.

Free radicals are classified as follows:

1. Reactive Oxygen Species (ROS)
2. Reactive Nitrogen Species (RNS)

3. Reactive Chlorine Species (RCS)

Each of these could result in oxidative stress by means of their high reactivity especially the ROS and RNS which are more abundant in macromolecules and are involved in almost all the major metabolism in the system (Somogyi *et al.* 2007).

### 2.3.1 RHEUMATOID ARTHRITIS AND OXIDATIVE STRESS IN HEART AND BRAIN

As a prototype of chronic inflammatory Rheumatoid arthritis is an autoimmune disease that has been linked to oxidative stress, a condition in which the pool of reactive oxygen species increases over time, either through increased production, decreased antioxidant defenses, or a combination of both, implying a compromise in redox signaling. (Agca & Heslinga, 2016).

The exact mechanisms by which oxidative stress contributes to the initiation and perpetuation of local (in the articular milieu) and systemic inflammation in rheumatoid arthritis, especially in the early stages, are still unknown. it becomes easy to admit the potential cross-talk between oxidative stress and RA, as such autoimmune disease characteristically represents an entity of chronic systemic inflammation (Garcia-Gonzalez, 2015). Despite this almost self-evident interaction, few studies have been dedicated to understanding the in-depth mechanisms by which redox imbalance may contribute to even fewer research focusing on the investigation of oxidative biomarkers in autoimmune arthritis, with the creation of the proinflammatory milieu seen in RA and vice versa. (Quiñonez-Flores, 2016).

Rheumatoid arthritis is a systemic disease and in addition to affecting the joints, it evokes inflammatory and oxidative alterations in other organs, such as lungs, liver and heart (McInnes & Schett, 2011). In the serum of individuals with rheumatoid arthritis and in the extra-articular tissues of animal models of rheumatoid arthritis, oxidative stress indicators are altered (Stamp & Khalilova, 2012).

Inflammation in rheumatoid arthritis also affects the brain where it causes fatigue and reduced cognitive function (McInnes & Schett, 2011). Patients with severe rheumatoid arthritis have been shown to have cerebral atrophy and other anatomical changes (Wendt & Sá-Nakanishi, 2012). In the brains of rats with adjuvant-induced arthritis, levels of reactive oxygen species (ROS), nitrogen oxides (NO), lipoperoxides, and protein carbonyl groups are higher, notably in the mitochondria, where the transmembrane potential is also higher (Wartolowska & Hough, 2015). These alterations are accompanied by decreased levels of reduced glutathione (GSH) and diminished activities of antioxidant enzymes. Additionally, the activity of the pro-oxidant enzyme xanthine oxidase (XO) and

the pro-inflammatory enzyme iNOS are increased in the brain of arthritic rats (Wartolowska & Hough, 2015). As evidenced by increased isoprostane and prostaglandin levels in affected patients' synovial fluid, free radicals at the site of inflammation play an important role in both the initiation and progression of this syndrome. (Mahajan & Tandon, 2004).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS/DESIGN/METHODOLOGY

### 3.1 MATERIALS/APPARATUS USED

i. Dissecting set
ii. Blender
iii. Mortar & pestle
iv. Gloves
v. Muslin Cloth
vi. Funnel
vii. Filter Paper
viii. Beaker
ix. Dry oven
x. Rotary evaporator
xi. Refrigerator
xii. Spatula
xiii. Water bath
xiv. Carbonate buffer
xv. Mechanical homogenizer
xvi. Centrifuge
xvii. UV Spectrophotometer
xviii. Micro pipette
xix. Test-tubes
xx. Digital Vernier caliper
xxi. Insulin syringe (1ml),
xxii. Lithium heparin tubes
xxiii. Plain sterile tubes,

**Reagents Used:**

i. Distilled water
ii. Ethanol
iii. PBS (Phosphate Buffer Saline)
iv. Formaldehyde (2%),
v. Standard drug (Diclofenac),
vi. SOD Randox Kits
vii. MDA Randox Kits

### 3.2.0 COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

Fresh leaves of *Alafia barteri* were collected from Osun State, Nigeria. Botanical identification and authentication were done by Dr George at the Department of Botany, University of Lagos, Lagos State, Nigeria, where a voucher specimen number of **LUH 8657** was allotted to it.

### 3.2.1 PREPARATION OF LEAF & ROOT EXTRACTS

**Aqueous Extraction:** *Alafia barteri* leaves and root bark were air dried for four (4) weeks and then grinded to powder using the blender. 60g of the powdered leaves and grinded bark was extracted with 300mls of distilled water respectively and allowed to stand at room temperature for 72hours with constant stirring. The solutions were then filtered using a muslin cloth. The filtrates were collected into a clean beaker and concentrated using laboratory oven to dryness to get the pure extracts.

**Ethanol Extraction:** The root barks of *Alafia barteri* were chopped into smaller pieces and pounded into smaller pieces using mortar and pestle. 60g of the pounded barks were extracted with 300mls of distilled water and allowed to stand at room temperature for 72hours with constant stirring. The solution was then filtered using a cloth sieve, the filtrate was collected into a clean beaker. The filtrate is then concentrated using rotatory evaporator to get the pure extracts.

### 3.3.0 EXPERIMENTAL ANIMALS

A total number of 77 mice were randomly divided into 11 groups of 7 animals each. They were acclimatized in clean and dry cages for seven days with regular supply of feed and water.

Experimental Design

Group 1: Normal control
Group 2: Arthritic control

Group 3: Aqueous leaf extract (200mg)

Group 4: Aqueous leaf extract (400mg)

Group 5: Ethanol leaf extract (200mg)

Group 6: Ethanol leaf extract (400mg)

Group 7: Standard drug (Diclofenac) (10mg/kg)

Group 8: Aqueous root extract (200mg)

Group 9: Aqueous root extract (400mg)

Group 10: Ethanol root extract (200mg)

Group 11: Ethanol root extract (400mg

### 3.3.1 INDUCTION OF FORMALDEHYDE AND PAW DIAMETER

Arthritis was induced by the injection of 0.1mls of formaldehyde into the right hind paw of each mouse.

**Figure 3.1:** During Induction

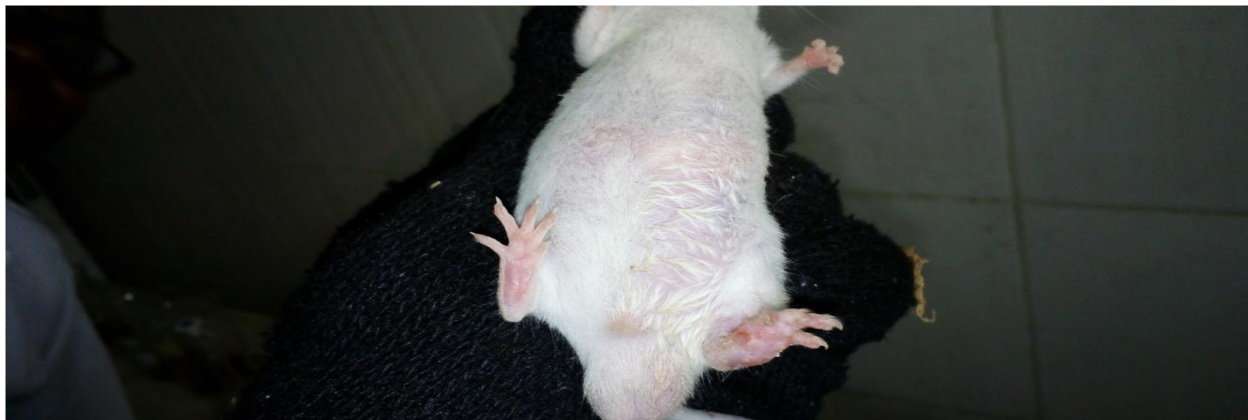

**Figure 3.2:** Swollen paw after Induction

### 3.4.1 Tissue Preparation

Oral administration took place for 10 days after which the mice were sacrificed with the heart and brain tissues harvested. The harvested tissues (heart and brain) were rinsed in ice-cold 0.1 M PBS (pH 7.4) and homogenized using Ultra Turrax homogenizer toobtain a cloudy homogenate. The homogenates were centrifuged at 12,000*g* for 10 minutes to obtain the supernatants which were separated and refrigerated at 4$^{o}$C for analysis of oxidative stress parameters.

## PROCEDURE

### 1. Assessment of Lipid Peroxidation (LPO)

The level of oxidative damage on the tissues was estimated by quantifying the amount of thiobarbituric acid reactive substances (TBARS) present in the sample using the method described by Varshney and Kale (1990).

**Principle** : Malondialdehyde (MDA) which is produced from the peroxidation of membrane fatty acid and food products under acidic condition reacts with the chromogenic reagent, 2-thiobarbituric acid (TBA), to give off a pink colored complex with maximum absorbance at 532 nm.

Reagents Preparation for MDA Assay

1. Trichloroacetic acid (TCA, 30%): TCA (9 g) was dissolved in distilled water and made up to 30 mls with same.
2. Thiobarbituric acid (0.75%): This was prepared by dissolving 0.225 g of thiobarbituric acid (TBA) in 0.1 M HCl and made up to 30 ml with same.

3. Tris-KCl buffer (0.15 M, pH 7.4): KCl (1.12 g) and 2.36 g of Tris base were dissolved separately in distilled water and made up to 100 ml with same and pH was then adjusted to 7.4.

**Procedure:** In a test tube containing 0.4 ml of the each homogenate (heart or brain tissue), 1.6 ml of Tris-KCl buffer was added followed by 0.5 ml of 30% TCA. 0.5 ml of 0.75% TBA was then added and placed in a water bath for 45 minutes at 80ºC. The heated test tube was removed, placed on ice and centrifuged at 3000g. The clear supernatant was collected and record the absorbance measured against a reference blank of distilled water at 532 nm. The level of MDA was determined in units/mg protein or gram tissue was computed with a molar extinction coefficient of 1.56 x 105 $M^{-1}cm^{-1}$.

