INVITRO ANTI-INFLAMMATORY ACTIVITY OF THE ETHANOL EXTRACT OF *SOLANUM AETHIOPICUM* (GARDEN EGG) LEAVES

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ABSTRACT

Inflammation is a complex biological response of vascular tissues to invasion by an infectious agent, antigen challenge, physical, chemical or traumatic damage. This is characterized by redness elevated heat, swelling, pain and loss of function. Ethanol extract of Solanum aethiopicum leaves was assessed for its invitro anti-inflammatory activity. The extract was subjected to qualitative and quantitative phytochemical screening. Invitro anti-inflammatory activity was evaluated using lipoxygenase assay, membrane stabilization assay, heat stabilization assay and protein denaturation activity at different concentrations. Aspirin, Diclofenac sodium, acetyl salicylic acid and linolenyl acid were used as standard drugs. Preliminary phytochemical screening showed that Saponins, phenolics, flavonoids, steroids, triterpenes, coumarins, glycosides and alkaloids were present in the plants leaves extract. The results showed that S. aethiopicum ethanol extract at a concentration range of 10 - 50µg/ml significantly ($p<0.05$) protects the heat induced protein denaturation. At the concentration of 30 and 40µg/ml, S. aethiopicum showed significant ($p<0.05$) heat stabilization action. The plant extract also exhibited significant ($p<0.05$) inhibition of lipoxygenase enzymes (IC_{50} 89.84) when compared to the linolenyl acid standard (IC_{50} 56.60). The results obtained in the present study indicated that ethanol extracts of S. aethiopicum can be a potential source of anti-inflammatory agents.

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CHAPTER ONE

1.0 INTRODUCTION

1.1. Background Information of the Study.

Inflammation is a complex biological response of vascular tissues to invasion by an infectious agent, antigen challenge, physical, chemical or traumatic damage. Although inflammation is a defence mechanism, the complex events and mediators involved in the inflammatory reactions can induce, maintain or aggravate many diseases (Ferrero-Miliani, Nielson, Andersen & Girardin, 2007).

It is a protective response of the body to eliminate the initial cause of cell injury (diluting, destroying and neutralizing the harmful agents), remove the damaged tissue and generate new tissue It involves a well-organized cascade of fluid and cellular changes within living tissue (Kumar, Bajwa, Singh & Kalia, 2013).

It is characterized by redness, swollen joint that is warm to touch, joint pain, its stiffness and loss of joint function (Shrestha, Ravi & Talib, 2013). Inflammation is either acute or chronic inflammation. Acute inflammation may be an initial response of the body to harmful stimuli. In chronic inflammation, the inflammatory response is out of proportion resulting in damage to the body (Pilotto, Sancarlo, Addante, Scarcelli & Franceschi, 2010).

Inflammation is an essential response provided by the immune systems that ensures survival during infection and tissue injury. Inflammatory responses are essential for the maintenance of normal tissue homeostasis. The molecular mechanism of inflammation is quite a complicated process which is initiated by the recognition of specific molecular patterns associated with either infection or tissue injury. The entire process of the inflammatory response is mediated by several

key regulators involved in the selective expression of pro-inflammatory molecules. Prolonged inflammations are often associated with severe detrimental side effects on health.

Alterations in inflammatory responses due to persistent inducers or genetic variations are on the rise over the last couple of decades, causing a variety of inflammatory diseases and pathophysiological conditions (Afser 2011).

Some drugs such as aspirin, indomethacin and diclofenac are used to reduce inflammation. They are called NSAIDs (Nonsteroidal anti-inflammatory drugs). They possess anti-inflammatory action for treating several inflammatory conditions including rheumatoid arthritis, osteoarthritis and musculoskeletal disorders, but prolonged use of NSAIDs has some associated adverse effects such as nausea, fluid retention, bleeding and gastric lesion.

Therefore, new drugs from medicinal plants that can be used to reduce inflammation and are devoid of these side effects are being researched on as alternatives to NSAIDS

Garden egg (*Solanum aethiopicum*) also known as African eggplant, Ethiopian eggplant or scarlet eggplant is a vegetable crop belonging to the family Solanaceae. West African species bear edible fruits; they include S. aethiopicum, S. melogena, S. macrocarpon, and S. muricatum (Prohens, Blanca & Neuz, 2005).

The genus Solanum includes both the edible and non-edible species. Solanum, a widespread plant genus of the family Solanaceae, has over 1000 species worldwide with at least 100 indigenous species in Africa and adjacent islands; these include a number of valuable crop plants and some poisonous ones. It is represented in Nigeria by some 25 species including those domesticated with their leaves, fruits or both eaten as vegetables or used in traditional medicine. Among them are two African eggplants, S. aethiopicum L. (Ethiopian eggplant) and S. macrocarpon L. (Gboma eggplant), which are widely cultivated in Nigeria and across the African continent.4-6 African eggplants, also called garden eggs (Hausa: Dauta; Igbo: afufa or añara; Yoruba: igba), are highly valued constituents of the Nigerian foods and indigenous medicines; they are commonly

consumed almost on daily basis by both rural and urban families. The family is one of the largest and most important families of vegetable grown for their edible form. They are native to sub-Saharan Africa and are essentially tropical in origin. *S. aethiopicum* is of high edible quality. Depending on the type, either the leaves and young shoots or the fruits or both are eaten; they may be consumed raw, dried, cooked or in salad form. They can also be boiled or fried as ingredient of stews, soups and vegetable sauces. The fruit's seeds have large endosperm, and are grown mainly for food and medicinal purposes (Anosike, Obidoa & Ezeanyika, 2012).

The eggplants form part of the traditional sub-Saharan African culture. The fruits is said to represent blessings and fruitfulness, are offered as a token of goodwill during visits, marriages and other social events. Wide variations exist within the vegetative and fruit characters both within and between the African eggplant species including variations in characters like diameter of corolla, petiole length, leaf blade width, plant branching, fruit shape, and fruit colour. Their uses in indigenous medicine range from weight reduction to treatment of several ailments including asthma, allergic rhinitis, nasal catarrh, skin infections, rheumatic disease and swollen joint pains, gastro-oesophageal reflux disease, constipation, dyspepsia.. Several studies support the folkloric use of the plants in local foods and medicinal preparations; for instance, different researchers have reported significant analgesic, anti-inflammatory, anti-asthmatic, anti-glaucoma, hypoglycaemic, hypolipidemic, and weight reduction effects of eggplants, particularly S. melongena, on test animals and humans (Bello, Muhammad & Gammaniel, 2005) and (Igwe, Aunyili & Ogbogu, 2003). These pharmacological properties have been attributed to the presence of certain chemical substances in the plants, such as fibre, ascorbic acid, phenols, anthocyanin, glycoalkaloids and α-chaconine (sanchez-Mata, Yokoyama & Hong 2010).

Some observations and oral reports though, show that people with high consumption of garden egg have relief in arthritic pains and swelling. Lack of scientific data to support these claims prompted this study which was therefore aimed at assessing the possible anti-inflammatory

activity of garden egg (*S. aethiopicum*) in both acute and chronic inflammatory. This natural "defence" process brings increased blood flow to the area, resulting in an accumulation of fluid. That is Inflammation is a pattern of response to injury, in which cells and exudates accumulate in irritated tissues and tend to protect from further damage.

In this study, analyses were carried out on the indigenous eggplants, *S. aethiopicum* and their anti-inflammatory activity was checked.

1.2. Statement of Problem.

Nonsteroidal anti-inflammatory drugs (NSAIDs) possess anti-inflammatory action for treating several inflammatory conditions including rheumatoid arthritis, osteoarthritis and musculoskeletal disorders, but prolonged use of NSAIDs has some associated adverse effects such as nausea, fluid retention, bleeding and gastric lesion.

Therefore, new anti-inflammatory drugs from medicinal plants devoid of these side effects are being researched on as alternatives to NSAIDS

In spite of our dependence on modern medicine and the tremendous advances in synthetic drugs, a large number of the world population (approximately 80% of people) cannot afford products of the western pharmaceutical industry and have to rely upon the use of traditional medicine which are mainly derived from plant material

1.3. Justification of the Study

Report has shown that *S. aethiopicum* fruits possesses ulcer protecting properties against experimentally induced ulcers in rats. They are used to treat colic; severe pain resulting in periodic spasm in an abdominal organ and blood pressure. Other reports on the pharmacological activity

of the plants show that it has purgative, sedative and anti-diabetic effect, but none has reported the anti-inflammatory potential of the leaves extract. Therefore this research is aimed at checking the possible invitro anti-inflammatory activity of the leaves.

1.4. SCOPE OF THE STUDY

The scope of the study is to evaluate the invitro anti-inflammatory activity of *S. aethiopicum* by checking heat stabilization, membrane stabilization, inhibition of protein denaturation and anti-lipoxygenase activity of the plant extract.

1.5. AIM OF THE STUDY

To evaluate the invitro anti-inflammatory activity of *S. aethiopicum*

1.6. OBJECTIVE OF THE STUDY

The objectives of the study are to:

i. Carry out quantitative phytochemical analysis on leaves extract of *S. aethiopicum.*
ii. Qualitatively determine the phytochemicals present in the *S. aethiopicum* leaves extract
iii. Estimate the heat stabilization capacity of the ethanoic extract of *S. aethiopicum* leaves
iv. Estimate membranes stabilization property of the ethanoic extract of *S. aethiopicum* leaves.
v. Evaluate the lipoxygenase inhibition activity *S. aethiopicum,*
vi. Determine the protein denaturation capacity of the ethanoic extract of S. *aethiopicum* leaves.

CHAPTER TWO

LITERATURE REVIEW

Recent studies have shown that Solanum. melongena skin which is a family of *S. aethiopicum* contains phenols, including caffeic acid, quinic acid, cinnamic acid, and chlorogenic acid, and flavonoids such as nasunin and quercetin (Helmja, Vaher, Pussa, & Kaljurand, 2009; Shen et al., 2005).

Sun (2014) discovered isoscopoletin, grossamide, and cannabisin F have been found in the root of eggplants and that Solanoflavone, a biflavonol glycoside has been isolated from the aerial part of white eggplant.

Eggplant peel has an abundant anti-oxidant anthocyanin and was demonstrated to prevent oxidative stress and inhibit angiogenesis as observed by (Azevedo et al., 2007; Azuma et al, 2008; Jing et al., 2015; Matsubara, Kaneyuki, Miyake, & Mori, 2005; Noda, Kneyuki, Igarashi, Mori, & Packer, 2000).

(Liu, Luo, & Kong, 2011) isolated Phenylethyl cinnamides from *S. melongena* root which have shown to possess inhibitory activity against alpha-glucosidase

Additionally, a significant reduction in cholesterol, triglycerides, and free fatty acids was observed in the serum and liver of rats treated with flavonoids from S. melongena fruit, indicating hypolipidemic effects (Sudheesh, Presannakumar, Vijayakumar, & Vijayalakshmi, 1997).

Furthermore, Das et al., (2011) observed that raw or grilled eggplant fruits exhibit cardio protection against ischemia-reperfusion injury in rats.

In addition, oral administration of water extract of *S. melongena* fruits (SMWE) was reported to significantly inhibit cutaneous anaphylactic reactions and tumour necrosis factor alpha (TNF-α) secretions from mast cells in rats (Lee et al., 2001).

