# A STUDY ON SCREENING AND QUANTIFICATION OF PHYTOCHEMICALS IN THE AQUEOUS EXTRACT OF *Vernonia amygdalina* LEAF

A RESEARCH PROJECT REPORT SUBMITTED TO THE SCHOOL OF PHARMACY KAMPALA INTERNATIONAL UNIVERSITY, WESTERN CAMPUS

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IN

PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR'S OF PHARMACY

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APRIL, 2010

# DEDICATION

I dedicate this project to my beloved mother, who endlessly advised me on how to go on in life. May the almighty God richly bless you and give you many more years.

# DECLARATION

I, **NAMAKULA EDITH** hereby declare that this research was done by me and that it has never been published by anyone before.

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NAMAKULA EDITH

SUPERVISOR:

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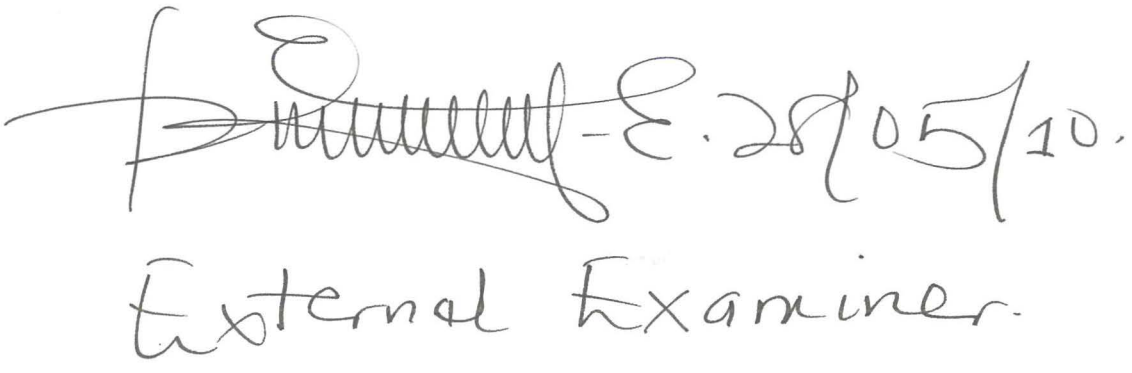

# ACKNOWLEDGEMENT

I acknowledge the following;

- The almighty God for giving me the energy to type and print the work
- My beloved mother for the financial support she has continuously given me.
- My supervisor for the advice he has continuously provided
- The university staff especially the library for enabling me to use the internet for long hours.

May the almighty God continuously bless you all.

# DEFINITIONS

- Phytochemical-health-protecting compounds that are found as components of plant tissues
- Quantification-act of determining the amount of a substance
- Screening-to separate or cut off from the inconveniences, danger or injury
- Extraction-removal of soluble material from an insoluble residue, either liquid or solid, by treatment with a liquid solvent.

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# ABBREVIATIONS

BHT-Butylated hydroxytoluene

MAPKS- mitogen-activated protein kinases

PBMC-peripheral blood mononuclear cells

NMR-nuclear magnetic resonance

HPLC-high performance liquid chromatography

HPTLC-high performance thin layer chromatography

TLC-thin layer chromatography

PC- paper chromatography

MeOH-methanol

AST -aspsrtate aminotransferase

I.p-intraperitoneal

Nm-nanometer

Ng-nanogram

LD50-lethal dose in 50% of study population

Mm/Kg-milligrams per kilogram

Mg/L- milligram per kilogram

Ml-milliliter

V.a-*vernonia amygdalina*

LDL-low density lipoprotein

HDL-high density lipoprotein.

# ABSTRACT

***Vernonia amygdalina*** (*V.amygdalina*) is a tropical plant whose leaves have been proven to have medicinal benefits. It has been used by natives as a remedy against constipation, malaria fever, high blood pressure and many bacterial infections, due to its proven antimicrobial activity. Recently, *V.amygdalina* has been used as an ethinomedicinal solution to persistent fever, headache, and joint pain associated with AIDS. Previous studies of the aqueous leaf extracts have reported saponins, steroids, flavonoids and sesquiterpene lactones being present in the crude leaf extract and probably responsible for the activities.

This study examined the components of the crude extract, the extractive values of different solvents, along with the quantities of some of the different phytochemicals in the leaf aqueous extract. Obtained data revealed the presence of numerous phytochemicals; water, ethanol and chloroform extractive values are 24%w/w, 12%w/w and 4%w/w; and percentage content of some crude phytochemicals present are 54%w/w flavonoid, 29%w/w triterpenoids, 5%w/w Saponins, 14%w/w tannins and 4%w/w alkaloids.

# CHAPTER ONE: INTRODUCTION

## 1.1 BACKGROUND

Most developing countries are endowed with vast resources of medicinal and aromatic plants. Furthermore, these people are precluded from the luxury of access to modern therapy, mainly due to economic reasons. The demands of the majority of the people in the developing countries for medicinal plants have been met by indiscriminate harvesting of spontaneous flora including those in forests. As a result many plant specie have become extinct and some are endangered. The pharmaceutical industry has come to consider traditional medicine as a source for identification of bio-active agents that can be used in preparation of synthetic medicines. (Prescott et al, 2000; Wink, 1999)

The natural product industry in Europe and United states is equally interested in traditional medicine. In Europe and America where Phytomedicine industry is shriving, extracts from medicinal plants are sold in purified form for the treatment and prevention of all kinds of diseases. We are at the stage where traditional medicine is considered more for its capacity to generate other medicines than for its own sake. In many cases research undertakings and the commercial use stemming from that research have always relied on the information provided by the local communities that, in many cases, have hardly benefited from the research results (Schulz et al, 1998; Aranya et al, 2006).

Modern health care has never been and probably never will be adequately and equitably provided anywhere in Africa, due to financial limitations related to rapid population growth, political instability and poor economic reform, to mention but a few. Hence, the majority of the people lack access to health care and even where it is available, the quantity is largely below acceptable levels. The situation is further exacerbated by severe financial constraints, the high debt burden, instability, high inflation rates, declining real income and deterioration of growth rates. (Tyler, 1999)

Phytomedicine is the use of plants and plant materials for human and animal medicines. Plants have always been a source of important medicines since the time immemorial and there is the need for better understanding of biological effects of medicinal plants in-vivo before formulation into dosage. These activities involve biological testing of plant extracts and the isolation of their active components and ultimately, their clinical

validations. This focuses on well defined plant extracts which are used for specific illnesses. (Kaufman et al, 1999)
For many medicinal plants including *V.amygdalina*, current interest is primarily focused on phytochemistry, pharmacognosy and horticulture as well as characterization, separation and subjection of their possible bioactive compounds to detailed structural analysis. (Brinskin, 2000)

Several species of *Vernonia*, including *V. calvoana*, *V. amygdalina*, and *V. colorata*, are eaten as leaf vegetables. Common names for these species include **bitterleaf**, **ewuro**, **ndole** and **onugbu**. They are common in most West African and Central African countries. They are one of the most widely consumed leaf vegetables in Cameroon, where they are key ingredients of ndole stew. The leaves have a sweet and bitter taste. They are sold fresh or dried, and are a typical ingredient in egusi soup.

In North America, of the 17 species of Vernonia (e.g., *V. altissima*, *V. fasciculata*, *V. flaccidifolia*) all have the same effective properties as a blood purifier and uterus one, containing sesquiterpene lactones, which helps also to prevent atheroscelosis.

*V. galamensis* is used as an oilseed in East Africa. It is grown in many parts of Ethiopia, especially around the city of Harar, with an average seed yield of 2 to 2.5 ton per ha. It is reported that the Ethiopian strains of *Vernonia* have the highest oil content, up to 41.9% with up to 80% vernolic acid, and is used in paint formulations, coatings plasticizers, and as a reagent for many industrial chemicals.

*Vernonia* species are used as food plants by the larvae of some Lepidoptera species including *Coleophora vernoniaeella* (which feeds exclusively on the genus) and *Shinia regia* (which feeds exclusively on *V. texana*).