2. **Assay of Superoxide Dismutase (SOD) Activity**

**Principle:** Superoxide dismutase activity was determined by its ability to inhibit the auto-oxidation of SOD reagent determined by the increase in absorbance at 480nm as described by Sun and Zigma (1978). The reaction mixture (3ml) contained 2.95ml 0.05M sodium carbonate buffer pH 10.2 and 0.02ml of heart and brain supernatants respectively. 0.03ml of 2mM SOD reagent was added, which was used to initiate the reaction. The absorbance was read at a regular interval of 1 min for 5 min at 480nm. $\varepsilon = 4020 M^{-1}cm^{-1}$ (Zou et al., 1986)

Activity of SOD = $\Delta A/\min \times TV/\varepsilon \times SV$

Where ΔA = change in absorbance

TV = total volume

SV = sample volume

ε = molar extinction

3. **Estimation of Reduced Glutathione**

The reduced glutathione (GSH) content of heart tissue and brain tissue as non-protein sulphybydryls was estimated according to the method described by Sedlak and Lindsay (1968). To the sample, 1ml of 10% TCA was added and centrifuged at 3000rpm. 5ml of the respective supernatants were treated with 0.5ml of Ellmans reagent and 3.0ml of phosphate buffer (0.2M, pH 8.0). The absorbance was then read at 412nm against the reagent blank.

Concentration of GSH = $\Delta A \times TV/\varepsilon \times SV$

$\varepsilon = 1.34 \times 10^4 M^{-1} cm^{-1}$

where

$\Delta A$ = change in absorbance

TV = total volume

SV = sample volume

$\varepsilon$ = molar extinction

**3.5.0 Statistical Analysis**

Results obtained from this study was analyzed by one-way analysis of variance (ANOVA) using the Graph Pad Prism Software (GPPS 7.0)

# CHAPTER FOUR

## RESULTS

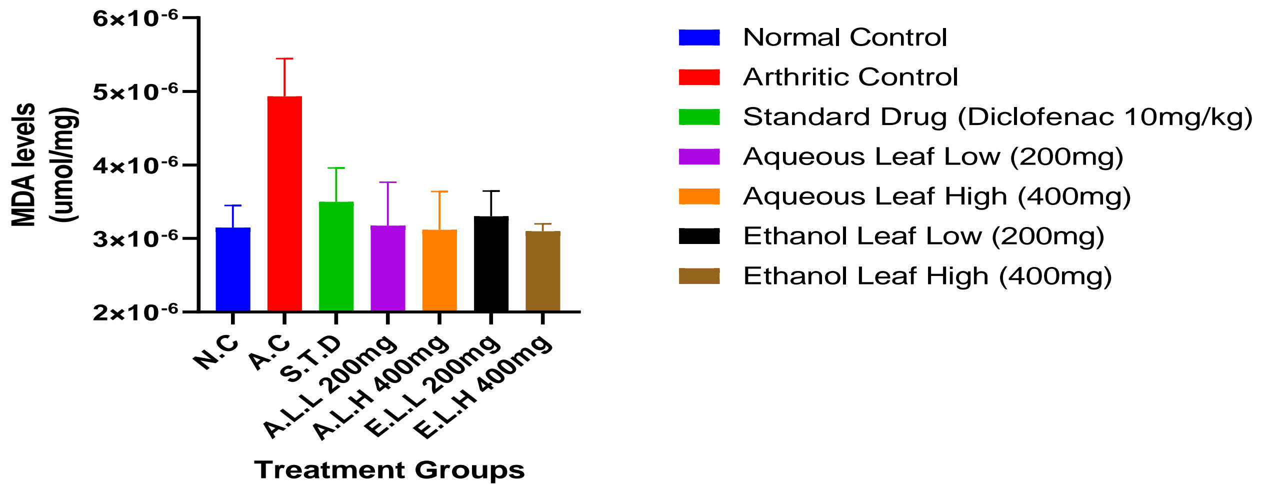


**Figure 4.1**: MDA levels of brain tissue homogenate of formaldehyde induced arthritic mice administered with aqueous and ethanol leaf extracts of *Alafia barteri.*

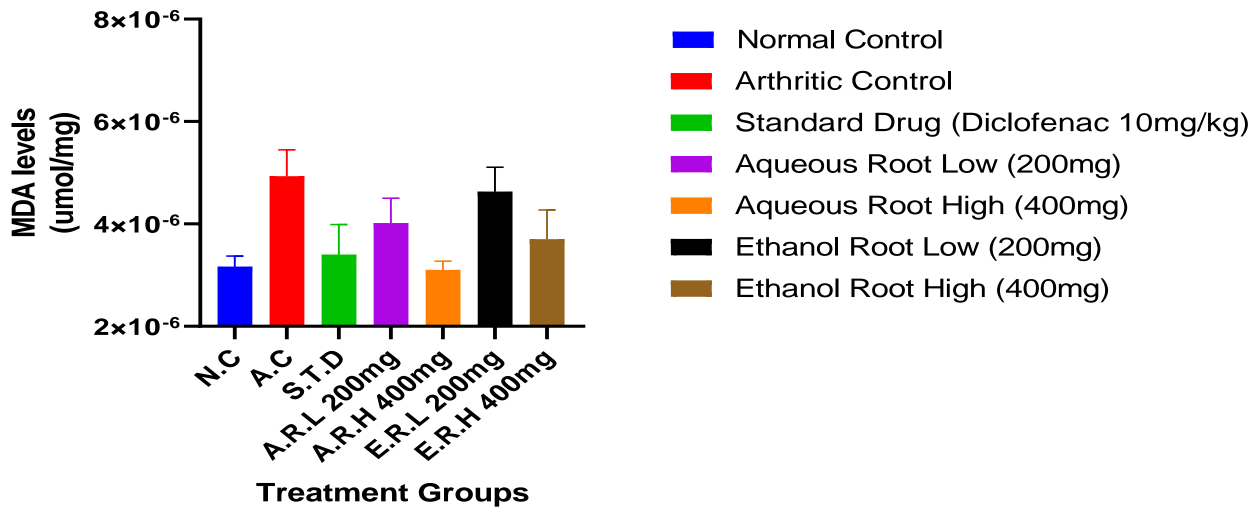


**Figure 4.2:** MDA levels of brain tissue homogenate of formaldehyde induced arthritic mice administered with aqueous and ethanol root extract of *Alafia barteri.*

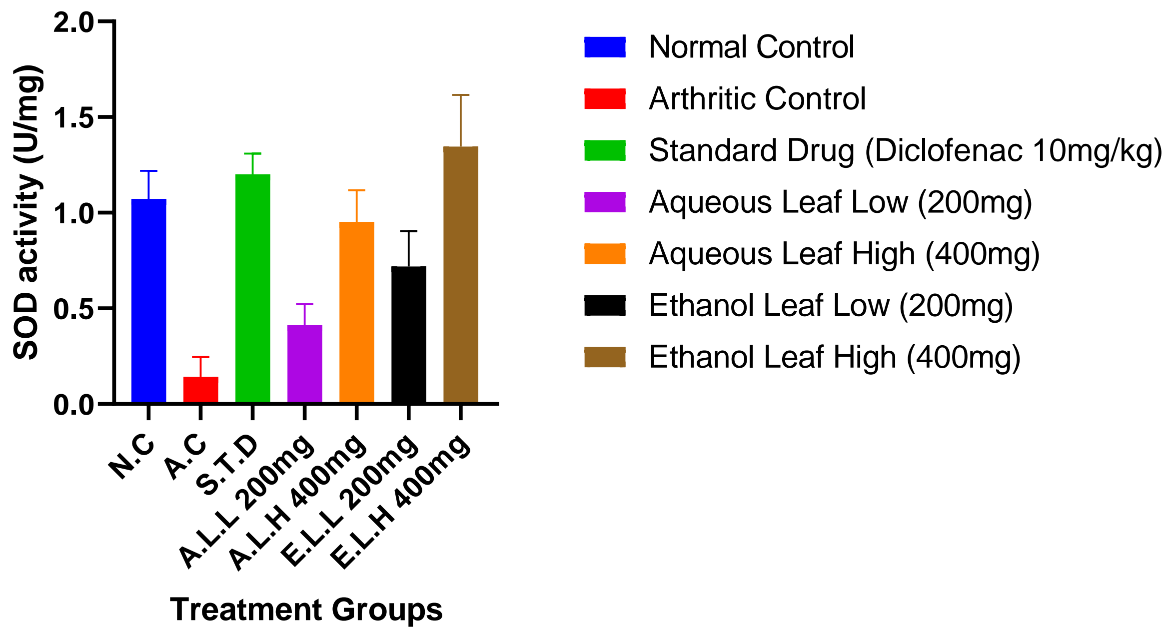


**Figure 4.3:** SOD activity of brain tissue homogenate of formaldehyde induced arthritic mice administered with aqueous and ethanol leaf extract of *Alafia barteri*.

