KAMPALA INTERNATIONAL UNIVERSITY -WESTERN CAMPUS

SCHOOL OF PHARMACY

COMPARATIVE PHYSICOCHEMICAL, PHYTOCHEMICAL AND ACUTE TOXICITY STUDIES OF *Ocimum gratissimum* AND *Ocimum suave* SPECIES IN WESTERN UGANDA

RESEARCH SUBMITTED TO THE SCHOOL OF PHARMACY KAMPALA INTERNATIONAL UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF PHARMACY DEGREE

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MARCH 2013

DECLARATION

I do declare that this research project is my own original finding. It has never been presented before for any other award.

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

Sign..... 13.05.2013.....

NALUWUGE ANNET **Date**

(AUTHOR)

CERTIFICATION

A research report submitted to the school of pharmacy, Kampala international university in partial fulfillment of the requirements for the award of bachelor of pharmacy degree

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DATE: 20 .03.2013.

DEDICATION

This report is dedicated to all research scientists whose tireless efforts are directed towards discovering the unknown about medicinal plants.

ACKNOWLEDGEMENT

The researcher registers her sincere gratitude to the Almighty God for granting her the ability and necessities that were required in this study.

Special thanks go to Mr. Ajayi Abayomi for all his precious time and efforts in the supervision, technical advise and guidance throughout the study.

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Sisters: Betty, Margret, Jackie and Tina,

Great friends: Nakiwewa Julian, Kyebale Romeo, Niwenyesiga Faith, Ntale Ismail and all her colleagues for their constructive ideas.

May God bless them abundantly.

ABSTRACT

Ocimum gratissimum and *Ocimum suave* belong to the family lamiaceae. Folkore medicine claims their use in many conditions.

Leaves of *O gratissimum* and *O suave* were extracted in aqueous methanol by maceration, extract was filtered and evaporated using the rotary evaporator and dried to a constant weight in an oven. Phytochemical analysis was carried out to determine the active constituents and Quantification tests were carried out to obtain the amount of phytochmicals in each extract. Standard procedures were used to determine the physicochemical properties of the two leaves. Acute toxicity studies were evaluated on laboratory rats, aiming at ascertaining the acute toxicity profile of the two leaves extract. This research is expected to contribute to the knowledge of acute toxicity of *O gratissimum* and *O suave* and their physicochemical and phytochemical composition.

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CHAPTER ONE

INTRODUCTION

Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body.

The genus Ocimum belong to the family lamiceae, they are widespread over Asia, Africa and Central & Southern America. The colors of the leaves vary from bright green to purple-green and sometimes almost black. The genus Ocimum is ranked high among some of the astonishing herbs for having enormous medicinal potentialities. Previous studies show that there are large numbers of species and varieties falls in this genus (Grayer et al., 2002). There about 60 or more species of *Ocimum* and numerous varieties belonging to the family lamiaceae e.g *Ocimum gratissimum*, *Ocimum basilicum*, *Ocimum americanum* and others (Martin and Salguerio, 1999; Mandal and Pattnaik, 2000).

Ocimum gratissimum commonly known as fever leaf and tea bush share a synonym with *O suave Wild* and are generally known as Omujaja in Uganda.

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

Background

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 MM 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 African countries 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 are relied upon by 65–80% of the World's population for their primary health care needs.

The plant may be considered as a biosynthetic laboratory, not only for primary metabolites such as carbohydrates, proteins and lipids that are utilized as food. But also for multitude of compounds like cardiac glycosides, alkaloids , tannins, saponins ,steroids, flavonoids, Reducing sugar, volatile and fixed oils etc, the exert physiological effect .The compounds that are responsible for therapeutic effect are usually the secondary metabolites.

O. gratissimum and O. suave have been used extensively in the traditional system of medicine in many countries. In the Northeast of Brazil, *O. gratissimum* 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 *et al., 2003)*. *Ocimum suave* is used traditionally for treatment of stomachache, cough and influenza, as a perfume, an insect repellent particularly against mosquitoes and a grain protectant (Hassanali *et al.,* 1990).

Problem statement

The practice of traditional medicine, which is deep rooted in rural areas, continues unabated alongside conventional medicine because of ease of availability, inaccessibility of health centre's 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.

Although there are more different species of *ocimum* which belong to one family (Lamiaceae) their therapeutic effects may differ. The establishment of variable constituents in these leaves may be responsible for their differences in therapeutic activities.

Objectives

Broad Objective

The purpose of the study is to compare the physicochemical and phytochemical composition of the leaves of *ocimum suave* and *ocimum gratissimum*, and their acute toxicity profile.

Specific objectives

1. To extract phytochemicals *O:suave* and *O.gratissimum* leaves.
2. To determine the physicochemical parameters in the leaves of the two *ocimum* species.
3. To carry out Quantification of the phytochemicals in the two *ocimum* species.
4. To evaluate the acute toxicity profile of *Ocimum suave* and *O.gratissimum* leaf extracts.

Justification

Medicinal plants have been the subject of intense research due to their potential as sources of commercial drugs or as lead compounds in drug development and isolation. Various aspects of the photochemistry and photobiology 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 these plants as a consequence of their chemical constituents. The dose that will be obtained from this study will help understand the desired therapeutic effects of the drug while minimizing the risk of toxic effects. It will also ensure documentation of phytochemical constituents and physicochemical composition for monograph information about the plants grown in Uganda. Moreover the process of standardization can be achieved by stepwise physicochemical and phytochemical studies. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy.