*V. amygdalina* is well known as a medicinal plant with several uses attributed to it, including for diabetes, fever reduction, and recently a non-pharmaceutical solution to persistent fever, headache, and joint pain associated with AIDS (an infusion of the plant is taken as needed). The roots of *V. amygdalina* have been used for gingivitis and toothache due to its proven antimicrobial activity

## Scientific classification

| | |
|---|---|
| Kingdom: | Plantae |
| phylum: | Angiosperms |
| subphylum: | Eudicots |
| class: | Asterids |
| Order: | Asterales |
| Family: | Asteraceae |
| Genus: | *Vernonia* |
| Species: | ***V. amygdalina*** |

## Binomial name

***Vernonia amygdalina***

Delile

***Vernonia amygdalina***, a member of the Asteraceae family, is a small shrub or a small tree up to 5meters high that grows in the tropical Africa. The leaves measure on average 5x15 cm are simple and entire (or minutely toothed) obovate-oblanceolate, finely glandular below and displaying few lateral nerves. The flowers occur in copious corym-base panicles, white, fragrant, and usually bee-infested.*V. amygdalina* is commonly called bitter leaf because of its bitter taste. The leaves may be consumed either as a vegetable (macerated leaves in soups) or aqueous extracts as tonics for the treatment of various illnesses. In the wild, chimpanzees have been observed to instinctively ingest the leaves when suffering from parasitic infections. Many herbalists and naturopathic doctors recommend aqueous extracts for their patients as treatment for emesis, nausea, diabetes, loss of appetite-induced abrosia

The leaves are reputed to be effective remedies for gastro-intestinal disorders, and as a general tonic. An aqueous decoction of the leaves has been used for the treatment of fevers. The dried leaves are chewed for the same purpose, and by pregnant women to check nausea. The fresh leaves are however, believed to be arbotifacient. The bitterness of the leaves is usually reduced by boiling, and by soaking in water followed by several washings with fresh water. The peeled stem is used for cleaning the teeth and the bark is administered for venereal diseases and diarrhea. The plant is added in very minute quantities in several other remedies, but it is not clear whether it is used to impart a bitter taste to the medication or included for therapeutic purposes. The leaves are ingredients in purgative enemas, diuretic mixtures, antihelmintic preparations and topical lotions for parasitic skin diseases. The bitterness of the leaves is often exploited by nursing mothers to assist in weaning by rubbing the juice on their breasts. Soup preparation with the washed leaves is believed to improve lactation.

*Vernonia amygdalina* extracts may help suppress, delay, or kill cancerous cell in many ways, such as:

- Induction of apoptosis as determined in cell culture and animal studies
- Enhanced Chemotherapy Sensitivity - *V. amygdalina* extracts may render cancerous cells to be more sensitive to chemotherapy.
- Inhibition of the growth or growth signals of cancerous cells.

- Suppression of metastasis of cancerous cells in the body by the inhibition of NFκB is an anti-apoptotic transcription factors as demonstrated in animal studies.
- Reduction of oestrogen level in the body by the suppression of Aromatase activity. The Involvement of blood estrogen level in the etiology of estrogen receptor (ER) positive breast cancer has been widely reported. Additional source of estrogen production in humans besides the ovary and adrenal gland is the conversion of testosterone to estrogen in a reaction catalyzed by Aromatase. Many studies have shown positive correlations between blood estrogen levels and breast cancer risks. Therefore, compounds that inhibit Aromatase activity are used for the treatment of breast cancer.
- Antioxidants - *V. amygdalina* may provide anti-oxidant benefits.
- Enhancement of the immune system - Many studies have shown that *V. amygdalina* extracts may strengthen the immune system through many cytokines (including NFκB, pro inflammatory molecule) regulation.

Studies conducted using streptozosin-induced diabetic laboratory animals showed that *V. amygdalina* administration decreased blood glucose by 50% compared to untreated diabetic animals.

## 1.2 PROBLEM STATEMENT

Herbalists and ethnics use crude leaf extract without knowing the constituents of the plant. There is also, limited information that can be provided by trained medical personnel in this perspective. As a consequence, the use of these herbal products lack the required standards due to undefined quantities of phytochemicals (bioactive or non-bioactive) and absence of quantitative data to assist in regulation of product quality.

## 1.3 PURPOSE OF STUDY

The purpose of this study is to identify phytochemicals present in the extract of *V. amygdalina* leaf and to quantitatively evaluate the leaf aqueous extract with respect to the extractive values and some identified phytochemicals present in the crude aqueous extract (used by natives for malaria therapy). This will assist in defining the herbal product both in providing information and for regulatory purposes.

## 1.4 OBJECTIVES

Specific objectives:

To identify *V. amygdalina* plant and collect the leaves

To dry and powder the leaves

To extract the powdered leaves using aqueous solvent

To extract the powdered leaves using ethanol

To screen the aqueous and alcoholic extracts for phytochemicals

To determine the extractive values of the powdered leaves using different solvents

To quantify some identified phytochemicals in the crude aqueous extract of the powdered leaves.

## 1.5 JUSTIFICATION OF STUDY

- The study will be a contribution to the pool of information available to researchers and health professionals with regards to the properties and magnitude of certain constituents of the preparation.
- Due to the need for regulation, provided data can serve as control tools for the quality of herbal preparation.
- Policy which is the enforcement pillar for regulation will be ably facilitated by the contribution of this study as well as, similar studies.
- Education of end users as well as traditional healers will be made much easier using information from this study.

# CHAPTER TWO: LITERATURE REVIEW

## 2.0 GENERAL LITERATURE

Phytochemical screening of *V.amygdalina* revealed the presence of saponins, sesquiterpene, and flavonoids (Igile et al 1994). Strong antioxidant activities have been reported for flavonoids from *V.amygdalina* and, its saponins have been reported to elicit antitumoral activities in leukemia cells (Jisaka et al 1993). Peptides from *V.amygdalina* are known to be potent inhibitor of mitogen-activated proteins kinases, which are crucial for breast tumor growth and also represents a key regulatory point for the tumour (Izeybigie 2003; Izeybigie and Ernest 2005). However, there is dearth of scientific data to support the folkloric use of this plant in the treatment of hypertension or related vascular diseases in Nigerian herbal homes.

Phytochemical screening of the plant showed the presence of flavonoids, terpenoids, saponins, tannins and reducing sugars.

Several stigmastene-type saponins such as vernoniosides A1, B1, A2, A3, B2, D2, A4 and C have been identified in the leaves (Ohigashi et al, 1991; Jisaka et al, 1992; Kamperdick et al, 1992). Philipson et al 1993 reported the antiplasmodial effects of some sesquiterpene and steroidal constituents of V.a and some were also active against *plasmodium falciparum* in vitro.

The antioxidant activities of luteolin, luteolin 7-O-beta-glucuroniside and luteum 7-O-beta-glucoside, flavonoid compounds isolated from the leaves of V.a have been reported using coupled oxidation of beta-carotene linoleic acid (Igile et al, 1994).

Flavonoids occurring in leaves of V.a were identified by chemical and spectroscopic techniques. The most abundant compound was luteolin-7-O-beta-glucuronoside. The antioxidant activity of the 3 flavones was determined by measuring the coupled oxidation of beta-carotene and linoleic acid. It was shown that luteolin was significantly a more potent antioxidant that the synthetic antioxidants BHT at the same concentrate (15mg/ml). The two glycosides showed similar activities but significantly lower activities than luteolin or BHT.

According to the journal of natural products, several secondary metabolites have been isolated from the leaves of V.a, including antimalarial and insect antifeedant

sesquiterpene lactones (1,3,4), bitter and non-bitter stigmastane-type glycosides (5-10), and flavones (11)

In a continuation of the investigation into the bioactive constituents of *V.amygdalina*, isolation and chemical characterization to two new stigmastane-type steroidal glycosides, as well as vernoniosides A3 were reported previously by Jisaka et al.

The three compounds isolated from the MeOH extract of *V.amygdalina*, comprised the glycosides, 1, 2 and vernoniosides A3. Glucose was the only sugar identified by TLC after enzymatic hydrolysis of each glycoside. All three substances were shown to contain hexose unit by Fabms, which was identified as beta-D-glucopyranoside by h1-and131C-NMR data.

NMR data led to the identification of the aglycone moiety of these compounds as a C-29 stigmastane-type sterol.