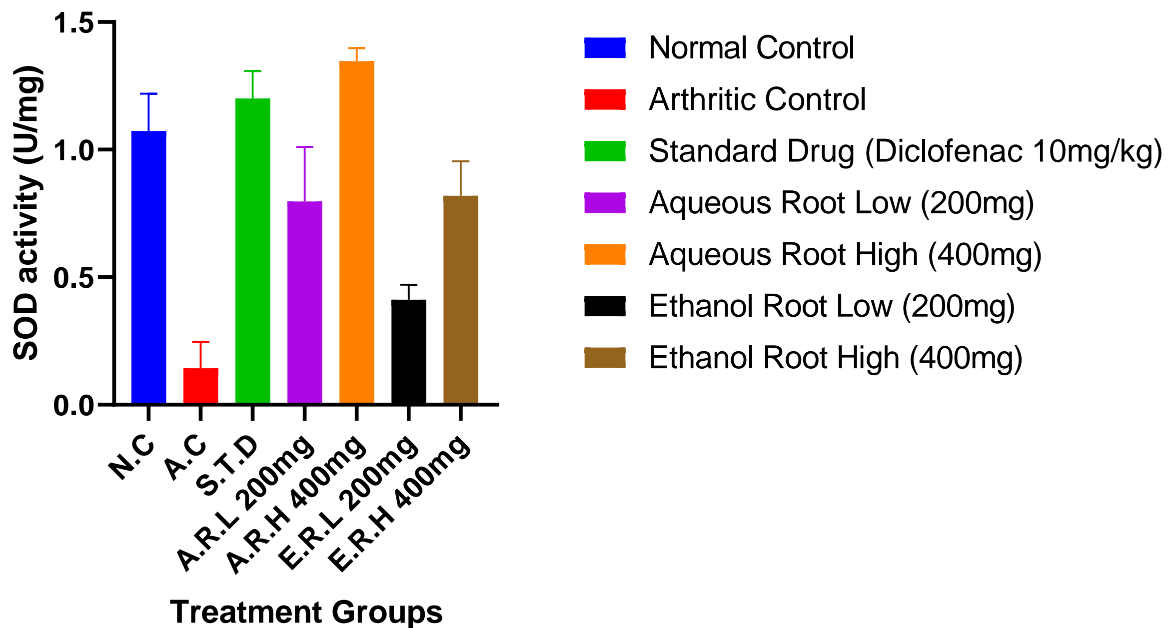


**Figure 4.4**: SOD activity of brain tissue homogenate of formaldehyde induced arthritic mice administered with aqueous and ethanol root extract of *Alafia barteri*.

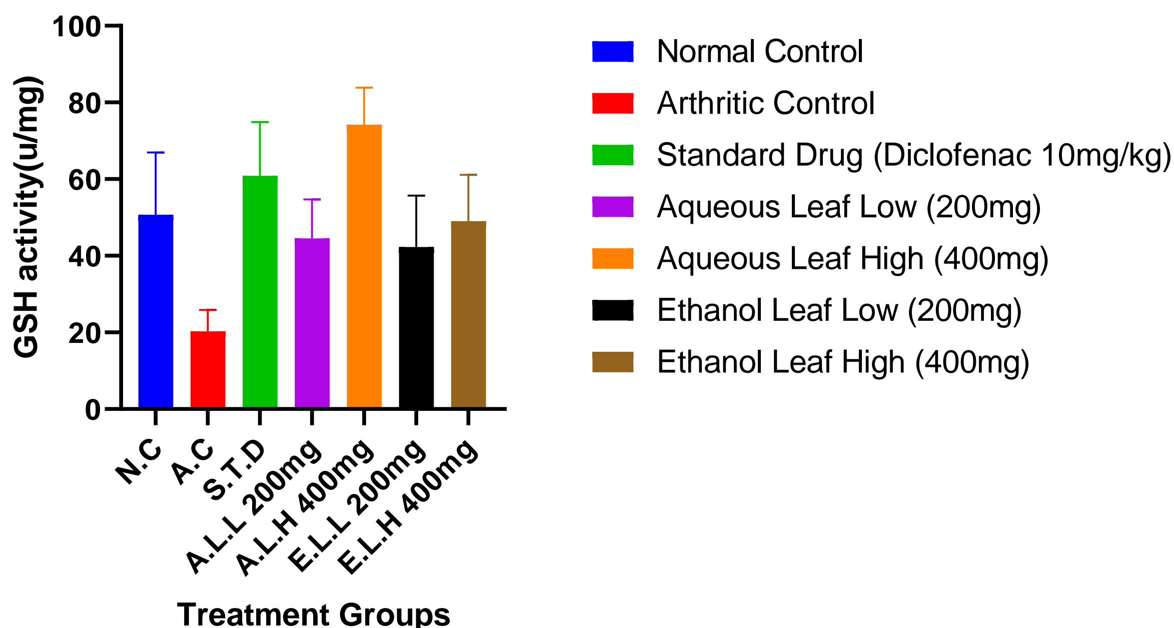


**Figure 4.5:** GSH activity of brain tissue homogenate of formaldehyde induced arthritic mice administered with aqueous and ethanol leaf extract of *Alafia barteri*.

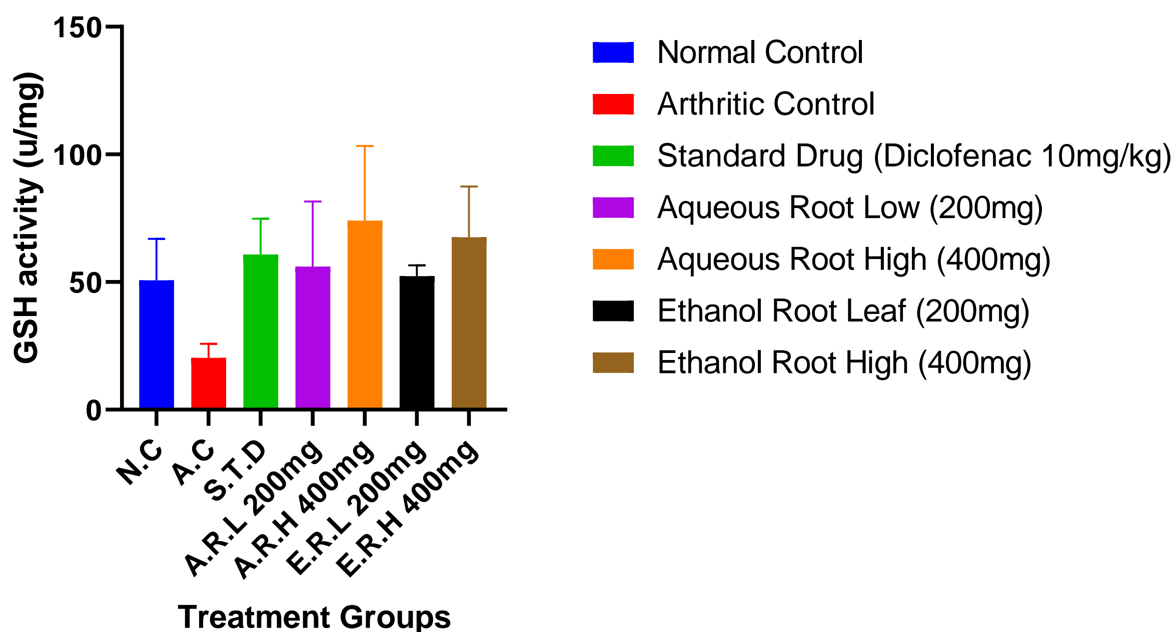


**Figure 4.6:** GSH activity of brain tissue homogenate of formaldehyde induced arthritic mice administered with aqueous and ethanol root extract of *Alafia barteri*.

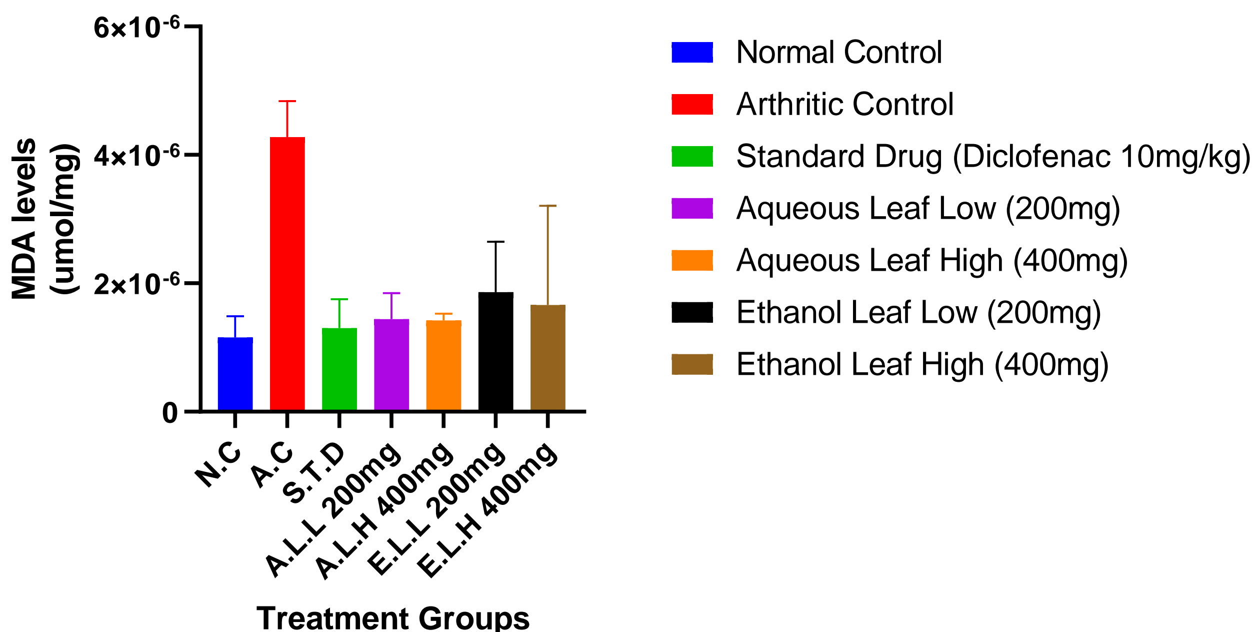


**Figure 4.7:** MDA levels of heart tissue homogenate of formaldehyde induced arthritic mice administered with aqueous and ethanol leaf extract of *Alafia barteri*.