CHAPTER TWO
LITERATURE REVIEW

Ocimum gratissimum and *Ocimum suave*

Ocimum gratissimum is an aromatic shrub from Asia and Africa is commonly known as fever leaf in general and other treatment alone or in combination with other plant. The parts of plant used are flowers and leaves. The flowers and the leaves of this plant are rich in essential oils so it is used in preparation of teas and infusion.

In the coastal areas of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhea. In the Savannah areas decoctions of the leaves are used to treat mental illness. (Akinmoladun AC, 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 II, Omodamiro OD, Nwanna IJ 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 like viz., the leaves are rubbed between the palms and sniffed as a treatment for blocked nostrils, they 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 rectum. (Matasyoh LG,et al 2007). In India, the whole plant has been used for the treatment of sunstroke, headache, and influenza, as a diaphoretic, antipyretic and for its anti-inflammatory activity. (Prajapati ND, et al 2003, Oliver B, 1980, oTa^nia Ueda, Nakamura RR, Mendonca F, *et al 2006).*The plant is commonly used in folk 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 TT, Salau AO 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, Omodamiro OD, Nwanna IJ 2005). 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 increases the body's resistance to stress, trauma, anxiety and fatigue. Moses Ssenoga, a naturopathic (natural medicine) doctor at Mukago Sanitarium in Mbuya, recommends the herb for 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. Senegal's recipe, the infusion of O.gratissimum leaves is used as pulmonary antisepticum, antitussivum and antispamodicum. (Ngassoum MB, 2003).*O. gratissimum* is associated with chemo-preventive, anti-carcinogenic, free radical scavenging, radio protective and numerous others pharmacological use. (Gupta SK, Prakash J, Srivastava S 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 M, Sheteolu AO, et al 1996). Earlier reports have shown the smooth muscle contracting lipid soluble principles, and ant mutagenic activity in organic solvent extracts of *O. gratissimum* leaves.(Onajobi FD 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. 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).

In Lower Moshi villages, investigations were done to establish whether whole plant and plant products derived from local areas can be used in combination with the bed nets to provide protection against malaria vectors and nuisance biting insects. Before starting such an investigation, an ethno botanical survey was conducted to understand the common knowledge, attitude and practices, of local people, on the use of plant products for protection against mosquitoes and other biting insects (Curtiset et al, 1998).

At Lower Moshi, *Ocimum suave, Ocimum kilimandscharicum*, *Azadirachta indica*, *Eucalyptus globules and Lantana camara* plants are common and known to have provided protection against mosquitoes. These aromatic plants, *Ocimum suave* (OS) *and Ocimum kilimandscharicum* (OK) locally known as a broom "Ufagio" in the Kiswahili language, belong to the family Lamiaceae and are the focus of this study. Several plants of this family have been proven to have insecticidal and repellent effects, used widely against blood- feeding arthropods and those feeding on crops (Curtiset et al, 1998).

Although, treated mosquito nets have been proved to be effective in reducing child morbidity and mortality, there are still operational problems slowing down the scaling up of Insecticides Treated bed Nets (ITN) usage such as seasonal variation of ITN use in the community, equity and access constraints, low rates of net re-treatment with insecticides and reports of insecticide resistance in malaria mosquitoes. With such problems facing the existing control measures against vector- borne diseases, there is a need to look for alternative and supplementary means to support existing control measures. Alternative, cost- effective and environmentally friendly bio-products such as plant repellents can potentially be improved to supplement existing vector-control measures (Palsson et al, 1999).

Although there are many plant species used traditionally for protection against blood- feeding insects, there are few studies to illustrate their protective efficacy and/or contribution to disease control. Following a survey conducted, OS and OK were the most common plants used as insects repellent by local communities at Lower Moshi, north-eastern Tanzania. This study evaluates deterrence, and feeding inhibition effects of *Ocimum suave* (OS), *Ocimum kilimandscharicum* (OK), *Azadirachta indica* (AI) *Eucalyptus globules* (EG) and *Lantana camara* (LC) on three mosquito species, *Anopheles arabiensis* (Patton), *Anopheles . Gambia* (Giles) and *Culex quinquefasciatus* (Say) in the field and experimental huts (Palsson, 1999).

The use of repellent materials from plants against nuisance insects is common with great potential to complement existing malaria control programmes and this requires evaluation in the field. Ocimum plant species, *Ocimum suave* (Wild) and *O. kilimandscharicum* (Guerke) materials and their essential oils extracted by steam distillation were evaluated in the field and experimental huts for repellence, feeding inhibition effects against three mosquito

species, *Anopheles arabiensis* (Patton), *Anopheles. gambiae* (Giles) and *Culex quinquefasciatus* (Say). The protective effect of essential oils from Ocimum plants were compared with N, N-diethly-3- methylbenzamide (DEET), a standard synthetic repellent. Also, the protective effect of fumigation by burning of repellent plants; *Ocimum suave, Ocimum kilimandscharicum, Azadirachta indica, Eucalyptus globules* and *Lantana camara* were tested in experimental huts and selected local houses (Palsson, 1999).

TOXICITY STUDIES

Acute toxicity refers to the effects on the whole body of a single dose of a chemical (or several doses within a 24- hour period), usually manifested over a period of 14 days. Determination of acute oral toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. Acute toxicity data are used to provide a rough guide for dose selection among many other factors.

