PHYTOCHEMICAL SCREENING, QUANTIFICATION AND THE ANTI-INFLAMMATORY ACTIVITY OF THE METHANOLIC EXTRACT OF *Ocimum gratissimum* LEAVES

BY

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BPH/0010/71/DF

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APRIL 2012

DECLARATION

I do declare that this is my own work and it has never been presented to any university or any other institution for the award of a degree, diploma or any other qualification what so ever.

Where the work of other people has been included, acknowledgement to this has been made in accordance to the text and references.

This study has never been submitted before for either publication or award of any kind and also take note that any open criticism and changes to this work without the notification of the author is welcome.

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CERTIFICATION

I certify that this work is an original research carried out by **FELISTER RUGALABAMU** of the school of pharmacy, Kampala International University-Western campus, Ishaka -Bushenyi, Uganda.

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DEDICATION

I dedicate this research to all the scientists who have dedicated their precious time and resources in carrying out the research on medicinal plants. I support you firmly.

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I remain indebted to all that have contributed to my reaching to this end. Their hard work patience and tolerance.

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To my beloved classmates we have come along way.

ABSTRACT

Ocimum gratissimum belongs to the family lamiaceae. Folkore medicine claims its use in anti-inflammatory condition. The aim of this work is to extract, analyze and quantify the Phytoactive constituents and evaluate the anti – inflammatory effect. Extraction of the plant material was done in aqueous by maceration and then successively extracted in hexane, chloroform and methanol by soxhlet apparatus. Preliminary phytochemical scrfeening was carried out to determine the active constituents in different solvent extract. Phytochemical quantification was done in the crude powder to determine the amount of saponins, tannins, flavonoids and alkaloids. Anti-inflamatory activities of the methanolic extract of *Ocimum gratissimum* was evaluated in the egg albumin – induced rat paw oedema.

The phytochemical screening shows the presence of flavonoids in all solvent extracts and some selective secondary metabolites in other solvent extract. The crude powder show highest amount of saponins and moderate amount of tannins, alkaloids and flavonoids. The methanolic extract at 100 - 400 mg/kg demonstrated a dose dependent and significant inhibition ($p<0.05$) of oedema in the egg albumin – induced oedema in rats.

This research contributed to the knowledge of the phytochemical present and the anti-inflammatory activity of *Ocimum gratissimum* leaves.

. **Key words:** Anti-inflammatory, Ocimum gratissimum, flavonoids, egg albumin.

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CHAPTER ONE

1.0 INTRODUCTION

Medicinal plants have been of great important to the health of individual and communities. The medicinal value of these plants had some chemical active substances that produce a definite physiological action on the human body. Nature has served as a rich repository of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources, notably of plant origin (Cowan, 1999) .Herbal medicine, based on their traditional uses in the form of powders, liquids or mixtures, has been the basis of treatment for various ailments in India since ancient times. The use of herbs as complementary and alternative medicine has increased dramatically in the last 20–25 years (Rios and Recio, 2005). According to World Health Organization (WHO, 2005) traditional medicines was relied upon by 65–80% of the World's population for their primary health care needs. Moreover, emergence of multiple drug resistant strain of microorganisms due to indiscriminate use of antibiotics to treat infectious diseases has generated a renewed interest in herbal medicine (Chopral *et al*., 1997). The beneficial health effects of many plants, used for centuries as seasoning agents in food and beverages, have been claimed for preventing food deterioration and as antimicrobials against pathogenic microorganisms (Ahmad I, *et al*.,1998).

1.1 Background

O. gratissimum has been used extensively in the traditional system of medicine in many countries. In the Northeast of Brazil, it was used for medicinal, condiment and culinary purpose. The flowers and the leaves of this plant were rich in essential oils so it was used in preparation of teas and infusion (Rabelo *et al*., 2003).

Ocimum gratissimum was commonly known as fever leaf but was divided into different native names in different part of the country. In Nigeria Yoruba language, it was known as Efirin nla, Nchannwu in Ibo, Bunsuru daji in Hausa, Ireru in Ebira, Ebavbokho in Benin, ufuo-yibo in Urhobo and ntion in Efik (Iwu, 1993).

It was used in the treatment of epilepsy in the coastal area of Nigeria (Osito, 1992), High fever (Oliver, 1980), and Diarrhea (Oliver 1980; Sofgwora, 1993). The plant was also used to treat typhoid fever and diabetes (Adjanahoun *et al* .,1991; Igoli *et al*., 2002; Tor-Anyin *et al*., 2003). Today, basil was used mainly as a culinary herb. It's medicinal value was not as widely appreciated in Western World. In France it was used in perfumes and cosmetics (Ross, 2003).

Inflammation was a normal protective response to tissue injury that was caused by physical trauma, noxious chemicals or microbiological agents (Tripathi.K.D, 2004). The practice of traditional medicine, which was deep rooted in rural areas, continues unabated alongside conventional medicine because of ease of availability, inaccessibility of health centres and also due to social cultural factors. Western style healthcare provided by the government is often not readily available and many regions remain completely underserved. Consequently, most communities still use herbal remedies as readily and cheaply available alternatives. The flowers and the leaves of Ocimum gratissimum were rich in essential oils. It is used in preparation of teas and infusion. (Rabelo *et al., 2003)*

1.2 PROBLEM STATEMENT

In Africa and other countries, 80% of the population depends on traditional medicine for treating various infectious and chronic condition (WHO 2008).*Ocimum gratissimum* was among the

traditional medicine used for treating infectious diseases. There were more different species of ocimum which belong to one family (Lamiaceae), however there therapeutic effect may differ. The establishment of valuable constituents of *O. gratissimum* may give anti inflammatory activity.

1.3 PURPOSE OF THE STUDY

The purpose of the study is to quantify the Phytochemical constituent in OG leaves and to evaluate the anti-inflammatory effect of the Methanolic extracts.

1.4 SPECIFIC OBJECTIVE

- To extract *O. gratissimum* leaves in different solvent.
- To test for the presence of different phytochemicals in the leaves of *O. gratissimum*.
- To carry out Quantification test to find out the quantity of the phytochemicals in. *O.gratissimum.*
- To evaluate the anti-inflammatory effect of *O. gratissimum* leaves extract.

1.5 JUSTIFICATION

Medicinal plants have been the subject of intense research due to their potential as source of commercial drugs or as lead compounds in drug development and isolation of *O. gratissimum*. Various aspects of the phytochemistry and phytobiology of natural products including their potential as their therapeutic agents have been reviewed. This study will provide scientific evidence to back the traditional use of this plant as a consequence of their chemical constituents.