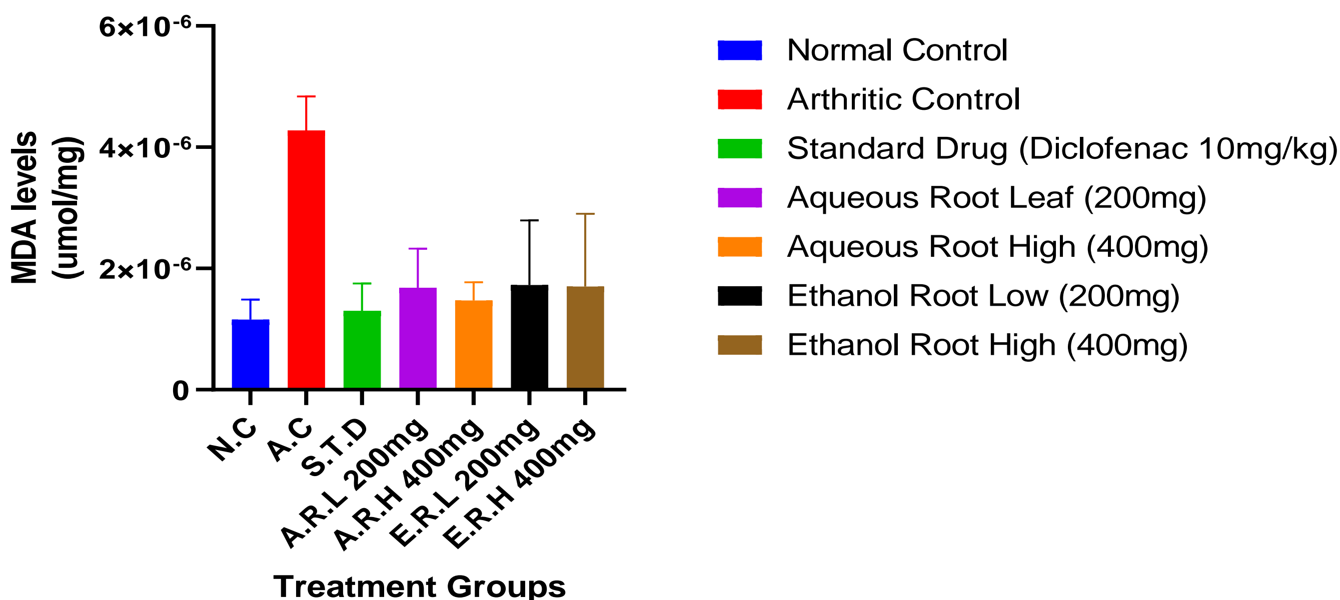


**Figure 4.8:** MDA levels of heart tissue homogenate of formaldehyde induced arthritic mice administered with aqueous and ethanol root extract of *A.barteri*

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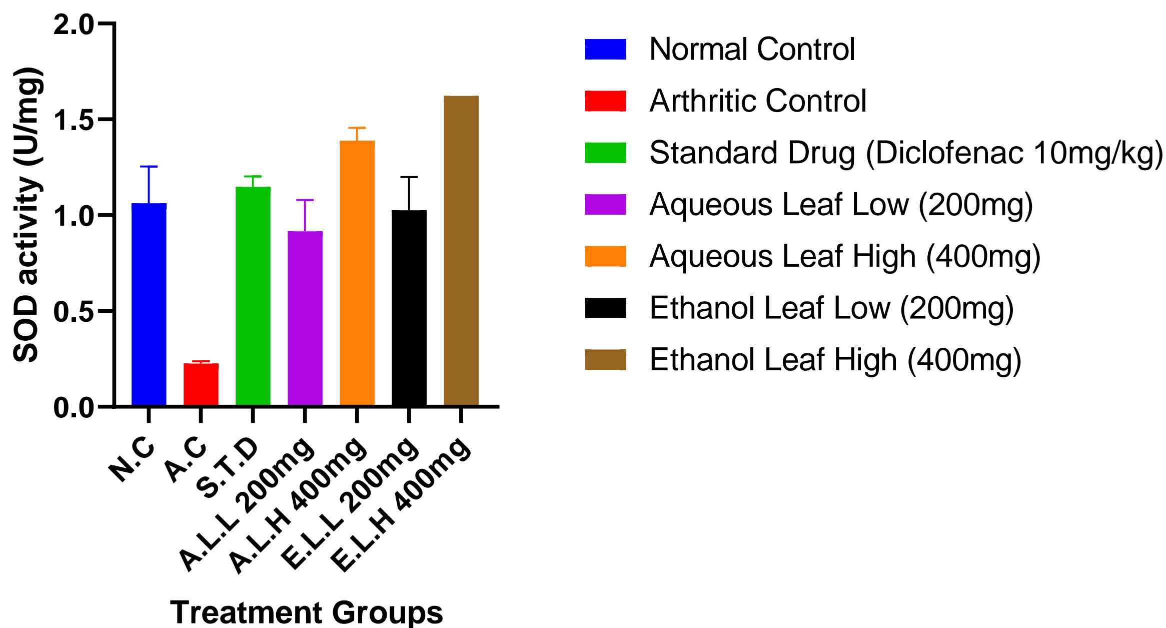


**Figure 4.9:** SOD activity of heart tissue homogenate of formaldehyde induced arthritic mice administered with aqueous and ethanol leaf extract of *Alafia barteri*.

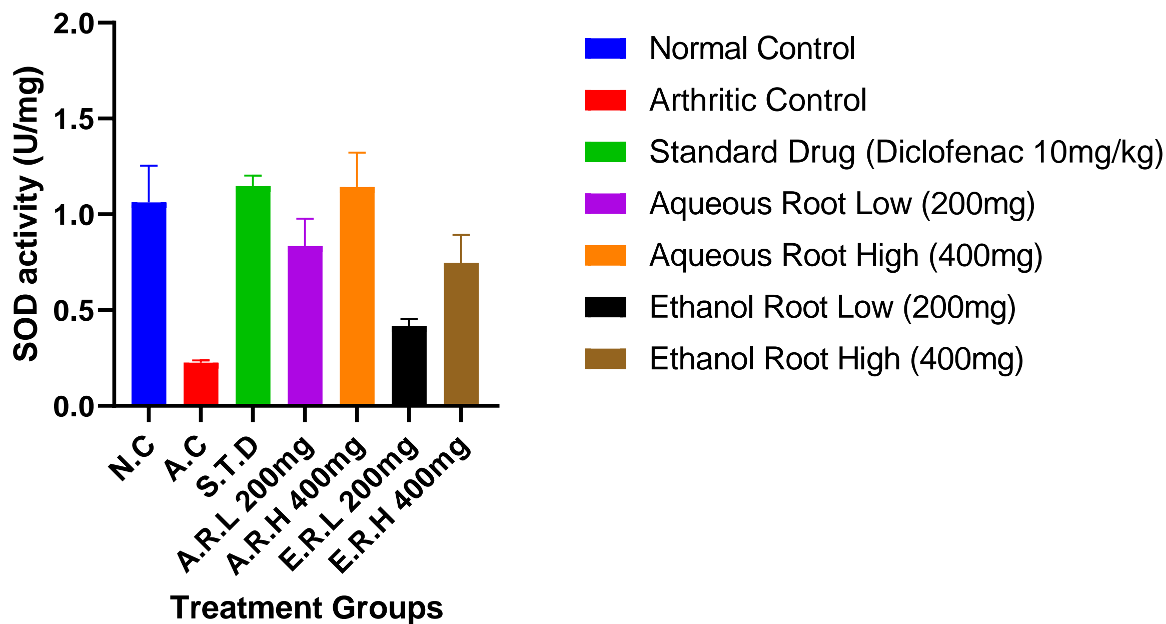


**Figure 4.10:** SOD activity of heart tissue homogenate of formaldehyde induced arthritic mice administered with aqueous and ethanol root extract of *Alafia barteri*.

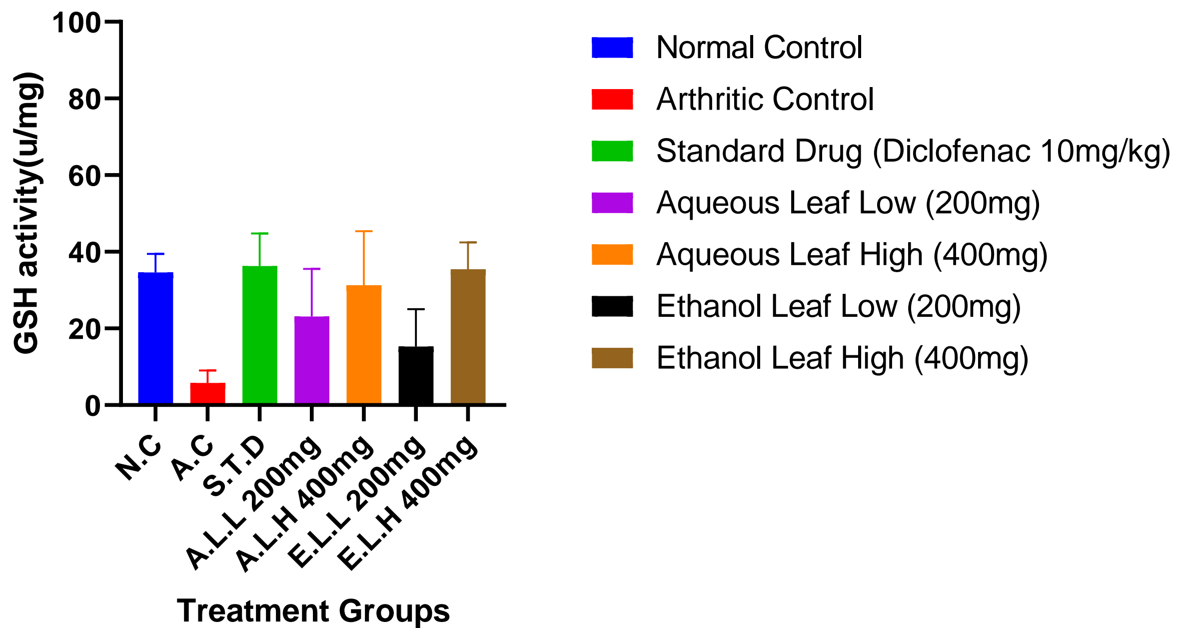


**Figure 4.11:** GSH activity of heart tissue homogenate of formaldehyde induced arthritic mice administered with aqueous and ethanol leaf extract of *Alafia barteri*.