CHAPTER THREE

METHODOLOGY

3.1Study design

The design of the study was experimental involving laboratory analysis and use of experimental animals.

3.2 Setting of the study

The study was carried out in the Laboratory of Kampala International University-Western Campus, Ishaka, Bushenyi District, Uganda.

3. 3 Plant Identification and Harvest

The two plants were collected with the assistance of Mr Pascal (Kampala International University, School of pharmacy laboratory assistant) and be taxonomically identified by a botanist from the Makerere University Kampala Herbarium. Herbarium samples were prepared and deposited in the school of pharmacy herbarium.

3.4 Extract preparation

The leaves of *O gratissimum and O suave* plants were collected and dried under shade, then ground into powder. 5 successive extractions of powdered leaves were carried out in 80% methanol. 100g of powdered leaves was macerated in 500ml of 80% Methanol and shaken for 48 hours on a laboratory rotator. The extract was filtered and concentrated in a Rotary evaporator (Buchi Rotavapor R-124) under reduced pressure and dried to a constant weight in oven at 40°C. Each extract was dried in separate containers in order to determine the mean % yield of extraction.

Percentage yield of extraction from the Methanol extraction was calculated as follows

Mean % Yield = Mean weight of extract + container – weight of container alone X 100

Weight of the initial extract

The extract were pulled together and stored in a refrigerator until ready for use.

3.5 PHYTOCHEMICAL SCREENING

The standard methods described by Evans (2002), Harborne (1998), Odebiyi and Sofowora (1978) and Sofowora (1982) were applied in this phytochemical screening of the individual constituents of each extract. The presence of the compounds was rated as positive (+) or negative (-) in the two extracts.

3.5.1 Test for Saponins:

Frothing Test: An aliquot of the extract was boiled with 5ml distilled water for two minutes. The mixtures were cooled and mixed vigorously and left for three minutes. The formation of frothing indicated the presence of saponins.

3.5.2 Test for tannins:

i. To 2ml of test solution, add 2 ml of distilled water and a pinch of lead acetate, formation of white precipitate indicate the presence of tannins.

ii. An aliquot of the extract was treated with 15% ferric chloride test solution. A resultant blue color indicate the presence of hydrolysable tannin.

iii. An aliquot of the extract dissolved in distilled water and filtered. The filtrate was boiled with 2% Hcl solution. Red precipitate observed indicated the presence of phlobotannins.

3.5.3 Test for Terpenoids/Steroids:

i. **Salkowski Test:** To an aliquot of the extract, 2ml of chloroform were added and thoroughly mixed, warmed in water for 30 minutes and 3ml concentrated sulphuric

acid carefully, added to form a layer. The formation of reddish-brown coloration, at the interface indicate presence of terpenoids (Sofowora, 1982).

3.5.4 Test for alkaloids:

i. **Mayer's test:** To an aliquot of the extract, 2ml of 1% HCl was added and stirred on steam bath. 2- 3 drops of Mayer's reagent were added to 1 ml of the filtrate. The reaction which turned opalescent was considered low positive (+), turbid was fairly positive (++) and abundant precipitate was highly positive (+++).

ii. **Wagner's Test**: The procedure was the same as in the previous tests to obtain the acid solution. Two or three drops of Wagner's reagents were added. The results were classified in the same way.

3.5.6 Test for flavonoids:

i. **Shinoda Assay**: The aliquot in ethanol was diluted with 1 mL of concentrated hydrochloric acid and a small strip of metal magnesium added. After 5 min of reaction, 1 mL amyl alcohol was added, mixed the phases and let them rest until they separated again. The extract aliquot in water was preceded the same way from the addition of concentrated hydrochloric acid.

The assay was considered positive for the presence of flavonoids, when the amyl alcohol turns dark yellow, orange, red or brown.

ii. To 2 ml of the alcoholic solution of the crude extract, 4 drops of 2% lead acetate solution were added. The development of yellow or orange color was taken as an indication of the presence of Flavonoids.

iii. To an aliquot of the extract filtrate, 5 ml of dilute ammonia solution was added followed by addition of concentration sulphuric acid. A yellow colouration indicated the presence of Flavonoids. The yellow colouration disappeared on standing.

3.5.7 Test for Reducing Sugar

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

3.5.8 Test for Cardiac glycosides

A drop of ferric chloride solution was added to 2ml glacial acetic acid. This solution was added to an aliquot of the extract. The mixture was underlayed with 1 ml concentrated sulphuric acid. A brown ring of the interphase indicated a deoxy-sugar characteristic of cardenolides. A violet ring also appeared below the brown ring while in the acetic acid layer, a greenish ring formed just gradually throughout the thin layer indicating the presence of cardiac glycosides.

3.6 PHYTOCHEMICAL QUANTIFICATION

3.6.1 Estimation of alkaloids contents

The alkaloid content of each extract was determined by the method of Harborne (1973). About 500mg of each sample was weighed into a 50ml beaker, and a 20ml of 10% acetic acid in ethanol was added, and allowed to stand for 4hrs. This was filtered using whatmann No.42 filtered paper, and concentrated in water bath to 1/2 (10 ml) of the original volume. Then concentrated NH4OH was added drop wise to each extract until the precipitate was complete. The suspension was allowed to settle and the precipitate collected, washed with NH4OH and then filtered again. The residue was dried and weighed. The percentage alkaloid was then calculated. Each experiment was performed in triplicate.

