PHARMACOGNOSTIC AND ACUTE TOXICITY STUDIES OF *MOMORDICA BALSAMINA* (LINN) FAMILY (CUCURBITACEAE)

BY

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DECLARATION

I declare that the work in the thesis entitled ***"Pharmacognostic and Acute Toxicity Studies of Momordica balsamina Linn"*** was entirely performed by me in the Department of Pharmacognosy and Drug Development under the supervision of Dr. H. Ibrahim and Dr. (Mrs.) H. Nuhu.

The Information obtained from the literature has been duly acknowledged in the text and a list of references provided. No part or whole of this dissertation was previously presented for award of another Degree or Diploma at any University.

_____ _____

Aliyu Abdulazeez ***Date***

CERTIFICATION

This thesis “Pharmacognostic and Acute Toxicity Studies of *Momordica balsainina (*Linn), (Family Cucurbitaceae)” by ***Aliyu Abdulazeez*** meets the regulations governing the award of the degree of Master of Science Pharmacognosy, Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my Late Mother (Alhaja Hawwau Titilayo, Morenike Esuu) for her love and care, when alive, and her spiritual upliftment even after death. My late father (Alhaji Aliyu, Adeshina Adigun) and my late brother (Dr. A.A. Aliyu), may their souls rest in perfect peace, Ameen.

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ABSTRACT

The Plant *Momordica balsainina* Linn (balsam apple) belongs to the tar. cucurbitraceae. The study carried out was designed to reveal the pharmacognostic characters of the plant for its proper identification and to evaluate the toxicity of the plant extract.

Detailed rnacroscopical, microscopical and phytochemical studies were carried out on the leaves, stern and roots of the plant Anatomical features, stomata parenchyma cells, trichomes, starch grains, calcium oxalate etc were investigated. Various phytochemical tests carried out on the powdered sample (of the leaves, stern and roots of the plant) revealed the presence of carbohydrate, alkaloids, tannins, flavonoids, saponins and steroids. Ca-oxalate stomata, starchgranis, trichomes were among the microscopical features revealed.

Paper and thin Layer chromatographic analysis were carried out to confirm the presence of compounds like alkaloids etc.

Quantitative procedures carried out gave the following results:

- Moisture content *15.67%* w/v
- Ash value 17.61%w/v
- Acid insoluble-ash-value 2.33% w/v
- Alcohol soluble-extractive value *5.00%* w/v
- Water soluble extractive value 10.00% w/v

Acute toxicity of the plant extract was determined using locke-method. The results of the toxidity tests showed that the water extract of the plant is safe for use or1ally with an LD50 of 20,000mgkg-l body weight (P0), but the extract was found to be toxic intraperitoneally with an LD50 of 1000mg kg-i (I.P.). This study has given some pharmacognostic standards for the genuine crude drug. The pharmacognostic standards are necessary for proper identification of the plant, and necessary for detection of its adulteration or substitution. It has also shown that the plant caused no signs of acute oral toxicity.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Traditional Medicine

This refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises to treat, diagnose and prevent illnesses or maintain well being (WI-TO, revised 2003).

Traditional medicine has maintained its popularity in all regions of the developing world, and its use is rapidly spreading in industrialized countries

However, scientific evidence from randomized clinical trial is only strong for many uses of acupuncture, some herbal medicines and for some of the manual therapies. Further research is needed to ascertain the efficacy and safety of several other practices and medicinal plants.

1.2 Dysmenorrhea

Dysmenorrhea is the medical term for pain during menstruation (period pain). Painful period may be caused by a number of factors. Cause of painful periods may include: **Tipped uterus or retroverted uterus: A** tipped uterus may cause pain during intercourse also.

Endometriosis: The tissue lining the uterus, the endornetrium, may grow outside the uterus causing pain during periods.

Hormonal changes and unbalances: A hormone produced by cells in the uterine lining called prostaglandin causes uterine contractions. Women with severe dysrnenorrhia have higher prostaglandin levels in their menstrual fluid than other women.

Adenomyosis: A condition where the lining of the uterus (endorneteriurn) grows into the muscle of the uterus

Fibroids: Non malignant growths in the uterus can cause pain during periods.

Pelvic inflammatory disease (PID)

Intrauterine device (IUD): some women may experience increased bleeding cramping and backache with their periods (Wright *et al,* 2003).

Pain during menstruation, dysmenorrhia, may be caused by other reasons as well, these could be identified by health care professionals.

Painful menstruating affect about 50% of menstruating women and 10% are incapacitated by up to 3 days (Hacker, *et* a! 2004). Painful menstruation is the leading cause of lost time from school and work among women of child-bearing age. This pain may precede menstruation by several days or may company it, and it usually subsides as menstruation tapers off.

Primary dysmenorrhia refers to menstruating pain that occurs in otherwise

healthy women (Wright et al 2003). This type of pain is not related to any specific problems with the uterus or other pelvis organs.

Secondary Dysrnenorrhia is menstrual pain that is attributed to some underlying disease process or structural abnormality either within or outside the uterus (for example pelvic inflammatory disease.),

The incidence of menstrual pain is greater in women in their late teens d twenties, then decline with age. Some women experience increased menstrual pain in their thirties and forties as their endocrine systems prepare for enopause by decreasing hormones levels and thus fertility. It does not appear be effected by child bearing Wright et al (2003).

The cramping associated with dysmenorrhia usually begins a few hours before he start of bleeding and may continue for a new days. The water extract of *momordica Balsamina* was used traditionally, in the treatment of the: period in experienced by young girls.

1.3 Antispasmodics

The plant prevents or eases muscle spasms in both skeletal muscles (the muscles that move the bones) and smooth muscles (the muscles related to many of the body's involuntary functions, such as digestion). Many sedatives, nervine or hypnotics herbs also have antispasmodic effects. Those antispasmodic herbs with an affinity for the respiratory system are useful for asthma etc.

Mechanism of action of antispasmodics

The exact mechanism of action of these drugs is often poorly understood. It is not clear whether the medication actually decrease muscle spasm or whether they exert other effects, like inhibition of the transmission of both monosynaptic and polysynaptic reflexes at the spinal cord level, possibly by hyperpolarisation of primary afferent fibre terminals with resultant relief of muscle spasticity. Barbiturates exert their effects through the central nervous system (CNS). (Roy R. Reeves et al, 2005).

1.5 Phytochemistry

Phytochemical investigations of a plant involve the extraction of the plant material, separation and isolation of the constituents and characterization of the isolated compounds.

Phytochernistry is an important aspect of drug-development that is of interest to the pharmacist. It helps, through standard methods, to determine the constituents contained in a crude drug, thereby establishing or otherwise the justification of the use of the crude drug (or plant) by the traditional herbal practitioners for the treatment of certain ailments and conditions.

The active constituents of plant are usually or frequently alkaloids or glycosides. Other groups such as carbohydrates, fats and oils and proteins are of dietary importance and many others (such as starches, gums etc)

are used in pharmacy, as pharmaceutical necessities but lack pharmacological actions.

1.5 Chromatography

Of the various methods of separating and isolating plant constituents, the ‘chromatographic procedure’, originated by Tswett, is one of the most useful techniques of general application. All finely divided solids have the power to adsorb other substances on their surfaces to a greater or lesser extent; similarly, all substances are capable of being adsorbed, some much more readily than others. The phenomenon of selective adsorption is the fundamental principle of chromatography, the general principle of which may be described with references to one of T wett’s original experiments.