The aqueous extract of the fruits also inhibited protease- activated receptor 2 (PAR2) agonist induced inflammation by reducing myeloperoxidase activity and TNF-α production in paw edema in mice (Han et al., 2003).

According to Korean folk medicine, people consumed water-containing ashes of eggplant stalks to treat gastritis, stomatitis, and gastric ulcers, and rubbed their skin with a slice of eggplant stalk for frostbites, skin warts, and skin burns. A mixture of eggplant stalk ashes and oil was used to treat acne.

The anti-inflammatory effect of the plant on egg albumin-induced oedema and cotton pellet induced granuloma in rats has also been reported. (Anosike et al., 2012).

Report has shown that *S. aethiopicum* possesses ulcer protecting properties against experimentally induced ulcers in rats. They are used to treat colic; severe pain resulting in periodic spasm in an abdominal organ and blood pressure. (Smith & sortin 2004)

Other reports on the pharmacological activity of the plant show that it has purgative (Saba, Dina, Adedapo & Akhiromen, 2003), sedative and anti-diabetic effects (Ezegwu & Okonta, 2004), but none have reported on its anti-inflammatory activity.

2.1 INFLAMMATION

Inflammation is a protective strategy evolved in higher organisms in response to detrimental results such as microbial infection, tissue injury and other noxious conditions. It is an essential immune response by the host that enables the removal of harmful stimuli as well as the healing of damaged tissue. Acute inflammation has therefore been considered as a part of innate immunity, the first line of host defence against foreign invaders and danger molecules. Mankind has known the classical symptoms of inflammation for hundreds of years, which include redness, pain, swelling and heat (Medzhitov, 2008).

However, emerging literature suggests that inflammation operates as a much-sophisticated system than ever thought at the molecular level. The entire course of inflammation comes with many

different processes involved in its initiation, regulation and resolution. Nowadays a diverse range of inflammations have been identified, with many different forms initiated by numerous stimuli and governed by various regulatory mechanisms. Due to its extensive and widespread nature, inflammation is believed to have an Impact on every aspect of normal human physiology and pathology. The current concept on inflammation has grown significantly over the years because of the vast expansion of the field in more divergent directions. As a result, we are far from being able to fully comprehend the consequence of inflammation in human health and diseases.

Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane 1995).

It is a complex process, which is frequently associated with pain and involves occurrences such as: the increase in vascular permeability, increase of protein denaturation and membrane alterations (Umapathy et al., 2010).

However, if inflammation is not treated it leads to onset of diseases like vasomotor rhinorrhoea, rheumatoid arthritis and atherosclerosis (Henson & Murphy 1989).

Inflammation is the body's normal protective response to an injury, irritation, or surgery. This natural "defence" process brings increased blood flow to the area, resulting in an accumulation of fluid. That is, Inflammation is a pattern of response to injury, in which cells and exudates accumulate in irritated tissues and tend to protect from further damage. As the body mounts this protective response, the symptoms of inflammation develop. These include: Swelling, Pain and Increased warmth and redness of the skin.

Inflammation can be acute or chronic. When it is acute, it occurs as an immediate response to trauma (an injury or surgery)—usually within two hours. When it is chronic, the inflammation reflects an ongoing response to a longer-term medical condition, such as arthritis.

Chronic inflammation may result from failure of the recovery phase of acute inflammation, or may occur as a distinct process from the outset, because of the nature of the irritant. Although it shares many characteristics of the acute inflammatory response, chronic inflammation is a biologically distinct pattern of response to an irritant. It may be divided into nongranulomatous and granulomatous chronic inflammation; the term granuloma refers to a localized collection of activated macrophages and their derivatives. Many common and clinical important disease states, such as rheumatoid arthritis, asthma tuberculosis, leprosy, schistosomiasis, chronic hepatitis, thyroiditis and multiple sclerosis, are examples of chronic inflammation and its consequences.

Inflammation is not the same as infection. Infections are caused by bacteria, fungus, and viruses and infections sometimes produce inflammation. However, infection and inflammation are treated very differently. Your foot and ankle surgeon can best determine the cause of your inflamed tissue.

2.2 TYPES OF INFLAMMATION

2.2.1 Acute inflammation

Acute inflammation is usually has becoming within minutes or at most hours after tissue injury, and may be characterized by the classical symptoms of redness, heat, oedema (Toth 2014).

It's a short term process. It is characterized by the exudation of fluids (kumar & collins 1999) and plasma proteins and the migration of leukocytes, most importantly neutrophils into the injured area. This acute inflammatory response is useful to the defense mechanism aimed at killing of bacteria, virus and parasites while still facilitating wound repairs. Acute inflammation is the initial response of the body to injurious stimuli and is achieved by increased movement of plasma and leukocytes from the blood into the injured tissues. The process of acute inflammation is initiated by cells already present in the tissues. This is characterized by marked vascular changes, including vasodilatation and increased capillary permeability which are induced by the actions of the various inflammatory mediators (Okoli et al., 2007)

2.2.2 Chronic inflammation

Chronic inflammation is of a more prolonged duration and histological by the presence of lymphocytes and macrophages, resulting in fibrosis and tissue necrosis. The chronic inflammation increases the development of the degenerative diseases such as rheumatoid arthritis, atherosclerosis, heart disease, Alzheimer, asthma, acquired immunodeficiency disorder (AIDS), cancer, congestive heart failure, multiple sclerosis, diabetes, infections, gout, IBDinflammatory bowel disease, aging and other neurodegenerative CNS depression, Chronic inflammation also has been implicated as part of the cause of the muscle loss that occurs with aging (Toth 2014) all of which are associated with immunopathological that appears to play a key role in the onset of the condition (Dalgleish & O'Byrne 2002).

2.3 THE MECHANISMS OF INFLAMMATION

This Involve a series of events in which the metabolism of arachidonic acid plays an important role. It can be metabolized by the Cyclooxygenase (COX) pathway to prostaglandins and thromboxane A2, or by the 5-lipoxygenase (5-LOX) pathway to hydroperoxy-eicosatetraenoic acids (HPETE's) and leukotrienes (LT's), which are important biologically active mediators in a variety of inflammatory events. Upon appropriate stimulation of neutrophils, arachidonic acid is cleaved from membrane phospholipids and can be converted to leukotrienes and prostaglandins through 5-LOX or COX pathways respectively. Inhibition of 5-LOX and COX-leads to decreased production of LTs and PGs, such a drug would have the potential to provide anti-inflammatory and analgesic effects with a reduction in the gastrointestinal side-effects. Furthermore, inflammatory processes also involve reactive oxygen species started by leukocyte activation. Therefore, screening of antioxidant properties may provide important information about the potential activity of a drug on inflammatory processes (Anoop & Bindu, 2015).

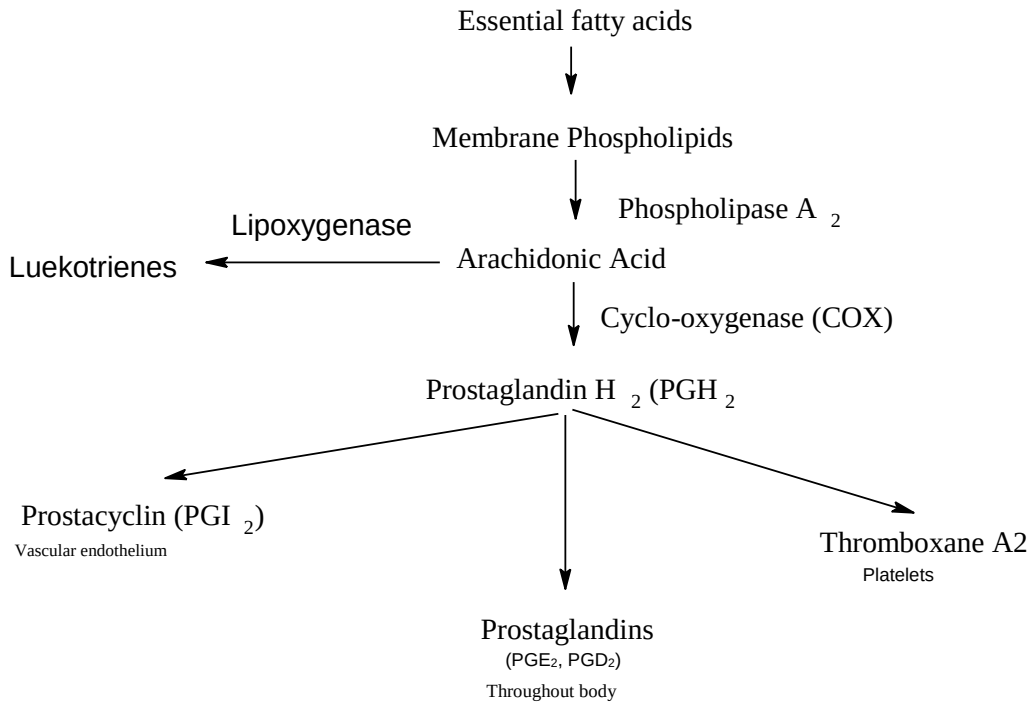


Figure 1: mechanism of inflammation

2.4. Medicinal Plants

The term medicinal plants include various types of plants used in herbalism and some of these plants have medicinal activities. Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Davidson-Hunt 2000). These medicinal plants are considered as rich sources of ingredients which can be used in drug development and synthesis. Besides that, these plants play a critical role in the development of human cultures around the whole world.

Medicinal plants are plants that contain active components which have been used over the years in the traditional medical practices for the treatment of various diseases (Ajibesin, Bala & Umoh, 2011).

They are the sources of many important drugs of the modern world. Many of these indigenous medicinal plants are used as spices and food plants; they are also sometimes added to foods meant for pregnant mothers for medicinal purposes (Akinpelu & Onakoya, 2006).

Medicinal plants are different types of plants used in the study of the medicinal and therapeutic use of plants and medicinal activities. In drug development and synthesis, medicinal plant can be used because it contains a high resource of ingredients. These medicinal plants also have a strong role in human values development all over the world (Mantry, 2014).

2.5 Secondary Metabolites

Secondary metabolites are metabolic intermediates or products which are not essential to growth and life of the producing plants but rather required for interaction of plants with their environment and produced in response to stress. Examples include Terpenes, Phenolics, and Glycosides, flavonoids, saponins and Alkaloids.

Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavours, and other industrial materials (Jian, Lawrence & Robert, 2005).

2.5.1 Terpenes

They constitute large class of natural products which are composed from isoprene unit. Terpenes are only hydrocarbons while Terpenoids are oxygenated hydrocarbons. The general molecular Formula of terpenes are multiple of $(C_5H_8)_{n,\ where}$ 'n' is number of linked isoprene units. Hence, terpenes are also termed as isoprenoid compound (Ruby & Rana 2015).

2.5.2 Phenolics

Phenolic is chemical compound which is characterized by the presence of aromatic ring structure bearing one or more hydroxyl groups. Phenolics are most abundant secondary metabolites of plants ranging from simple molecules such as phenolic acid to highly polymerized substances such as tannins (Ruby & Rana 2015).