3.6.2 Estimation of tannin content

Tannin content was determined by the method of Van Burden and Robinson (1981). 500mg of each sample extract was weighed into a 50ml plastic bottle,50ml distilled water added, shaken

for 1hr in a mechanical shaker, filtered into a 50ml volumetric flask, and made up to the mark with distilled water. Then 5.0ml of each sample was mixed with 2.0ml of 0.1M FeCl3 in 0.1N HCl and 0.008M potassium ferricynide added, allowed to stand for 10 minutes and the absorbance read at 120nm in spectrophotometer. Each experiment was performed in triplicate.

3.6.3 Estimation of saponin content

Saponin content was determined by the method of Obadoni and Chuko (2001). About 500mg of each extract was mixed with 50ml of 20% ethanol, and incubated in a water bath at 55oC for 4hrs with stirring.

The mixture was then filtered and the extract re-extracted with 50ml of 20% ethanol. The combined extract was concentrated to 40ml in a water bath at 90oC. The concentrate was then transferred into a 200ml separatory funnel and 20ml diethyl ether added, and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded; the purification process was repeated then 60ml of n-butanol added. The combined n-butanol extracts was washed twice with 10ml of 5% aqueous NaCl, and the remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight, and the percentage saponnin content calculated.

3.6.4 Estimation of flavonoid content

The flavonoid content of the extracts was estimated by the method of Boham and Kopicalabyazen (1974). About 500mg of each sample was extracted repeatedly with 10ml of 80% methanol. The whole solution was then filtered through Whatmann No.42 filter paper. The filtrate was transferred into a crucible and evaporated to dryness on a water bath, and weighed. The percentage flavonoids content was then calculated.

3.7 PHYSICOCHEMICAL PROPERTIES

Standard procedure as documented in the official literature was adopted in determining the alcohol, aqueous extractive values using the dried leaf powder (World Health Organization, 2003). The total ash value and acid insoluble ash value (using 0.1 M hydrochloric acid), was determined according to Kokate (2000). All experiments were performed in triplicate.

3.7.1 Determination of Extractives

Determination of water-soluble extractives – Pulverize the leaves of *O gratissimum and O suave*, pass through a No.2 sieve and mix well.

3.7.1.1 Cold extraction method – 4.0 g of the powdered sample was accurately weighed and placed in a 250– 300-mL conical flask with a stopper. 100 mL of water was accurately added; stopper inserted and extracted for 24 h. The sample was shaken frequently during the first 6 hours and then allowed to stand for 18 h. Filtered rapidly through a dry filter. 20 mL of the filtrate was accurately transferred to an evaporating dish, previously dried to constant weight, and evaporated to almost dryness on a water bath, then dried at 105 °C for 3 h. Cooled in a desiccator for 30 min, and then weighed immediately and accurately. The percentage of water-soluble extractives with reference to the dried sample was calculated.

3.7.1.2 Hot extraction method – 2.0–4.0 g of the powdered sample, accurately weighed, was placed in a 100– 250-mL conical flask with a stopper. 50–100 mL of water was accurately added, stopper inserted and the sample was weighed, allowed to stand for 1 h. A reflux condenser was attached to the flask and boiled gently for 1 h, then cooled to room temperature and weighed, readjusted to the original weight with water. Shaken and filtered through a dry

filter. 25 mL of the filtrate transferred accurately to an evaporating dish, previously dried to constant weight and evaporated to dryness on a water bath, then dried at 105 °C for 3 h. Cooled in a desiccator for 30 min, and then weighed immediately and accurately. The percentage of water-soluble extractives with reference to the dried CMM sample was calculated.

3.7.1.3 Determination of ethanol-soluble extractives –

Cold extraction method – Proceeded as in section 1(a) except by using ethanol (70%) in lieu of water as solvent.

Hot extraction method – Proceeded as in section 1(b) except by using ethanol (70%) in lieu of water as solvent.

3.7.2 Determination of Ash Values

4g in triplicate of the powdered samples was ignited to ash in a crucible, cooled in a dessicator for up to 24 hours and weight of total ash was taken. The ash was then dissolved in 200mls of distilled water and stirred. The mixture was filtered using a known weight of filter paper and the residue obtained was dried together with the filter paper and the weight of the insoluble residue taken. The results were subtracted from the total ash to obtain the amount of water soluble ash in the drug.

The percentage dry ash was calculated as follows;

$$\% \text{ Ash} = \frac{\text{Weight of total ash}}{\text{Initial weight of powder}} \times 100$$

3.7.3 Moisture Content

4g in triplicate of the powdered sample was weighed into weighed crucibles, and then dried in the oven at 100degrees until constant weight. Then it was allowed to cool in a dessicator. Loss in weight was recorded as moisture content.

3.8 ACUTE TOXICITY STUDY

To investigate the acute toxicity profile of the methanolic extract of *O. gratissimum* and *O. suave* leaves. This is Consistent with the goals of toxicology (Doull 1996), this evaluation aims to identify and characterize the adverse effects that can be produced in biological systems by a single oral exposure to *Ocimum gratissimum* and *O.suave* leaf extracts and later to use this information and that from further studies to predict the type and severity of responses in man under other exposure situations.