Adsorption chromatography has proved particularly valuable in the isolation and purification of plant constituents like vitamins, horniones, alkaloids, glycosides, etc.

In 1894 Consden, *et al* introduced a method of partition chromatography using strips of filter paper as carriers for the analysis of amino acids mixtures. The technique was extended to all classes of natural products, and although to a large extent, replaced by thin layer chromatography

(TLC), it remains the method of choice for the fractionation of some groups of substances (Trease and Evans, 1987).

The solution of mixture to be separated is applied as a spot near-one end of a prepared paper strip. The paper is then supported in an air tight chamber, which has an atmosphere saturated with solvent and water. The most satisfactory solvents are those that are miscible with water; such as phenol,

n-butanol and amyl alcohol. The paper may be dipped in the solvent mixture, so that the solvent front travels up the paper (ascending technique) or the trough of solvent may be supported at the top of the chamber, in which case the solvent travels down the paper (descending technique). As the solvent moves, the components also move along the paper at a varying rates, depending mainly, on the differences in their partition coefficients between the aqueous (hydration shell of cellulosefibres), and organic phases. After the chromatographic paper strips have been dried, the positions of the separated components can be revealed by the use of suitable developing agents or U.V. light. The ratio between the distance travelled on the paper by a component of the test solution and the distance travelled by the solvent is termed the Rf value and under standard conditions, which may include; temperature solvent-type (or type of moisture used where more than one solvents are

used and in their ratio as mobile phase), adsorbents etc. this is constant for the particular compound.

The quantity of substance present determines the size of the spot with any one solvent and can be made the basis of quantitative evaluations.

1.5.1 Chromatographic Methods

Paper Chromatography

The use of paper chromatography in the study of soluble plant pigments or constituent has become wide spread since (Bate-Smith, 1948) description of its application to the identification and separation of anthocyanins, flavones and their glycosides.

The general procedures employed was as follows:- The solution containing the substance or mixtures to be tested (or identified) was applied as a spot near one corner of a sheet of paper (Whatman No. 1) and hung from a trough containing suitable solvent. Development of the chrornatogram was allowed to continue until the solvent front has progressed to a point near the opposite edge of the paper, which was then removed and dried. After drying the paper was examined in ordinary light, and under ultra-violet light, and if desired, sprayed with suitable reagents and re-examined.

Thin-layer chromatography (TLC)

This involves the use of a glass or plastic plate thinly-coated with an absorbent in the chrornatographic process. This is another method used in the separation of the chemical constituents of powdered sample of Momordica balsarnina plant.

The advantages of Thin-layer chromatography over paper chromatography include:

1. Separation can be effected more rapidly with smaller quantities of the mixture.
2. Separated spots are more compact and more clearly demarcated from one another.
3. Nature of the support-paper, glass or plastic is often, such that drastic reagents such as concentrated sulphuric acid, which would destroy a paper-chromatogram can be used for the location of the separated substances on the TLC.

Preparation of thin layer plates

A slurry of silica-gel (30 **g)** was prepared with 60 ml of distilled water and applied to glass plates (20 x 20 cm) by means of **a** leveler, with the spreader (jobbing), set at *0.25* mm. The plates were air- dried for twenty-four (24) hours then stored in desiccator oven till they were required for use.

Solvents selection

The selection of solvent used for the development of the chromatogram depends upon the solubility—characteristics of the substances to be separated. Appropriate solvents were selected for each of the constituents to be chromatographed, and the chromatography was conducted for all the constituents (discovered) of the plant *Momordica balsamina* example Alkaloids Saponins, steroids, tannins, and flavonoids. Example of solvents includes:

1. Water

Water has the very useful property of moving glycosides but leaving aglycones at or very near the origin (Roberts et al 1953). While water gives relatively poor separations, so far as measurements of $\mathbf{R_F}$ are concerned, its ability to bring about gross separations of groups of compound types and to remove by virtue of their rapid movement the very water-soluble sugars, makes it a valuable solvents to be used in combination with organic solvents.

2. Butanol-water-Organic Acid

Butanol-water-Organic Acid mixtures of varying proportions move most flavonoid compound with an excellent range of RF values and with good separations and definition of spots (Paech, K. *et al 1955).*

3. Mixtures of Hydrocarbon solvents, water and methanol

This mixtures (Lindstedt, 195 Oh) give excellent separations and convenient R_F values for the non glycosidic flavonoid and silbene derivatives found in pine heart woods.

Solvent system

The solvent strength parameter E° is defined as the adsorption energy per unit of standard sorbent. The higher the mobile phase strength (greater interaction with the sorbent), the greater will be the RF (value) of the solute in simple liquid-adsorption TLC. Usually, one tries to select a mobile phase so as to obtain RF value of 0.3 - 0.7 particularly if well-separated zones are desired.

In practice, however, single - solvent mobile phases usually do not give separation, although they might give proper mobility. A more precise approach to separations based on three mobile phases and how to modify them can be obtained b y understanding the theories of mobile phase strength and selectivity. Varying the selectivity (changing interactions) can affect the separation. Solvent strength is dependent on its reactivity with the sorbent. With a large number of sorbents available, one has to resort to some trial and error to find the best mobile phase for separating the components of interest.

Colour reactions and spraying reagents

Many compound that possess distinct colours in the crystalline form are only faintly coloured or nearly colourless when present in micrograms quantities (example quantities applied on the chromatographic paper) on a paper chromatogram. Some compounds are even colourless, and therefore, not visible on the paper. The development of the constituent spots on the paper can be accomplished by viewing them under the ultraviolet light and marking the areas made visible by the radiation and where necessary, by exposing the paper to the vapours of certain compound (like ammonia, in the case of flavonoids), after viewing it under visible and ultra-violet light.

Or by spraying the paper with Liebermann Burchard reagent for steroids selected to produce coloured reaction products with the constituents on the chromatogram, and again observing the colours in visible and ultra-violet light. Only the an thocyamins chaleones, and aurones posses deep colours necessary to define them clearly to the naked eye when present in trace amounts on the chrornatogram. (Paech, K. *et al* 1955). The constituent were identified one after the other using this method.

Reagents for visualization

The commonly used procedure for visualization is to spray the chromatogram after it is developed with the detection reagent.

There are a few reagents used generally for the visualization of organic compounds. The most widely used is sulphuric acid, which sometimes reacts immediately or upon heating, ‘to form brown or black charred areas. For very uncreative substance 50 % nitric acid can be added to the concentrated acid to increase its oxidizing capabilities.

Adsorbent and solvent systems

Silica- gel was the adsorbent used for the chromatography of the constituents, while solvents used were, however, different for different chemical constituents.

Silica — gel has become the most widely used sorbent for TLC since being described (Kirchner *et al.,* 1951). A function of the surface hydroxyl contents (Beesely), which is responsible for selective adsorption onto the particle. The higher the water uptake, the more active the sorbent.

The process of plate activation is used in an attempt to control the hydroxyl (i.e. water) content of the layer in order that reproducible separations will be obtained. It is noted that conditioning of an activated TLC plate to 40% relative humidity (6.67% water by weight) gives a more reproducible chromatographic system and seems to suggest the deactivation of very active sites and a more uniform hydrogen — bonding mechanism.

Other types of absorbents include:

- Kieselguhr
- Alumina
- Cellulose
- Magnesium silicate

Alumina (Aluminum oxide) is also widely used as a sorbent chemically it is basic, and for a given layer-thickness, it will not separate quantities of material as large as can be separated on silica gel. Alumina is more chemically reactive than silica gel and care must be exercise with some compounds and compound classes to avoid, decomposition or rearrangement of these substances during sample application, storage before development or during development.