2.5.3 Glycosides (Heavily modified sugar molecules)

They are formed from a simple sugar and another compound by replacement of a hydroxyl group in the sugar molecule. Many drugs and poisons derived from plants are glycosides

2.5.4 Flavonoids

Flavonoids or bioflavonoids (from the Latin word flavus meaning yellow, their colour in nature) are a class of plant and fungus secondary metabolites**.** Flavonoids are polyphenolic compounds that occur ubiquitously in foods of plant origin. Over 4000 different flavonoids have been described, and they are categorized into flavonols, flavones, catechins, flavanones, anthocyanidins and isoflavonoids. Flavonoids are a group of plant metabolite thought to provide health benefits through cell signally pathway and anti-oxidant effects. These molecules are found in a variety of fruits and vegetables. Flavonoids are abundant in plants, in which they perform several functions. They are essential pigment for producing the colours to attract pollinating insect. They have been shown to exert antimicrobial, mutagenic, anti-inflammatory, and antioxidant activities. Flavonoids showed anti-inflammatory activities by inhibiting both the cyclooxygenase and lipooxygenase pathways (Mona et al., 2014)**.**

2.5.5 Alkaloids

They are small, heavily derivate of amino acid derived from plant and they have basic characteristics, hence the term alkaloids from alkali. They contain nitrogen bases heterocyclic ring within their molecule. Some alkaloids such as isoquinoline, indole and diterpene are known to have good anti-inflammatory activity; this is based on their ability to prevent the synthesis or the action of some proinflammatory cytokines. (Mona et al., 2014)

2.5.6 Saponins

They are plant steroids, (often glycosylated)**.** According to Mona et al (2014), Saponins are steroid or triterpene glycosides which are widely distributed in the plant kingdom that include a large number of biologically active compounds. Saponins isolated from about 50 plants showed anti-inflammatory activity against several experimental models of inflammation in mice and rats. Mechanisms considered included indirect (many saikosaponins) and direct (saikosaponin and ginsenosides) corticomimetic activity, inhibition of glucocorticoid degradation (glycyrrhizin), inhibition of enzymatic formation and release of inflammation mediators.

2.6 Inflammatory Mediators

In appreciating the inflammatory process, it is important to understand the role of chemical mediators. These are substances that tend to direct the inflammatory response. These inflammatory mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes/macrophages. They are triggered by bacterial products or host proteins. Chemical mediators bind to specific receptors vascular permeability, neutrophil chemotaxis, stimulate smooth muscle contraction, have direct enzymatic activity ,induce pain or mediate oxidative damage. Most mediators are short - lived but cause harmful effects. Examples of chemical mediators include vasoactive amines (histamine, serotonin), arachidonic acids (prostaglandins, leukotrienes) and cytokines (tumour necrosis factor and interleukin -1) (Smith & Sortins 2004).

As in the case in acute inflammation, the ordered process of cellular accumulation and activation in chronic inflammation is dependent upon the sequential release of chemical mediators of inflammation. Some of these are preformed and stored in the granules of platelets and mast cells; some, such as complement components, are generated by activation of plasma enzyme cascades; but the majorities are newly synthesized by cells of the tissue or by previously recruited

inflammatory cells. Prominent among the latter group are relatively small protein molecules, collectively referred to as cytokines, which act as potent biological signals for cellular migration and activation (Wakefield & Lloyd, 1992).

In chronic inflammation, cytokines play critical roles in macrophage and T-cell recruitment, activation and local replication; in the survival of inflammatory cells by inhibition of apoptosis of an immune response; and in the induction of granulation tissue and fibrosis (Jackson, Seed, Kircher, Willoughby & Winkler 1997).

Cytokines exert their effects by binding to cell membrane receptors on the same cell (autocrine action), adjacent cells (paracrine action) or remote cells (acting as a hormone). The families of cytokines include molecules referred to as chemokines, interleukins, interferons, colony stimulating factors and growth factors, many of which are important in chronic inflammation. During inflammation, circulating cells attach to the vascular endothelium and migrate between endothelial cells. When stimulated by cytokines, endothelial cells regulate the recruitment of leucocytes via sets of surface adhesion molecules that tether the two cells together. Activation of the endothelial cells induces a variety of cytokines, as well as adhesion molecules. Acute inflammation is initially characterized by recruitment of neutrophils, in part mediated by the activity of the so-called a chemokines such as interleukin. This is followed by T-cell and monocyte accumulation, believed to be mediated by other chemokines (Rand et al., 1996). Additional chemokines exhibit relative specificity for eosinophils or basophils, which are frequently associated with allergic disorders (Adams & Lloyd, 1997).

Cytokines secreted by macrophages are critical to the development of the repair response that parallels chronic inflammation, because of their role in the induction of granulation tissue.

2.7 TREATMENT OF INFLAMMATION

To reduce inflammation and the resulting swelling and pain, injured tissue needs to be properly treated. The earlier you start treatment, the better. Treatment for acute inflammation consists of "R.I.C.E." therapy—which stands for **R**est, **I**ce, **C**ompression, and **E**levation**.**

For acute inflammation in the foot or ankle, your foot and ankle surgeon will recommend the following:

2.7.1 Rest

Stay off of your foot as much as possible to prevent further injury. In some cases, complete immobilization may be required. Your doctor will decide whether you will need crutches and whether movement of your foot or ankle is appropriate.

2.7.2 Ice

Icing, which decreases blood flow to the tissue, thus reducing swelling and pain, should be continued until your symptoms resolve. Wrap ice cubes—or a bag of frozen peas or corn—in a thin towel and place the pack on the injured area for 20 minutes of each hour you're awake. If your skin turns blue or white, discontinue icing for a few hours. Two cautions: ***Never*** apply ice or frozen bags directly to your skin. And ***never*** leave an ice pack on your injury while you sleep.

2.7.3 Compression

Keep the inflamed area compressed by wrapping it in an elastic bandage or stocking. Compression prevents additional fluid accumulation and helps reduce pain. Wrap the bandage more firmly at the toes and less firmly at the calf. If your toes tingle or your foot throbs, the wrapping may need to be loosened. If the tingling or throbbing continues after loosening the wrap, contact your doctor as soon as possible.

2.7.4 Elevation

Keeping the foot elevated reduces the swelling by allowing excess fluid to drain to the heart. The proper way to elevate your foot is to keep it level with or slightly above the heart. Place one or two pillows under your calf, and make sure your hip and knee are slightly bent. Never keep your leg extended straight out. In addition to the above measures, your foot and ankle surgeon may prescribe a Nonsteroidal anti-inflammatory drug (NSAID), such as ibuprofen, or another type of medication.

2.8 SOLANUM AETHIOPICUM

The garden egg (*S. aethiopicum* L.) is a cultivated eggplant, which is a popular traditional vegetable in tropical Africa. This species is grown for its leaves and fruits.

The fruits of *S. aethiopicum* are consumed fresh. It could also be steamed, pickled, boiled or used in preparing stews with other vegetables or meats, while young leaves are often used in soups and with other vegetables (Anosike et al., 2012).

S. aethiopicum has been domesticated and is cultivated majorly in Africa and consequently abundant mostly in Central and West Africa. *S. aethiopicum* has also been introduced into the Caribbean and South America and is grown in some parts of southern Italy (Chinedu, Olasumbo & Eboji 2011).

The juice obtained by the maceration of the leaves is applied in the treatment of uterine complaints in various parts of Africa. In addition, the leaf extracts serves as an anti-emetic and sedative and to treat tetanus associated with miscarriages (Chinedu et al., 2011).

The garden egg (*S. aethiopicum*) can be regarded as a brain food because it accommodates the anthocyanin phytonutrient found in its skin called nasunin which is a potent antioxidant and free radical scavenger that has been revealed to prevent the destruction of cell membrane, that can promote cancer and lessening free radical damage in joints, primary factor in rheumatoid arthritis (Anosike et al., 2012).

High crude fibre, low fat and low dry matter may be helpful in preventing such diseases as constipation, carcinoma of the colon and rectum, diverticulitis and atherosclerosis. It may partly account for the weight reduction effect of eggplant (Chinedu et al., 2011).

It is also high in potassium, a necessary salt that helps in maintaining the function of the heart and regulate blood pressure.

2.8.1 CLASSFICATION OF *S. AETHIOPICUM*

Kingdom - plantae

Phylum - angiosperm

Class - magnoliopsida

Order - solanales

Family - solanaceae

Genus - solanum

Specie - aethiopicum

2.8.2 HEALTH BENEFIT OF *S. AETHIOPICUM*

1. **It fights cancer:** Studies have shown that Polyphenols in eggplant have anti-cancer effects. The anthocyanin and chlorogenic acid that are contained in garden egg help protect cells from damage caused by free radicals. Thus preventing tumour growth and the spread of cancer cells. It was also shown that the anticancer action of anthocyanin appears to include preventing new blood vessels from forming in the tumour, reducing inflammation, and blocking the enzymes that help cancer cells to spread.
2. **It helps during pregnancy:** It is packed with vitamins, minerals, and other nutrients which make it an essential part of the daily ration of every pregnant woman. Given the fact that the baby is taking much of the useful nutrients for himself, women often suffer from the lack of individual components in their body. So, eating this plant can be an excellent solution to such situations.

3. **It helps in weight reduction**: Lifestyle experts confirm that when it comes to weight management and loss, dietary fibres are one of the most important factors because they act as "bulking agents" in the digestive system. The compounds in garden egg do not only increase satiety, they also reduce appetite. Observation has also shown that eggplant, as it is also known, help reduce calorie intake by making a person feel fuller for longer.
4. **It prevents liver disorder and help control sugar level:** Studies have shown that the importance of garden egg to the liver cannot be overemphasized because the antioxidants in garden egg help protect the liver from certain toxins; thereby making it a remedy for liver issues. However, research has also shown that garden egg helps control sugar level; making it a great dietary option for diabetic patients. It has the ability to reduce glucose absorption in the body and lower blood sugar levels. It also possesses low soluble carbohydrates, which assist in this regard.
5. **It helps to improve vision**: The cream-colour flesh of garden egg has a pleasantly bitter taste and this is due to the presence of small amounts of nicotinoid alkaloids it contains. Its spongy consistency helps to protect from poor vision caused by glaucoma as well as improving overall vision.

Figure 2: *Solanum aethiopicum* plant

2.9. NON- STEROIDAL ANTI-INFLAMMATORY DRUGS

The Non-Steroidal Anti-inflammatory Drugs (NSAIDS) are the classes of drugs that reduce pain, decrease fever, prevent blood clots and in higher doses decrease Inflammation. They are widely used for the treatment of minor pain and for the management of edema and tissue damage resulting from inflammatory diseases (Grosser, Fries and FitzGerald 2006). NSAIDS such as aspirin, ibuprofen and diclofenac that exhibit non-selective COX inhibition represents some of the most widely prescribed NSAIDS to relieve short term fever, pain and Inflammation (Inotai, Rojkovich & Meszaros, 2010).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1 COLLECTION AND IDENTIFICATION OF THE PLANT MATERIAL

The leaves of *S. aethipicum* were collected in fresh condition from Owode area in Ogun state Nigeria on the 23rd of January 2019 and further identified by Botanist at the University of Lagos. With identification number: 8381

3.2. PREPARATION OF EXTRACTS

The plant material was rinsed and air dried at room temperature then grounded into a uniform powder using a blender and weight. The plant was grounded and 100g was macerated in ethanol for 72hrs filtered with a white cloth and then concentrated in a rotary evaporator at an optimum temperature of 40-50 degree Celsius

3.3 PHYTOCHEMICAL SCREENING

3.3.1. QUALITATIVE PHYTOCHEMICAL SCREENING

Phytochemical analysis extract was carried out using the method described by Odebiyi & Sofowora (1978) for the detection of saponins, tannins, phenolics, alkaloids, steroids, triterpenes, phlobatannins, phenolics, fixed oils and fat, photosterols, glycosides and flavonoids.