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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Plant

2.1.1 *Ocimum gratissimum*

O. gratissimum is an aromatic shrub from Asia and Africa, a herbaceous plant which belongs to the Labiatae family. The plant is indigenous to tropical areas especially India and it is also in West and East Africa. The plant was found throughout the tropics and subtropics and its greatest variability occurs in tropical Africa and India (Aruna, 1990). In South East Asia, it was cultivated as a home garden crop but it was grown on a commercial scale in Vietnam.

In Nigeria, it is found in the Savannah and coastal areas. It is cultivated in Ceylon, South Sea Islands, and also within Nepal, Bengal,Chittagong and Deccan(Nadkarni KMand Indian Materia 1999). It is known by various names in different parts of the world. In India it is known by its several vernacular names, the most commonly used ones being Vriddhutulsi (Sanskrit), Ram tulsi (Hindi), Nimma tulasi (Kannada). In the southern part of Nigeria, the plant is called "effinrin-nla" by the Yoruba speaking tribe. It is called "Ahuji" by the Igbos, while in the Northern part of Nigeria, the Hausas call it "Daidoya" (Effraim, 2003). In Uganda, it is known as omujaja. There about 60 or more species of Ocimum and numerous varieties belonging to the family lamiaceae e.g *Ocimum gratissimum, Ocimum basilicum, Ocimum americanum, Ocimum sanctum, Ocimum suave* and others (Martin and Salguerio, 1999; Mandal and Pattnaik, 2000).

Taxonomically classified as:

Kingdom: Plantae

Unranked : Angiospermae

Unranked: Eudicots

Unranked: Asterids

Order : Lamiales

Family:Lamiaceae

Genus: Ocimum

Species: O. gratissimum

Binomial name: Ocimum gratissimum

O. gratissimum is a shrub up to 1.9m in height with stems that are branched. The leaves measure up to 10 x 5 cm, and are ovate to ovate-lanceolate, sub-acuminate to acuminate at apex, cuneate and decurrent at base with a coarsely crenate, serrate margin, pubscent and dotted on both the sides. The leaves show the presence of covering and glandular trichomes. Stomata are rare or absent on the upper surface while they are present on the lower surface. Ordinary trichomes are few, while the long ones up to 6-celled are present on the margins mostly; the short ones which are 2 celled, are mostly found on the lamina. Petioles are up to 6 cm long and racemes up to 18 cm long. The peduncles are densely pubescent. Calyx is upto 5mm long, campanulate and 5-7 mm long, greenish white to greenish-yellow in colour. Nutlets are mucilaginous when they are wet (Bhat, 2003). On the 2 surfaces of the leaf epidermal cells are typical of irregular contours, and diacytic stomata, secretory glands most abundant in the leaf, are also present in simple pluricellular hairs on the leaf veins. The cross section shows the epidermis monoestratificada (beam), a layer of parenchyma fenced in sub-epidermal position, followed by parenchymal pond, and finally the epidermis monoestratificada lower (García *et al.*, 1998).

2.1.2 Traditional use

O. gratissimum has been used extensively in the traditional system of medicine in many countries. In the North east of Brazil, it is used for medicinal, condiment and culinary purpose.

The flowers and the leaves of this plant are rich in essential oils so it is used in preparation of teas and infusion (Rabelo, 2003).

It was used for a variety of reasons. *O. gratissimum* was a vegetable plant of wide nutritional and medicinal applications in Nigeria and in some other parts of the world. In culinary, it was used in salads, soups, pastas, vinegars and jellies in many parts of the world. The Thai people are popularly known to use it in food flavouring.

In the coastal areas of Nigeria, the plant is use in the treatment of epilepsy, high fever and diarrhea. In the Savannah areas decoctions of the leaves are used to treat mental illness. (Akinmoladun, *et al.* 2007). *O. gratissimum* is used by the Ibos of Southeastern Nigeria in the management of the baby's cord, to keep the wound surfaces sterile, it is also used in the treatment of fungal infections, fever, cold and catarrh. (Ijeh *et al.*, 2005) Brazilian tropical forest inhabitants use a decoction of *O.gratissimum* roots as a sedative for children. (Cristiana M *et al.*, 2006). People of Kenyan Sub Saharan African communities' use this plant for various purposes ike viz., the leaves are rubbed between the palms and sniffed as a treatment for blocked nostrils, hey are also used for abdominal pains, sore eyes, ear infections, coughs, barrenness, fever, convulsions, and tooth gargle, regulation of menstruation and as a cure for prolapse of the ectum. (Matasyoh et al., 2007). In India, the whole plant has been used for the treatment of unstroke, headache, and influenza, as a diaphoretic, antipyretic and for its anti-inflammatory ctivity. (Prajapati *et al.*, 2003, Oliver B, 1980, oTa^nia Ueda *et al., 2006).* The tribal's of Nigeria use the leaf extract in treatment of diarrhea, while the cold leaf infusions are used for the elief of stomach upset and hemorrhoids. (Kabir *et al.*, 2005). The plant is commonly used in olk medicine to treat different diseases such as upper respiratory tract infections, diarrhea, and

headache, diseases of the eye, skin diseases, pneumonia, cough, fever and conjunctivitis (Adebolu and Salau, 2005). Formulations of the leaf essential oil of *O. gratissimum* (Ocimum oil) have been incorporated in a variety of bases as topical antiseptics and for use in the treatment of minor wounds, boils and pimples. *O. gratissimum* and *Xylopia aethiopica* in combination are used in the preparation of potions and teas for women during peuperium. (Ijeh II *et al.*, 2005). In traditional medicine, the leaves have been used as a general tonic and anti-diarrhea agent and for the treatment of conjunctivitis by instilling directly into the eyes; the leaf oil when mixed with alcohol is applied as a lotion for skin infections, and taken internally for bronchitis. The dried leaves were snuffed to alleviate headaches and fever among other uses (Iwu , 1993).