Kieseighur (Diatomaceous earth) is a chemically neutral sorbent that does not separate or resolve very well as either alumina or silica gel. It is used mainly as the support for the stationary phase in partition chromatography. Cellulose is used as a sorbent in TLC (Thin Layer Chromatography) when it is convenient to perform a given paper chromatographic separation by TLC in order to decrease the amount of time necessary for the separation and increase the sensitivity of detection.

Other substances used as sorbents include a variety of ion-exchange cellulose powders, polyamide powder, etc.

R1 Value

The R value is a convenient way to express the position of a substance on developed chromatogram. It is calculated as the ratio.

$$R_F = \frac{dis\tan ce\, of\ \ compound\ \ from\, origin}{dis\tan ce\ \ of\ \ solvent\ \ front\, origin}$$

R_F values are between 0 and 0.999 and without units. Distance is measured to the centre of the sample zone or spot.

It is desired to express positions relative to the position of another substance

X. The R can be calculated.

$$R_F = \frac{dis\tan ce\ \ of\ \ compound\ \ from\, origin}{dis\tan ce\ \ of\ \ reference\, compound\ x\ \ from\, origin}$$

It is possible for values to be greater than I. The R thus is the “relative retention value”. Under proper conditions, it is also possible to measure both the area of a spot and its density, using “photo densitometer”.

Ultra-violet light

Under the ultra-violet light different chemical groups attached to different structures of the constituents, or the composition (Structurally) of the constituent themselves absorb the ultra-violet radiation and give certain colouration on the chromatogram, that is distinct to that particular class of compound. This featured the basis for the-identification of different classes

of chemical compound under the ultra-violet radiation.

1.6 Extraction

Extraction Procedure

French Apothecary Nicholas Lemsey 1645-1715, extended the use of extraction process and made use of alcohol as a solvent. Alcohol has since then, been a general solvent for many plant constituents (which are usually dried before extraction), except for most fixed oils, where, water immiscible solvents are used e.g. Light petroleum-ether (for extraction of essential oils and fixed oils and steroids), while Ether and chloroform are used for extraction of alkaloids and Quinones. Extraction of Organic bases (example alkaloids) usually necessitates basification of the plant material if a water immiscible solvent is to be used. For aromatic acids and phenols, acidification may be required.

Extraction, itself, may be performed by continuous or repeated maceration with agitation, percolation or by continuous extraction (for example, soxhlet apparatus).

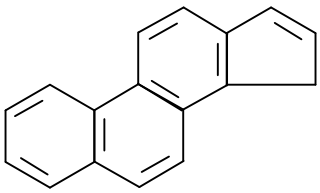
a. Alkaloids

Alkaloids comprise a major group of naturally occurring compounds. Many of which have important medicinal properties, chemically the alkaloids are

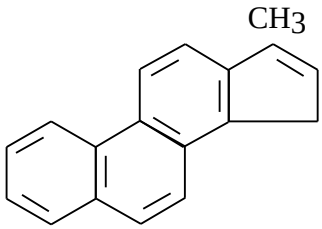
complex nitrogenous bases, in which the nitrogen is frequently contained in a heterocyclic ring system. The alkaloids are precipitated from neutral or slightly-acid solution by a number of reagents. It is these types of reaction (that leads to precipitation of alkaloids) that is used in preliminary identification of alkaloids in crude powdered drugs. However care must be taken as other nitrogenous compound or bases will give the same reaction (result) example Proteins and biological Amines.

b. Steroids

Included in the steroids are the sterols, from which the name steroid was derived. Examples of these are Vitamin D., Bile-acids, sex hormones etc. Their structures are based on 1, 2, cyclopent-anophen anthrene skeleton. (See fig. a).



(a)



(b)

Steroids give among other products, Diel's hydrocarbon, on hydrogenation with selenium at 360°C (Finar, 1991). Thus, steroids can be defined as any compound which gives Diel's hydrocarbon when distilled with selenium.

Sterols, likewise are group of crystalline steroidal alcohol containing between 27 and 30 carbon atoms and posses a 2 - β- hydroxyl group and an exocyclic double bond, usually in *5,* 6 positions together with a side

chain which exhibit various degrees of branching and unsaturation (Dence, 1980).

The sterols can be classified into two:

1. Animal sterols for example cholesterol, sex hormones, bile-acids and hormones of adrenal cortex
2. Plant sterols (example digitoxin). The basic difference between animal and plant sterols is the presence of an additional alkyl group in the C - 17 side chain of the plant sterols. example cardiac glycosides, digitoxin.

Steroids occurs generally in conjugated form with one or more sugar residue, including glucose, xylose, galactose, fructose etc.

They (steroids and sterols) are of tremendous pharmaceutical importance. Particularly in the manufacture of steroidal homones and contraceptives. Steroids could exist in conjugation with sugar moiety (as in saponin glycosides).

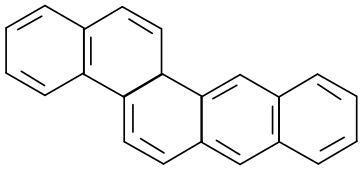
The aglycone moiety of saponin-glycosides are also know as *Sapogenins.* Structurally, Saponins could be classified into two groups:

(i) Steroidal saponins

(ii) Triterpenoid saponins

Steroidal saponins give Diel's hydrocarbon on dehydrogenation with selenium at 360^{o}C, and have upto about twenty-seven (C-27) carbon atoms. They are never found as free saponins in plant materials.

Triterpenoid saponins give mainly naphthalene or picine-derivative on dehydrogenation with selenium at 360^{o}c and have about thirty (C-30) carbon atoms in their structure.



(Picine)

The sugar portion may consist of 1 - *5* sugar units. Including glucose, xylose, galactose, fructose etc.

Some of its pharmaceutical importance include its possession of surface tension, which is used as an emulsifying property in pharmaceutical industries.

It tends to alter the permeability of cell - wall, hence exert general toxicity in all organized tissues.

Saponins

Steroidal saponins are glycosides: Glycoside, are a large group of naturally occurring plant products, that on hydrolysis (by either acides or enzymes) yield one or more sugars (glycone) and a none – sugar moiety (the aghycone). Thus investigation of glycoside containing drugs involves the characterization of the aglycone residue.

Saponins share, in varying degrees, two common characteristics:-.

(i) They foam in aqueous solution.

(ii) An intravenous injection of saponins result in haemolysis (Olaniyi, 1993).

Saponin glycosides

These are group of compounds with basic nucleus of steroidal or triterpenoidal type. They are mostly of medicinal importance e.g. vitamin N; cardiac — glycoside, sex hormones etc. These glycosides have common basic properties that can be used as basis for their detection in drugs. All saponins are haemolytic in nature.

Saponins are glycosides and they share in varying degrees two common characteristics:

a. form in aqueous solution

b. Injection results in haemolysis (Olaniyi, 1993).

The Aglycone moiety of the saponin glycoside is known as the sapogenins structurally saponins can be divided into two groups:

a Steroidal - (C-27) saponin (consists of about 27 carbon atoms)

b Trtierpenoidal saponin (C-30) (made up of up to about 30 carbon atoms).

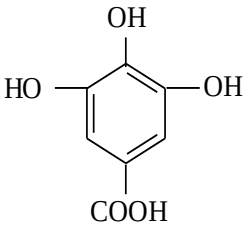
Both types have a glycosidic linkage at carbon atom C-3.