3.3.1.1 Alkaloids

To a test tube $1cm^3$ of 1%HCl was added to $3cm^3$ of the extracts in the test tube. The mixture was heated for 20 minutes, cooled and filtered. The filtrate was used in the following tests: 2 drops of Wagner's reagent was added to $1cm^3$ of the extracts. A reddish brown precipitate indicates the presence of alkaloids

3.3.1.2. Tannins

Freshly prepared 10% KOH ($1cm^3$) was added to $1cm^3$of the extracts. A dirty white precipitate indicated the presence of tannins.

3.3.1.3. Phenolics

Five percent FeCl (2 drops) $_3$ was added to 1cm^3 of the extracts in a test tube. A greenish precipitate indicates the presence of phenolics.

3.3.1.4 Glycosides

H_2SO (1cm of 50%) $_4$ was added to 1cm^3 of the extracts, the mixture was heated in boiling water for 15 minutes. 10cm^3 of Fehling's solution was added and the mixture boiled. A brick red Precipitate indicates the presence of glycosides.

3.3.1.5 Saponins

Frothing test: 2cm^3 of the extract in a test tube was vigorously shaken for 2 minutes. Frothing indicates the presence of saponins.

3.3.1.6. Flavonoids

NaOH (1cm) was added to 3cm^3 of the extracts. A yellow colouration indicates the presence of flavonoids.

3.3.1.7 Steroids

Salakowsti test: 5 drops of concentrated H_2SO_4 was added to 1cm^3 of the extracts. Red colouration indicates the presence of steroids.

3.3.1.8 Phlobatannins

The extract (1cm) was added to 1% HCl. A red precipitate indicates the presence of phlobatannins.

3.3.1.9. Triterpenes

Acetic anhydride (5 drops) was added 1cm^3 of the extracts. A drop of concentrated H_2SO_4 was then added and the mixture was steamed for 1 hour and neutralized with NaOH followed by the addition of chloroform. A blue green colour indicates the presence of triterpenes.

3.3.1.10. Phytosterols (Finar 1986)

Liberman-burchard's test: 50mg is dissolved in 2ml acetic anhydride. To this, one or two drops of conc .H2SO4 is added slowly along the sides of the test tube. An array of colour changes shows the presence of phytosterols.

3.3.1.11. Fixed oils and fats (kokate, 1999)

A small quantity is processed between two filter papers; oil stain on the paper indicates the presence of fixed oil.

3.3.1.12. Terpenoids

Aqueous extract of the sample (5ml) is mixed with 2ml of CHCl3 in a test tube 3ml of con. H2SO4 is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

3.3.1.13. Amino acid (Yasuma and Ichikawa 1953)

Two drops of ninhydrin solution (10mg of ninhydrin in 200ml of acetone) are added to two ml of aqueous filtrate. A characteristic purple colour indicates the presence of amino acids.

3.3.2 QUANTITATIVE PHYTOCHEMICAL SCREENING

3.3.2.1 Estimation of total phenolic content

The total phenolic content of sample was estimated according to the method of Makkar et.al (1997). The aliquots of the extract was taken in a test tube and made up to the volume of 1 ml with distilled water. Then 0.5ml of Folin-Ciocalteu reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially to the test tube. Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40mins. and the absorbance was recorded at 725 nm against the reagent blank. Using Gallic acid monohydrate, a standard curve was prepared. The linearity obtained was in the range of 1-10 μg/ml. using the standard curve, the total phenolic content was calculated and expressed as Gallic acid equivalent in mg/g of extract.

3.3.2.2 Determination of Total flavonoid assay

Total flavonoid content was measured by aluminium chloride colorimetric assay. 1ml of extracts or standard solution of Quercetin (500µg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To the above mixture, 0.3 ml of 5% $NaNO_2$ was added. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added. At 6min, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510nm. Total flavonoid content of the flower was expressed as percentage of Quercetin equivalent per 100g of fresh mass.

3.3.2.3. Determination of coumarin

About 5ml of 5N NaOH was added to the solution for 1 ml of the extract (0.5 g in 1ml of ethanol), the solution was heated at 80^OC for 5 min and cooled, 0.75 ml of 5 N H_2SO_4 was added and mixed thoroughly, 0.25 g of anhydrous $NaHCO_3$, was the added and mix then transferred to the extractor. The flask was rinsed with distilled water and transferred to the extractor and made up to 50 ml and then extracted for 3 hrs. The inner tube was transfer to the pet ether in the extractor to the extraction flask. 20 ml of water was added to the pet ether extract and the pet ether was carefully evaporated in a water bath at 50-55 0C.

The aqueous solution was added to a volumetric flask; and made up to 50 ml with continuous mixing. 25 ml was pipette into a flask and 1% Na_2CO_3 was added to the solution, it was heated in the water bath at 85^OC for 15 min and cooled. 5 ml of the diazonium solution was added, and allowed to stand for 2 hours. The absorbance was read at 540 nm against reagent blank. The cumarin content was calculated from standard curve (Willard & Karl 1937).

3.3.2.4. Determination of Alkaloids:

The quantitative determination of alkaloids was done by distillation and titrimetric methods as described by Henry (1973). Briefly, 2g of finely ground sample was weighed into 100ml beaker and 20mls of 80% absolute alcohol added to give a smooth paste. The mixture was transferred to

a 250ml flask and more alcohol added to make up to 1g of magnesium oxide was then added. The mixture was digested in a boiling water bath for an hour and half under a reflux air condenser with occasional shaking. The mixture was filtered while hot through a Buchner funnel. The residue was poured back into the flask and re-digested for another thirty minutes with 50ml alcohol after which the alcohol was evaporated. Distilled water was added to replace the lost alcohol. When all alcohol has evaporated, 3 drops of 10% HCl was added. The whole solution was later transferred into 250ml volumetric flask; 5ml of Zinc acetate solution and 5ml of potassium ferricyanide solution were thoroughly mixed together to give a homogenous mixture. The flask was allowed to stand for a few minutes, filtered through a dry filter paper and 10ml of the filtrate was transferred into a separating funnel and the alkaloids present were extracted vigorously by shaking with five successive portions of chloroform. The residue obtained was dissolved in 10ml of hot distilled water and transferred into a Kjeldahl tube with the addition of 0.2g of selenium for digestion to a clear colourless solution. The clear colourless solution was used to determined Nitrogen using Kjeldahl distillation apparatus the distillate was back titrated with 0.01N HCl and the titre value obtained was used to calculate the % Nitrogen using the formulae:

$$\%N = \frac{\text{Titre value} \times \text{Atomic mass of Nitrogen} \times \text{Normality of HCl}}{\text{Weight of sample (mg)}} \times 100$$

% Alkaloid = % Nitrogen × 3.26

Where 3.26 is a constant

3.3.2.5. Determination of Glycosides:

10ml of extract was pipette into a 250ml conical flask. 50ml Chloroform was added and shaken on a Vortex Mixer for 1 hour. The mixture was filtered into a conical flask. 10ml pyridine and 2ml of 2% sodium nitroprusside were added and shaken thoroughly for 10 minutes. 3ml of 20% NaOH was later added to develop a brownish yellow colour.

Glycoside standard of concentration ranging from 0-5mg/ml were prepared from 100mg/ml stock glycoside standard. The series of standards 0-5mg/ml were treated similarly like the sample above. The absorbance of sample as well as standards were read on a spectronic 21D Digital spectrophotometer at a wavelength of 510nm. % Glycoside was calculated using the formula:

$$\% \text{ Glycoside} = \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

3.3.2.6. Determination of Saponins:

The spectrophotometric method of Brunner (1984) was used for the analysis of saponins. Briefly, 1g of the finely ground dried sample was weighed into a 250ml beaker and 100ml of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing. Thereafter, the mixture was filtered through a Whatman No. 1 filter paper into a 100ml beaker containing 20ml of 40% saturated solution of $MgCO_3$. The resulting mixture was again filtered to obtain a clear colourless solution. One millilitre of the colourless filterate was pipette into a 50ml volumetric flask and 2mlof 5% $FeCl_3$ solution was added and made up to the marked level with distilled water. This was then allowed to stand for 30 minutes for a blood red colour to develop. 0-10ppm saponins standard was prepared from saponins stock solution. The standard solutions were treated similarly with 2ml of 5% $FeCl_3$ solution as earlier described. The absorbance of the samples as well as standard saponin solutions was read after colour development using a Jenway V6300 spectrophotometer at wavelength of 380nm.

Percentage saponin was calculated using the formula:

$$\% \text{ saponin} = \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

3.3.2.7. Determination of Steroids

0.05g of sample extract was weigh into a 100ml beaker. 20ml of chloroform-methanol (2:1) mixture was added to dissolve the extract upon shaking for 30 minutes on a shaker. The whole mixture is mixed until free of steroids. 1ml of the filtrate was pipette into a 30ml test tube and 5ml of alcoholic KOH was added and shaken thoroughly to obtain a homogenous mixture. The mixture was later placed in a water bath set at 37˚C-40˚C for 90 minutes. It was cooled to room temperature and 10ml of petroleum ether added followed by the addition of 5ml distilled water. This was evaporated to dryness on the water bath. 6ml of Liebermann Buchard reagent was added to the residue in dry bottle and absorbance taken at a wavelength of 620nm on a spectronic 21D digital spectrophotometer. Standard steroids of concentration of 0-4mg/ml were prepared from 100mg/ml stock steroid solution and treated similarly like the sample as above.

% steroid was calculated using the formula:

$$\% \text{ steroids} = \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

3.3.2.8. Determination of Triterpenes:

0.50g of sample was weighed into a50ml conical flask and 20ml 0f 2:1 chloroform-methanol mixture was added, shaken thoroughly and allowed to stand for 15 minutes. The supernatant obtained was discarded, and the precipitate was re-washed with another 20ml chloroformmethanol mixture for re-centrifugation.

The resultant precipitate was dissolved in 40ml of 10% Sodium Dodecyl Sulphate (SDS) solution.1ml of 0.01M ferric chloride solution was added to the above at 30 seconds intervals; shaken well, and allowed to stand for 30 minutes. Standard triterpenes of concentration range 05mg/ml were prepared from 100mg/l stock triterpenes solution from sigma-Aldrich chemicals,

U.S.A. The absorbance of sample as well as that of standard concentrations of triterpenes were read on a digital spectrophotometer at a wavelength of 510nm.