According to Biolin International official site in African Journal of Biotechnology vol.2, No.12, Dec, 2003 pp.662-671

Earlier investigations on *V.amygdalina* showed that purified chloroform fractions identified as vernodaline, vernolide and vermomygdine elicited cytotoxic effects in human carcinoma narsopharynx cells with IC50 values of 1.8, 2.0 and 1.5 micrograms per ml respectively (Kupchan et al, 1969). It was concluded that the activities were dependant on their possessions of the alpha-methylyl-lactone group as part of their structures.

Subsequently Jisaka et al, 1993 demonstrated that vernodaline elicited antitumoural activities in leukemia cells pp 388 and C-1210 with IC50 values of 0.11 and 0.17 micrograms/ml for vernodaline and 0.131 and 0.11 micrograms for vernolide respectively. Recently Irevbigie (2003) isolated some peptides (edotides) from the aqueous extract of *V.amygdalina*. The peptides were shown to be potent inhibitor of MPKS which are crucial for breast tumour growth and also represent a key regulatory point for tumour growth. The antiestrogen breast cancer drug (tamoxifen) has also been shown to modulate MAPK activity (Atanascov et al, 2002; Mandlekar and Kong, 2001). This indicates that edotides from V.a may be considered as alternative to tamoxifen.

Furthermore extracts from *V.amygdalina* have also been suggested to have cell growth inhibitory effects in prostate cancer cell line (PC-3) and no effect on normal human peripheral blood mononuclear cells (Izevbigie, 2003).

As part of a continuing study of the Nigerian flora a chemical analysis of the flavonoids of some plants of the family papilionaceae prominent in traditional medicine was undertaken. The flowers of the selected plants were extracted and the flavonoids were detected and identified by standard methods. In any such biochemical/chemotaxonomic study where a sizeable number of plants are analysed and in which small differences in type and quantity of each sample are critical, a fast and accurate method of determining the composition becomes paramount. In this work, the determination of the concentration of each flavonoid as a factor of absorbance on a simple single cell photoelectric colorimeter (Seagull Electric Institute Model-1) is reported. The results obtained for*Lonchocarpus cyanescens* genus Lonchocar pus were consistently satisfactory, sensitive and economical compared with the standard thin layer procedure. As far as the authors know, this is the first report of this method in flavonoid work.

## 2.1 EXTRACTIVE VALUES

The determination of water-soluble or ethanol-soluble extractive is used as a means of evaluating drugs the constituents of which are not readily estimated by other means. In certain cases extraction of the drug is by maceration, in others by a continuous extraction.

In maceration, the powdered crude drug is left to soak in the solvent, so that cellular substances dissolve. The material is shaken periodically during 2-14 days in a hermetically closed flask. Then the liquid is filtered pressing the crude drug through a cloth and washing the residue with a small volume of the filtrate.

Solid-liquid extraction where a soluble constituent is extracted from a solid by the means of a solvent (Trease and Evans, 1987) (c)

## 2.2 TANNINS

This is a class of products that give rise to the astrigency and bitterness in plants and food. It comprises of water-soluble polyphenolic compounds, which may have a high molecular weight. They are broadly divided into hydrolysable tannins which are formed by esterification of sugars simple phenolic acids that are shikimate derived, and non-hydrolysable tannins or condensed tannins which occur due to polymerisation reactions between flavonoids.The hydrolysable tannins may be hydrolysed with base to simple

acids and sugars. Tannins can bind to proteins hence used to tan leather, clarify beer and as astringent preparations in pharmacy.

Tannins are also used traditionally, internally for the protection of inflamed surfaces of mouth and throat. They act as antidiarrheals and have been used as antidotes in poisoning by heavy metals, alkaloids and glycosides. Recent studies have concentrated on the antitumour activity of tannins and it has been shown that, to exhibit a strong activity, ellagitannin monomer units having galloyl groups at O-2 and O-3 positions on the glucose core, as in tellimagrandins are required. Anti-HIV activity has also been demonstrated (Heinrich, 2004)

## 2.3 SAPONINS

Plant materials containing saponins have long been used in many parts of the world for their detergent properties like the root of *Saponaria officinalis* in Europe and bark of *Quillaia saponaria* in South America. Such plants contain a high percentage of glycosides known as saponins which are characterized by their property of producing a froathing aqueous solution. They also have haemolytic properties and when injected into the blood stream, are highly toxic. As glycosides they are hydrolysed by acids to give an aglycone and various sugars and related uronic acids. According to the structure of the aglycone, two kinds of saponin are recognised-the steroidal saponins and the pentacyclic triterpenoid types.Steroidal saponins are less widely distributed in nature than the pentacyclic triterpenoid type. Steroidal saponins are of great importance owing to their relationship with such compounds as sex hormones, cortisone, diuretic steroids, vitamin D and cardiac glycosides. Some are starting materials for the synthesis of these compounds (Heinrich, 2004)

## 2.4 ALKALOIDS

Alkaloid (alkali-like). Typical alkaloids are derived from plant sources, they are basic, contain one or more nitrogen atoms and have a marked physiological action on man or other animals. In the plant alkaloids may exist in the free state, as salts or as amine or alkaloid N-oxides. Not only are alkaloidal substances often administered in solution, but also the differences in solubility between alkaloids and their salts provide methods for the isolation of alkaloids from the plant and their separation from the non-alkaloidal

substances also present. Free bases are frequently sparingly soluble in water but soluble in organic solvents; with salts the reverse is often the case, these being usually soluble in water but sparingly soluble in organic solvents.

Alkaloids are divided into many classes;

- Pyridine,piperidine and pyrrolidine alkaloids
- Phenylalkylamine alkaloids
- Quinoline alkaloids
- Isoquinoline alkaloids
- Indole alkaloids
- Tropane alkaloids
- Xanthine alkaloids
- Imidazole alkaloids

Generally, alkaloids are used widely in humans that is to say; Nicotine is formulated into chewing gum as an aid to cessation of smoking in products such as Nicorette, they alleviate fatigue, Castanopermine from *Castanospernum australe* inhibits alpha-glucosidase enzyme involved in glycoprotein processing, which is important in the formation of viral coating, abnormalities of which stop infection of white blood cells in HIV. Ephedra treats colds, asthma and other bronchial conditions; cactus contains Peyote which induces vivid dreams and hallucinations. Colchicine treats gout; Cinchona contains quinine which treats malaria and quinidine which treats type 1 cardiac arrhythmias. Opium contains morphine which is an excellent analgesic and a smooth muscle relaxant. Codeine suppresses cough while thebain is included in veterinary sedatives like etorphine.Papaverine is an antispasmodic and also treats male impotence while emetine is an expectorant. Ergometrine expels placenta after birth and also increases contractions and relieves migraine.

In plants, alkaloids are thought to help in development especially during grafting and also participate in metabolic processes. They are thought to have a role in defence of plant against singlet oxygen which is damaging to all living organisms and is produced in plant tissues in the presence of light (Heinrich, 2004)

## 2.5 FLAVONOIDS

The flavonoids are derived from a C6-C3 (phenylpropane) unit which has as its source shikimic acid and a C6 unit derived from the polyketide pathway. Flavonols may further be oxidised to yield anthocyanins which contribute to the brilliant blues of flowers and the dark colour of red wine. It is likely that these compounds have high ecological importance in nature as colour attractants to insects and birds as an aid to plant pollination.

Flavonoids might have important dietary significance because, being phenolic compounds, they are strongly antioxidant hence can ameliorate cancer and heart disease. The glycosides are generally soluble in water and alcohol, but insoluble in organic solvents. They dissolve in alkalis giving yellow solutions which on addition of acid become colourless. The flavonoids act as anti-inflammatory and anti-allergic. They have antithrombotic and vasoprotective properties, for inhibition of tumour promotion and as a protective for the gastric mucosa. These effects have been attributed to the influence of flavonoids on arachidonic acid metabolism. Many flavonoid-containing plants are diuretic. Some have antitumour, antibacterial or antifungal properties (Heinrich, 2004)

## 2.6 TRITERPENOIDS

These are C-30-derived terpenoids with an exceptionally wide distribution including man, bacteria, soft corals and amphibia. The triterpenoids include steroids-degraded triterpenoids which are used as sex hormones. Others are sterols. They are components of resins and resinous exudates from plants. Resins act as physical barriers to attack by fungi and bacteria after tree damage.