A testimonial of the uses of Ocimum gratissimum was cited below

"Having battled stomach problems for long, Georgina was advised to abandon caffeinated drinks for herbal tea. A naturopathic (natural medicine) doctor recommended that she takes ocimum (popularly known as omujaja). After a week of routine drinking of the herb, her health was okay. Dr. Deepak Patel, a herbalist in Kampala, advises that one dries the herb and stores it in its natural state. "Preservatives, when added to the herb, destroy its natural contents and introduce toxins. Keeps blood pressure in check Relieves or prevents spasms (stiffness of muscles).Eases tension, Boosts appetite, Cleanses the blood, Lowers blood sugar and keeps diabetes in check, Lowers stress and prevents insomnia (sleep disorder).It is an anti inflammatory remedy that keeps disease at bay, curbs cholesterol. It is a natural herb product that ncreases the body's resistance to stress, trauma, anxiety and fatigue. Moses Ssenoga, a naturopathic (natural medicine) doctor at Mulago Sanitarium in Mbuya, recommends the herb or the treatment of flu and asthma. Ssenoga says fresh leaves of the herb when crushed or squeezed provide relief from an insect bite or sting. A glass of omujaja helps relieve stress,

tension and calms high blood pressure. Ssenoga says fresh leaves of the herb when crushed or squeezed provide relief from an insect bite or sting. Ssenoga's recipe, the infusion of O.gratissimum leaves is used as pulmonary antisepticum, antitussivum and antispamodicum. (Ngassoum MB, 2003)".

2.1.3 Constituents

The flowers and the leaves of this plant are rich in essential oils so it is used in preparation of teas and infusion. The main active contituents of O.gratissimum is essential oils. The essential oil of *Ocimum gratissimum* contains eugenol and shows some evidence of antibacterial activity.(silva *et al*., 2010).The Major constituents of essential oils are hydrocarbons, esters, terpenes, lactones, phenols, aldehydes, acids, alcohols, and ketones. Among these, the oxygenated compounds (alcohols, esters, aldehydes, ketones, lactones, phenols) are the principal odor source. They are more stable against oxidizing and resinifying influences than other constituents. On the other hand, unsaturated constituents like monoterpenes and sesquiterpenes have the tendency to oxidize or resinify in the presence of air and light. The knowledge of individual constituents and their physical characteristics, such as boiling point, thermal stability and vapor-pressure-temperature relationship, is of paramount importance in technology development of oxygenated compounds.

Variation of chemical composition of the essential oil of *O. gratissimum* eugenol type was studied for 11 h during the daytime. Microwave oven technique was used for the serial extraction and the obtained oils were analysed by GC/MS. A considerable variation was observed in eugenol yield 98% at 12.00 a.m. to 11% at 05.00 p.m. These results show the influence of the

solar light on eugenol production and can be useful to indicate the optimal time for collection of the plant (Vasconcelos Silva MG *et al.*, 1999)

2.1.4 Pharmacological Studies

The essential oil of *Ocimum gratissimum* contains eugenol and shows some evidence of antibacterial activity. (Silva *et al.*, 2010). Although, conventional antibiotics have been very useful in orthodox medicine, it has been argued by many that its concomitant use with herbal extracts was not desirable as one normally antagonizes the activity of the other. The ethanolic extract of the leaves of *Ocimium gratisimum* L. (Lamiaceae), used in traditional medicine for the treatment of several ailments such as urinary tract, wound, skin and gastrointestinal infections (Nweze and Eze, 2004). A test on guinea pigs ileum found evidence that the essential oil relaxes the muscles of the small intestine, consistent with the traditional use of the plant to treat gastrointestinal disorders (Socorro *et al.*, 2002). A study on rats also found evidence that a leaf extract of the plant prevented diarrhea (Veronica *et al.*, 1999). The ethanolic extract of *Ocimum gratissimum* produced a significant and sustained increase in the sexual activity of normal male mice, without any adverse effects. "Thus, the resultant aphrodisiac activity of the extract lends support to the claims for its traditional usage in sexual disorders (Pande and Pathak, 2009). *Ocimum gratissimum* has anti-fertility effects in male mice. (Obianime et al., 2010). *Ocimum gratissimum* ethanolic extracts showed a hepatoprotective effect. (Surana, 2010). Leaf extract of *O. gratissimum* showed antidiabetic properties in streptozocin-induced in diabetic rats. (Muhamed *et al.*, 2007).

A polyherbal preparation of a water extract obtained from the leaves of *Gongronema latifolia*, *Vernonia amygdalina* and *Ocimum gratissimum* showed analgesic activity. (Iroanya *et al*., 2009). *O. gratissimum* has mosquito-repellent and mosquitocidal potential (Oparaocha *et al*., 2010). *O. gratissimum* is associated with chemo-preventive, anti-carcinogenic, free radical scavenging, radio protective and numerous others pharmacological use. (Gupta *et al*., 2002). *O. gratissimum* is used to treat different diseases, e.g., upper respiratory tract infections, diarrhea, headache, ophthalmic, skin diseases, pneumonia, and also as a treatment for cough, fever and conjunctivitis. (Ilori *et al*., 1996). Earlier reports have shown the smooth muscle contracting lipid soluble principles, and antimutagenic activity in organic solvent extracts of *O. gratissimum* leaves.(Onajobi, 1986) This medicinal plant has also potential role as antibacterial, antifungal, antimicrobial, anthelmintic, and in vitro antidermatophytic agent(T Nakamura CV *et al*., 1999). The aqueous leaf extract and seed oil showed anti-proliferative and chemo-preventive activity on HeLa cells (Prakash J.*et al*., 1999). Nangia-Makker *et al*.,2007 reported that, aqueous extract of *O. gratissimum* leaves inhibits tumor growth and angiogenesis by affecting tumor cell proliferation, migration, morphogenesis, stromal apoptosis and induction of inducible cyclooxygenase (COX-2). (Nangia-Makker P, *et al* .,2007).

2.1.5 Reported Anti-inflammatory activity

The inhibitory effect produced by chemical constituents of essential oils of three plants used in traditional medicine as anti-inflammatory and analgesic drug, *in vitro*, on soybean lipoxygenase L-1 and cyclooxygenase function of prostaglandin H synthase (PGHS), the two enzymes involved in the production of mediators of inflammation. The essential oils were extracted from plants *O.gratissimum* along with two more plants. Among the three essential oils,

O.gratissimum inhibited the two enzymes, cyclooxygenase function of PGHS and lipoxygenase L-1, with an IC50= 125 g/ml and 144 g/ml (Sahouo *et al.*, 2003).