The percentage of triterpenes was calculated using the formula:

$$= \frac{\text{Absorbance of sample} \times \text{Average gradient} \times \text{Dilution factor}}{\text{Weight of sample} \times 10{,}000}$$

3.4 Invitro Anti Inflammatory Assays

3.4.1 Red Blood Cell (RBC) Membrane Stabilization

RRBCs were prepared by collecting blood (5 ml) from healthy rat red blood cell. The blood collected was centrifuged, and the supernatant obtained was carefully pipetted out. The packed cells were then suspended in an equal volume of isosaline and centrifuged. The process was repeated 4 times until clear supernatant was observed. Then, with normal saline, a 10% HRBC suspension was prepared and kept at 4°C until use. The reaction mixture (4.5 ml) consisted of 2 ml hyposaline (0.25% w/v NaCl), 1 ml of isosaline buffer solution, pH 7.4 (6.0 g TRIS, 5.8 g NaCl, HCl to regulate the pH, and water to make 1000 ml), and varying volumes of the extract solution in isotonic buffer (concentration = 10 mg/ml) to make the volume to 4.0 ml. Then, 0.5 ml of 10% HRBC in normal saline was added. Two controls were performed. Control 1 included 1.0 ml of isosaline buffer instead of extract, and Control 2 included 1 ml of extract solution and without red blood cells. The mixture was incubated for 30 min at 56°C. After cooling the tubes for 20 min under running water, the mixture was centrifuged, and the absorbance of the supernatant was read at 560 nm.

The percentage of membrane stabilization was determined using the formula:

Extract absorbance value – control 1 absorbance value 100 Control 2 absorbance value − ×

The control 1 represents 100% RRBC lyses. Acetyl Salicylic acid was used as a standard.

3.4.2 Anti Lipoxygenase Activity

Anti-Lipoxygenase activity was studied using linoleic acid as substrate and lipoxidase as enzyme. Test samples were dissolved in 0.25ml of 2M borate buffer pH 9.0 and added 0.25ml of lipoxidase enzyme solution (20,000U/ml) and incubated for 5 min at 25^0C. After which, 1.0ml of linoleic acid solution (0.6mM) was added, mixed well and absorbance was measured at 234nm. Indomethacin was used as reference standard. The percent inhibition was calculated from the following equation,

$$\% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

A dose response curve was plotted to determine the IC50 values. IC50 is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

3.4.3 Heat induced haemolysis

The reaction mixture (2ml) consisted of 1 ml test sample of different concentrations (100 – 500 µg/ml) and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 ºC for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. The Percentage inhibition of Haemolysis was calculated as follows.

$$\text{Percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control.}} \times 100$$

3.4.4 Protein Denaturation Assay

The anti-inflammatory activity of *S. aethiopicum* was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima & Kobayashi 1968 and Sakat 2010 followed with minor modifications. The reaction mixture was consists of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The sample extracts were incubated at 37 ºC for 20 min and then heated to 51 º C for 20 min, after cooling the samples the turbidity was measured at 660nm. (UV Visible Spectrophotometer Model 371, Elico India Ltd) The experiment was performed in triplicate.

The Percentage inhibition of protein denaturation was calculated as follows:

$$\text{Percentage inhibition} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs control}} \times 100$$

3.5 Statistical Analysis

The experimental data obtained were expressed as mean ± S.E.M. the difference between the extract and standard were compared using one way analysis of variance (ANOVA) followed by Fisher LSD (control Vs. Test) and Duncan multiple range test using SPSS Software Version 20. $P<0.05$ was considered statistically significant. The $IC_{50\ values}$ were calculated using Microsoft excel version 2007.

CHAPTER FOUR
RESULT AND DISCUSSION

4.0. RESULT

4.1 PRELIMINARY PHYTOCHEMICAL SCREENING

Phytochemical screening was conducted on the leaf sample to test for the presence of certain phytochemicals and the following result was obtained.

TABLE 1: The Qualitative phytochemical analysis of ethanol extract of *S. aethiopicum* leaves extract

PHYTOCHEMICALS	
Saponins	+
Tannins	-
Phenolics	+
Flavonoids	+
Steroids	+
Terpernoids	-
Triterpenes	+
Coumarins	+
Glycosides	+
Alkaloids	+
Fixed oils	-

Key: + means test substance present, - means test substance absent

4.2 Quantitative Phytochemical Analysis

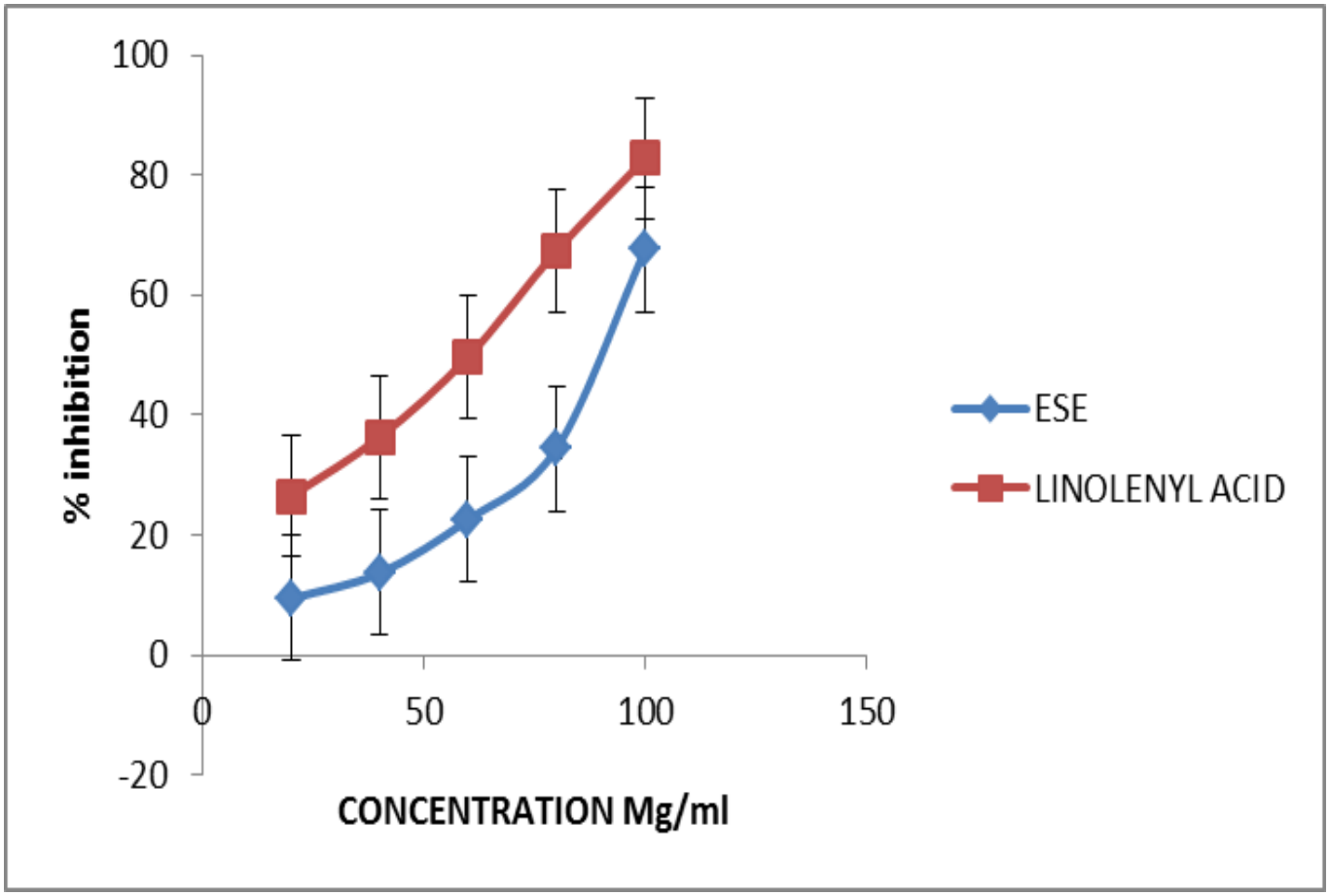
TABLE 2: Quantitative Phytochemical Analysis of ethanol extract of *S. aethiopicum* leaves extract

PHYTOCHEMICALS	CONCENTRATION(mg/100g)
Saponins	0.162±0.0089
Phenolics	24.77±0.064
Flavonoids	165.38±0.10
Steroids	46.71±0.09
Triterpenes	8.396±0.029
Coumarins	144.60±3.97
Glycosides	14.37±0.024
Alkaloids	48.88±0.020

Key: values are expressed in mean ± standard error.

4.3 Invitro Inflammatory Assays

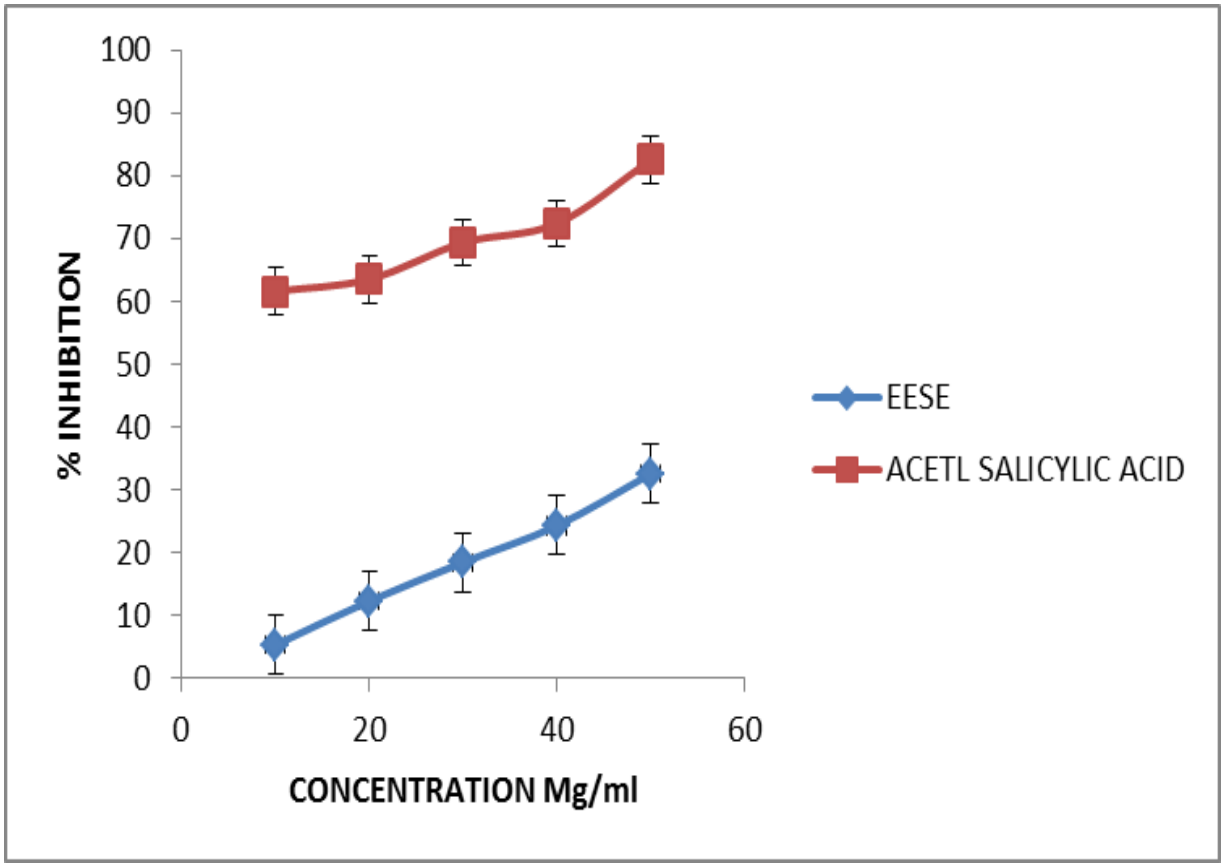
4.3.1. Anti-lipoxygenase activity of ethanol extract of *S. aethiopicum* leaves extract



ESE: Ethanol extract of *S. aethiopicum*

Figure 3: Anti-lipooxygenase activity of alcoholic extract of *S. aethiopicum* leaves extract and Linolenyl hydroxamic acid (control).The data represent the percentage inhibition of antilipooxygenase activity. Each point represents the values obtained from three experiments, performed in triplicate (mean± S.E.M).