The steroid and the triterpenoid s1ns can be distinguished by the thc that, while the steroid saponions give Diel's hydrocarbon on dehydrogenation with selenium at 360°C, the triterpenoidal saponins give, mainly, naphthalene (or picine) derivatives under the same conditions.

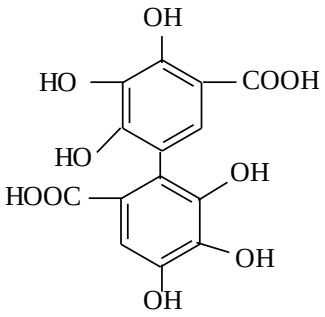
c. Tannins

These are chemical substance present in plant or their extracts, that have the ability to combine with protein of animals hides, prevent them from putrefaction and convert them to leather. There are two main types of Tannins: - Hydrolysable and condensed Tannins.

i. Hydrolysable Tannins: These are Tannins that may be hydrolyzed by acids or enzymes. They consist of several molecules of phenolic acids such as gallic and hexahydroxy diphenic acids which are united by ester linkages to a central glucose molecule.



Gallic acid



Hexahydroxy diphenic acid

They give blue colour with iron salts.

ii. Condensed tannins: Condensed tannins are not readily hydrolysable to simple molecules and they do not contain a simple sugar moiety. The solution of condensed tannins usually turn green with ferric chloride Solution.

Hydrolysable tannins may be hydrolysed by acids or enzymes and consist of several molecules of phenolic acids, such as gallic and hexahydroxy diphenicacids which are united by ester linkages to a central glucose molecule. Hydrolysable tannins give blue colour with iron salts.

Condensed tannins are not readily hydrolysed to simple molecules and they do not contain a simple sugar moiety. They are related to the avonoid pigments and have polymeric flavan-3-ol structures. On treatment with acids or enzymes, condensed tannins are. converted into red insoluble compound called phobaphenes. Their solutions turns green with ferric chloride solution.

e. Flavonoids

Flavonoids are also glycosides: They are group of plant glycoside with basic nucleus of phenolicpolymers (aglycone) linked to a sugar moiety (glycone) through a glycosidic bond. Similar to the condensed tannins, flavonoids have polymeric flavon-3-ol structure. On treatment with acid or enzymes, flavonoids yield phenolic aglycone and sugar moiety (glycone). The flavonoids are all structurally derived from the parent substance flavone which occurs in plants, (particularly, all vascular plant) and all flavonoids share a number of properties in common. There are over (10) classes of flavonoids.

They can be extracted with 70% alcohol (ethanol) and remain in aqueous layer, following partition of the extract with petroleum ether. Flavonoids are phenolic, hence, change in colour when treated with base ‘or Ammonia, thus they are easily detected on chromatograms (or in solution). Flavonoids contain conjugated aromatic system and thus show intense absorption band in UN and visible regions of the spectrum.

Some classes of flavonoids are recognized:

Anthrocyanins

Glycoflavones

Biflavonyls

Lenoco anthrocyanin

Chalcones and Aurones

Flavonols

Flavonones

Flavones

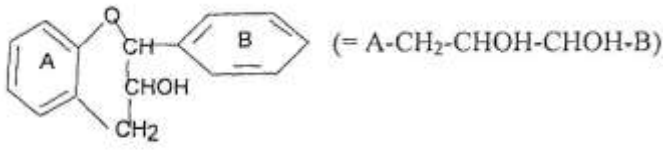
Isoflavonones

They are mainly water soluble compounds. Flavonoids are phenolic and are present in plants bound to sugar as glycosides, and any one flavonoid aglycone may occur in a single plant in several glycosidic combinations. Flavonoids have been detected in certain mutant strains of green alga chlarmydomonas specie (Moewus 1951) and (Kuhn *et al,* 1942, 1948, 1949). A lot is known about flavonoids distribution to support the statement that they occur throughout the higher plants and probably in all parts of the plant:- roots, bark, wood stems, leaves, flowers, fruits and seed.

The occurrence of flavones in insects has been demonstrated (Thomson, 1926), who isolated a yellow pigment from the wings of the “marbled white” butterfly.

Miscellaneous properties of flavonoid compounds that have been studied, before now, are the bacteriostatic action of anthocyanins (Blank and Suter, 1948) and chalcones (Shraufspatter et al., 1948) etc.

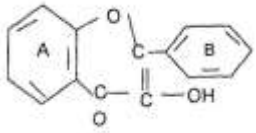
The range of structural variation found in the known compounds of the flavonoid Aglycone-type is associated, primarily, with variation in the oxidation level of the C3- portion of the molecule. The range of oxidation level extends from the highly reduced catechin type.



(= A-CH_2-CHOH-CHOH-B)

(Catechin)

To the highly oxidized flavonol



(= A-CO-O-CHOH-B)

(flavonol)

1.7 Aims and objectives

The aim and objectives of this work are as follows

- To study the microscopic, morphotogical, and anatomical features of the pahrmacognostic study) *Momordica Balsamina* which will be useful in producing the monography of the plant.
- To determine various evaluative parameters of the plant, such as, moisture content, ash value, acid insoluble ash value, extractive values etc.(phytochemical study)
- To determine the acute toxicity profile of the plant

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Family: Cucurbitaceae

A family of plant including the melon, cucumber, calabash etc. it is one of the most important families of food plant in the World. Most of the plants (in this family, like the *iviomordica balsarnina)* are annual vines with fairly large showery blossoms, characterized by commonly having five - angled stems and coiled tendrils. *Momordica balsamina* is considered as one of the most popular African herbs. (Herbal Africa, 2007).

Taxonomy

Kingdom	Plants
Sub Kingdom	Vascular plants
Super division	Spermatophyte (seed plants)
Division	Magnoliophyta (flowering plants)
Class	Magnoliopsida (Dicotyledons)
Sub class	Dillenidae
Order	Cucurbitales(violales)
Genus	*Momordica*
Species	*Balsamina*
Category	Vines and climbers

There are about 90 genera and over 700 species that are further characterized by commonly having five - angled sterns and coiled tendrils.

2.2 The Plant: *Momordica balsamina*

The plant grows quickly form seeds and is widely naturalized in the Tropics. It is commonly called *garahuni* by the Hausas and *garafuni* by the Fulanis (of Northern Nigeria). The Yoruba (in the South-Western part of Nigeria) call it 'efmrinwere' (Ondo). *vIoinordica balsamina* has various other names worldwide as follows:

Country	**Name**
Chinese	Jiao ku gua
English	Balsam apple, Southern balsam pear (different from balsam pear), African cucumber,
French	Cucumbre balsamite, Margose, Courgette africaine
German	Balsarnapfel
Italian	Pomo meraviglia
Japanese	Not Reishi
Portuguese	Balsamina de-purga
Poland	Balsamina de purga, Balsamina pequena
Spanish	Balsamina
Uganda	Bombo

(Jeffrey, C. 1967)

Momordica balsainina is widespread throughout the drier parts of tropical Africa. It is mainly found on sandy soils, from sea-level up to 1600m altitude. It occurs in woodland, wooded grassland, on river banks and in dry river beds. *Momordica balsainina* is mainly collected from the wild, but widely cultivated as well. It is planted close to homesteads, often growing over fences and huts. *Momordica balsamina* is widespread and not in danger of genetic erosion. Transfer of genes within the genus is possible, allowing improvement of notably *Momordica charantia.(Discover* nature 011669)

2.2.1 Uses

The leaves and young fruits of *Momordica balsamina* are cooked and eaten as a vegetable in Cameroon, Sudan and Southern Africa. The bitter young fruits have been reported widely as edible, whereas the ripe fruits cause vomiting and diarrhoea, and can be poisonous. The bright red fruit pulp is eaten in Namibia. (Herbal Africa, 2007).