2.2 GENERAL METHODS OF EXTRACTION O MEDICINAL PLANT

2.2.1 Maceration

In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing (Sukhdev *et al.*, 2008)

2.2.2 Infusion

Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs. (Sukhdev *et al.*, 2008)

2.2.3 Digestion

This is a form of maceration in which gentle heat is used during the process of extraction. It is used when moderately elevated temperature is not objectionable. The solvent efficiency of the menstruum is thereby increased. (Sukhdev *et al.*, 2008)

2.2.4 Decoction

In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water-soluble, heat-stable constituents. This process is typically used in preparation of Ayurvedic extracts called "quath" or "kawath". The starting ratio of crude drug to water is fixed, e.g. 1:4 or 1:16; the

volume is then brought down to one-fourth its original volume by boiling during the extraction procedure. Then, the concentrated extracts fi ltered and used as such or processed further. (Sukhdev *et al.*, 2008)

2.2.5 Percolation

This is the procedure used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used (Figure
1). The solid ingredients are moistened with an appropriate amount of the specified menstruum and allowed to stand for approximately 4 h in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstruum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h. The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional menstruum is added as required, until the percolate measures about three-quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to the percolate. Sufficient menstruum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting. (Sukhdev *et al.*, 2008)

2.2.6 Hot Continuous Extraction (Soxhlet)

In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus. The extracting solvent in flasks A is heated and its vapors condense in condenser D. The condensed extractant drips into

the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into fl ask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This affects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale. (Sukhdev *et a.,l* 2008)

2.2.7 Aqueous Alcoholic Extraction by Fermentation

Some medicinal preparations of Ayurveda (like *asava* and *arista*) adopt the technique of fermentation for extracting the active principles. The extraction procedure involves soaking the crude drug, in the form of either a powder or a decoction (*kasaya*), for a specified period of time, during which it undergoes fermentation and generates alcohol in situ; this facilitates the extraction of the active constituents contained in the plant material. The alcohol thus generated also serves as a preservative. If the fermentation is to be carried out in an earthen vessel, it should not be new: water should first be boiled in the vessel. In large-scale manufacture, wooden vats, porcelain jars or metal vessels are used in place of earthen vessels. Some examples of such preparations are *karpurasava, kanakasava, dasmularista*. In Ayurveda, this method is not yet standardized but, with the extraordinarily high degree of advancement in fermentation technology, it should not be difficult to standardize this technique of extraction for the production of herbal drug extracts. (Sukhdev *et al.*, 2008)

2.2.8 Counter-current Extraction

In counter-current extraction (CCE), wet raw material is pulverized using toothed disc disintegrators to produce fine slurry. In this process, the material to be extracted is moved in one direction (generally in the form of fine slurry) within a cylindrical extractor where it comes in contact with extraction solvent. The further the starting material moves, the more concentrated the extract becomes. Complete extraction is thus possible when the quantities of solvent and material and their flow rates are optimized. The process is highly efficient, requiring little time and posing no risk from high temperature. Finally, sufficiently concentrated extract comes out at one end of the extractor while the marc (practically free of visible solvent) falls out from the other end. (Sukhdev *et a.,l* 2008)

2.2.9 Ultrasound Extraction (Sonication)

The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitations. Although the process is useful in some cases, like extraction of rauwolfia root, its large-scale application is limited due to the higher costs. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules. (Sukhdev *et al.*, 2008)

2.2.10 Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is an alternative sample preparation method with general goals of reduced use of organic solvents and increased sample throughput. The factors to consider include temperature, pressure, sample volume, analyte collection, modifier (co solvent)

addition, flow and pressure control, and restrictors. Generally, cylindrical extraction vessels are used for SFE and their performance is good beyond any doubt.

The collection of the extracted analyte following SFE is another important step: significant analyte loss can occur during this step, leading the analyst to believe that the actual efficiency was poor. (Sukhdev *et al.*, 2008)

There are many advantages to the use of CO2 as the extracting fluid. In addition to its favorable physical properties, carbon dioxide is inexpensive, safe and abundant. But while carbon dioxide is the preferred fluid for SFE, it possesses several polarity limitations. Solvent polarity is important when extracting polar solutes and when strong analyte-matrix interactions are present. Organic solvents are frequently added to the carbon dioxide extracting fluid to alleviate the polarity limitations. Of late, instead of carbon dioxide, argon is being used because it is inexpensive and more inert. The component recovery rates generally increase with increasing pressure or temperature: the highest recovery rates in case of argon are obtained at 500 atm and 150°c\ (Sukhdev *et al.*, 2008)

The extraction procedure possesses distinct advantages:

i) The extraction of constituents at low temperature, which strictly avoids damage from heat and some organic solvents.

ii) No solvent residues.

iii) Environmentally friendly extraction procedure

The largest area of growth in the development of SFE has been the rapid expansion of its applications. SFE finds extensive application in the extraction of pesticides, environmental samples, foods and fragrances, essential oils, polymers and natural products. The major deterrent

in the commercial application of the extraction process is its prohibitive capital investment. (Sukhdev *et al.*, 2008)

2.2.11 Solid Phase Micro-Extraction

Solid phase micro-extraction (SPME) was developed in the 1990s by Professor J. Pawliszyn to provide a quick and solventless technique for the isolation of analytes from a sample matrix. The traditional methods by which the analytes of interest were isolated are typically timeand labor-intensive and involve multistep procedures, which could reduce sensitivity. Also, the use of solvents can be hazardous to the operators' health and can damage the environment.SPME was developed from the technique of solid phase extraction, but the sorbing material is permanently attached to the fiber, allowing reuse of the extracting phase. SPME uses a small volume of sorbent, typically dispersed on the surface of small fi bers, to isolate and concentrate analytes from the sample matrix. After contact with the sample, analytes are absorbed or adsorbed by the fi ber phase (depending on the nature of the coating). After the extraction step, the fibers are transferred, with a syringe like handling device, to the analytical instrument, for separation and quantify cation of the analytes. This technique integrates sampling, extraction and sample introduction, and is a simple way of performing on-site monitoring. Applications of this technique include environmental monitoring, fragrance drug analysis, and in-laboratory and on-site analyses. (Sukhdev *et al* .,2008)

2.3 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The term liquid chromatography (LC) refers to a range of chromatographic systems, indicating liquid-solid, liquid-liquid, and ion-exchange and size exclusion chromatography. Glass column

chromatography is an example of classic liquid column chromatography in which the mobile phase percolates under gravity through a glass column filled with a finely divided stationary phase. Liquid chromatography has overtaken gas chromatography, as high performance liquid chromatography (HPLC) systems now provide features such as:

i) High resolving power

ii) Fast separation

iii) Continuous monitoring of column effluent

iv) Qualitative and quantitative measurements and isolation

v) Automation of analytical procedures and data handling. (Sukhdev *et al.*, 2008)