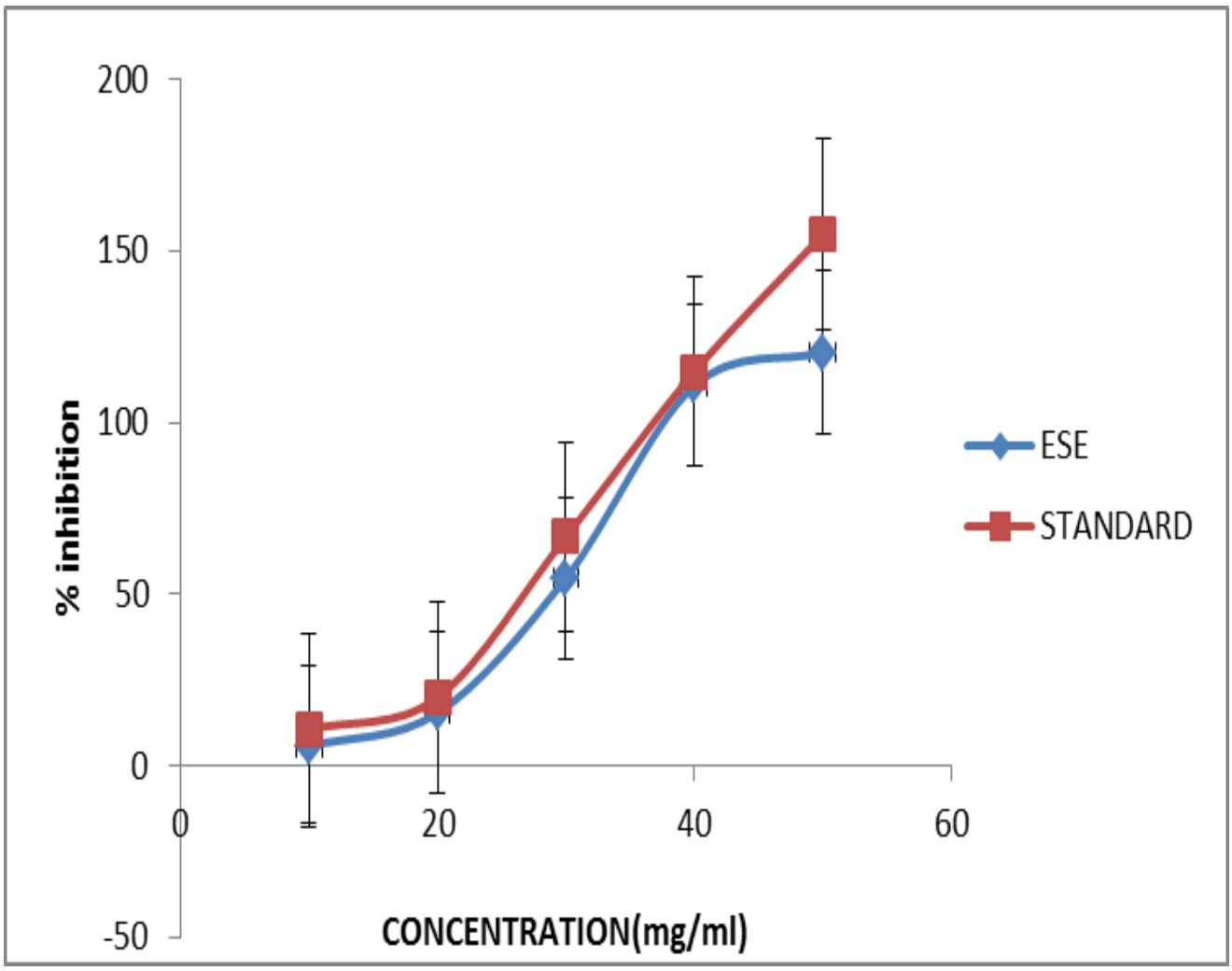
4.3.2 Membrane stabilization assay



ESE: Ethanol extract of *S. aethiopicum*

Figure 4: membrane stabilization activity of alcoholic extract of *S. aethiopicum* leaves extract and Acetyl Salicylic acid (control). The data represent the percentage membrane stabilization capacity off the plant sample. Each point represents the values obtained from three experiments, performed in triplicate (mean± S.E.M).

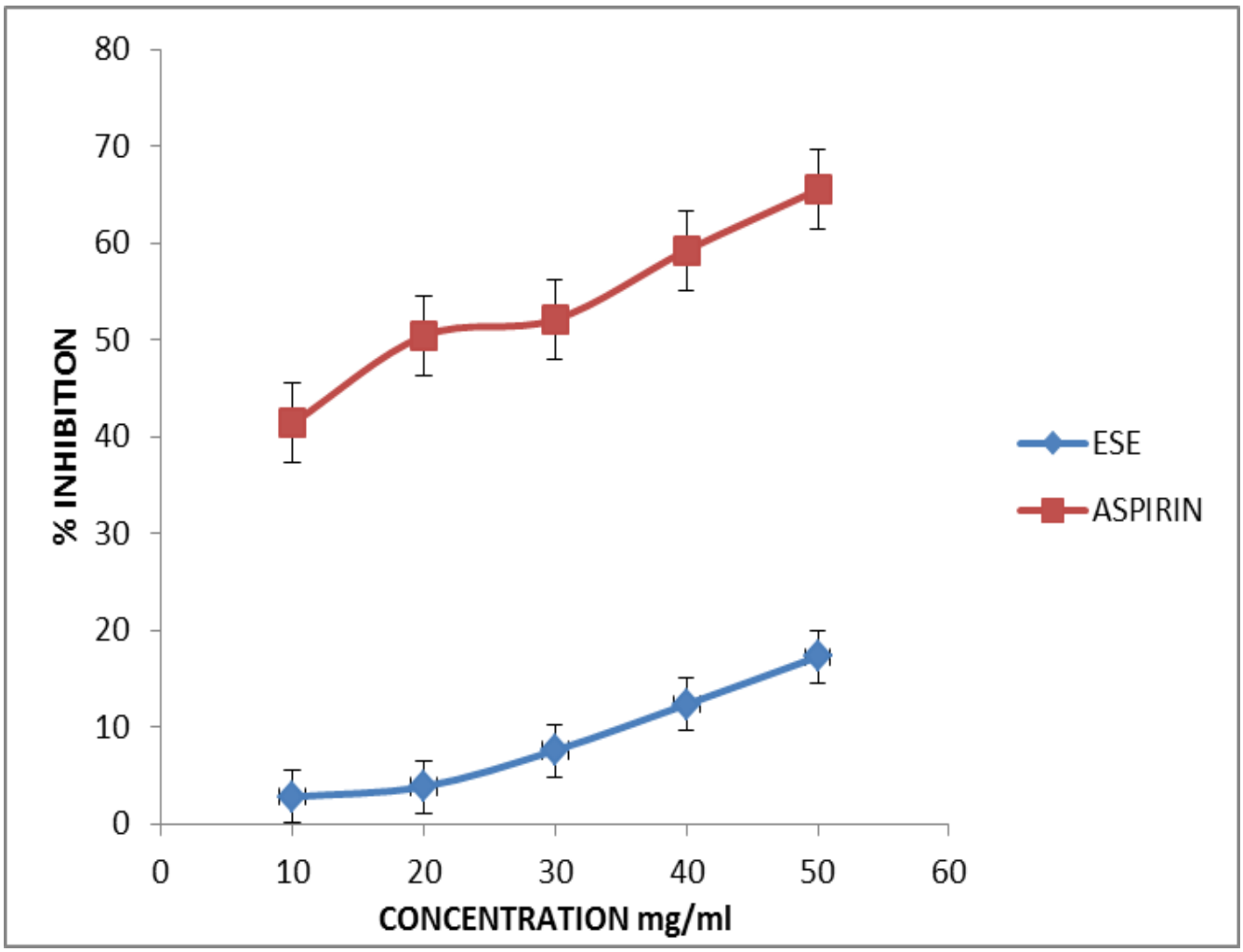
4.3.3 Heat Stabilization



ESE: Ethanol extract of *S. aethiopicum*

Figure 5: heat stabilization capacity of the alcoholic extract of *S. aethiopicum* leaves extract and Aspirin (control). The data represent the percentage heat stabilization ability of the plant sample. Each point represents the values obtained from three experiments, performed in triplicate (mean± S.E.M).

4.3.4 Protein Denaturation Assay



ESE: Ethanol extract of *S. aethiopicum*

Figure 6: Protein denaturation capacity of alcoholic extract of *S. aethiopicum* leaves extract and Aspirin (control). The data represent the percentage denaturation ability of the plant sample. Each point represents the values obtained from three experiments, performed in triplicate (mean± S.E.M).

The result of lipoxygenase inhibition indicated that the IC_{50} values of the extract was significantly higher than that of the standard (linolenyl acid) which 89.84 µg/ml and 56.60µg/ml respectively. The Standard significantly inhibited the activity of lipoxygenase. However, it was discovered that at a concentration of 100µg/ml, the standard, linolenyl acid exhibited the highest percentage Inhibition of lipoxygenase at 82.76.6±0.26% (figure 3).

The result of membrane stabilization indicated that the IC_{50} values of the extract was significantly higher than that of the standard (acetyl salicylic acid) which 77.38 µg/ml and 9.17µg/ml respectively. The Standard significantly inhibited the activity of Albumin Denaturation. However, it was discovered that at a concentration of 50µg/ml, the standard, acetyl salicylic acid exhibited the highest percentage of membrane stabilization at 82.52± 0.16% (figure 4).

The result of heat stabilization showed that the plant sample and the standard exhibited highest percentage of heat stabilization at 50µg/ml (33.43 ±0.97 and 153.51±0.87 respectively) and that the IC_{50} value of the extract was significantly higher than that of the standard which is 26.50µg/ml and 23.91µg/ml respectively. The Standard significantly stabilizes heat (figure 5).

The result of inhibition of protein denaturation indicates that the IC_{50} value of the extract was significantly higher than that of the standard (Aspirin) which is 127.06µg/ml and 23.52µg/ml respectively. The aspirin Standard significantly stabilizes heat. However, it was discovered that at a concentration of 50µg/ml, the standard, exhibited the highest percentage of inhibition of protein denaturation at 65.50± 0.06% (figure 6).