Terpenoid compounds of these plants have high antimicrobial activity oxidation of triterpenoids gives cholesterol which is a component of cell membranes and gallstones in animals. Also anti-inflammatory drugs contain products of triterpenoid breakdown-hydrocortisone; oral contraceptive norethisterone is from triterpenoids. Horse chestnut treats gastric ulcers (Heinrich, 2004)

# CHAPTER THREE: METHODOLOGY

## 3.1: SETTING OF STUDY

The study was carried out at Kampala international university, western campus in Ishaka, Uganda.

## 3.2: PLANT IDENTIFICATION AND COLLECTION

The plant was collected in Ishaka after identification of the leaves by Dr. Byarugaba Dominic. The fresh *Vernonia amygdalina* leaves were collected during the mid-morning hours from plants which had flowers.

## 3.3 EXTRACTION

Extraction was conducted using the method of the natives as adopted by Anoka et al (2008).

### 3.3.1: Materials

Leaf powder, metallic mortar and pestle, triple beam balance, distilled water, Whatman no. 1 filter paper, laboratory rotator ( Digisystem, Digisystem Laboratory Instruments Inc model, DSR-2800A)

### 3.3.2: Procedure

The leaves were shade dried (for a week) and then pounded to fine powder using a metallic mortar and pestle. 50g of the powder were weighed using a weighing balance (triple beam balance 700/800 series) and 500ml of warm distilled water (at $80^0$C) were added and then rotated continuously for 12 hours using a laboratory rotator at 110 rates per minute. This was left to stand for 24 hours and then filtered using small size filter paper. The filtrate was dried in the hot air oven at $70^0$C.

The above procedure was repeated using ethanol as solvent instead of water.

## 3.4: PHYTOCHEMICAL SCREENING (TREASE AND EVANS, 1987) a

### 3.4.1: Tannins

#### 3.4.1.1: Materials

Test tube, spatula, distilled water, water bath, Whatman No. 1 filter paper, 0.1% ferric chloride.

#### 3.4.1.2: Procedure

About 0.2g of the extract was mixed with water and heated on a water bath. The mixture was filtered and a few drops of 0.1% ferric chloride will be added to the filtrate.

### 3.4.2: Phlobatannins

#### 3.4.2.1: Materials

Test tube, spatula, distilled water, water bath, Whatman No. 1 filter paper, 2% hydrochloric acid solution.

#### 3.4.2.2: Procedure

0.5g of the extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% hydrochloric acid solution.

### 3.4.3: Saponins

#### 3.4.3.1: Materials

Test tube, spatula, distilled water, water bath.

#### 3.4.3.2: Procedure

0.2g of the extract was shaken with 5ml of distilled water and then heated to boil.

### 3.4.4: Flavonoids

#### 3.4.4.1: Materials

Test tube, spatula, distilled water, dilute NaOH, HCl

#### 3.4.4.2: Procedure

0.2g of the extract was dissolved in dilute NaOH and HCl added.

### 3.4.5: Steroids

#### 3.4.5.1: Materials

Test tube, spatula, distilled water, acetic anhydride, sulphuric acid.

#### 3.4.5.2: Procedure

2ml of acetic anhydride was added to 0.5g of the extract with 2ml $H_2SO_4$.

### 3.4.6: Terpenoids

#### 3.4.6.1: Materials

Test tube, spatula, chloroform, concentrated sulphuric acid.

#### 3.4.6.2: Procedure

0.5g of sample was dissolved in 1ml chloroform and 1ml concentrated $H_2SO_4$ carefully added to form a layer.

### 3.4.7: Cardiac glycosides

#### 3.4.7.1: Materials

Test tube, spatula, glacial acetic acid, ferric chloride solution, concentrated sulphuric acid.

#### 3.4.7.2: Procedure

0.5g of the extract was treated with 2ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was underplayed with 1 ml of concentrated $H_2SO_4$.

### 3.4.8: Reducing sugars

#### 3.4.8.1: Materials

Test tube, spatula, distilled water, Fehling's solution A and B, water bath.

#### 3.4.8.2: Procedure

0.5g of the extract was shaken with distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for 2 minutes.

### 3.4.9: Alkaloids

#### 3.4.9.1: Materials

Test tube, spatula, distilled water, Mayer's reagent, and Wagner's reagent.

#### 3.4.9.2: Procedure

0.5g of the extract was dissolved in distilled water and Mayer's reagent added.

Same was done but a few drops of Wagner's reagent was added instead of Mayer's reagent.

## 3.5: DETERMINATION OF EXTRACTIVE VALUES (Kokate, 2000) a:

### 3.5.1: Alcoholic Extractive Value Determination

#### 3.5.1.1: Materials

Leaf powder, 200ml beakers (5), 90% ethanol, parafilm, aluminium foil, laboratory rotator, evaporating dish, sensitive weighing balance, filter paper, hot air oven, triple-beam balance.

#### 3.5.1.2: Procedure

5g of powder was weighed and transferred into a 200ml beaker and macerated with100ml of 90% ethanol and covered with parafilm and aluminium foil and then shaken using a laboratory rotator at 100 rates per minute for 6 hours. The maceration was left to stand until the 24 hours elapsed. The maceration was filtered using small size Whitman No. 1 filter paper. 25ml of the alcoholic filtrate was put in previously weighed evaporating dish and dried at $105^0$C. The weight of the dish and the dried extract after completing drying was obtained. The difference in weight gave the mass of the extract in 25ml. The weight of the extract in 100ml was calculated. The experiment was carried out five times simultaneously.

### 3.5.2: Aqueous extractive value Determination

#### 3.5.2.1: Materials

Leaf powder, 200ml beakers (5), chloroform, distilled water, parafilm, aluminium foil, laboratory rotator, evaporating dish, sensitive weighing balance, hot air oven, filter paper

#### 3.5.2.2: Procedure

Chloroform water BPC was prepared and used in place of alcohol as below.

5g of powder was weighed and transferred into a 200ml beaker and macerated with100ml of chloroform water and covered with parafilm and aluminium foil and then shaken using a laboratory rotator at 100 rates per minute for 6 hours. The maceration was left to stand until the 24 hours elapsed. The maceration was filtered using small size Whitman No. 1 filter paper. 25ml of the aqueous filtrate was put in previously weighed evaporating dish and dried at $105^0$C. The weight of the dish and the dried extract after

completing drying was obtained. The difference in weight gave the mass of the extract in 25ml. The weight of the extract in 100ml was calculated. The experiment was carried out five times simultaneously.

### 3.5.3: Chloroform extractive value determination

#### 3.5.3.1: Materials

Leaf powder, 200ml beakers (5), chloroform parafilm, aluminium foil, laboratory rotator, evaporating dish, sensitive weighing balance, hot air oven, filter paper.

#### 3.5.3.2: Procedure

5g of powder was weighed and transferred into a 200ml beaker and macerated with100ml of pure chloroform and covered with parafilm and aluminium foil and then shaken using a laboratory rotator at 100 rates per minute for 6 hours. The maceration was left to stand until the 24 hours elapsed. The maceration was filtered using small size Whitman No. 1 filter paper. 25ml of the chloroform filtrate was put in previously weighed evaporating dish and dried at $105^0$C. The weight of the dish and the dried extract after completing drying was obtained. The difference in weight gave the mass of the extract in 25ml. The weight of the extract in 100ml was calculated. The experiment was carried out five times simultaneously.

## 3.6: PHYTOCHEMICAL QUANTIFICATION

### 3.6.1: Quantification of total tannins (Kokate, 2000) b

#### 3.6.1.1: Materials

Spatula, 200ml beakers (5), Sensitive weighing balance, 100ml measuring cylinder, Laboratory rotator, Whatman no 1 filter papers (5), Hot air oven, Distilled water, Petroleum ether, Separatory funnels (5), Potassium dichromate solution (7%), leaf powder.