There has been tremendous growth in this technique since 1964 when the first HPLC instrument was constructed by Csaba Horvath at Yale University. For the isolation of compounds, preparative mode HPLC (prep-HPLC) can be used in pharmaceutical development for trouble-shooting purposes or as part of a systematic scale-up process. The importance of prep-HPLC in pharmaceutical production as a purification tool has been increasing. Chromatographic separation can remove impurities of different polarity and can reduce the content of an enantiomer in a racemic mixture.In both of these instances, crystallization may be used to prepare the pure product. Bench to pilot scale production of natural products needs some form of automation: thus, developing well-automated preparative chromatographic methods is a necessary but demanding task. Innovations in micro-analytical to preparative HPLC played an important role in the progress of natural product chemistry. HPLC is used routinely in phytochemistry to pilot the preparative-scale isolation of natural products and to control the final purity of the isolated compounds. The development of hyphenated techniques related to this

efficient separation technique in the past 20 years has provided powerful new tools such as LC/UV-photodiode array detection, LC/mass spectrometry (LC/MS) and LC/NMR. The combination of high separation efficiency of HPLC with these different detectors has made possible the acquisition of data on an LC peak of interest within a complex mixture. (Sukhdev *et al.*, 2008)

2.3.1 Theoretical Aspects of HPLC

Separation of chemical compounds is carried out by passing the mobile phase, containing the mixture of the components, through the stationary phase, which consists of a column packed with solid particles.

The cause for retention is physical and chemical forces acting between the solute and the two phases, on the chromatographic column. The reason for retention is the difference in the magnitude of forces; this results in the resolution and hence separation of the individual solutes. The separation of compounds occurs by distribution of solutes between the two phases. (Sukhdev *et a.,l* 2008)

2.4 MEDIATORS OF INFLAMMATION

Inflammation is a response of a tissue to injury, often injury caused by invading pathogens. It is characterized by

- increased blood flow to the tissue causing
- increased temperature,
- redness,
- swelling, and

- Pain.

2.4.1 Plasma cascade systems

- The complement system, when activated, creates a cascade of chemical reactions that promotes opsonization, chemotaxis, and agglutination, and produces the MAC.
- The kinin system generates proteins capable of sustaining vasodilation and other physical inflammatory effects.
- The coagulation system or *clotting cascade* which forms a protective protein mesh over sites of injury.
- The fibrinolysis system, which acts in opposition to the *coagulation system*, to counterbalance clotting and generate several other inflammatory mediators.

2.4.2 Plasma derived mediators

Name	Produced by	Description
Bradykinin	*Kinin system*	A vasoactive protein which is able to induce vasodilation, increase vascular permeability, cause smooth muscle contraction, and induce pain.
C3	*Complement system*	Cleaves to produce *C3a* and *C3b*. C3a stimulates histamine release by mast cells, thereby producing vasodilation. C3b is able to bind to bacterial cell walls and act as an opsonin, which marks the invader as a target for phagocytosis.
C5a	*Complement*	Stimulates histamine release by mast cells, thereby producing

	system	vasodilation. It is also able to act as a chemoattractant to direct cells via chemo taxis to the site of inflammation.
Factor XII (*Hageman Factor*)	*Liver*	A protein which circulates inactively, until activated by collagen, platelets, or exposed basement membranes via conformational change. When activated, it in turn is able to activate three plasma systems involved in inflammation: the kinin system, fibrinolysis system, and coagulation system.
Membrane attack complex	*Complement system*	A complex of the complement proteins C5b, C6, C7, C8, and multiple units of C9. The combination and activation of this range of complement proteins forms the *membrane attack complex*, which is able to insert into bacterial cell walls and causes cell lysis with ensuing death.
Plasmin	*Fibrinolysis system*	Able to break down fibrin clots, cleave complement protein C3, and activate Factor XII.
Thrombin	*Coagulation system*	Cleaves the soluble plasma protein fibrinogen to produce insoluble fibrin, which aggregates to form a blood clot. Thrombin can also bind to cells via the PAR1 receptor to trigger several other inflammatory responses, such as production of chemokines and nitric oxide. Abbas A.B.; Lichtman A.H. (2009)

Cell derived mediators

Name	Type	Source	Description
Lysosome granules	*Enzymes*	Granulocytes	These cells contain a large variety of enzymes which perform a number of functions. Granules can be classified as either *specific* or *azurophilic* depending upon the contents, and are able to break down a number of substances, some of which may be plasma-derived proteins which allow these enzymes to act as inflammatory mediators.
Histamine	*Vasoactive amine*	Mast cells, basophils, platelets	Stored in preformed granules, histamine is released in response to a number of stimuli. It causes arteriole dilation and increased venous permeability.
IFN-γ	*Cytokine*	T-cells, NK cells	Antiviral, immunoregulatory, and anti-tumour properties. This interferon was originally called macrophage-activating factor, and is especially important in the maintenance of chronic inflammation.
IL-8	*Chemokine*	Primarily macrophages	Activation and chemo attraction of neutrophil, with a weak effect on monocytes and eosinophils.

Leukotriene B4	*Eicosanoid*	Leukocytes	Able to mediate leukocyte adhesion and activation, allowing them to bind to the endothelium and migrate across it. In neutrophil, it is also a potent chemo attractant, and is able to induce the formation of reactive oxygen species and the release of lysosome enzymes by these cells.
Nitric oxide	*Soluble gas*	Macrophages, endothelial cells, some neurons	Potent vasodilator, relaxes smooth muscle, reduces platelet aggregation, aids in leukocyte recruitment, direct antimicrobial activity in high concentrations.
Prostaglandins	*Eicosanoid*	Mast cells	A group of lipids which can cause vasodilation, fever, and pain.
TNF-α and IL-1	*Cytokines*	Primarily macrophages	Both affect a wide variety of cells to induce many similar inflammatory reactions: fever, production of cytokines, endothelial gene regulation, chemo taxis, leukocyte adherence, activation of fibroblasts. Responsible for the systemic effects of inflammation, such as loss of appetite and increased heart rate. (Abbas A.B.; Lichtman A.H. (2009)

CHAPTER THREE

3.0 METHODOLOGY

3.1 Plants Material

Dried leaves of *Ocimum gratissimum* were ground into powder and store in air tight container prior to extraction.

3.2 Chemicals and Reagents

Laboratory grade solvents: n- hexane, Chloroform, Ethanol, Methanol, Petroleum Ether, Diethyl ether, Ethyl acetate, Ethanol, and ether.

3.3 PREPARATION OF THE EXTRACT

3.3.1 Maceration

The dry material was crushed into powder, then, 100g of the powder was weighed into an empty beaker, and then soaked in 500mls of distilled water and then shaken for 48 hours. The extract was sieved and the juice was filtered using a clean white cotton cloth. The filtrate was put into cleaned and dried beakers and were placed in the water bath using retort stand and evaporated at 30°C and then dried in the oven at 40c. The gummy extract was put into already weighed small plastic dish and weighed in an electronic sensitive weighing balance. The percentage yield was calculated and then stored in the refrigerator for further studies.