4.4 DISCUSSION

Inflammation is a protective response of the body to eliminate the initial cause of cell injury (diluting, destroying and neutralizing the harmful agents), remove the damaged tissue and generate new tissue It involves a well-organized cascade of fluid and cellular changes within living tissue (Kumar, Bajwa, Singh & Kalia, 2013).

The ethanoic extract of the leaves of *S. aethiopicum* can be a source of herbal medicine to efficiently treat various human diseases. The result of phytochemical screening revealed that *S. aethiopicum* is abundantly rich in saponins, phenolics, flavonoids, steroids, triterpenes, coumarins, glycosides and alkaloids. This result is in accordance to the result of Anosike et al., 2012). Therefore, the presence of this phytochemicals might be responsible for the reported anti-inflammatory activities of the plant.

The establishment of new invitro test systems has stimulated the screening of plants aiming to find leads for the development of new drugs. The plant lipoxygenase pathway is in many respects equivalent to the 'arachidonic acid cascades' in animals. For this reason, the in vitro inhibition of lipoxygenase constitutes a good model for the screening of plants with anti-inflammatory potential. LOXs are sensitive to antioxidants and the most of their action may consist in inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy or lipid peroxy- radical formed in course of enzyme peroxidation. This can limit the availability of lipid hydroperoxide substrate necessary for the catalytic cycle of LOX.

S. aethiopicum leaves extract was checked at 20, 40, 60, 80, 100µg/ml concentrations, it showed increase in lipoxygenase inhibition respectively. From these result, the strongest inhibition was obtained at concentration 100µg/ml. The standard linolenyl hydroxamic acid showed the highest inhibition at a concentration of 100µg/ml. At the concentration of 20 and 40 µg/ml, *S. aethiopicum* showed significant difference ($p > 0.05$) when compared with control (Table 3). The results obtained from our studies on *S. aethiopicum* have shown a potential anti-inflammatory activity.

The ethanol extract of *S. aethiopicum* leaves extracts inhibited the lipoxygenase enzyme activity. This indicates that plant *S. aethiopicum* is more useful in studies of inflammation and in various related physiological studies, aging and diseases such as cancer, neurological disorder etc. This is in accordance with the findings of leelaprakash who checked the invitro anti-inflammatory activity of methanol extract of *Enicostemma axillare*

The RBC membrane stabilization has been used as a method to study the invitro anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may well stabilize lysosomal membranes. Stabilization of lysosomal is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil, such as bacterial enzymes and proteases, which causes further tissue inflammation and damage upon extra cellular release. The lysosomal enzymes released during inflammation produce a various disorders. The extra cellular activity of these enzymes are said to be related to acute or chronic inflammation. The nonsteroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. In this study, the standard acetyl salicylic acid exhibited the highest erythrocyte membrane stabilization activity with IC_{50} of -9.17 (Table 4).The result revealed that the concentration is dose-dependent and the extract showed the maximum Inhibitory activity. Fawole et al., (2010) reported that plants rich in flavonoids are known to possess anti-inflammatory activity.

The extract was effective in the stabilization of heat at different concentrations. The results showed that *S. aethiopicum* at concentration 30 and 40µg/ml protect significantly ($p<0.05$) the erythrocyte membrane against lysis induced by heat (table 5). Aspirin 100µg/ml offered a significant ($p<0.05$) protection against damaging effect of heat solution. This is in accordance with the findings of Leelaprakash and Mohan in 2010 that checked the invitro anti-inflammatory activity of methanol extract of *Enicostemma axillare*

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Denaturation of proteins is a well-documented because most biological proteins lose their biological function when denatured. Protein Denaturation involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding (Bagad, Umarkar, Tatiya & Surana, 2011). In this study, the standard acetyl salicylic acid exhibited high protein denaturation inhibition activity with IC_{50} of 23.52 (Table 6). The inhibitory effect of different concentrations of *S. aethiopicum* leaf extract on protein denaturation is shown in (Table 6). The leaf extract showed significant inhibition of albumin denaturation in a dose-Dependent manner.

In the present study, results indicate that the ethanol extracts of *S. aethiopicum* possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols,

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

In this study, results indicate that the ethanol extracts of *S. aethiopicum* leaves has potent anti-inflammatory activities. From the result, the extract was able to reduce the activities of lipoxygenase enzymes and stabilize heat better than inhibition of protein denaturation and membranes stabilization. This study gives us the idea that *S. aethiopicum* can be used in development of potent anti-inflammatory drugs in inhibition of lipooxygenase enzymes and heat stabilization.

5.2 RECOMMENDATION

i. Further research should be carried out to isolate the active ingredient of anti-inflammation in *S. aethiopicum* leaves.

ii. Comparative studies should be done between different parts of the plant to determine which part of the plant has more anti-inflammatory properties.

iii. Invivo anti-inflammatory activity of the plant should examined to ascertain the plants effectiveness in anti-inflammation.

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APPENDIX

STATISTICAL ANALYSIS

ANTI LIPOXYGENASE ACTIVITY

Table 3: Effect of ethanoic extract of *S. aethiopicum* leaves extract on Anti-lipoxygenase activity

CONC.µg/ml	%INHIBITION OF SAMPLE	%INHIBITION OF STANDARD
20	9.41 ^{b} ±0.64	26.50^{c}±0.24
40	13.64 ^{b} ±0.15	36.35^{c} ±0.15
60	22.55 ^{b} ±0.29	49.79 ^{c}±0.67
80	34.45 ^{b} ±0.28	67.40^{c} ±0.70
100	67.62^{b} ±0.00	82.76^{c} ±0.26
IC_{50}	89.84	56.60

Values are represented as mean ± SEM (N=3).p< 0.05 considered as IC_{50} significant when compared to the Standard drug (Diclofenac).

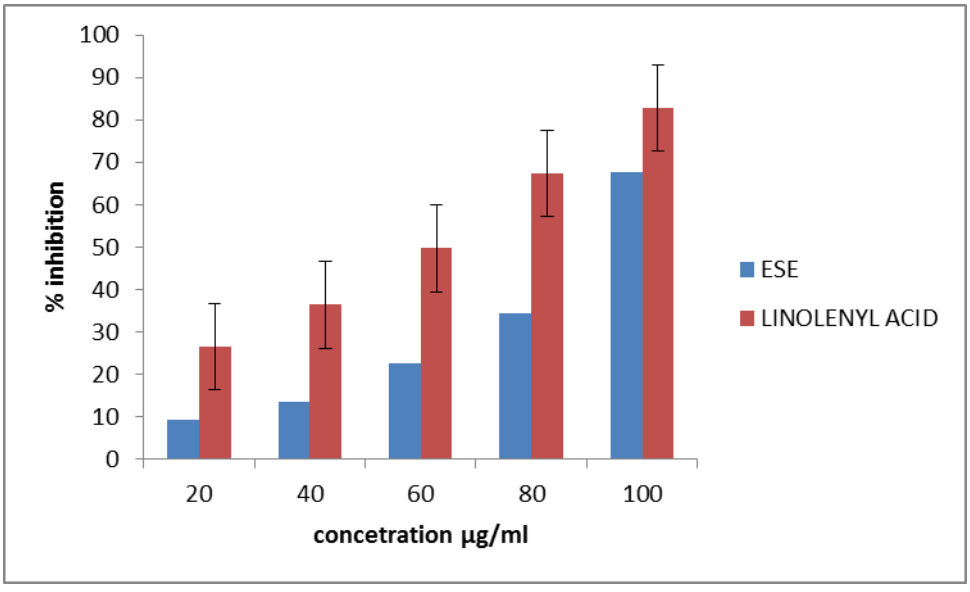

Figure 7: Chart of Antilopoxygenase activity of ethanoic extract of *S. aethiopicum* leaves and linolenyl acid (control).

MEMBRANE STABILIZATION

Table 4: Effect of ethanoic extract of *S. aethiopicum* leaves extract on % membrane stabilization of animal cells

CONC.µg/ml	%INHIBITION OF SAMPLE	%INHIBITION OF STANDARD
10	5.29^{a} ±0.53	61.60^{c} ±0.57
20	12.28^{a} ±0.16	63.51^{c} ±0.26
30	18.43^{a} ±0.24	69.34^{c} ±0.03
40	24.28^{a} ±0.16	72.38^{c} ±0.10
50	32.54^{a} ±0.35	82.52^{b} ±0.16
IC_{50}	77.38	-9.17

Values are represented as mean ± SEM (in triplicates).$p < 0.05$ considered as IC_{50} significant when compared to the Standard drug (acetyl salicylic acid).

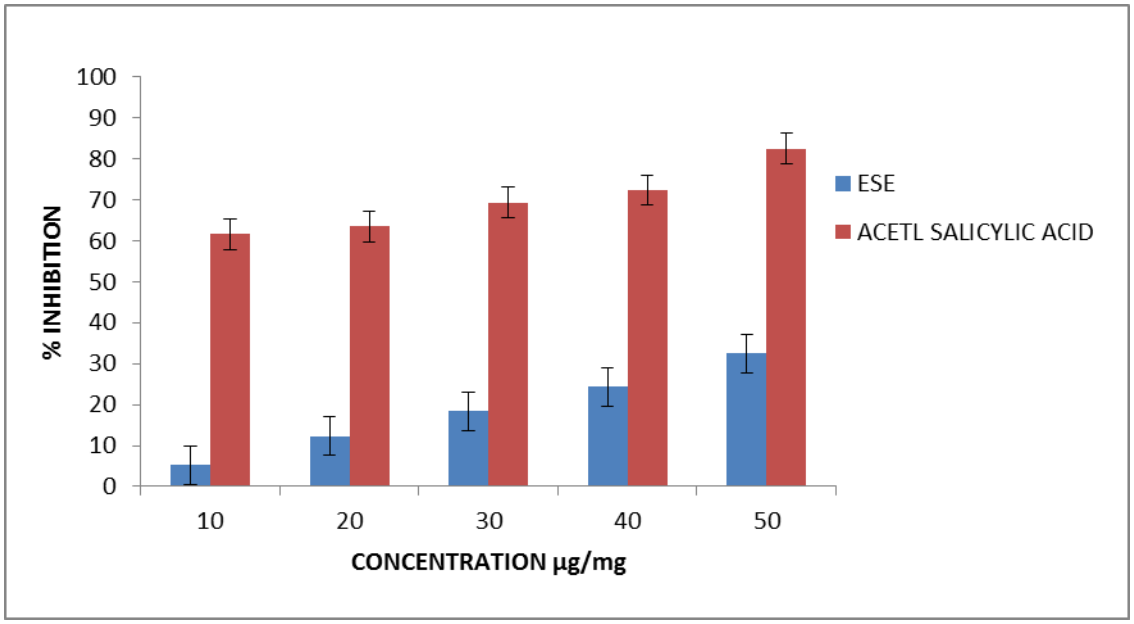

Figure 8: Chart of membrane stabilization capacity of ethanolic extract of *S. aethiopicum* leaves and acetyl salicylic acid (control).