#### 3.6.1.2 Procedure

10g of the herbal sample was weighed in a 200ml beaker using a sensitive weighing balance and macerated with 100ml of distilled water and then rotated on a laboratory rotator for 20hours and left to stand until the 24 hours will elapse. The maceration was filtered using small size Whatman No.1 filter paper. The filtrate was dried in the hot air

oven at $70^0$C and the extract weighed. The weighed extract was dissolved with minimum amount 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 $80^0$C. The solid was weighed to determine the weight of total tannins. The procedure was done 5 times simultaneously.

### 3.6.2: Quantification of total alkaloids (Kokate, 2000) c

#### 3.6.2.1: Materials

Spatula, 1000ml beakers (10), Sensitive weighing balance, 100ml measuring cylinder, Laboratory rotator, Whatman no 1 filter papers (5), Hot air oven, Distilled water, Separatory funnels (5), 2% aqueous acetic acid, concentrated ammonia, chloroform, leaf powder.

#### 3.6.2.2: Procedure

100g of the plant powder was weighed using a sensitive weighing balance and macerated in 400ml 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 until the 24 hours elapsed. The maceration was filtered and the maceration washed through the filter paper with 2 successive portions of 100ml of distilled water. The filtrate and the filtered maceration washings were bulked together and the volume noted and transferred to a beaker of known weight and then evaporated to dryness at a temperature of $80^0$C.

The weight of the dry extract was determined using a sensitive balance and the extract redissolved 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 $75^0$C, filtered and the filtrate basified with excess ammonia (concentrated) 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 were collected and combined in a beaker of known weight and evaporated to dryness in a hot air oven at $75^0$C. The weight of the gummy extract was determined using a sensitive balance. This was done 5 times simultaneously.

### 3.6.3: Quantification of total Saponins (Obadoni and Ochuko, 2000)

#### 3.6.3.1: Materials

Spatula, 200ml beakers (5), Sensitive weighing balance, 100ml measuring cylinder(3), Laboratory rotator, Whatman no 1 filter papers (5), Hot air oven, Distilled water, Separatory funnels (5), 5% sodium chloride solution, n-butanol, diethyl ether, leaf powder.

#### 3.6.3.2: Procedure

20g of the plant powder was weighed in a 200ml beaker and macerated with 200ml of distilled water and rotated on a laboratory rotator for 15 hours and was left to stand until the 24 hours elapsed. The maceration was filtered using Whatman No.1 small size filter paper and the filtrate reduced to 40ml over a water bath at about $90^0$C. The concentrate was transferred into a 250ml separatory funnel. 20ml of diethyl ether was added and shaken vigorously every 15 minute for 1 hour. The aqueous layer was recovered and 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 $70^0$C. The residue was weighed to determine the quantity of saponins present. The procedure was done 5 times simultaneously.

### 3.6.4: Quantification of total flavonoids (Harbone, 1998) a

#### 3.6.4.1: Materials

Spatula, 200ml beakers (5), Sensitive weighing balance, 100ml measuring cylinder, Laboratory rotator, Whatman no 1 filter papers (5), Hot air oven, Distilled water, Separatory funnels (5),ethyl acetate, amyl alcohol, leaf powder.

### 3.6.4.2: Procedure

20g of the plant powder were weighed using a sensitive balance and 200ml of distilled water were added. The mixture was shaken continuously for 12 hours on a laboratory rotator at a speed of 100 rates per minute and then left to stand until the 24 hours elapsed. 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 then determined. The extract was then be extracted twice with 20ml portions of ethyl acetate. The solution was then extracted with 10ml of amyl alcohol. The ethyl acetate shall be dried in a hot air oven at $70^0$C and weighed to find out the amount of flavonoids present. This was done 5 times simultaneously.

## 3.6.5: Quantification of total triterpenoids (Harbone, 1998) b

### 3.6.5.1: Materials

Spatula, 200ml beakers (5), Sensitive weighing balance, 100ml measuring cylinder, Laboratory rotator, Whatman no 1 filter papers (5), Hot air oven, Distilled water, Separatory funnels (5), leaf powder,2M sulphuric acid, chloroform.

### 3.6.5.2: Procedure

20g of the plant powder was weighed using a sensitive balance and 200ml of distilled water was added. The mixture was shaken continuously for 12 hours on a laboratory rotator at a speed of 100 rates per minute and then left to stand until the 24 hours shall elapse. 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 then determined. The extract was then redissolved in a minimum amount of distilled water and acidified with 2M $H_2SO_4$ and then extracted with 30ml of chloroform 3 times. The extract was dried in a hot air oven at a temperature of $70^0$C and weighed to get total phenolics. The quantity of triterpenoids was got by subtracting the quantity of flavonoids from the answer. This was done 5 times simultaneously.

# CHAPTER FOUR: RESULTS AND DISCUSSION

## 4.10: RESULTS

**TABLE 1**

### 4.1.1: PHYTOCHEMICAL SCREENING

| PHYTOCHEMICAL | ALCOHOLIC EXTRACT | AQUEOUS EXTRACT |
|---|---|---|
| Tannins | + | + |
| Phlobatannins | + | + |
| Saponins | + | + |
| Flavonoids | + | + |
| Steroids | + | – |
| Terpenoids | + | + |
| Cardiac glycosides | + | + |
| Reducing sugars | – | + |
| Alkaloids | + | + |

Note: - means absence

+ means presence

**TABLE 2**

### 4.1.2: EXTRACTIVE VALUES

| SERIAL NUMBER | TYPE OF EXTRACTIVE VALUE | EXTRACTIVE VALUE (%W/W) |
|---|---|---|
| 1 | ETHANOLIC | 12.651± 0.30 |
| 2 | AQUEOUS | 24.781± 0.77 |
| 3 | CHLOROFORM | 4.835 ± 0.34 |

Refer to tables $E_V1$, $E_V2$, $E_V3$ in appendix 1

TABLE 3

## 4.1.3: PHYTOCHEMICAL QUANTIFICATION

| SERIAL NUMBER | PHYTOCHEMICAL | %W/W CONTENT IN AQUEOUS EXTRACT |
|---|---|---|
| 1 | Tannins | 14.2 ± 2.20 |
| 2 | Alkaloids | 2.48 ± 0.12 |
| 3 | Saponins | 5.79 ± 0.05 |
| 4 | Flavonoids | 54.30 ± 4.60 |
| 5 | Triterpenoids | 29.45 ± 6.90 |

Refer to tables for tannins, alkaloids, Saponins, Flavonoids, Triterpenoids in appendix 2

# 4.2: DISCUSSION

## 4.2.1: PHYTOCHEMICAL SCREENING

Phytochemical screening of the plant showed the presence of steroids, flavonoids, tannins, triterpenes etc. The leaf is known to exhibit medicinal activity as well as physiological activity (journal of natural products). The result of the screening showed the presence of steroids in the alcoholic extract but not in the aqueous extract suggesting, the absence of this phytochemical in aqueous preparation of the natives. This is understandable, considering the insolubility of sterols in aqueous medium. Reducing sugars were found in the aqueous extract and not in the alcoholic extract because the sugars are soluble in water (hydrophilic nature) but insoluble in organic solvents such as alcohol. The presence of flavonoids and tannins which is known to have antibacterial and antifungal activities (Jisaka et al 1993) supports the use by natives for microbial implicated infectious diseases.

The leaf aqueous extract exhibited the presence of saponins, which explains the foaming and emulsifying nature (Sun et al., 2008) of the extract. Alkaloids, cardiac glycosides and triterpenoids were also, among the identified phytochemicals.

## 4.2.2: EXTRACTIVE VALUES

Aqueous extractive value is 24%w/w, alcoholic is12%w/w and chloroform is 4%w/w. This means that there are more aqueous soluble constituents in the leaves than substances soluble in organic solvents.

Extractive value though highly variable, has found use in the control of organic adulteration in crude (herbal) drug regulation (Trease and Evans, 2005).

## 4.2.3: QUANTIFICATION

The obtained data showed the plant leave aqueous extract contains 14.2%W/W of tannins. This concentration is quite high and hence suggests that this could be a ready source for tannins to the lather tanning industry and beer factories for hop alternative (Heinrich, 2004). The plant can be used to make tablets, a combination with activated charcoal which can be an antidote of heavy metals, alkaloids and glycoside poisoning, because of the constituent of tannins.