Medicinal use of *Momordica balsamina* is widespread and diverse. Common, widespread uses are as an antihelmintic (fruits, seeds and leaves), against fever and excessive uterine bleeding (leaves), and to treat syphilis, rheumatism, hepatitis and skin disorders. Other uses are as an abortifacient, aphrodisiac and latogenic, and in treating diabetes.

In many African countries the fruit is taken as a purgative and vermifuge, whereas the leaves are steeped in water and taken to treat diarrhoea and dysentery. Steeped leaves used as enema are said to have strong astringent properties. The seed is used internally as an antihelmintic, especially in Democratic Republic of Congo. In West Africa the plant is used as a febrifuge either by washing or drinking. Yellow fever and jaundice are treated by an enema of the entire plant in water or eye instillation of leaf sap. The plant is used as on aphrodisiac and in local treatment of gonorrhoea. Preparations made from stems and leaves are used to treat yawns. A decoction is applied to boils, ulcers, septic swellings ond infected feet. Paster made from pulverized plants is used to treat malignant ulcers, breast cancer and skin parasites such as filaria and guinea worms. The leaves are taken to treat menstrual problems and the roots are used against syphilis and rheumatism and as on abortifacient. Crushed leaves are used together with other drugs to relieve heart problems, example tachycardia. Manifold medicinal uses are also reported from Asia and the Americas, example to treat cancer, diabetes, psoriasis and many infectious diseases. It is especially renowned as a remedy for diabetes mellitus, just by eating it regularly as a vegetable (Herbal Africa, 2007).

Where *Momordica balsam ma* is consumed regularly, there are no occurrences of osteoporosis. It is the traditional and conventional consensus that this can be attributed to *Momordica balsamina.* It is rich in vitamin A, vitamin C, calcium and iron an in their atura1 fon, these vitamins and minerals are easily absorbed by the system, strengthening :he bones thereby avoiding osteoporosis. Following a proper diet with the right amounts frninerals, vitamins and regular intake of Mornordica balsamina will reduce the risk of)steoporosis. Mornordica balsamina high concentration of calcium and other bone-friendly minerals and vitamins help the body increase bone density. (Herbal Africa 2007).

The bitter taste of all parts *of Momordica balsamina* may be caused by cucurbitacins, in many other Cucurbitaceae, but may also be caused by saponins. The ribosome activating protein momordin II has been isolated, as well as the caffeic acid ester Jsnlarinic acid, which is of pharmaceutical interest because of its anti-inflammatory, antiviral, antibacterial and antioxidant activities.

It was used many years ago, in the treatment of Portugal Army's Paludism (a yellow very sickness). (Herbal Africa 2007).

M*omordica* is a well known food item in South-Eastern Asia, where it is commonly called *bitter melon.* The taste is quite bitter and it is consumed by the native pu1ation, mainly, because it is said to have a therapeutic cooling action. The antispasmodic action of *Momordica balsam ma* that is employed in the treatment of period-pain in young ladies was reported by Scafforth *et al.,* (1980).

Traditionally, different parts of the plant have been used for different purposes in different parts of the World. In the Indian Peninsular, the roots are used as abortifacient, the roots and fruit are used for the same purpose in some parts of Nigeria and Ghana. The whole plant is sometimes used as an ingredient in aphrodisiac preparation (Akinniyi *et al.,* 1986 and Sofowora 1982). The Wallofs, in Senegal, have used the fruits as purgative agents (Sofowora, 1982). The fruits and leaves are used for the treatment of wounds in Nigeria and Syria as haernostatic antiseptic. The whole plant is used as sponge in treating skin diseases, such as, scabies, psoriasis, and other cutaneous diseases and as tranquilisers (Akinniyi *ci' al.,* 1986 and Sofowora 1982) in the treatment of mental illness. The aqueous extract of *Momordica balsamina* has also been used in reducing and reliving 'period-pain' in young girls (Scaforth *et al.,* 1980). The plant is traditionally used against piles, burns, chapped-hands etcetera. It is applied externally against malignant ulcers (Dalziel, *1958).* It is believed to

be of use in detoxification of the body and in counteracting the effect of over- indulgence in fatty and spicy food. It is used to increase energy and stamina. It is also recommended for sufferers of osteoporosis. Post-natal mothers eat the leaves to boost or stimulate milk production (from their breasts) in Zaria-City (Northern part of Nigeria). It has an antidiabetic effect but no definite assurance of insulin-like properties. It has been shown to have antiviral and anticancer potential. While antiviral and the anticancer effects may not be effective when consumed orally, the blood sugar regulating effect appears to be attained by consuming the melon or its dried extracts. The fleshy portion of the fruit is the edible part and is mainly cooked in soups. The root is used in the treatment of urethral discharge and the young leaves and tendrils are used by *Pedi* as an anti-emetic. The water extract of *Momordica balsam ma* leaves possess potential antinociceptive (analgesic) and anti inflammatory activities in rats. (Y. Karumi *et al* 2003).

2.2.2 Properties *of Momordica balsamina* Leaf Extract

The composition of the leaves per 100 g is: water 89.4 g, protein 3.0 g, fat 0.1 g, carbohydrate 3.6 g, fibre 0.9 g, Ca 340 mg, Mg 87.1 mg, P 27.7 mg, Fe 12.7 mg, Zn 0.9 mg, thiamin 0.01 mg, riboflavin 0.09 mg, niacin 0.7 mg, ascorbic acid 0.4 mg. The composition of the fruits per 100 g is: water 89.4 g, protein 2.0 g, fat 0.1 g, carbohydrate *5.1* g, fibre 1.8 g, Ca *35.9 m*g, Mg 41.2 mg, P *35.8* mg, Fe 2.6 mg, Zn 1.0 mg, thiamin 0.04 mg, riboflavin

0.06 mg, ascorbic acid *0.5* mg (Arnold, *et al 1985).*

Another study, conducted in Nigeria, assessed the nutritive value *of Moinordica balsamina* L. leaves by analysing their proximate composition, amino acid profile and mineral constituents. The results showed that the plant leaves had high moisture content (71 .00± 0.95% fresh weights). The concentration of estimated crude protein and available carbohydrates on dry weight (DW) basis were 11.29 0.07% and *39.05* ± 2.01% respectively. The leaves also have high ash (18.00 ± *0.56%* DW) and crude fibre (29.00 ± 1.23% DW) contents; while crude lipid (2.66 ± 0.13% DW) and energyvalue (191.16 kcal/100g DW) were low.