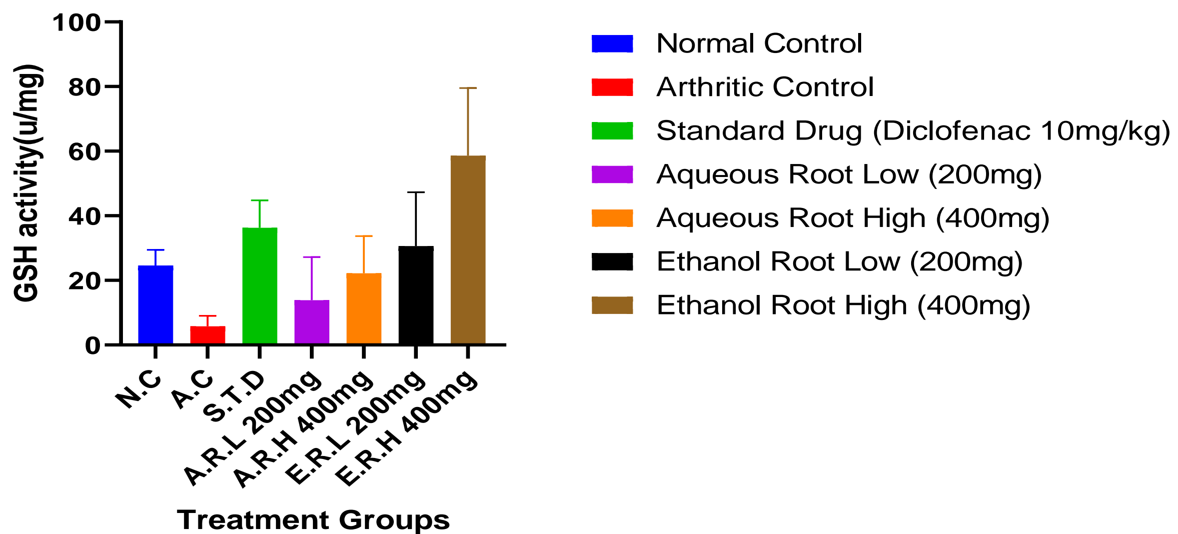


**Figure 4.12:** GSH activity of heart tissue homogenate of formaldehyde induced arthritic mice administered with aqueous and ethanol root extract of *Alafia barteri*.

Figure 4.1 revealed a significant decrease in brain MDA levels of arthritic mice on administration of *Alafia barteri* aqueous and ethanol leaf extracts at 400mg/kgbwt. The aqueous root extract at 400mg/kg bwt caused a significant reduction in brain and heart MDA levels when compared to the arthritic control group. (Figure 4.2). The aqueous leaf extract at 400mg/kg significantly reduced heart MDA levels followed by the ethanol extracts, though diclofenac had more effect. GSH activity of the heart increased on administering EL and ER at a dose of 400mg/kg but in comparison with the standard drug. The root extracts also significantly increased brain GSH activity during this study. Administration of the aqueous root and ethanol leaf extracts significantly increased the heart and brain SOD levels.

### 4.2 DISCUSSION

Malondialdehyde, a white liquid, is a highly reactive chemical that arises as an enol. (Nair & O'Neil, 2008). It occurs naturally and is a marker for oxidative stress. During excessive oxidative, Malondialdehyde (MDA) increases and total antioxidant capacity (TAC) decreases in body (Farahnaz, 2013). During this study, oral administration of *Alafia barteri* extract increased SOD and GSH levels, leading to the elimination of ROS which has the potential to restore the oxidative level back to normal, while it decreased the MDA level in the brain and heart of the arthritic mice. Superoxide dismutase (SOD) which is an anti-oxidant help the body to remove the superoxide radicals by converting it to hydrogen peroxide ($H_2O_2$). Glutathione peroxidases are also involved in the removal of $H_2O_2$.An increase in SOD and GSH in the body than that of MDA will bring about low or no oxidative stress occurring.

The aqueous and ethanol leaf and root extracts of the plant help in sufficient increase in SOD and GSH activity due to the presences of bioactive constituents such as flavonoid, terpenoids, saponin, tannins, steroid and cardiac glycoside which are antioxidant agents, this concur with the report of Makajuola *et al*, 2016. The presence of these secondary metabolites has contributed to its medicinal value as well as physiological activity. Anti-allergic, anti-inflammatory, anti-microbial, anticancer, and anti-diarrheal properties have all been discovered in flavonoids. Tannins are antioxidants, antimicrobials, anti-inflammatory agents, and diuretics.

# CHAPTER FIVE

## 5.1 CONCLUSION

This study reveals that the aqueous and ethanol leaf and root extract of *Alafia barteri* possesses anti-oxidant activities as determined by the inhibition or reduction of MDA levels and increase in the levels of antioxidant enzymes: SOD and GSH, hence validating its claim as a plant with antioxidant activities.

## 5.2 RECOMMENDATION

The result of this study shows the anti-oxidative and anti-arthritic properties of *Alafia barteri* plant which makes it suitable and recommendable for the treatment of arthritis. The plant should be recommended in arthritis researches to help improve the standard of human life.

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**APPENDIX**

**WEIGHT OF MICE**

Each mouse were weighed and grouped according to similar weight. After which the average of the mice according to each group was taken.

**Weight of mice**

| CAGE 1: NORMAL CONTROL | CAGE 2: ARTHRITIC CONTROL | CAGE3:AQUEOUS LEAF LOW DOSE (200MG) | CAGE4:AQUEOUS LEAF HIGH DOSE (400MG) |
|---|---|---|---|
| N:23.53<br>H:25.89<br>B:25.79<br>T:28.56<br>HB:24.86<br>HT:28.03<br>BT:28.88<br>AVERAGE WEIGHT:28.88 | N: 31.02<br>H:31.37<br>B:31.16<br>T:30.52<br>HB:30.01<br>HT:31.77<br>BT:30.21<br>AVERAGE WEIGHT: 30.21 | N:36.40<br>H:35.64<br>B:35.57<br>T:35.45<br>HB:36.65<br>HT:36.63<br>BT:35.52<br>AVERAGE WEIGHT :35.98 | N:33.95<br>H:33.67<br>B:32.06<br>T:33.64<br>HB:33.42<br>HT:33.81<br>BT:33.47<br>AVERAGE WEIGHT:33.43 |
| CAGE 5: ETHANOL LEAF LOW DOSE (200MG) | CAGE 6: ETHANOL LEAF HIGH DOSE (400MG) | CAGE 7: STANDARD DRUG(DICLOFENAC) (10MG/KG) | |
| N:36.18<br>H:38.25<br>B:37.52<br>T:35.52<br>AVERAGE WEIGHT:36.87g | N:33.90<br>H:32.33<br>B:33.12<br>T:34.81<br>HB:34.89<br>AVERAGE WEIGHT: 33.61g | N:20.06<br>H:22.99<br>B:29.25<br>T:28.72<br>HB:28.02<br>AVERAGE WEIGHT:25.80 | |

**LIPID PERIOXIDATION OF BRAIN TISSUSE OF THE MICE**

**ABSORBANCE READING USING SPECTOPHOTOMETER**

| GROUP 1 NORMAL CONTROL (N.C) | ABORBANCE READING | CONCENTRATION |
|---|---|---|
| N.C BACK 1 | 0.4836 | 3.1e-$^{6}$ |
| N.C HEAD | 0.5304 | 3.4e-$^{6}$ |
| N.C BACK & TAIL | 0.468 | 3e-$^{6}$ |
| N.C NEUTRAL | - | - |

| GROUP 2 ARTHRITIC CONTROL (A.C) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| A.C BACK & TAIL | 0.858 | 5.5e-$^{6}$ |
| A.C NEUTRAL | 0.702 | 4.5e-$^{6}$ |
| A.C BACK | 0.7488 | 4.8e-$^{6}$ |
| A.C TAIL | - | - |

| GROUP 3 AQUEOUS LEAF Low (A.L.L) | ABSORBANCE READING | |
|---|---|---|
| A.L.LOW BACK & TAIL | 0.4368 | 2.8e-$^{6}$ |
| A.L.LOW BACK | 0.5304 | 3.4e-$^{6}$ |
| A.L.LOW HEAD & TAIL | 0.6084 | 3.9e-$^{6}$ |
| A.L.LOW HEAD & BACK | 0.406 | 2.6e-$^{6}$ |

| GROUP 4 AQUEOUS LEAF High (A.L.H) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| A.L.HIGH HEAD | 0.593 | 3.8e-$^{6}$ |
| A.L.HIGH BACK & TAIL | 0.468 | 3e-$^{6}$ |
| A.L.HIGH HEAD & BACK | 0.406 | 2.6e-$^{6}$ |
| A.L.HIGH BACK | 0.546 | 3.5e-$^{6}$ |
| A.L.HIGH NEUTRAL | 0.421 | 2.7e-$^{6}$ |