The aqueous extract contains 5.755%W/W as saponins which supports the study by Heinrich, 2004, in the use of the herb as detergent by natives.

The aqueous extract of the plant leaves also contains 2.48%W/W alkaloids, 54%W/W flavonoids and 29.5%W/W triterpenoids. Such estimated quantities as in this study is a relevant contribution to the defining of actual quantity of constituents, in the eventual dosing system of the herbal product.

## 4.3: LIMITATIONS

- There is limited available equipment and reagents for quantification of phytochemicals hence, some phytochemicals were not quantified.
- Lack of reference materials (text books and internet facility).
- Limited time to pursue the study beyond the present scope.

### 4.4: CONCLUSION

From the obtained data the use of *V. amygdalina* as a herbal remedy can be maximized. The presence of the identified phytochemicals can assist in identifying or projecting possible interactions. The obtained quantitative values could serve as a contributory tool in quality monitoring of the herbal product as used with in the locality. Based on these facts, substantial information can be provided for data compilation in eventual herbal pharmacopoeia, for crude drugs.

### 4.5: RECOMMENDATIONS

- Other quantitative estimations of other phytochemicals should be done to enable possible standardization of dosing.
- Further study should be done on the bioactivity or non-bioactivity on some of the identified phytochemicals.
- Other quantitative evaluations such as ash value establishment to support quality monitoring of product.

# CHAPTER FIVE

## 5.0 REFERENCES


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# APPENDIX 1

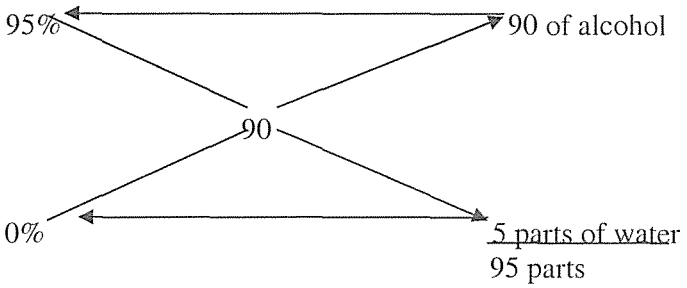
## 1 .1 EXTRACTIVE VALUES

### 1.1.1 ALCOHOLIC EXTRACTIVE VALUES

Preparation of 90% alcohol

Alcohol available = 95% v/v

Using allegation media;




Volume of alcohol needed =520ml

Volume of concentrated alcohol=$\frac{90}{95}$x520

=493ml

Diluted up to 520ml with distilled water

**A1:**

25ml =0.1594g

100ml=$\frac{0.1594 \times 100}{25}$

=0.6376g

5g =0.6376g

100g =$\frac{0.6376 \times 100}{5}$

=12.752%w/w

**A2:**

25ml =0.1508g

100ml $= \frac{0.1508 \times 100}{25}$

$= 0.6032g$

5g $= 0.6032g$

100g $= \frac{0.6032 \times 100}{5}$

$= 12.064\%w/w$

**A3:**

25ml $= 0.1600g$

100ml $= \frac{0.1600 \times 100}{25}$ g

$= 0.64g$

5g of powder $= 0.64g$ of extract

100g of powder $= \frac{0.64 \times 100}{5}$ g of extract

$= 12.80\%w/w$

**A4:**

25ml $= 0.1590g$

100ml $= \frac{0.1590 \times 100}{25}$ g

$= 0.6360g$

5g of powder $= 0.6360g$ of extract

100g of powder $= \frac{0.6360 \times 100}{5}$ g of extract

$= 12.72\%w/w$

**A5:**

25ml $= 0.1615g$

100ml =0.1615x100 g
25
=0.6460g

5g of powder =0.6460g of extract

100g of powder = 0.6460x100 g of extract
5
=12.92%w/w

**Average value** = 12.725+12.064+12.80+12.72+12.92
5
=12.65%w/w

**TABLE $E_V1$**

| SERIAL NUMBER | WT OF EMPTY DISH (g) | WT OF DISH + EXTRACT (g) | WT OF EXTRACT (g) | EXTRACTIVE VALUE (%W/W) | DEVIATION (g) |
|---|---|---|---|---|---|
| A1 | 48.1792 | 48.3386 | 0.5158 | 12.064 | 0.10 |
| A2 | 44.9838 | 45.1346 | 0.5158 | 12.064 | 0.58 |
| A3 | 48.1801 | 48.3401 | 0.1600 | 12.800 | 0.15 |
| A4 | 73.2500 | 73.4090 | 0.1590 | 12.720 | 0.07 |
| A5 | 29.4830 | 29.6445 | 0.1615 | 12.920 | 0.27 |
| AVERAGE | | | | **12.514±0.30** | **1.17** |

### 5.1.1.2 AQUEOUS EXTRACTIVE VALUES

Preparation of chloroform water

From the pharmaceutical codex;

Chloroform water:

Chloroform----------2.5ml

Purified water----------to 1000ml

Shake frequently until solution is effected

1000ml of solution=2.5ml of chloroform

1ml of solution =2.5/1000ml of chloroform

100ml of solution =$\frac{2.5x100}{1000}$ ml of chloroform

=0.25ml of chloroform

Volume of solution needed=520ml

100ml =0.25ml of chloroform

520ml =$\frac{0.25x520}{100}$ ml of chloroform

=1.3ml of chloroform

Therefore 1.3ml of chloroform was drawn and distilled water added up to the 520ml mark.

**B1**

25ml =0.292g

100ml =$\frac{0.292x100}{25}$ g

=1.168g

5g of powder =1.168g of extract

100 of powder =$\frac{1.168x100}{5}$ g of extract

=23.36%w/w

**B2**:

25ml =0.3096g

100ml =$\frac{0.3096x100}{25}$ g

=1.2384g

5g of powder =1.2384g of extract

100g of powder =$\frac{1.2384x100}{5}$ g of extract

=24.77%w/w

**B3:**

25ml =0.3208g

100ml =$\frac{0.3208 \times 100}{25}$ g

=1.2832g

5g of powder =1.2832g of extract

100g of powder =$\frac{1.2832 \times 100}{5}$ g of extract

=25.66%w/w

**B4**:

25ml =0.3119g

100ml =$\frac{0.3119 \times 100}{25}$ g

=1.2476g

5g of powder =1.2476g of extract

100g of powder =$\frac{1.2476 \times 100}{5}$ g of extract

=24.95%w/w

**B5**:

25ml =0.3145g

100ml =$\frac{0.3145 \times 100}{25}$ g

=1.2580g

5g of powder =1.2580g of extract

100g of powder =$\frac{1.2580 \times 100}{5}$ g of extract

=25.16%w/w

**Average value** =$\frac{25.16+24.95+25.66+24.77+23.36}{5}$

=24.78%w/w

**TABLE $E_V2$**

| LABEL | WT OF EMPTY DISH (g) | WT OF DISH + EXTRACT (g) | WEIGHT OF EXTRACT (g) | EXTRACTIVE VALUE(%W/W) | DEVIATION (g) |
|---|---|---|---|---|---|
| B1 | 34.9479 | 35.2399 | 0.2920 | 23.360 | 1.41 |
| B2 | 32.0786 | 32.3882 | 0.3096 | 24.768 | 0.01 |
| B3 | 36.9583 | 37.2791 | 0.3208 | 25.664 | 0.88 |
| B4 | 30.1408 | 30.4536 | 0.3119 | 24.952 | 0.17 |
| B5 | 35.9085 | 36.2230 | 0.3145 | 25.160 | 0.38 |
| AVERAGE | | | | **24.781±0.77** | **2.85** |