3.8.1 Animals and experimental design

Twenty (25) female Wistar rats (weighing 100–150 g) were obtained from the Pharmacology dept animal facility. During the acclimatization period, clinical observations as well as body weight measurements of the animals were conducted and only healthy ones were selected. The rats were assigned into groups including a control group by the stratified random method according to their body weight. The route of administration was by oral gavage in accordance with the main route of intake of *Ocimum gratissimum* and *O suave* decoction by humans for medicinal purposes. Five wistar rats constituted a group. Five groups including the control group were established. A single oral low dose (LD) of 2 000 mg/kg b.w. and a single oral high dose (HD) of 5 000 mg/kg b.w. of the methanolic extracts of *Ocimum gratissimum* and *O suave* leaves were administered. The observation period was 14 days post administration.

The effect of Extracts on general behavior was observed; on locomotor activity, writhing response, fighting, convulsion, tremor, exophthalmos, ptosis, piloerection, tail elevation, traction, motor incoordination, muscle tone, catalepsy, righting reflex, pain response, pinna reflex, skin color, respiration, lacrimation, salivation, diarrhea, vocalization and death. One night-fasted animals were administered with the drugs, General actions about 23 items were indicated as "+" or "—" after specific times. The observation was hourly for up to six hour post administration.

Over the course of 2 weeks, observation regarding the variation of physical states, weights, intake of feed & water, once a day or more by the naked eyes was taken.

Body weights were measured before dosing on the day of administration and on the 4^{th}, 7^{th} 10^{th} and 14^{th} day of observation.

3.9 STATISTICAL ANALYSIS OF DATA

All data generated was presented as Mean ± Standard Error of the Mean (SEM) and statistical comparison was performed using descriptive statistics and ANOVA-repeated measures. P less than 0.05 were considered as statistically significant.

3.10 ETHICAL CONSIDERATIONS

3.10. 1Laboratory animal acquisition and maintenance

Female Wistar rats (weighing 150–200 g) were used for this study. The animals were bred and housed in the Animal Facility of the dept of Pharmacology & Toxicology, Kampala International University-Western Campus. The animals were kept in a cage lined with sawdust, at room temperature with adequate ventilation, under a naturally illuminated environment with 12 hours of light and 12 hours of darkness. They were fed with standard diet (Nuvita $^{(R)}$ Animal Feed Ltd, Jinja Uganda) and had access to clean drinking water *ad libitum*.

The animal experiments were conducted according to the National Institute of Health Guide for the care and use of laboratory animals (NIH, 1996) and ethical guidelines for investigation of experimental pain in conscious animals (Zimmerman, 1993).

3.10.2 Research Approval

The study was carried out after submission of research proposal and approval of the proposal by research sub-committee of the School of Pharmacy.

3.11 Dissemination of findings

A copy of the research shall be submitted to the office of the dean of school of pharmacy. Another copy will be left in the library of KIU-WC. The research findings will be presented at the annual Pharmacy Students Association conference.

3.12 Limitations to the study

- Delays caused by lack of availability of research materials e.g. Reagents in the laboratory.
- Lack of equipment; for example personal computer.
- Frequent power black outs; this might hamper the undertaking of the study.

3.10 Time Frame: The whole study took approximately 6 months.

CHAPTER FOUR

RESULTS

4.1. PERCENTAGE YIELD

Ocimum suave Methanolic Extract [OSME] gave more yield of extraction than *Ocimum gratissimum* Methanolic Extract [OGME].

Table 4.1: results of % yield of extraction

Extract	Mean % Yield
Ocimum Gratissimum Methanol Extract[OGME]	1.86
Ocimum Suave Methanol Extract [OSME]	2.23

4.2. PHYTOCHEMICAL SCREENING RESULTS

Results revealed presence of saponnins, tannins, Terpenoids/steroids, alkaloids, flavonoids, reducing sugars, cardiac glycosides and lactone coumarins.

Table 4.2: Results of phytochemical screening

Substance	**Test/Reagent**	**OGME**	**OSME**
Saponins	Frothing test	+	++
Tannins	1.Lead acetate	+	+
	2.15% ferric chloride	++	+
	3.2% Hcl	-ve	-ve
Terpenoids/Steroids	1.Salkowski test	+	+++
Alkaloids	1.Mayer test	+	+
	2.Wagners test	+	+
Flavonoids	1.Shinoda Assay	+	+
	2.2% lead acetate	++	+
	3. Dilute ammonia solution.	+	+
Reducing sugars	Iodine	+	+
Cardiac glycosides	Ferric chloride + galacial acetic acid.	++	+
Lactone coumarins	Balijet test	++	+

+ (positive) indicates presence of the phytochemical.

-ve (negative) indicates absence of the phytochemical.