3.3.2 Soxhlet Extraction

Successive sohxlet extraction using laboratory grade N-hexane, Chloroform and Methanol was carried out in a sohxlet apparatus. 25g of powder was weighed into an empty beaker using a beam balance and then fixed into a soxhlet (thimble) then hexane 125 ml was poured into the percolator, 100mls was put in the bottom flask and 25mls was to soak the extract. This procedure

was repeated four times with Hexane, chloroform and methanol successively in order to extract 100mg of dried powder. Each solvent extract was pooled together, concentrated over the water bath at 40^0C and dried in the oven. % yield of extraction was calculated as follows;

$$\frac{\text{Weight of empty beaker + extract} - \text{weight of empty beaker}}{\text{Weight of dried powder}} \times 100$$

3.4 PHYTOCHEMICAL SCREENING

The standard described by Trease and Evans (1996) was applied in this phytochemical screening of the individual constituents of the extract. The presence of the compounds to be tested was rated as positive (+) or negative (-). These compounds included: tannins, phlobatannins, saponins, terpenoids, flavonoids, alkaloids and reducing sugar. Some phytochemical tests were performed according to the literature by Nayak and pereira for detecting the presence of different chemical constituents was used as follows:-

3.4.1 Screening for saponins:

300mg of extract was boiled with 5ml water for two minutes. The mixture was cooled and mixed vigorously and left for three minutes. The formation of frothing indicated the presence of saponins.

3.4.2 Screening for tannins:

300mg of the extract, sodium chloride is added to make to 2% strength. Then it is filtered and mixed with 1% gelatin solution. Precipitation indicated the presence of tannins.

3.4.3 Screening for Terpenoids:

300mg of extract was mixed with 5mls chloroform and warmed for 30 minutes. The chloroform solution was then treated with a small volume of concentrated sulphuric acid and mixed properly. The appearance of red color indicated the presence of terpenoids.

3.4.4 Screening for alkaloids:

300mg of extract was digested with 2M HCL. Acidic filtrate was mixed with amyl alcohol at room temperature, and examined the alcoholic layer for pink colour which indicated the presence of alkaloids.

3.4.5 Screening for flavonoids:

The presence of flavonoids was determined using 1% aluminium chroride solution in methanol, concentrated HCL, magnesium turns and potassium hydroxide solution, OR Too small quantity of the methanol extract dissolved in ethanol and hydrolysed with 10% sulphuric acid and cooled. Extract with diethyl ether and divided into three portion in three separate test tubes, Add 1ml diluted sodium carbonate,1ml of 0.1m sodium hydroxide and 1ml of diluted ammonia solution to the three test tubes .In each test tube, development of yellow color demonstrate the presence of flavonoids.

3.4.6 Screening for steroids:

300mg of the extract was mixed with 2ml sulphuric acid then 2mls acetic anhydride was added to mixture color change from violet to blue or green was observed.

3.4.7 Screening for Reducing sugar

5-10 of iodine was added to 5ml of the test solution and the mixture was boiled in a water bath over a Bunsen burner.A dark blue color developed indicating presence of reducing sugar.

3.4.8 Screening for phlobatannins

300mg of the extract was dissolved in distilled water and filtered.The fitrate was boiled with 2% Hcl solution. Red precipitate was observed.

3.5 PYTOCHEMICAL QUANTIFICATION

3.5.1 Quantification of total tannins

10g of the sample powder was weighed in a 200ml beaker using a weighing balance and there after macerated with 100ml of distilled water, rotated on a rotator for 20hours and left to stand for 24 hours.

The maceration was filtered using small size Whatman No.1 filter paper and the filtrate dried in a hot air oven at 70^0C.The dried extract was weighed and recorded.

The residue was dissolved with 5mls of distilled water and then extracted repeatedly with 20ml of petroleum ether while shaking at 15 minutes intervals for 1 hour. This purification process was repeated and there after tannins precipitated with 7% potassium dichromate.

The precipitate was washed with 10 ml of petroleum ether and then dried in the hot air oven at a temperature of 80^0C. The solid was weighed to determine the weight of total tannins.

3.5.2 Quantification of total alkaloids

10g of the sample powder was weighed using a sensitive weighing balance and macerated in 100ml of distilled water with continuous shaking on a laboratory rotator at a speed of 200 rates per minute for 16 hours and then left to stand for 24 hours.

The maceration was filtered and then washed through the filter paper with 2 successive portions of 100ml of distilled water. The filtrate and the filtered maceration washings was bulked together and the volume noted and then transferred to a beaker of known weight, evaporated to dryness at a temperature of 80^0C.

The weight of the dry extract was determined using a sensitive balance and recorded. The extract was re-dissolved with distilled water to one tenth of the initial volume. This was acidified with excess 2% aqueous acetic acid and allowed to stand for 2 hours.

The acidified mixture was concentrated to half its original volume with a water bath at a temperature of 75^0C, filtered and the filtrate basified with concentrated ammonia and centrifuged using a centrifuging machine model 800 at 1000 rates per minute for 30 minutes.

The obtained solid was dissolved in 10ml of 2% aqueous acetic acid solution, basified with minimum concentrated ammonia until a precipitate was seen. The mixture was extracted with 2 successive 20ml portions of chloroform with vigorous shaking once every 15 minutes for 1 hour and the extraction repeated.

The aqueous portions was collected in a beaker of known weight and evaporated to dryness in a hot air oven at 75^0C. The weight of the gummy extract was determined using a sensitive balance.

3.5.3 Quantification of total saponin

10g of the sample powder was weighed in a 200ml beaker and macerated with 100ml of distilled water and rotated on a laboratory rotator for 15 hours and was left to stand for 24 hours. The maceration was filtered using Whatman No.1 small size filter paper and the filtrate reduced to 40ml over a water bath at about 90^0C.

The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously every 15 minute for 1 hour. The aqueous layer was recovered while the ether layer discarded.

The purification process was repeated and then, 60ml of n-butanol added to extract repeatedly. The combined n-butanol extract was washed twice with 10ml of 5% aqueous NaCl and the remaining solution heated in the hot air oven at 70^0C.

The residue was weighed to determine the quantity of saponins present.

3.5.4 Quantification of total flavonoids (Harbone)

10g of the sample powder was weighed using a sensitive balance and 100ml of distilled water shall be added. The mixture will be shaken continuously for 12 hours on a laboratory rotator at a speed of 100 rates per minute and then left to stand for 24 hours.