HEAT STABILIZATION

Table 5: Effect of ethanolic extract of *S. aethiopicum* leaves extract on heat stabilization

CONC.µg/ml	%INHIBITION OF SAMPLE	%INHIBITIONOF STANDARD
10	6.69^{a}±0.19	10.46^{c}±0.07
20	13.37^{b}±0.39	19.94^{c} ±0.06
30	20.06^{b} ±0.58	65.71^{c} ±0.61
40	26.75^{b} ±0.78	114.58^{c} ±0.85
50	33.43^{b} ±0.97	153.51^{c} ±0.87
IC_{50}	26.50	23.91

Values are represented as mean ± SEM (in triplicates).$p < 0.05$ considered as IC_{50} significant when compared to the Standard drug.

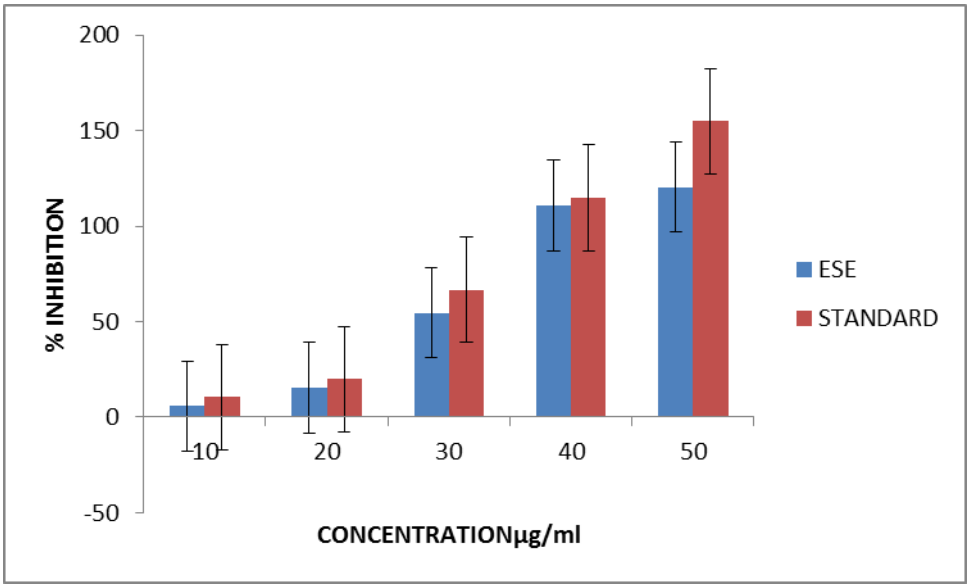

Figure 9: Chart of heat stabilization capacity of ethanolic extract of *S. aethiopicum* leaves and control.

INHIBITION OF PROTEIN DENATURATION

Table 6: Effect of ethanolic extract of *S. aethiopicum* leaves extract on inhibition of protein denaturation

CONC. µg/ml	% INHIBITION OF SAMPLE	% INHIBITION OF STANDARD
10	30.78[a]±0.60	41.35[a] ±0.17
20	41.83[a] ±0.05	50.40[a] ±0.05
30	45.58[a] ±0.08	52.10[a] ±0.02
40	50.32[a] ±0.18	59.23[a] ±0.12
50	61.25[a] ±0.16	65.50[a] ±0.06
IC50	127.06	23.52

Values are represented as mean ± SEM (in triplicates).p< 0.05 considered as IC50 significant when compared to the Standard drug (aspirin).

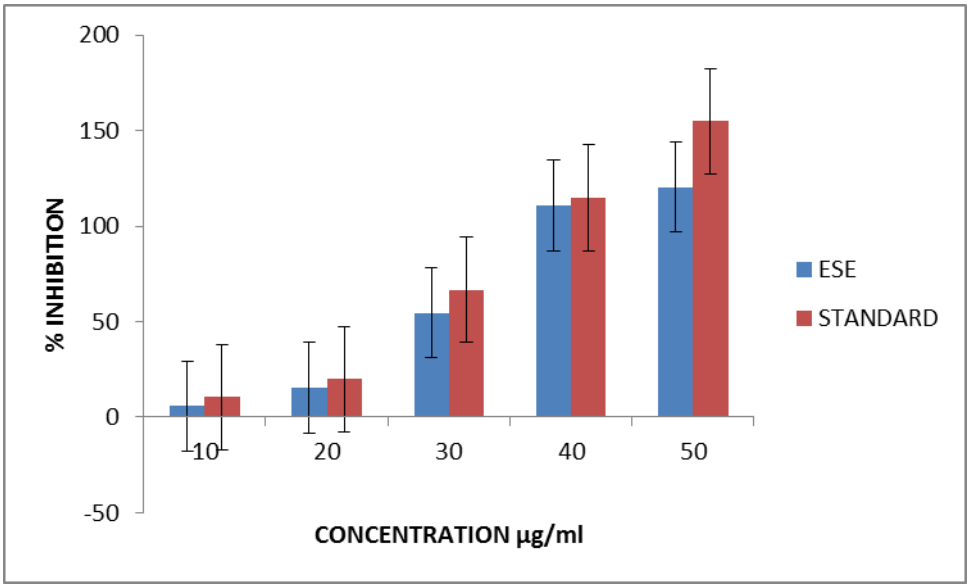

Figure 10: Chart of protein denaturation inhibition of ethanolic extract of *S. aethiopicum* leaves and aspirin (control).

ANTI LIPOXYGENASE ANOVA

				Sum of Squares	df	Mean Square	F	Sig.
A1	Between Groups	(Combined)		505.716	2	252.858	371.250	.000
		Linear Term	Contrast	291.976	1	291.976	428.684	.000
			Deviation	213.740	1	213.740	313.816	.000
	Within Groups			2.043	3	.681		
	Total			507.759	5			
A2	Between Groups	(Combined)		731.460	2	365.730	3800.136	.000
		Linear Term	Contrast	515.906	1	515.906	5360.545	.000
			Deviation	215.554	1	215.554	2239.727	.000
	Within Groups			.289	3	.096		
	Total			731.748	5			
A3	Between Groups	(Combined)		1156.723	2	578.362	4681.796	.000
		Linear Term	Contrast	738.483	1	738.483	5977.966	.000
			Deviation	418.240	1	418.240	3385.625	.000
	Within Groups			.371	3	.124		
	Total			1157.094	5			
A4	Between Groups	(Combined)		2032.647	2	1016.323	3482.324	.000
		Linear Term	Contrast	1085.990	1	1085.990	3721.030	.000
			Deviation	946.657	1	946.657	3243.618	.000
	Within Groups			.876	3	.292		
	Total			2033.522	5			
A5	Between Groups	(Combined)		2654.487	2	1327.244	3548.754	.000
		Linear Term	Contrast	229.107	1	229.107	612.582	.000
			Deviation	2425.380	1	2425.380	6484.925	.000

	Within Groups			1.122	3	.374	
	Total			2655.609	5		

				Sum of Squares	Df	Mean Square	F	Sig.
A1	Between Groups	(Combined)		3234.423	2	1617.211	1223.771	.000
		Linear Term	Contrast	3170.077	1	3170.077	2398.850	.000
			Deviation	64.346	1	64.346	48.692	.006
	Within Groups			3.964	3	1.321		
	Total			3238.387	5			
A2	Between Groups	(Combined)		2627.341	2	1313.670	7095.033	.000
		Linear Term	Contrast	2624.171	1	2624.171	14172.948	.000
			Deviation	3.169	1	3.169	17.118	.026
	Within Groups			.555	3	.185		
	Total			2627.896	5			
A3	Between Groups	(Combined)		2637.359	2	1318.680	10613.190	.000
		Linear Term	Contrast	2591.830	1	2591.830	20859.944	.000
			Deviation	45.529	1	45.529	366.435	.000
	Within Groups			.373	3	.124		
	Total			2637.732	5			
A4	Between Groups	(Combined)		2798.886	2	1399.443	9133.403	.000
		Linear Term	Contrast	2313.941	1	2313.941	15101.836	.000
			Deviation	484.945	1	484.945	3164.971	.000

	Within Groups			.460	3	.153		
	Total			2799.345	5			
A5	Between Groups	(Combined)		3346.519	2	1673.260	3962.852	.000
		Linear Term	Contrast	2497.844	1	2497.844	5915.751	.000
			Deviation	848.675	1	848.675	2009.952	.000
	Within Groups			1.267	3	.422		
	Total			3347.786	5			

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				Sum of Squares	df	Mean Square	F	Sig.
A1	Between Groups	(Combined)		1483.373	2	741.687	2.991	.193
		Linear Term	Contrast	1483.372	1	1483.372	5.982	.092
			Deviation	.001	1	.001	.000	.999
	Within Groups			743.980	3	247.993		
	Total			2227.353	5			
A2	Between Groups	(Combined)		2173.328	2	1086.664	3.006	.192
		Linear Term	Contrast	2173.327	1	2173.327	6.012	.092
			Deviation	.001	1	.001	.000	.999
	Within Groups			1084.544	3	361.515		
	Total			3257.872	5			
A3	Between Groups	(Combined)		1982.921	2	991.460	3.000	.192

		Linear Term	Contrast	1982.921	1	1982.921	6.000	.092
			Deviation	.000	1	.000	.000	1.000
	Within Groups			991.392	3	330.464		
	Total			2974.313	5			
A4	Between Groups	(Combined)		2195.120	2	1097.560	2.993	.193
		Linear Term	Contrast	2195.119	1	2195.119	5.986	.092
			Deviation	.001	1	.001	.000	.999
	Within Groups			1100.200	3	366.733		
	Total			3295.320	5			
A5	Between Groups	(Combined)		2316.822	2	1158.411	2.986	.193
		Linear Term	Contrast	2316.818	1	2316.818	5.972	.092
			Deviation	.004	1	.004	.000	.998
	Within Groups			1163.774	3	387.925		
	Total			3480.596	5			

				Sum of Squares	df	Mean Square	F	Sig.
A1	Between Groups	(Combined)		26.204	2	13.102	168.567	.001
		Linear Term	Contrast	23.482	1	23.482	302.108	.000
			Deviation	2.722	1	2.722	35.025	.010

	Within Groups			.233	3	.078		
	Total			26.437	5			
A2	Between Groups	(Combined)		41.778	2	20.889	57.761	.004
		Linear Term	Contrast	15.491	1	15.491	42.835	.007
			Deviation	26.287	1	26.287	72.687	.003
	Within Groups			1.085	3	.362		
	Total			42.863	5			
A3	Between Groups	(Combined)		2308.225	2	1154.112	1288.241	.000
		Linear Term	Contrast	135.747	1	135.747	151.523	.001
			Deviation	2172.478	1	2172.478	2424.959	.000
	Within Groups			2.688	3	.896		
	Total			2310.912	5			
A4	Between Groups	(Combined)		9926.803	2	4963.401	3477.359	.000
		Linear Term	Contrast	25.938	1	25.938	18.172	.024
			Deviation	9900.865	1	9900.865	6936.545	.000
	Within Groups			4.282	3	1.427		
	Total			9931.085	5			
A5	Between Groups	(Combined)		15719.082	2	7859.541	4686.295	.000
		Linear Term	Contrast	1165.548	1	1165.548	694.964	.000
			Deviation	14553.534	1	14553.534	8677.625	.000
	Within Groups			5.031	3	1.677		
	Total			15724.113	5			