### 1.1.3 CHLOROFORM EXTRACTIVE VALUES

**C1**:

25ml =0.0602g

100ml $= \frac{0.0602 \times 100}{25}$ g

$=0.2408$g

5g of powder =0.2408g of extract

100g of powder $= \frac{0.2408 \times 100}{5}$ g of extract

$=4.816$%w/w

**C2**:

25ml =0.0581g

100ml $= \frac{0.0581 \times 100}{25}$ g

$=0.2324$g

5g of powder =0.2324g of extract

100g of powder $= \frac{0.2324 \times 100}{5}$ g

$=4.648$%w/w

**C3**:

25ml =0.0576g

100ml =$\frac{0.0576 \times 100}{25}$ g

=0.2384g

5g of powder =0.2384g of extract

100 g of powder =$\frac{0.2384 \times 100}{5}$ g of extract

=4.768%w/w

**C4**:

25ml =0.0570g

100ml =$\frac{0.0570 \times 100}{25}$ g

=0.2280g

5g of powder =0.2280g of extract

100g of powder =$\frac{0.2280 \times 100}{5}$ g of extract

=4.560%w/w

**C5**:

25ml =0.0673g

100ml =$\frac{0.0673 \times 100}{25}$ g

=0.2692g

5g of powder =0.2692g of extract

100g of powder =$\frac{0.2692 \times 100}{5}$ g of extract

=5.3845w/w

**Average value** =$\frac{5.384+4.560+4.768+4.648+4.816}{5}$

=4.835%w/w

**TABLE $E_V3$**

| LABEL | WT OF EMPTY DISH (g) | WT OF DISH + EXTRACT (g) | WT OF EXTRACT (g) | EXTRACTIVE VALUE (%W/W) | DEVIATION (g) |
|---|---|---|---|---|---|
| C1 | 36.2323 | 36.2925 | 0.0602 | 4.816 | 0.19 |
| C2 | 31.1920 | 35.2501 | 0.0581 | 4.648 | 0.43 |
| C3 | 31.3958 | 31.4534 | 0.0576 | 4.768 | 0.06 |
| C4 | 35.6597 | 35.7087 | 0.0570 | 4.560 | 0.27 |
| C5 | 34.6003 | 34.6676 | 0.0673 | 5.384 | 0.55 |
| AVERAGE | | | | **4.835±0.34** | **1.49** |

# APPENDIX 2

## 2.0 QUANTIFICATION

## 2.1 QUANTIFICATION OF TOTAL TANNINS

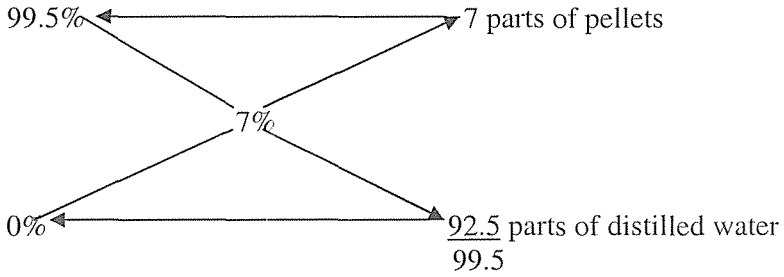
Preparation of strong potassium dichromate solution

From the pharmaceutical codex;

Potassium dichromate solution =7% solution

Salt ($K_2Cr_2O_7$) =99.5%

Using allegation media;




Weight of dichromate pellets=$\frac{7x100}{99.5}$ g

=7.035g

Distilled water was added up to 100ml mark.

**T1**:

1.18g of extract =0.15g of tannins

100g =$\frac{0.15x100}{1.18}$ g

=12.7%w/w

10g of powder =0.15g of tannins

100g =$\frac{0.15x100}{10}$ g

=1.5%w/w

**T2**:

1.216g of extract =0.212g of tannins

100g $= \frac{0.212 \times 100}{1.216}$ g

$= 17.4\%$w/w

10g of powder =0.212 g of tannins

100g $= \frac{0.212 \times 100}{10}$ g

$= 2.12\%$w/w

**T3**:

1.138g of extract =0.124 g of tannins

100g $= \frac{0.124 \times 100}{1.138}$ g

$= 10.89\%$w/w

10g of powder =0.124 g of tannins

100g $= \frac{0.124 \times 100}{10}$ g

$= 1.24\%$w/w

**T4**:

1.118g of extract =0.170g of tannins

100g $= \frac{0.170 \times 100}{1.118}$ g

$= 15.2\%$w/w

10g of powder =0.170g of tannins

100g $= \frac{0.170 \times 100}{10}$ g

$= 1.7\%$w/w

**T5**:

1.007g of extract=0.148g of tannins

100g $= \frac{0.148 \times 100}{1.007}$ g

$= 14.69\%$w/w

10 g of powder =0.148g of tannins

100g $= \frac{0.148 \times 100}{10}$ g

$= 1.48\%$w/w

**Average value per extract** $= \frac{12.70+17.40+10.89+15.20+14.69}{5}$

$= 14.20\%$w/w

**Average value per powder** $= \frac{1.50+2.12+1.24+1.70+1.48}{5}$

$= 1.61\%$w/w

**TABLE OF TOTAL TANNINS**

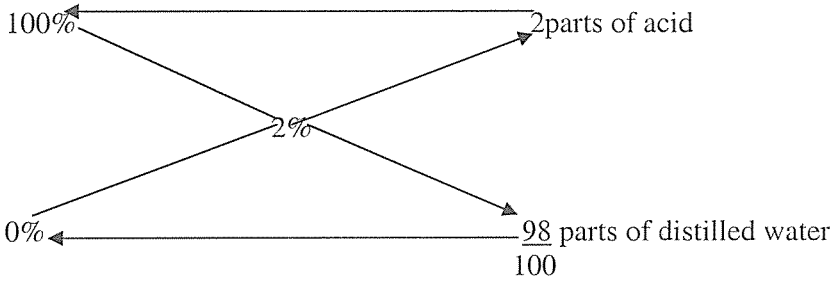
| LABEL | WT OF EXTRACT (g) | WT OF TANNINS (g) | % W/W CONTENT IN AQUEOUS EXTRACT (g) | %W/W CONTENT IN POWDER (g) | DEVIATION (g) |
|---|---|---|---|---|---|
| $T_1$ | 1.180 | 0.150 | 12.70 | 1.50 | 1.5 |
| $T_2$ | 1.216 | 0.212 | 17.40 | 2.12 | 3.2 |
| $T_3$ | 1.138 | 0.124 | 10.89 | 1.24 | 3.3 |
| $T_4$ | 1.118 | 0.170 | 15.20 | 1.70 | 1.0 |
| $T_5$ | 1.007 | 0.148 | 14.69 | 1.48 | 0.5 |
| AVERAGE | | | **14.20±2.22** | **1.61** | **9.4** |

## 2.2 QUANTIFICATION OF TOTAL ALKALOIDS

Preparation of 2% acetic acid

Concentration available =100%

Using allegation media;




Volume of acetic acid needed = 500ml

Volume of acid $= \frac{2 \times 500}{100}$

$= 10ml$

Distilled water was added up to 500ml mark

**A1**:

10.172g of extract =0.260g of alkaloids

100g $= \frac{0.260 \times 100}{10.172}$ g

$= 2.6\%w/w$

100g of powder =0.26 g of alkaloids

$= 0.26\%w/w$

**A2**:

9.616 g of extract =0.225g of alkaloids

100g $= \frac{0.225 \times 100}{9.616}$ g

$= 2.4\%w/w$

100g of powder =0.225g of alkaloids

$= 0.23\%w/w$

**A3**:

9.232g of extract =0.233g of alkaloids

100g =$\frac{0.233 \times 100}{9.232}$ g

=2.52%w/w

100g of powder =0.233g of alkaloids

=0.23%w/w

**A4**:

10.632 g of extract =0.248g of alkaloids

100g =$\frac{0.248 \times 100}{10.632}$ g

=2.33%w/w

100 g of powder =0.248 g of alkaloids

=0.25%w/w

**A5**:

10.304 g of extract =0.27g of alkaloids

100g =$\frac{0.27 \times 100}{10.304}$ g

=2.625w/w

100g of powder = 0.27g of alkaloids

=0.27%w/w

**Average value per extract** =$\frac{2.60+2.34+2.52+2.33+2.62}{5}$

=2.48%w/w

**Average value per powder** =$\frac{0.26+0.23+0.23+0.25+0.27}{5}$

=0.25%w/w

TABLE OF TOTAL ALKALOIDS

| LABEL | WT OF EXTRACT (g) | WT OF ALKALOIDS (g) | % W/W CONTENT INAQUEOUS EXTRACT | DEVIATION (g) | %W/W CONTENT IN POWDER |
|---|---|---|---|---|---|
| A1 | 10.172 | 0.260 | 2.60 | 0.12 | 0.26 |
| A2 | 9.616 | 0.225 | 2.34 | 0.14 | 0.23 |
| A3 | 9.232 | 0.233 | 2.52 | 0.04 | 0.23 |
| A4 | 10.632 | 0.248 | 2.33 | 0.15 | 0.25 |
| A5 | 10.304 | 0.270 | 2.62 | 0.14 | 0.27 |
| AVERAGE | | | **2.48±0.12** | **0.59** | **0.25** |