| GROUP 5 ETHANOIC LEAF Low (E.L.L) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| E.L.L BACK & TAIL | 0.546 | $3.5e\text{-}^{6}$ |
| E.L.L HEAD | 0.546 | $3.5e\text{-}^{6}$ |
| E.L.L HEAD & BACK | 0.452 | $2.9e\text{-}^{6}$ |

| GROUP 6 ETHANOIC LEAF High (E.L.H) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| E.L.H BACK | 0.468 | $3e\text{-}^{6}$ |
| E.L.H NEUTRAL | 0.484 | $3.1e\text{-}^{6}$ |
| E.L.H BACK & TAIL | 0.499 | $3.2e\text{-}^{6}$ |

| GROUP 7 AQUEOUS ROOT Low (A.R.L) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| A.R.L BACK & TAIL | 0.671 | $4.3e\text{-}^{6}$ |
| A.R.L BACK | 0.624 | $4e\text{-}^{6}$ |
| A.R.L NEUTRAL | 0.499 | $3.2e\text{-}^{6}$ |

| GROUP 8 AQUEOUS ROOT High | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| A.R.H TAIL | 0.468 | $3e\text{-}^{6}$ |
| A.R.H BACK & TAIL | 0.515 | $3.3e\text{-}^{6}$ |
| A.R.H BACK | 0.468 | $3e\text{-}^{6}$ |
| A.R.H HEAD & BACK | 0.655 | $4.2e\text{-}^{6}$ |
| A.R.H NEUTRAL | 0.718 | $4.6e\text{-}^{6}$ |
| A.R.H HEAD, BACK & TAIL | 0.592 | $3.8e\text{-}^{6}$ |

| GROUP 9 ETHANOIC ROOT LOW (E.R.L) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| E.R.L NEUTRAL | 0.749 | 4.8e-6 |
| E.R.L BACK | 0.639 | 4.1e-6 |
| E.R.L TAIL | 0.780 | 5e-6 |

| GROUP 10 ETHANOIC ROOT HIGH (E.R.H) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| E.R.H BACK | 0.686 | $4.4e^{-6}$ |
| E.R.H TAIL | 0.608 | $3.9e^{-6}$ |
| E.R.H NEUTRAL | 0.484 | $3.1e^{-6}$ |

| GROUP 11 STANDARD DRUG (STD) | ABSORBANCE | CONCENTRATION |
|---|---|---|
| STD TAIL | 0.562 | $3.6e^{-6}$ |
| STD NEUTRAL | 0.406 | $2.6e^{-6}$ |
| STD BACK | 0.624 | $4e^{-6}$ |

**LIPID PERIOXIDATION OF HEART TISSUE OF THE MICE**

**ABSORBANCE READING USING SPECTOPHOTOMETER**

| GROUP 1 NORMAL CONTROL (N.C) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| N.C BACK 1 | 0.203 | $1.3e^{-6}$ |
| N.C HEAD | 0.172 | $1.1e^{-6}$ |
| N.C BACK & TAIL | 0.114 | $7.3e^{-7}$ |
| N.C NEUTRAL | 0.232 | $1.5e^{-6}$ |

| GROUP 2 ARTHRITIC CONTROL (A.C) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| A.C BACK & TAIL | 0.624 | $4e^{-6}$ |
| A.C NEUTRAL | 0.668 | $4.4e^{-6}$ |
| A.C BACK | 0.780 | $5e^{-6}$ |
| A.C TAIL | 0.577 | $3.7e^{-6}$ |

| GROUP 3 AQUEOUS LEAF Low (A.L.L) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| A.L.LOW BACK & TAIL | 0.172 | $1.1e^{-6}$ |
| A.L.LOW BACK | 0.218 | $1.4e^{-6}$ |
| A.L.LOW TAIL | 0.234 | $1.5e^{-6}$ |
| A.L.LOW HEAD & TAIL | 0.328 | $2.1e^{-6}$ |

| A.L.LOW HEAD & BACK | 0.172 | $1.1e^{-6}$ |
|---|---|---|

| GROUP 4 AQUEOUS LEAF High (A.L.H) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| A.L.HIGH HEAD | 0.172 | $1.3e^{-6}$ |
| A.L.HIGH BACK & TAIL | 0.218 | $1.4e^{-6}$ |
| A.L.HIGH HEAD & BACK | 0.218 | $1.4e^{-6}$ |
| A.L.HIGH BACK | 0.249 | $1.6e^{-6}$ |
| A.L.HIGH NEUTRAL | 0.218 | $1.4e^{-6}$ |

| GROUP 5 ETHANOL LEAF Low (E.L.L) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| E.L.L BACK & TAIL | 0.390 | $2.5e^{-6}$ |
| E.L.L HEAD | 0.115 | $7.4e^{-7}$ |
| E.L.L HEAD & BACK | 0.296 | $1.9e^{-6}$ |
| E.L.L TAIL | 0.359 | $2.3e^{-6}$ |

| GROUP 6 ETHANOL LEAF High (E.L.H) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| E.L.H BACK | 0.045 | $2.9e^{-7}$ |
| E.L.H NEUTRAL | 0.055 | $3.5e^{-7}$ |
| E.L.H BACK & TAIL | 0.468 | $3e^{-6}$ |

| GROUP 7 AQUEOUS ROOT Low (A.R.L) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| A.R.L BACK & TAIL | 0.296 | $1.9e^{-6}$ |
| A.R.L BACK | 0.109 | $7e^{-7}$ |
| A.R.L NEUTRAL | 0.374 | $2.4e^{-6}$ |
| A.R.L HEAD & TAIL | 0.359 | $2.3e^{-6}$ |

| GROUP 8 AQUEOUS ROOT High | ABSORBANCE READING | CONCENTRATION |
|---|---|---|

| A.R.H TAIL | 0.218 | 1.4e-$^{6}$ |
|---|---|---|
| A.R.H BACK & TAIL | 0.296 | 1.9e-$^{6}$ |
| A.R.H BACK | 0.218 | 1.4e-$^{6}$ |
| A.R.H HEAD & BACK | 0.187 | 1.2e-$^{6}$ |
| A.R.H NEUTRAL | 0.203 | 1.3e-$^{6}$ |
| A.R.H HEAD, BACK & TAIL | 0.234 | 1.5e-$^{6}$ |

| GROUP 9 ETHANOL ROOT LOW (E.R.L) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| E.R.L NEUTRAL | 0.265 | 1.7e-$^{6}$ |
| E.R.L BACK | 0.499 | 3.2e-$^{6}$ |
| E.R.L TAIL | 0.203 | 1.3e-$^{6}$ |
| E.R.L BACK & TAIL | 0.109 | 7e-$^{7}$ |

| GROUP 10 ETHANOL ROOT HIGH (E.R.H) | ABSORBANCE READING | CONCENTRATION |
|---|---|---|
| E.R.H BACK | 0.265 | 1.7e-$^{6}$ |
| E.R.H TAIL | 0.078 | 5e-$^{7}$ |
| E.R.H NEUTRAL | 0.452 | 2.9e-$^{6}$ |

| GROUP 11 STANDARD DRUG (STD) | ABSORBANCE | CONCENTRATION |
|---|---|---|
| STD TAIL | 0.296 | 1.9e-$^{6}$ |
| STD NEUTRAL | 0.125 | 8e-$^{7}$ |
| STD BACK | 0.187 | 1.2e-$^{6}$ |
| STD HEAD & TAIL | 0.203 | 1.3e-$^{6}$ |

**SUPEROXIDE DISMUTASE ASSAY (SOD) OF BRAIN TISSUSE OF THE MICE ABSORBANCE READING USING SPECTOPHOTOMETER AT 480nm FOR 5 MINUTES INTERVAL.**

| GROUP 1 NORMAL CONTROL (N.C) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| N.C BACK 1 | 0.344 | 0.133 | 0.517 | 0.571 | 0.373 | 0.984 |
| N.C HEAD | 0.822 | 0.253 | 0.410 | 0.884 | 0.350 | 1.058 |
| N.C BACK & TAIL | 0.017 | 0.027 | 0.175 | 0.220 | 0.080 | 0.965 |
| N.C NEUTRAL | 0.063 | 0.458 | 0.578 | 0.191 | 1.294 | 1.285 |

| GROUP 2 ARTHRITIC CONTROL (A.C) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| A.C BACK & TAIL | 0.275 | 0.257 | 0.255 | 0.213 | 0.427 | 0.456 |
| A.C NEUTRAL | 0.208 | 0.084 | 0.028 | 0.053 | 0.049 | 0.225 |
| A.C TAIL | 0.051 | 0.086 | 0.052 | 0.086 | 0.044 | 0.177 |

| GROUP 3 AQUEOUS LEAF Low (A.L.L) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| A.L.LOW BACK & TAIL | 0.428 | 0.330 | 0.346 | 0.400 | 0.505 | 0.431 |
| A.L.LOW BACK | 0.210 | 0.217 | 0.229 | 0.230 | 0.241 | 0.293 |
| A.L.LOW HEAD & TAIL | 0.353 | 0.354 | 0.253 | 1.130 | 0.657 | 0.512 |
| A.L.LOW HEAD & BACK | - | | | | - | |