The mixture was filtered using Whatman No.1 small size filter paper and then dried in the hot air oven for 20 hours. The weight of the extract was determined.

The extract was re-extracted twice with 20ml portions of ethyl acetate and thereafter extracted with 10ml of amyl alcohol. The ethyl acetate was dried in a hot air oven at 70^0C and weighed to determine the amount of flavonoids present.

3.6 EVALUATION OF ANTI-INFLAMMATORY ACTIVITIES BY THE EGG-ALBUMIN INDUCED RAT PAW OEDEMA

Fresh raw egg-albumin was used as an in vivo model to induce acute inflammation (Winter *et al*., 1963) according to the method described by Adzu *et al* ..2003.

Rats were divided into five groups (n= 5) and pretreated as follows: group 1 received 10ml/kg normal saline (control group), groups 2-4 received 100, 200 and 400 mg/kg of Methanolic extract of *Ocimum gratissimum* leaves, respectively, while group 5 received 10mg/kg

indomethacin. All treatments were administered orally. Oedema was induced 30 min later in all the rats by sub-plantar injection of 0.1 ml of raw egg albumin to the left hind paw.

Oedema formation was taken as increase in paw circumference measured by wrapping a white cotton thread around the injected paw. Initial paw sizes were taken before injection of egg albumin. while subsequent measurements were taken at an interval of 30 min for a total of 120 min. Results were expressed as mean suppression of inflammation compared as with the pre-egg albumin injection value for each rat.

3.7 STATISTICAL ANALYSIS OF DATA

All data generated were presented as Mean ± Standard Error of the Mean (SEM) and statistical comparison of means was performed using student's t- test in Microsoft EXCEL and SPSS software. $P < 0.05$ was considered as statistically significant.

CHAPTER 4

RESULTS

4.1 Yield of Extract

The percentage yield of the maceration of *Ocimum gratissimum* leaves in distilled water for 48 hours was calculated to be 12.7%.

In the Soxhlet extraction, 100g of powdered *Ocimum gratissimum* leaves successively extracted in n- Hexane, Chloroform and methanol gave a percentage yield as presented in Table 4.1 below.

Table 4.1: Percent Yield of different solvent soxhlet extraction of *Ocimum gratissimum* leaves.

EXTRACT	PERCENTAGE YIELD
Hexane	3.8%
Chloroform	3.2%
Methanol	7.7%

4.2 Phytochemical Constituents in Different Solvent Extract of *Ocimum gratissimum*

The phytochemical screening carried out in the different solvent extract following standard procedures gave indication for the presence or absence of secondary metabolites extracted by the different solvents. The results are presented in table 4.2 below.

Table 4.2 Preliminary Phytochemical Test for Different Solvent Extract of *Ocimum gratissimum* leaves.

PHYTOCHEMICAL CONSTITUENTS	AQUEOUS	HEXANE	CHLOROFORM	METHANOL
Alkaloids	+	–	–	+
Flavonoids	+	+	+	+
Saponins	+	–	–	+
Tannins	+	–	–	+
Reducing sugars	–	–	+	+
Terpenoids	+	+	+	–
Steroids	–	+	+	+
Phlobatannins	+	–	–	–

+ = Presence of constituents; - = Not detected

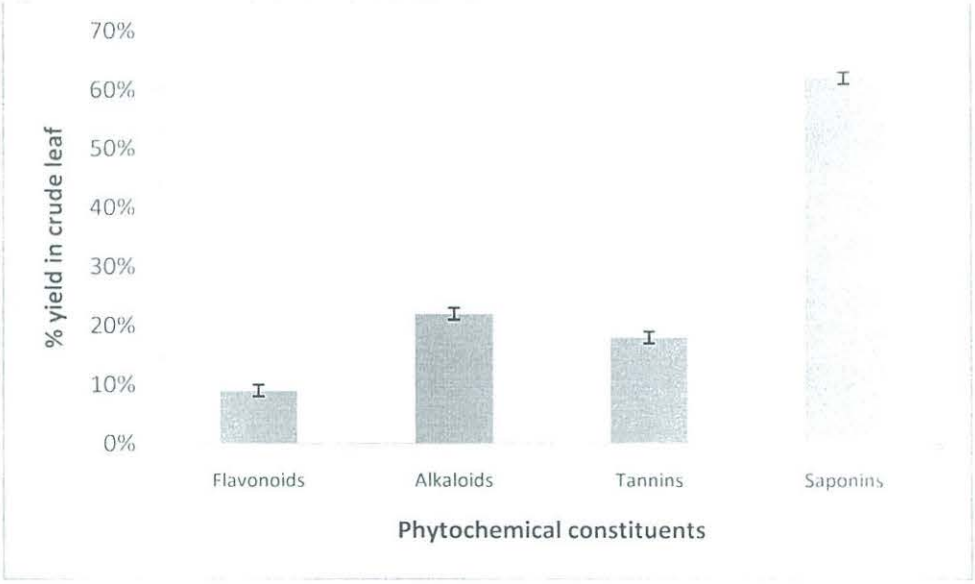
4.3 Quantification of Phytochemical Constituents

The percentage yield of the phytochemical quantification from 10g of crude powder of *Ocimum gratissimum* is as presented in Table 4.3:

Table 4.3: Percent Yield of Quantified Phytochemical Constituent in crude powder of *Ocimum gratissimum* leaves.

PHYOCHEMICAL CONSTITUENTS	PERCENTAGE YIELD IN CRUDE LEAF
Flavonoids	9%
Alkaloids	22%
Tannins	18%
Saponins	62%

Fig 4.1: Quantification of Phytochemical Constituents in powdered *Ocimum gratissimum* leaves.



4.4: **Evaluation of anti-inflammatory activities of Methanolic Extract of *Ocimum gratissimum* in egg albumin - induced oedema in the hind paw of rats.**

The egg albumin experiment to determine the anti-inflammatory in the methanolic extract gave a dose dependent inhibition of oedema in the 30 and 60 mins post albumin injection. Oedema peaked in the 60 min in the control group and declines gradually till the 120 min.

The Mean and SEM of the paw circumference is presented in Table 4.4, Increase or decrease in paw circumference in presented in table 4.5 and Fig 4.2 below.

Table 4.4: Anti-inflammatory effect of *Ocimum gratissimum* Methanolic extract (OGME) on egg albumin –induced oedema in rats.