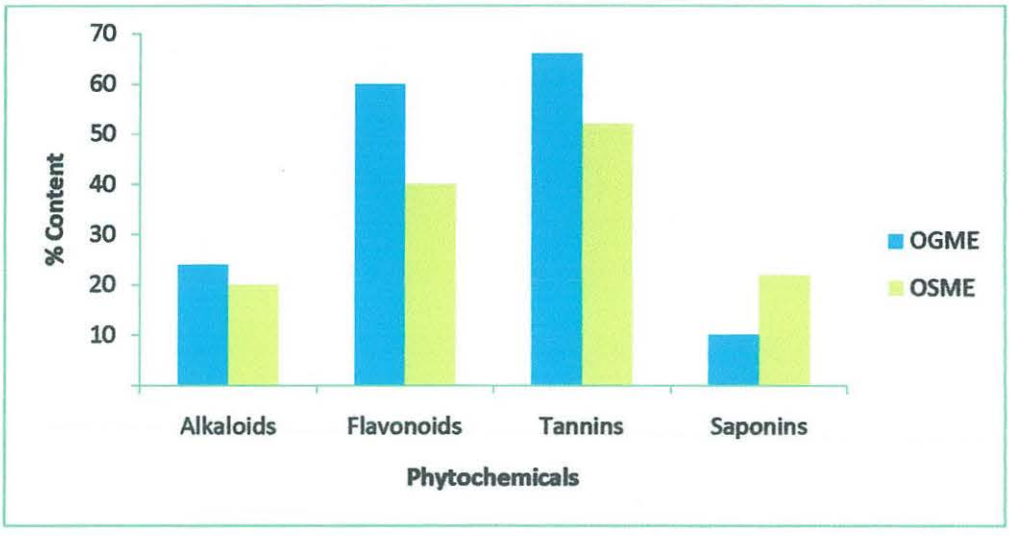
4.3. PHYTOCHEMICAL QUANTIFICATION

Results revealed that *Ocimum gratissimum* contained more alkaloids, Flavonoids and tannins compared to *Ocimum suave*. Saponnins are more in OSME than in OGME.

Table 4.3: Phytochemical Content of *Ocimum gratissimum* methanolic extract (OGME) and *Ocimum suave* methanolic extract (OSME)

	OGME		OSME	
Phytochemicals	Mean ± SEM	% content	Mean ± SEM	% content
Alkaloids	0.12 ± 0.15	24	0.10 ± 0.03	20
Flavonoids	0.30 ± 0.10	60	0.20 ± 0.10	40
Tannins	0.33 ± 0.05	66	0.26 ± 0.04	52
Saponins	0.05 ± 0.03	10	0.11 ± 0.01	22

Fig 4.3: Percentages of Phytochemical contents in *Ocimum gratissimum* methanolic extract (OGME) and *Ocimum suave* methanolic extract (OSME).



4.4. PHYSICOCHEMICAL PROPERTIES

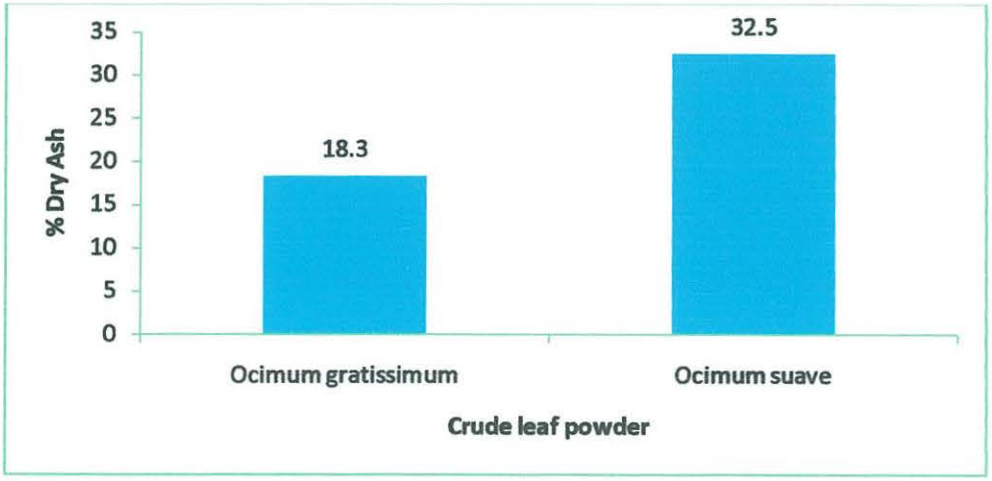
Moisture content, weight of ash, ash insoluble ,ash soluble, soluble extractive values in cold and hot water, cold and hot ethanol were assessed for the two extracts.

Table 4.4.1: Moisture content and Ash values of *Ocimum gratissimum* and *Ocimum suave* dried leaves. Values are Mean ± SEM.

Plants	Moisture content	weight of ash	Ash insoluble	Ash Soluble
Ocimum gratissimum	0.73 ± 0.07	0.73 ± 0.08	0.63 ± 0.15	0.40 ± 0.25
Ocimum suave	0.50 ± 0.37	1.30 ± 0.25	1.2 ± 0.21	0.23 ± 0.09

Mean moisture content value for *O.gratissimum* was high compared to *O.suave* . Water insoluble mean ash value for *O.suave* was greater than for *O.gratissimum* and the reverse is true for the ash soluble values.

Fig 4.4.1: Percentage dry ash determined from dried leaves of *Ocimum gratissimum* and *Ocimum suave*



The % dry ash content of 32.5% for *O.Suave* was greater than for *O.gratissimum* with 18.3%.

Table 4.4.2: Soluble Extractives in *Ocimum gratissimum* and *Ocimum suave* dried leaves.