Seventeen amino acids (isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, valine, alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, proline and serine) were detected in *Momordica balsamina* (Table 2.1) with glutamic acid, leucine and aspartic acid being the predominance amino acids. Isoleucine, leucine, valine and aromatic acids were found to be higher than requirement pattern for children, while sulphur containing amino acids are the only limiting amino acids for adults. (WHO/ FAO/UNU *(1985).*

The study also gave the mineral composition of the leaves as per l00g (DW) as follows:

K (1,320.00 mg),Na (122.49 mg), Calcium-Ca (941.00 mg), magnesium-

Mg (220.00 mg), P. (130.46 mg), Iron - Fe (60.30 mg), Copper - Cu *(5.44* mg), Manganese - Mn (11.60 mg) and Zinc - Zn (3. 18 mg). Comparing the leaves mineral contents with RDA values, the results indicated that the *Momordica balsarnina* leaves could be good supplement for some mineral elements particularly K, Ca, Mg, Fe, Cu and Mn. Table 2.2 shows the mineral concentrations of *Momordica balsamina* leaves. The leaves have low sodium with relatively high concentration of potassium. A (KJNa) ratio in diet is an important factor in prevention of hypertension and arteroscierosis, since potassium (K) depresses arid sodium (Na) enhances blood pressure. A K/Na ratio of 3-4 is considered the most adequate for the normal retention of protein during growth stage. The calculated K/Na ratio in the leaves was above the range needed for body use, but addition of sodium chloride in the diet prepared with this plant leaves is expected to bring the ratio within the range. (Pakistani Journal of Nutrition, 2006).

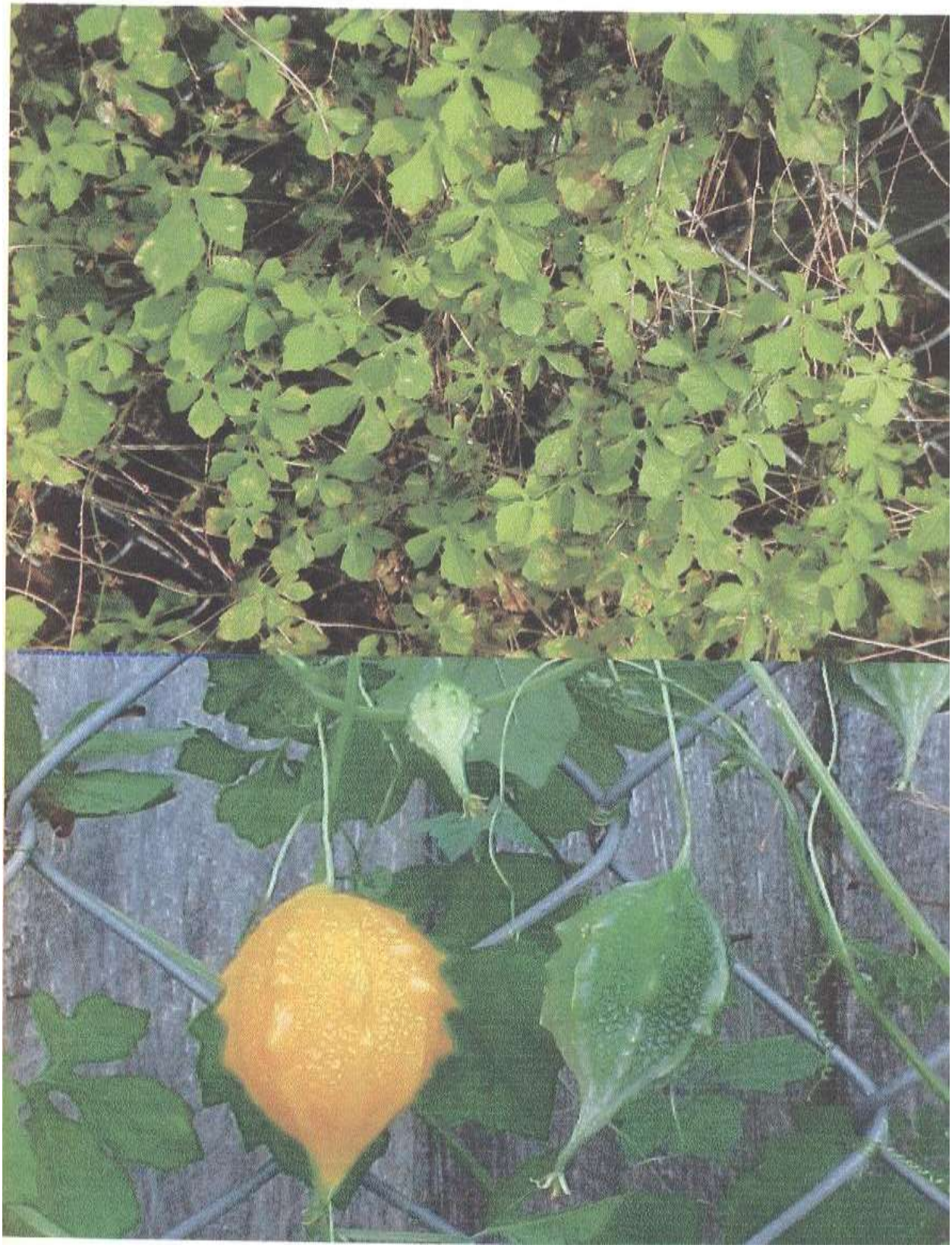


PLATE I: *MOMORDICA balsamina*

Table 2.1: Amino acid composition of Momordica balsamina L. Leaves

	Concentration (g/l00g protein)
Isoleucine	2.94
Leucine	8.38
Lysine	3.94
Methionine	0.90
Cysteine	*0.56*
Total Sulphur (Essential Amino Acids)	1.46
Phenylalanine	3.94
Tyrosine	2.62
Total Aromatic (Essential Amino Acids)	6.46
Threonine	3. 13
Valine	4.11
Histidine	2.50
Alanine	4.16
Arginine	4.87
Aspartic Acid	8.21
Glutamic acid	12.38
Glycine	4.66
Proline	3.21
Serine	4.00

* The date are mean of two replicates

Pakistan Journal of Nutrition (5, (6), 522-529, 2006

Table 2.2. Mineral Composition of *Momordica balsamina L.* Leaves

Element	**Concentration (mg/l00g DW)***
Potassium - **K**	1,320.00
Sodium-Na	122.49
Calcium- Ca	941.00
Magnesium- Mg	220.00
Phosphorus - P	130.46
Manganese - Mn	11.60
Iron - Fe	60.30
Copper - Cu	*5.44*
Zinc-Zn	3.18
K/Na	10.78
Ca/P	7.21

* The data are mean value of two replicates

Calcium and phosphorus are associated with each other for growth and maintenance of bones, teeth and muscles. The calcium level in the *Momordica balsamina* leaves was higher than the values reported in some green leafy vegetables. For good Ca to P intestinal absorption, Ca/P ratio should be close to unity. This ratio is high for favour of Ca. Thus,

Momoridica balsamina leaves appear to be good source of Ca but poor source of P.

Magnesium is an important mineral element in connection with circulatory diseases such as ischernic heart disease and calcium metabolism in bone. In this study, high Mg in the leaves could be as a result of its presence as component of chlorophyll. Zinc is involved in normal function of immune system. The leaves zinc content in the sample compared favourably to most values reported for green leafy vegetables. (Pakistani Journal of Nutrition, 2006).

Iron is an essential trace element for haemoglobin formation, normal functioning of the central nervous system and in the oxidation of carbohydrates, proteins and fats. From this study, *Moniordica balsamina* leaves had low iron content compared with other green leafy vegetables. The bio availability of iron is affected by the presence of anti- nutritional factors, the intestinal absorption of haeme-iron and non-haerne-iron differs (37% Vs. 5%), and about 90% of the iron in food is non-haeme-iron. This shows that only about 3.02mg of leaves iron will be absorbed. Nonetheless, the amount is more than adequate as 1 .00 mg/day of iron is suitable for adult human to maintain the daily balance of intake and excretion. The high percentage of iron in the sample could probably be the

reason for the use of this plant by the lactating mothers to regenerate lost blood. (Pakistani Journal of Nutrition, 2006).