Treatment	Dosage (mg/kg, p.o)	Paw circumference (cm)				
		0 min	30 min	60 min	90 min	120 min
Control	10 ml/kg	2.14± 0.02	2.74 ± 0.06	2.78 ± 0.09	2.58 ± 0.07	2.52 ± 0.1
OGME	100	2.14 ± 0.06	2.7 ± 0.04	2.56 ± 0.07	2.46 ± 0.04	2.36 ± 0.07
OGME	200	2.1 ±0.04	2.52 ±0.06	2.5 ± 0.05	2.42 ± 0.05	2.48 ± 0.05
OGME	400	2.18 ± 0.02	2.58 ± 0.07	2.5 ± 0.05	2.4 ± 0.04	2.32 ± 0.05
Indomethacin	10	2.12 ± 0.06	2.68 ± 0.06	2.5 ± 0.03	2.48 ± 0.04	2.36 ± 0.07

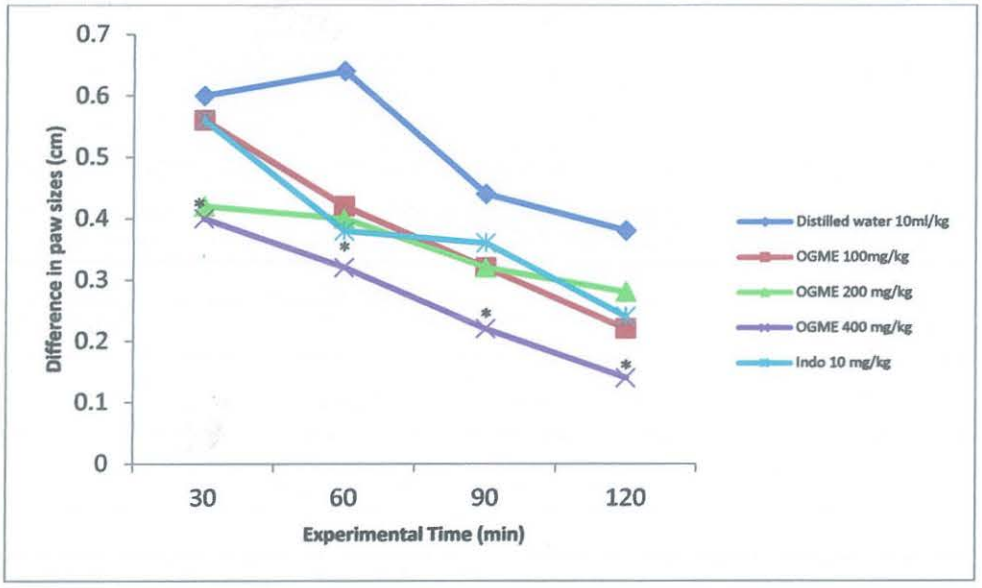
Results are Mean ± SEM (n =5)

Table 4.5: Difference paw circumference

Treatment	Dosage (mg/kg, p.o)	Difference in paw circumference (cm)			
		30 min	60 min	90 min	120 min
Control	10 ml/kg	0.6	0.64	0.44	0.38
OGME	100	0.56 (6.7)	0.42 (34.4)	0.32 (27.3)	0.22 (42.1)
OGME	200	0.42 (30)	0.4 (37.4)	0.32 (27.3)	0.28 (26.3)
OGME	400	0.4 (33.3)*	0.32 (50)*	0.22 (50)*	0.14 (63.2)*
Indomethacin	10	0.56 (6.7)	0.38 (40.6)	0.36 (18.2)	0.24 (36.8)

*P< 0.05 compared with the control, percentage inhibition is shown in parentheses.

Fig 4.2: Effect of OGME on egg albumin-induced acute oedema in rats.



Data were calculated as mean difference from initial paw circumference and after time t, (n = 5).*$P < 0.05$ vs. control.

CHAPTER 5

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

Ocimum gratissimum has been a popular herb mainly used has a folk medicine, meaning that it is collected and used by lay persons to treat the common ailments (Rios and Recio 2005). Its uses have been attributed to the presence of essential oils and flavonoid constituents. Flavonoids are known anti-inflammatory constituents present in many plant extracts. In our solvent extraction, flavonoids were present in all the solvent extracts of *Ocimum gratissimum* (Table 4.2). Other secondary metabolites found in the different solvent extracts are alkaloids, tannins, saponins, and steroids . Steroids was known to exert their anti-inflammatory effect by inhibiting phospholipase A2, a key enzyme of arachidonic acid metabolism, thereby stopping prostaglandin synthesis (Barar, 2000). Some plant steroids have also been shown to stabilize lysozomal membranes there by inhibiting the release of pro-inflammatory mediators (Okoye *et al.*, 2010). Alkaloids and flavonoids have also been shown to possess anti-inflammatory activity by inhibiting the action of arachidonic acid metabolism via the cyclooxygenase and 5-lipoxygenase pathways (Barik *et al.*,1992 and Hasare *et al.*,). Flavonoids also inhibit inflammation by lysozomal membrane stabilization and also by inhibiting migration of leucocytes to the site of inflammatory stimulus (Okoye and Osadebe, 2009).

The egg-albumin induced inflammatory reaction has been shown in two phases. The early phase, which begins at 30 minutes after administration of the albumin results from the release of histamine, serotonin and bradykinin; while the later phase (1 h after irritant administration) is associated with the release of mediators such as prostaglandins (Vinegar *et al.*, 1969 and

Ogonowski *et al.*, 1997). The result of the anti-inflammatory effect of *Ocimum gratissimum* shown in table 4.4 indicated that the methanolic extract exhibited significant inhibition of acute edema induced by egg albumen. The oedema peaked at 1hr and gradually declined to 2hrs.The methanolic extract of *Ocimum gratissimum* extract produces a pronounced dose-dependent anti-inflammatory effect in both phases of albumin induced oedema. From the above results, it can be suggested that ant-inflammatory effect of the plant extract on albumin induced oedema may be related to inhibition of the release of mediators such as prostaglandins (PGE2 and PGI2) which have been implicated as mediators of the inflammatory and pain responses.

5.2 CONCLUSION

The results of the present study have shown that the leaf of Ocimum gratissimum is rich in secondary metabolites –saponins, flavonoids, tannins and alkaloids and that the methanolic extract possesses significant anti-inflammatory activity in acute inflammation in rats thus, justifying its use by traditional medicine practitioners in the management of disease conditions associated with inflammation.

5.3 RECOMMENDATION

- ✓ Study anti-inflammatory activities in hexane and chloroform fraction
- ✓ Fractionate and study fraction in in vivo animal models
- ✓ Purification and characterization of the potent anti-inflammatory compounds
- ✓ Elucidation of the actual mechanism of anti-inflammatory mechanism of action.
- ✓ Standardize *Ocimum gratissimum* leaves extract for commercialization

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