| GROUP 4 AQUEOUS LEAF High (A.L.H) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| A.L.HIGH HEAD | 1.151 | 0.313 | 0.230 | 0.358 | 0.272 | 0.937 |
| A.L.HIGH BACK & TAIL | 0.266 | 0.334 | 0.300 | 0.135 | 0.256 | 0.83 |
| A.L.HIGH HEAD & BACK | 0.523 | 0.208 | 0.301 | 0.257 | 0.241 | 0.846 |
| A.L.HIGH BACK | 0.266 | 0.268 | 0.311 | 0.308 | 0.678 | 1.236 |
| A.L.HIGH NEUTRAL | 0.029 | 0.054 | 0.038 | 0.410 | 0.033 | 0.912 |

| GROUP 5 ETHANOL LEAF Low (E.L.L) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| E.L.L BACK & TAIL | 0.290 | 0.295 | 0.656 | 0.272 | 0.166 | 0.872 |
| E.L.L HEAD | 0.251 | 0.562 | 0.684 | 1.081 | 0.247 | 0.512 |
| E.L.L HEAD & BACK | 0.536 | 0.775 | 0.356 | 0.274 | 0.675 | 0.617 |

| GROUP 6 ETHANOL LEAF High (E.L.H) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| E.L.H BACK | 0.750 | 0.449 | 0.095 | 0.029 | 0.170 | 1.087 |
| E.L.H NEUTRAL | 0.424 | 0.026 | 0.065 | 0.870 | 0.617 | 1.693 |
| E.L.H BACK & TAIL | 0.028 | 0.308 | 0.023 | 0.730 | 0.064 | 1.189 |

| GROUP 7 AQUEOUS ROOT Low (A.R.L) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| A.R.L BACK & TAIL | 0.301 | 0.248 | 0.566 | 0.253 | 0.310 | 0.732 |
| A.R.L BACK | 0.370 | 0.445 | 0.349 | 0.323 | 0.311 | 0.977 |
| A.R.L NEUTRAL | 0.199 | 0.238 | 0.315 | 0.246 | 0.124 | 0.956 |

| GROUP 8 AQUEOUS ROOT High | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| A.R.H TAIL | 0.037 | 0.080 | 0.105 | 0.076 | 0.136 | 1.387 |
| A.R.H BACK & TAIL | - | | | - | - | - |
| A.R.H BACK | 0.201 | 0.249 | 0.398 | 0.408 | 0.187 | 1.316 |
| A.R.H HEAD & BACK | 0.624 | 0.161 | 0.385 | 0.235 | 0.229 | 1.289 |
| A.R.H NEUTRAL | 0.144 | 0.241 | 0.225 | 0.240 | 0.199 | 1.393 |
| A.R.H HEAD, BACK & TAIL | 0.282 | 0.628 | 0.251 | 0.217 | 1.154 | 2.616 |

| GROUP 9 ETHANOIC ROOT LOW (E.R.L) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| E.R.L NEUTRAL | 0.042 | 0.078 | 0.307 | 0.277 | 0.050 | 0.405 |
| E.R.L BACK | 0.016 | 0.169 | 0.878 | 0.077 | 0.274 | 0.474 |
| E.R.L TAIL | 0.870 | 0.504 | 0.012 | 0.019 | 0.889 | 0.357 |

| GROUP 10 ETHANOIC ROOT HIGH (E.R.H) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| E.R.H BACK | 0.041 | 0.261 | 0.223 | 0.807 | 0.497 | 0.968 |
| E.R.H TAIL | 0.241 | 0.266 | 0.241 | 0.339 | 0.369 | 0.784 |
| E.R.H NEUTRAL | 0.692 | 0.282 | 0.011 | 0.806 | 0.927 | 0.705 |

| GROUP 11 STANDARD DRUG (STD) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| STD TAIL | 0.311 | 0.170 | 0.237 | 0.175 | 1.003 | 1.076 |
| STD NEUTRAL | 0.248 | 0.301 | 0.410 | 0.951 | 1.001 | 1.259 |
| STD BACK | 0.298 | 0.196 | 0.150 | 0.551 | 0.209 | 1.267 |

**SUPEROXIDE DIMUTASE ASSAY (SOD) OF HEART TISSUSE OF THE MICE ABSORBANCE READING USING SPECTOPHOTOMETER AT 480nm FOR 5MINUTES INTERVAL**

| GROUP 1 NORMAL CONTROL (N.C) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| N.C BACK 1 | 0.119 | 0.288 | 0.248 | 0.106 | 0.644 | 1.175 |
| N.C BACK 2 | 0.153 | 0.187 | 0.167 | 0.692 | 0.835 | 1.046 |
| N.C HEAD | 0.033 | 0.173 | 0.521 | 0.348 | 0.141 | 1.324 |
| N.C BACK & TAIL | 0.048 | 0.094 | 0.104 | 0.188 | 0.282 | 0.902 |
| N.C NEUTRAL | 0.083 | 0.031 | 0.224 | 0.107 | 0.437 | 0.862 |

| GROUP 2 ARTHRITIC CONTROL (A.C) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| A.C BACK & TAIL | - | - | - | - | - | - |
| A.C NEUTRAL | 0.097 | 0.118 | 0.286 | 0.711 | 0.169 | 0.216 |
| A.C BACK | 0.040 | 0.053 | 0.416 | 0.206 | 0.182 | 0.226 |
| A.C TAIL | 0.342 | 0.324 | 0.362 | 0.359 | 1.085 | 0.237 |

| GROUP 3 AQUEOUS LEAF Low (A.L.L) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| A.L.LOW BACK & TAIL | 0.132 | 0.509 | 0.737 | 0.595 | 0.305 | 1.038 |
| A.L.LOW BACK | 0.145 | 0.076 | 0.111 | 0.236 | 0.692 | 0.923 |
| A.L.LOW TAIL | - | - | - | - | - | |
| A.L.LOW HEAD & TAIL | 0.119 | 0.539 | 0.357 | 0.539 | 0.805 | 0.631 |

| A.L.LOW HEAD & BACK | 0.141 | 0.148 | 0.187 | 0.899 | 0.963 | 1.009 |
|---|---|---|---|---|---|---|

| GROUP 4 AQUEOUS LEAF High (A.L.H) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| A.L.HIGH TAIL | 0.065 | 0.158 | 0.242 | 0.678 | 0.874 | 1.341 |
| A.L.HIGH BACK & TAIL | 0.275 | 0.198 | 0.276 | 0.269 | 0.217 | 1.358 |
| A.L.HIGH HEAD & BACK 1 | 0.218 | 0.171 | 0.146 | 0.261 | 0.864 | 1.466 |
| A.L.HIGH HEAD & BACK 2 | 0.207 | 0.157 | 0.133 | 0.451 | 0.810 | 0.974 |
| A.L.HIGH BACK | 0.115 | 0.148 | 0.203 | 0.214 | 0.256 | 0.423 |
| A.L.HIGH NEUTRAL | 0.125 | 0.119 | 0.122 | 0.167 | 0.202 | 0.231 |

| GROUP 5 ETHANOL LEAF Low (E.L.L) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| E.L.L BACK & TAIL | 0.190 | 0.204 | 0.205 | 0.188 | 0.472 | 0.916 |
| E.L.L HEAD | 0.249 | 0.293 | 0.215 | 0.945 | 0.350 | 1.226 |
| E.L.L HEAD & BACK | 0.060 | 0.467 | 0.627 | 0.115 | 0.183 | 0.937 |
| E.L.L TAIL | 0.297 | 0.161 | 0.172 | 0.187 | 0.850 | 1.659 |
| E.L.L BACK | 0.171 | 0.273 | 0.239 | 0.386 | 0.325 | 0.462 |

| GROUP 6 ETHANOIC LEAF High (E.L.H) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| E.L.H BACK | - | - | - | - | - | - |
| E.L.H NEUTRAL | 0.192 | 0.238 | 0.318 | 0.373 | 0.530 | 1.104 |
| E.L.H BACK & TAIL | 0.156 | 0.274 | 0.427 | 0.306 | 0.697 | 1.623 |

| GROUP 7 AQUEOUS ROOT Low (A.R.L) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| A.R.L BACK & TAIL | 0.171 | 0.155 | 0.190 | 0.182 | 0.438 | 0.816 |
| A.R.L BACK | 0.321 | 0.293 | 0.098 | 0.052 | 0.325 | 1.025 |
| A.R.L NEUTRAL | 0.017 | 0.067 | 0.120 | 0.256 | 0.380 | 0.684 |
| A.R.L HEAD & TAIL | 0.121 | 0.159 | 0.124 | 0.317 | 0.300 | 0.743 |
| A.R.L TAIL | 0.304 | 0.262 | 0.055 | 0.232 | 0.144 | 0.738 |

| GROUP 8 AQUEOUS ROOT High | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| A.R.H TAIL | 0.345 | 0.284 | 0.364 | 0.380 | 0.677 | 0.996 |
| A.R.H BACK & TAIL | 0.031 | 0.111 | 0.394 | 0.464 | 0.403 | 1.375 |
| A.R.H BACK | 0.138 | 0.069 | 0.413 | 0.626 | 0.613 | 1.246 |
| A.R.H HEAD & BACK | 0.408 | 0.576 | 0.983 | 0.290 | 0.280 | 1.124 |
| A.R.H NEUTRAL | 0.391 | 0.348 | 0.522 | 0.323 | 0.772 | 0.902 |
| A.R.H HEAD, BACK & TAIL | 0.367 | 0.371 | 0.320 | 0.362 | 0.421 | 1.062 |

| GROUP 9 ETHANOIC ROOT LOW (E.R.L) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| E.R.L NEUTRAL | 0.373 | 0.281 | 0.373 | 0.388 | 0.504 | 0.393 |
| E.R.L BACK | 0.135 | 0.314 | 0.383 | 0.337 | 0.825 | 0.407 |
| E.R.L TAIL | 0.418 | 0.252 | 0.306 | 0.275 | 0.294 | 0.472 |
| E.R.L BACK & TAIL | 0.409 | 0.392 | 0.381 | 0.303 | 0.379 | 0.396 |

| GROUP 10 ETHANOIC ROOT HIGH (E.R.H) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| E.R.H BACK | 0.428 | 0.411 | 0.358 | 0.326 | 0.792 | 0.892 |
| E.R.H TAIL | 0.510 | 0.292 | 0.358 | 0.368 | 0.626 | 0.748 |
| E.R.H NEUTRAL | 0.358 | 0.510 | 0.292 | 0.368 | 0.626 | 0.604 |

| GROUP 11 STANDARD DRUG (STD) | 1st. | 2nd. | 3rd. | 4th. | 5th. | concentration |
|---|---|---|---|---|---|---|
| STD TAIL | 0.066 | 0.123 | 0.200 | 0.165 | 0.908 | 1.186 |
| STD NEUTRAL | 0.064 | 0.115 | 0.547 | 0.937 | 0.226 | 1.109 |
| STD BACK | - | - | - | - | - | - |
| STD HEAD & TAIL | 0.263 | 0.552 | 0.770 | 0.843 | 0.866 | 1.809 |