Copper is an essential trace element in human body where it exists as an integral part of copper proteins ceruloplasmin, which is concerned with the release of iron from the cells into the plasma and is involved in energy metabolism. Manganese is another microelement essential for human nutrition, it acts as activator of many enzymes. The manganese content (1 1.60mg/bOg) is higher than some cultivated green leafy vegetables such as spinach *(0.5* mg/lO0 g), lettuce (0.3 mg/l00g) and 0.2 mg/l00g in cabbage. (Pakistani Journal of Nutrition, 2006).

Nutritional significant of mineral elements is usually compared with the standard recommended dietary allowance. The plant contains more than adequate levels of Na (for adults and children), Ca, Fe, Cu, Mn and Mg (for children). Thus, the plant leaves are a good source of such mineral elements particularly the micro elements. The leaves can be an important green leafy vegetables when used as a source of nutrients to supplement other major sources. (Pakistani Journal of Nutrition, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Collection and Identification

Samples of *Momordica balsamina* were collected from Zaria-City in the month of October, 2005.

The plant was collected from the local inhabitants living in Zaria town between month of October and May, this is the period during which the plant mainly flowers and fruits. It, however, flowers and fruits all the year when the plant is grown in its natural habitat.

The plant was identified in the field by the inhabitants and later confirmed at the Herbarium of Biological Sciences Department of Ahmadu Bello University, (A.B.U.), Zaria, and was given a herbarium No. 2718. A herbarium specimen was prepared and kept for further comparisons as may be needed, in future.

3.2 Preparation of Herbarium Specimen

The plant parts were arranged between two-pieces of blothing papers and inserted between more sheets of papers placed on a flat board. Another board was placed on top and pressed down with heavy objects. The wet outer papers were changed on alternate days until the specimen was

completely dried. The dried sample was removed and fastened (using celotape) onto a clean white sheet cut to herbarium size.

An information card indicating locality, botanical and vernacular names, habitat and date of collection was affixed on the sheet. These herbarium sheets were later placed in folded cardboard papers and were occasionally placed in the sun to prevent mould growth (University of Florida 2007)

3.3 Drying

Samples of the plant collected, were washed clean (off dirt's like sand under a slow running water etc). They were then dried at room temperature, in the shade. Some fresh samples, collected, were used for 'pharmacognostic' screening: like microscopy and chromocroscopy of leaf, stern and root.

Some portion of the dried samples were pulverized into fine powder and used for microscopical, extraction and toxicity studies.

3.4 Reagents

Conc. Hcl Acid, 2 % Hcl, conc-H2S04, PbS04, Draggendorff's reagent Meyers Reagent, Feel3 solution, NH3 solution, phioroglucinol, Acetic anhydride, N/50 Iodine.

Solvents

90% ethanol, chloroform, petroleum-ether, methylated spirit, ethyl acetate, n-hutanol, di-ethylether, Benzene, Acetone, Chloroform water (0.25%).

3.5 Apparatus

Microscope (compound), bath weighing- machine, desiccators, oven. camera-lucida, cricubles, flat-bottomed-flask, flat-bottomed-evaporating dish, mechanical-shaker, Test-tubes, conical-flasks, Beakers, separating funnels, measuring-cylinders, slides. Cover-slips, filter-papers, Ash- less-filter papers, chromatographic paper, chromatographic plate, Vaccum-pump, Bunsen-burner, stoppered conical-flask; watch-glass, filter-funnel, glass-rod, etc.

3.6 Animals

White Wister strain rats weighing between 124.30 to 21 4.40 g were used. The Animals were obtained from the Veterinary Department of Ahmadu Bello University Zaria and kept in good condition of laboratory use (in the Department of Pharmacology and Clinical Pharmacy of Ahmadu Bello University) for the study. They were fed on standard diet (grower mash

ECWA Feeds Nig. Ltd., Jos, Nigeria) and were given water ad-libiturn. The animals were given different marks on their bodies for identification.

3.7 Macroscopical Investigations of the Plant

The plant was observed macroscopically and using pharmacognostical standards, the eaves, stems flowers, seeds and fruits were consequently noted and described.

3.8 Microscopical Investigations of the Plant

The microscopy of the fresh plant, the transverse, longitudinal; and radial sections of the fresh plant parts (leave, stem and roots) and the powdered samples were carried out using standard methods (Trease and Evans, 1987).

A small quantity of the powdered sample or thin anatomical section was aced on a clean slide. A few drop of choral-hydrate solution was added and covered with a clean coverslip. The slide was heated, gently, over a busen-flarne for a few seconds. Overheating was avoided, as this could cause formation of chioral hydrate crystals, leading to false observation. When the tissue was sufficiently cleared, air bubbles, if any, were removed. A few drops of dilute glycerol were then added and the slide was

observed under a microscopy (Trease and Evans, 1987).

The following parts of the plant were observed under the microscope;

a.(i) Upper epidermal cells of the leaf

(ii) Lower epidermal cell of leaf (showing the stomata)

b. Transverse section of leaf (through the midrib)

c. Transverse section of a portion of stem

d. Transverse section of the root

e. Longitudinal section of a portion of stem

g. Longitudinal section of the root

3.8.1 Chemomicroscopy of powdered sample

A few drops of appropriate reagent were applied to the powdered sample and then observed under the compound microscope for the presence/absence of chemical substances such as starch Ca- oxalate, tannin.

a. Test for tannins

Powdered sample of the plant was treated with ferric chloride solutions and observed under the microscope for the presence of tannins (Trease and Evan 1987).

b. Test for starch

The powdered plant sample was treated with iodine solution and observed under the microscope for the presence of starch (Trease and Evans 1987).

c. Test for calcium - oxalate crystals

A sample of the dried plant powdered was cleared with chloralhydrate solution and observed under the microscope using glycerine as a mountant, for the presence of calcium oxalate crystals (Trease and Evans 1987). After addition of a few drops of concentrated sulphuric acid (80 %), the powdered sample was again viewed for the presence of calcium oxalate crystal (Trease and Evans, 1987).

Some structures, as seen in the dried powdered sample of the plant were drawn and labeled (seen result).

3.9 Quantitative Leaf Microscopy

3.9.1 Determination of palisade ratio

Pieces of the leaf about 2 mm square were cleared by boiling with chloral hydrate solution mounted and examined with a 4 mm objective. A camera lucida was arranged so that the epidermal cell and the palisade cells lying below them may be traced. A number of groups, each of four epidermal cells were traced and their outlines linked in to make them more conspicuous. The palisade cells lying beneath each group were then focused and traced. The palisade cells in each group were counted, (those being included in the count which are more than half - covered by the epidermal cells), the figure obtained was divided by 4 (This gave the palisade - ratio of that group (Trease and Evans, 1987).

3.9.2 Determination of stomatal number

Four fragments of the leaf from the middle of the lamina were cleared with chloral hydrate solution.

A camera lucida was set up and by means of a stage micrometer the paper was divided into squares of $1mm^2$ using a 16 mm objective. The stage micrometers were then replaced by the cleared preparation and stomata traced.

Prior to this, using fresh leaves, replicas of leaf surface were made which were satisfactory for the determination of stomata number and stomata index. An approximate 50% gelatin gel was liquefied on a water bath and smeared on a hot slide. The fresh leaf was added; the slide was inverted and cooled under a tap and after 15- 30mins. The specimen was stripped off. The imprint on the gelatin gave a clear outline of epidermal cells, stomata, and trichomes (Trease and Evans, 1987).