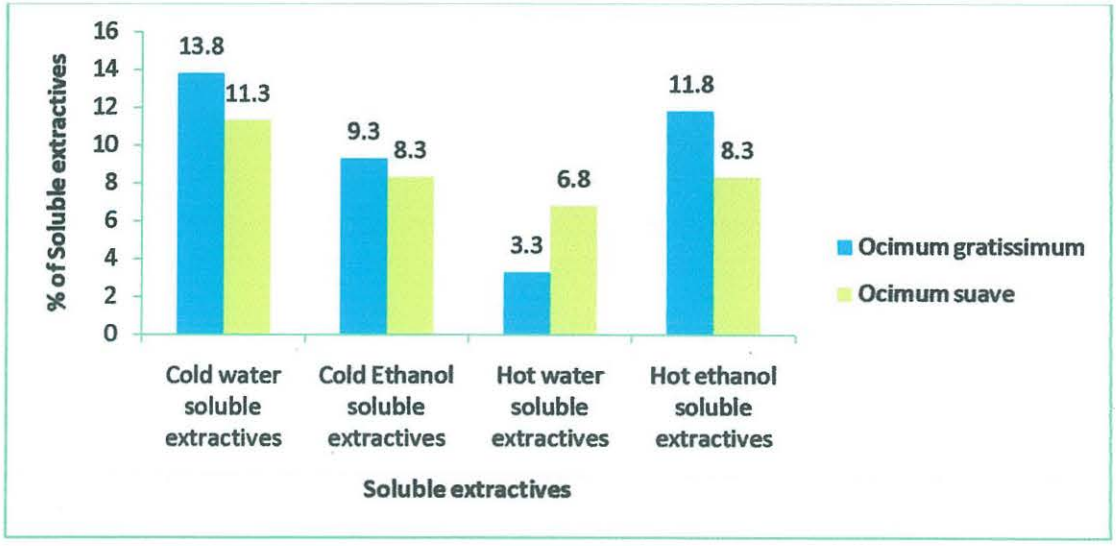
Values are Mean ± SEM.

Plants	Cold water soluble extractives	Cold Ethanol soluble extractives	Hot water soluble extractives	Hot ethanol soluble extractives
Ocimum gratissimum	0.55 ± 0.25	0.37 ± 0.17	0.13 ± 0.03	0.47 ± 0.07
Ocimum suave	0.45 ± 0.25	0.33 ± 0.09	0.27 ± 0.03	0.33 ± 0.12

The cold water yields higher values for *Ocimum gratissimum* than for *Ocimum suave*, while hot water yields higher values for O.S than O.G.

Both cold ethanol and hot ethanol give the same soluble extractive values for *ocimum suave*. Hot ethanol gives higher values of *ocimum gratissimum* than cold ethanol. Cold water gives the highest values for both samples compared to the other solvents.

Fig 4.4.2: Percentages of Soluble extractives in *Ocimum gratissimum* and *Ocimum suave* dried leaves.

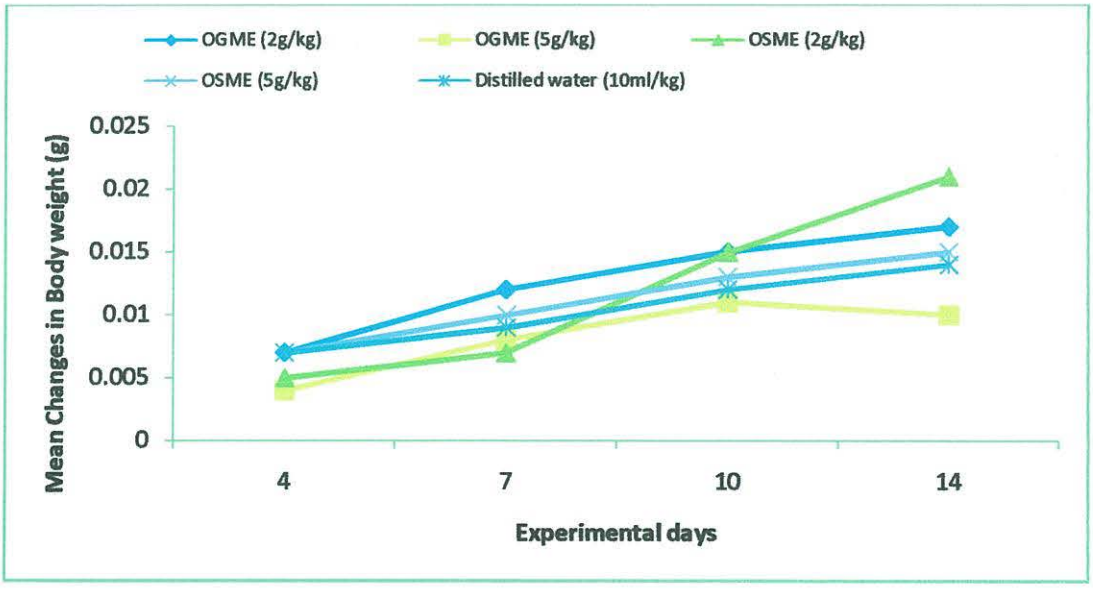


4.5. ACUTE TOXICITY

Table 4.5: Body Weight of experimental animals (kg), Values are Mean ± SEM

Treatments	Experimental days				
	0	4	7	10	14
)GME (2g/kg)	0.124 ± 0.005	0.131 ± 0.005	0.136 ± 0.005	0.139 ± 0.005	0.141 ± 0.005
)GME (5g/kg)	0.125 ± 0.006	0.129 ± 0.006	0.133 ± 0.006	0.136 ± 0.006	0.135 ± 0.006
)SME (2g/kg)	0.126 ± 0.006	0.131 ± 0.006	0.136 ± 0.006	0.141 ± 0.007	0.147 ± 0.005
)SME (5g/kg)	0.127 ± 0.006	0.134 ± 0.006	0.137 ± 0.006	0.140 ± 0.006	0.142 ± 0.007
)istilled water 10ml/kg)	0.129 ± 0.007	0.136 ± 0.007	0.138 ± 0.007	0.141 ± 0.006	0.143 ± 0.006

Fig 4.5: Mean Changes in Body Weight of experimental animals (kg)



There was loss in weight of the animals administered with *Ocimum gratissimum* Methanolic extract 5g/kg from day 10-14.