**ESTIMATION OF REDUCED GLUTATHIONE OF BRAIN TISSUSE OF THE MICE ABSORBANCE READING USING SPECTOPHOTOMETER AT 412nm.**

| GROUP 1 NORMAL CONTROL (N.C) | ABORBANCE READING | concentration |
|---|---|---|
| N.C BACK 1 | 0.163 | 30.4 |
| N.C HEAD | 0.372 | 69.378 |
| N.C BACK & TAIL | 0.255 | 47.558 |
| N.C NEUTRAL | 0.297 | 55.435 |

| GROUP 2 ARTHRITIC CONTROL (A.C) | ABORBANCE READING | Concentration |
|---|---|---|
| A.C BACK & TAIL | 0.113 | 21.075 |
| A.C NEUTRAL | 0.136 | 25.435 |
| A.C BACK | 0.077 | 14.361 |
| A.C TAIL | 0.148 | 27.602 |

| GROUP 3 AQUEOUS LEAF Low (A.L.L) | ABORBANCE READING | Concentration |
|---|---|---|
| A.L.LOW BACK & TAIL | 0.312 | 58.099 |
| A.L.LOW BACK | 0.259 | 48.375 |
| A.L.LOW HEAD & TAIL | 0.247 | 46.039 |
| A.L.LOW HEAD & BACK | 0.212 | 39.467 |

| GROUP 4 AQUEOUS LEAF High (A.L.H) | ABORBANCE READING | Concentration |
|---|---|---|
| A.L.HIGH HEAD | 0.379 | 70.773 |
| A.L.HIGH BACK & TAIL | 0.358 | 66.741 |
| A.L.HIGH HEAD & BACK | 0.457 | 85.168 |
| A.L.HIGH BACK | 0.158 | 29.467 |
| A.L.HIGH NEUTRAL | 0.112 | 20.888 |

| GROUP 5 ETHANOL LEAF Low (E.L.L) | ABORBANCE READING | Concentration |
|---|---|---|
| E.L.L BACK & TAIL | 0.276 | 51.474 |
| E.L.L HEAD | 0.260 | 48.49 |
| E.L.L HEAD & BACK | 0.144 | 26.856 |

| GROUP 6 ETHANOL LEAF High (E.L.H) | ABORBANCE READING | Concentration |
|---|---|---|
| E.L.H BACK | 0.220 | 40.959 |
| E.L.H NEUTRAL | 0.053 | 39.753 |
| E.L.H BACK & TAIL | 0.353 | 65.884 |

| GROUP 7 AQUEOUS ROOT Low (A.R.L) | ABORBANCE READING | Concentration |
|---|---|---|
| A.R.L BACK & TAIL | 0.135 | 25.178 |
| A.R.L BACK | 0.373 | 69.583 |
| A.R.L NEUTRAL | 0.444 | 82.806 |

| GROUP 8 AQUEOUS ROOT High | ABORBANCE READING | Concentration |
|---|---|---|
| A.R.H TAIL | 0.328 | 61.261 |
| A.R.H BACK & TAIL | 0.338 | 62.94 |
| A.R.H BACK | 0.644 | 120.125 |
| A.R.H HEAD & BACK | 0.444 | 82.806 |
| A.R.H NEUTRAL | 0.233 | 43.428 |
| A.R.H HEAD, BACK & TAIL | 0.072 | 13.428 |

| GROUP 9 ETHANOL ROOT LOW (E.R.L) | ABORBANCE READING | Concentration |
|---|---|---|
| E.R.L NEUTRAL | 0.276 | 51.474 |
| E.R.L BACK | 0.260 | 48.49 |
| E.R.L TAIL | 0.305 | 56.855 |

| GROUP 10 ETHANOL ROOT HIGH | ABORBANCE READING | Concentration |
|---|---|---|
| E.R.H BACK | 0.401 | 74.734 |
| E.R.H TAIL | 0.335 | 62.38 |
| E.R.H NEUTRAL | 0.483 | 90.125 |

| GROUP 11 STANDARD DRUG (STD) | ABORBANCE READING | concentration |
|---|---|---|
| STD TAIL | 0.251 | 46.812 |
| STD NEUTRAL | 0.401 | 74.805 |
| STD BACK | 0.327 | 60.968 |

**ESTIMATION OF REDUCED GLUTATHIONE OF HEART TISSUSE OF THE MICE ABSORBANCE READING USING SPECTOPHOTOMETER AT 412nm.**

| GROUP 1 NORMAL CONTROL (N.C) | ABORBANCE READING | concentration |
|---|---|---|
| N.C BACK 1 | - | - |
| N.C HEAD | 0.150 | 28.046 |
| N.C BACK & TAIL | - | - |
| N.C NEUTRAL | 0.114 | 21.19 |

| GROUP 2 ARTHRITIC CONTROL (A.C) | ABORBANCE READING | concentration |
|---|---|---|
| A.C BACK & TAIL | - | - |
| A.C NEUTRAL | 0.035 | 6.528 |
| A.C BACK | 0.022 | 1.123 |
| A.C TAIL | 0.042 | 7.833 |

| GROUP 3 AQUEOUS LEAF Low (A.L.L) | ABORBANCE READING | concentration |
|---|---|---|
| A.L.LOW BACK & TAIL | - | - |
| A.L.LOW BACK | 0.086 | 15.968 |
| A.L.LOW HEAD & TAIL | 0.201 | 37.46 |
| A.L.LOW HEAD & BACK | 0.086 | 16.039 |

| GROUP 4 AQUEOUS LEAF High (A.L.H) | ABORBANCE READING | concentration |
|---|---|---|
| A.L.HIGH TAIL | 0.119 | 22.274 |
| A.L.HIGH BACK & TAIL | 0.129 | 24.103 |
| A.L.HIGH HEAD & BACK | 0.255 | 47.558 |
| A.L.HIGH BACK | 0.040 | 7.460 |
| A.L.HIGH NEUTRAL | 0.041 | 7.647 |

| GROUP 5 ETHANOL LEAF Low (E.L.L) | ABORBANCE READING | concentration |
|---|---|---|
| E.L.L BACK & TAIL | 0.057 | 10.631 |
| E.L.L HEAD | 0.064 | 11.936 |
| E.L.L HEAD & BACK | 0.086 | 16.039 |
| E.L.L TAIL | 0.034 | 6.341 |

| E.L.L BACK | 0.169 | 31.519 |
|---|---|---|

| GROUP 6 ETHANOL LEAF High (E.L.H) | ABORBANCE READING | concentration |
|---|---|---|
| E.L.H BACK | - | - |
| E.L.H NEUTRAL | 0.163 | 30.444 |
| E.L.H BACK & TAIL | 0.216 | 40.426 |

| GROUP 7 AQUEOUS ROOT Low (A.R.L) | ABSORBANCE READING | concentration |
|---|---|---|
| A.R.L BACK & TAIL | 0.156 | 29.242 |
| A.R.L BACK | 0.034 | 6.341 |
| A.R.L NEUTRAL | 0.033 | 6.155 |
| A.R.L HEAD & TAIL | - | - |

| GROUP 8 AQUEOUS ROOT High | ABSORBANCE READING | concentration |
|---|---|---|
| A.R.H TAIL | 0.098 | 18.277 |
| A.R.H BACK & TAIL | 0.211 | 39.352 |
| A.R.H BACK | 0.081 | 15.107 |
| A.R.H HEAD | 0.032 | 5.968 |
| A.R.H NEUTRAL | - | - |
| A.R.H HEAD, BACK & TAIL | - | - |

| GROUP 9 ETHANOL ROOT LOW (E.R.L) | ABSORBANCE READING | concentration |
|---|---|---|
| E.R.L NEUTRAL | 0.143 | 26.67 |
| E.R.L BACK | 0.220 | 41.004 |
| E.R.L TAIL | 0.246 | 46.039 |
| E.R.L BACK & TAIL | 0.048 | 8.952 |

| GROUP 10 ETHANOL ROOT HIGH | ABSORBANCE READING | concentration |
|---|---|---|
| E.R.H BACK | - | - |
| E.R.H TAIL | 0.423 | 78.916 |
| E.R.H NEUTRAL | 0.199 | 37.087 |
| E.R.H HEAD, BACK & TAIL | 0.321 | 59.867 |

| GROUP 11 STANDARD DRUG (STD) | ABSORBANCE READING | concentration |
|---|---|---|
| STD TAIL | 0.146 | 27.274 |

| STD NEUTRAL | 0.236 | 44.103 |
|---|---|---|
| STD BACK | 0.201 | 37.558 |
| STD HEAD & TAIL | 0.042 | 7.833 |