## 2.3 QUANTIFICATION OF TOTAL SAPONINS

**S1**:

40ml of solution =1.143 g of saponins

20g of powder =1.143g of saponins

100g $= \frac{1.143 \times 100}{20}$ g

$=5.715\%$w/w

**S2**:

40ml of extract =1.16g of saponins

20g of powder =1.16g of saponins

100g $= \frac{1.16 \times 100}{20}$ g

=5.85w/w

**S3**:

40ml of solution =1.158g of saponins

20g of powder =1.158 g of saponins

100g $= \frac{1.158 \times 100}{20}$ g

$=5.79\%$w/w

S4:

40ml of solution =1.136 g of saponins

20g of powder =1.136 g of saponins

100g $= \frac{1.136 \times 100}{20}$ g

$= 5.68\%$w/w

S5:

40ml of solution =1.158g of saponins

20g of powder =1.158g of saponins

100g $= \frac{1.158 \times 100}{20}$ g

$= 5.79\%$w/w

**Average value** $= \frac{5.715+5.800+5.790+5.680+5.790}{5}$

$= 5.755\%$w/w

**TABLE OF TOTAL SAPONINS**

| LABEL | WT OF EMPTY BEAKER (g) | WT OF BEAKER +EXTRACT (g) | WT OF EXTRACT (g) | %W/W CONTENT IN POWDER | DEVIATION (g) |
|---|---|---|---|---|---|
| S1 | 106.664 | 107.807 | 1.143 | 5.715 | 0.04 |
| S2 | 103.863 | 105.023 | 1.160 | 5.800 | 0.04 |
| S3 | 104.721 | 105.879 | 1.158 | 5.790 | 0.03 |
| S4 | 107.615 | 108.751 | 1.136 | 5.680 | 0.07 |
| S5 | 109.065 | 110.207 | 1.158 | 5.790 | 0.07 |
| AVERAGE | | | **1.150** | **5.755±0.05** | **0.25** |

## 2.4 QUANTIFICATION OF TOTAL FLAVONOIDS

**F1**:

1.27 g of extract =0.673 g of flavonoids

100g $= \frac{0.673 \times 100}{1.27}$ g

=53%w/w

20g of powder =0.673g of flavonoids

100g $= \frac{0.673 \times 100}{20}$ g

=3.365%w/w

**F2**:

1.670 g of extract =0.971g of flavonoids

100 g $= \frac{0.971 \times 100}{1.670}$ g

=58%w/w

20g of powder =0.971g of flavonoids

100 g $= \frac{0.971 \times 100}{20}$ g

=4.855%w/w

**F3**:

1.66g of extract =0.761g of flavonoids

100g $= \frac{0.761 \times 100}{1.66}$ g

=45.84%w/w

20g of powder =0.761 g of flavonoids

100g $= \frac{0.761 \times 100}{20}$ g

=3.805%w/w

**F4**:

1.84 g of extract =1.039g of flavonoids

100g $= \frac{1.039 \times 100}{1.84}$ g

$= 56.47\%$w/w

20g of powder =1.039g of flavonoids

100g $= \frac{1.039 \times 100}{20}$ g

$= 5.195\%$w/w

**F5**:

1.94g of extract =1.129g of flavonoids

100g $= \frac{1.129 \times 100}{1.94}$ g

$= 58.2\%$w/w

20g of powder =1.129g of flavonoids

100g $= \frac{1.129 \times 100}{20}$ g

$= 5.645\%$w/w

**Average value per extract** $= \frac{53+58+45.84+56.47+58.2}{5}$

$= 54.3\%$w/w

**Average value per powder** $= \frac{3.365+4.855+3.805+5.195+5.645}{5}$

$= 4.573\%$w/w

**TABLE OF TOTAL FLAVONOIDS**

| LABEL | WT OF EXTRACT(g) | WT OF FLAVONOIDS (g) | % W/W CONTENT IN AQUEOUS EXTRACT | DEVIATION (g) | %W/W CONTENT IN POWDER |
|---|---|---|---|---|---|
| F1 | 1.270 | 0.673 | 53.00 | 1.3 | 3.365 |
| F2 | 1.670 | 0.971 | 58.00 | 3.7 | 4.855 |
| F3 | 1.660 | 0.761 | 45.84 | 8.4 | 3.805 |
| F4 | 1.840 | 1.039 | 56.47 | 2.1 | 5.195 |
| F5 | 1.940 | 1.129 | 58.20 | 3.9 | 5.645 |
| AVERAGE | **1.676** | **0.914** | **54.30±4.60** | **19.4** | **4.572** |

## 2.5 QUANTIFICATION OF TOTAL TRITERPENOIDS

**TR1;**

1.623g of extract = 1.401g of phenolics

100g = $\frac{1.401 \times 100g}{1.623}$

=86.32%W/W

Triterpenoids =86.32-53

=33.32%W/W

20g of powder =1.401g of phenolics

100g =$\frac{1.401 \times 100g}{20}$

=7%W/W

Triterpenoids =7-3.365

=3.64%W/W

**TR2:**

1.012 g of extract =1.003g of phenolics

100g $= \frac{1.003 \times 100}{1.012}$ g

=99.1%W/W

Triterpenoids =99.1-58

=41.1%W/W

20g of powder =1.003g of phenolics

100g $= \frac{1.003 \times 100}{20}$ g

=5.015%W/W

Tritepenoids = 5.015-4.855

=0.16%W/W

**TR3:**

1.335g of extract =1.13g of phenolics

100g $= \frac{1.13 \times 100}{1.335}$ g

=84.6%W/W

Triterpenoids =84.6-45.84

=38.8%W/W

20g of powder =1.13g of phenolics

100g $= \frac{1.13 \times 100}{20}$ g

=5.65%W/W

Triterpenoids =5.65-3.805

=1.85%W/W

**TR4:**

0.962g of extract =0.82g of phenolics

100g $= \frac{0.82 \times 100}{0.962}$ g

=85.2%W/W

Triterpenoids =85.2-56.47

=28.73%W/W

20g of powder =0.82 g of phenolics

100g = $\frac{0.82 \times 100g}{20}$

=4.1%W/W

Triterpenoids =4.1-5.195

=1.095%W/W

**TR5:**

1.781g of extract =1.131g of phenolics

100g =$\frac{1.131 \times 100}{1.781}$ g

=63.5%W/W

Triterpenoids =63.5-58.2

=5.3%W/W

20g of powder =1.131 g of phenolics

100g =$\frac{1.131 \times 100}{20}$ g

=5.655%W/W

Triterpenoids = 5.655-5.645

=0.01%W/W

**TABLE OF TOTAL TRITERPENOIDS**

| SERIAL NUMBER | WT OF EXTRACT (g) | WT OF PHENOLICS (g) | %W/W CONTENT IN AQUEOUS EXTRACT | %W/W CONTENT IN POWDER |
|---|---|---|---|---|
| T1 | 1.623 | 1.401 | 33.32±3.80 | 3.64 |
| T2 | 1.012 | 1.003 | 41.10±11.6 | 5.015 |
| T3 | 1.335 | 1.130 | 38.80±9.30 | 0.16 |
| T4 | 0.962 | 0.820 | 28.73±0.72 | 1.85 |
| T5 | 1.781 | 1.131 | 05.30±0.72 | 0.01 |
| AVERAGE | **1.343** | **1.097** | **29.45±6.90** | **1.35** |

## NOTE: ( Clark and Schkade)

Standard deviation= $\sqrt{\frac{\sum(x-\mu)^2}{N}}$

$\sum$- summation

$\mu$ - mean

$\sqrt{}$- square root

N- Sample size