CHAPTER FIVE

5.0 DISCUSSION

Extraction is removal of soluble material from an insoluble residue either liquid or solid, by treatment with a liquid solvent, the solvent used is in a position to dissolve appreciable quantities of desired substances. The controlling factor in the rate of extraction is normally the rate of diffusion of the solute through the liquid boundary layer or layers at the interface. (Trease and Evans Pharmacognosy).It is employed for materials for which as yet no suitable chemical or biological assay exists. (WHO, 1998).

Both extracts contained phytochmicals but in varying quantities. *Ocimum Suave* Methanolic Extract contained more of Saponins and Terpenoids/steroids than *Ocimum Gratissimum* Methanolic Extract. *Ocimum Gratissimum* Methanolic Extract contained more tannins, Flavonoids and lactone coumarins compared to OSME. The two extracts contained approximately equivalent amounts of alkaloids and reducing sugars.

Moisture content is achieved by heating the powder in an oven to a constant weight at 105°C. Moisture content should be minimized in order to prevent decomposition of crude drug either due to chemical change or microbial contamination. Presences of water or volatile materials do contribute to weight loss. For materials containing little volatile materials; direct drying to constant weight is used. (Trease and Evans Pharmacognosy). A small moisture content indicated by small weight loss for *O.suave* was probably due to the fact that it could be containing little amount of volatile materials than *O.gratissimum.*

Ash content of the drug is the residue remaining after incineration as a form of adulteration of which many times the plant is mixed with various substances like sand, soil, chalk powder or other different inorganic contents. Incineration is done to burn out all organic matter and ash

value is a criterion to judge the identity, quality or purity of crude drugs/plant material especially in the powdered form. (Trease and Evans Pharmacognosy).

Total ash values obtained for the two samples were within the normal limits not so high and not so low indicating to some extent the amount of care taken in the preparation of the drug. This could also be attributable to the first hand collection of the plant from a non polluted area and proper sorting out of the plant material to remove adulterants.

The significance of ashing the drug is to remove all traces of organic matter that may otherwise interfere with the analytical determination and the ash obtained normally consists of carbonate, phosphates and silicates of sodium, potassium, calcium and magnesium. The *total ash* method is designed to measure the total amount of material remaining after ignition. This includes both "physiological ash", which is derived from the plant tissue itself, and "non-physiological" ash, which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. The total ash usually consists mainly of carbonates, phosphates, silicates and silica. The *Water-soluble ash* is used to detect the presence of exhausted drug. The % dry ash content of 32.5% for *O.Suave* was greater than for *O.gratissimum* with 18.3% therefore O.S has more of the carbonates, phosphates, silicates and silica than O.G.

The low water soluble ash value indicates that there was low presence of exhausted drug in OS sample, while the high insoluble ash values could be due to presence of insoluble plant powdered materials like the midrib or stems of the leaves. O.G sample had high water soluble ash values thus high content of exhausted drug and low water insoluble ash values indicating low presence of insoluble plant powdered materials.

Values of solvent extractive indicated variability among the two samples using the same method of extraction. Variation could have been as a result of temperature which actually affects solubility. With cold maceration, variability can occur depending on the place the maceration was done and the temperature of that particular place

The soluble extractive values for *Ocimum gratissimum* are higher than for *Ocimum suave* using both cold ethanol, and cold water. Both cold ethanol and hot ethanol give similar soluble extractive values for *ocimum suave*. Hot ethanol gives higher values of *ocimum gratissimum* than cold ethanol while hot water yields more *Ocimum Suave* than *Ocimum gratissimum*. Duration of maceration and method of extraction used can bring about variability in results.

Generally, the administered extract showed no effect on the general behavior of the wistar rats. The loss in weight of the animals administered with *Ocimum gratissimum* Methanolic extract 5g/kg from day 10-14 might have been due to several reasons, like loss of appetite which is an acute toxic symptom.

5.1. CONCLUSIONS AND RECOMMENDATIONS

The extracts of both *Ocimum Suave* and *Ocimum gratissimum* contain phytochemicals in varying quantities .This validates the usage of *ocimum* leaves for medicinal purposes. Physicochemical studies like determination of extractive values, ash values and moisture content are vital to avoid misidentification and adulteration. These studies help in identification and authentication of the plant material. This information could be helpful in the identification and preparation of monographs on the two *ocimum* plants. The physico-chemical standards, such as ash values, extractive values, will be useful to identify the authenticity of the species even from

the crushed or powdered plant materials. The information obtained from the ash values and extractive values are useful during the time of collection and also during extraction process. Using these standards, the 2 plants can be differentiated from other related species.

Future studies can be done to isolate the different phytochmicals and identify them in order to develop phytodrugs from *Ocimum Suave* and *Ocimum gratissimum.* The chronic toxicity of *O.gratissimum* should also be studied.

We would recommend the local people around the region of South Western Uganda to utilize the two *ocimum* plant leaves, as their phytochemical constituents are a rich source of antioxidants which help the body to get rid of free radicals and prevent degenerative diseases. (Trease, G.E. and Evans, W.C.1989)

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