3.9.3 Determination of stomatal index

Pieces of leaf, other than extreme margin or midribs were cleared (with chloral hydrate solution) and mounted on the microscope. The lower surface was examined by means of a 4mm objective and an eye-piece containing 5mm square micrometer disc, number of epidermal cells and stomata were counted. The two guard cells and ostiole were considered as one unit of stomata, within the square. Any cell with at least half of its area

within the square grid was counted. Successive adjacent field were examined until about 400 (four hundred) cells had been counted (Trease and Evans, 1987). This was done four times.

3.9.4 Determination of vein-islet number

Parts of the central lamjna of fresh leaves of *Momordica balsam ma* were cleared in chloral hydrate solution in a test tube, using boiling water bath. Camera lucida was set up and by means of stage micrometer the paper was divided into squares of 1mm using a 4 mm objective lens. The stage micrometer was their replaced by the prepared slide of the cleared leaf parts and the veins were traced in the area of a square (2 mm x 2 mm), and counted. Each numbered area was completely enclosed by the veins, and those veins not completely enclosed were excluded (if cut by the top left hand side of the square). A range of values were obtained and the mean was taken as vein-islet number (Trease and Evans, 1987).

3.9.5 Determination of vein-islet termination number

Following the procedure for the determination of vein-islet number the veinislet and branch of a vein-islet terminations were counted per mm2. A range of values were recorded and the mean calculated, was noted as the vein-islet termination number.

3.10 Quantitative Evaluation of Crude Powder

Evaluation of crude powder of *Momordica balsamina* by the determination of some physical constants, was carried out, using specific standard procedures (Trease and Evans, 1987). The evaluative parameters determined include;

3.10.1 Determination of Ash value *of Momordica balsamina* Powder

A nickel-crucible was heated at ***105°C*** to a constant weight and its accurate weight was noted. The crucible was cooled and stored in a desiccator.

About two grams (2 g) of the powder of *Momordica balsam ma* was accurately weighed out into the crucible.

The crucible (with its content) was gently heated until it was moisture-free and was completely charred.

The heat was gradually increased until most of the carbon (black residue) was vapourised and finally heated strongly until the residue was free from carbon (i.e. almost white)

The crucible (with contents) was cooled and weighed. The weight was noted.

Heating and cooling was continued until the weight of the residue (ash) was constant.

The weight of the (Ash) was determined, by subtracting the weight of the crucible *from* the final weight (i.e. the weight of the crucible and the residue or ash).

3.10.2 Determination of Acid Insoluble Ash Value

A crucible was heated to a constant weight at 105°C and its accurate weight was noted then crucible was cooled. Two grams **(2** g) of the powder of *Momordica balsamina* was accurately weighed out into the crucible. The crucible (with its content) was gently heated until it was moisture-free and was completely charred. The heat was gradually increased until most of the carbon (black residue) was vapourized and finally heated strongly until the residue was free from carbon. The crucible (with contents) was cooled and weighed. Heating and cooling was continued until the weight of the residue (ash) was constant. The crucible (with the ash) was transferred to beaker containing 25 ml of dilute hydrochloric acid. The content (in the beaker) was boiled for five minutes and filtered through an ash-less filter paper.

The beaker and crucible were washed with water and the washings passed through the fitter paper. The washings were repeated three times,

and the residue was collected in the tip of the cone of the filter paper. The filter paper was dried along with the funnel in an oven at *105°C.* The filer paper with the residue was folded and kept into an accurately weighed and previously heated nickel crucible.

The crucible was heated gently until the filter paper was completely charred, and then heated strongly for a few minutes. The crucible was cooled and weighed, the final weight was noted. The weight of the residue (the acid insoluble residue) was determined by subtracting the weight of the crucible from the final weight.

The acid-insoluble ash-value was calculated (in percentage), with reference to the initial weight of the powdered drug (i.e. *Momordical balsarnina).*

3.10.3 Determination of Extractive Values

(1) Alcohol-soluble extractive value

Five gram (5g) of powdered plant was accurately weighed out into a 250m1 stopped conical flask.

A hundred (100) ml of 90% ethanol was added. The flask was shaken on a mechanical shaker for six (6) hours and the allowed to stand for 18 hrs. The extract was filtered.

The beaker and crucible were washed with water and the washings passed through the fitter paper. The washings were repeated three times, and the residue was collected in the tip of the cone of the filter paper. The filter paper was dried along with the funnel in an oven at 105°C. The filer paper with the residue was folded and kept into an accurately weighed and previously heated nickel crucible.

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3.10.3 Determination of Extractive Values

(1) Alcohol-soluble extractive value

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A heated flat-bottomed evaporating dish was accurately weighed and the

weight noted.

Twenty milliliters (2Oml) of the filtrate (i.e. extract above) was taken into the tarred flat bottomed evaporating dish, and evaporated to dryness on a hot plate.

The residue was dried to a constant weight at 105°C in an oven, and the final weight noted. The weight of the residue (obtained from twenty-20 ml-milliliters extract) was calculated by subtracting the weight of the evaporating dish from the final weight (of the residue and crucible). Alcohol -extractive value was calculated (in percentage) with reference to the initial weight of the powdered plant

(ii) Water soluble extractive value

The procedure for (alcohol soluble extractive value) was repeated but using chloroform-water (instead of ethanol).

Chloroform-water (0.25 of w/v chloroform in distilled water (Trease and Evans, 1987). This was repeated 3 times.

3.10.4 Determination of Moisture Content by *"Loss on (drying method"*

This was carried out by the loss on drying method, and was repeated three times.

An evaporating dish was heated to a constant weight. The weight was noted. The evaporating dish was allowed to cool, and then three (3) grams

of the powdered plant was accurately weighed into the dish. The evaporating-dish (with contents) was placed in an oven at 105°C and dried to a constant weight. (This was achieved by checking the weight at thirty minutes interval, after an initial drying of one hour, until two consecutive same weights were obtained) (Trease and Evans, 1987).

3.11 Preliminary Phytochemical tests of *Momordica balsainina* Powder

In the phytochemical investigations of *Momordica balsamina,* special attention was paid to detection and extraction of its chemical constituents because of their uses in ethnomedicines. Chemical tests using standard and established methods were carried out to detect the presence of various chemical constituents present in the plant powder. Using standard and established methods, the phytochemical screening of *Momordica balsamina* was carried out in three stages:

(a) The preliminary phytochemical tests for detection of chemical constituents present in the dried powder of *Momordica balsamina.*

(b) Extraction of chemical constituents (from the powdered crude sample of *Momordica balsamina,* using standard and documented procedures.

(c) Chromatography of flavonoids and other constituents of the plant, that were isolated. Identification of certain groups of compounds (or

plant constituents) may be achieved from the results and observation of simple physical and chemical tests. Since most of these drugs are complex chemicals, polymers etc, elucidation of their constituents requires hydrolysis as an initial step of investigation, followed by specific tests that will yield specific results for particular groups of compounds or constituents.

Preliminary tests in accordance to standardized and recognized method (Trease and Evans), were carried out in the identification of chemical constituent, of dried powder of *Momordica balsamina* Linn.

After phytochemistry, then chromatography was conducted Chromatographic procedures were further carried out to ascertain (establish) presence of the five chemical constituents that were discovered in the powdered sample of the plant.

The Preliminary Phytochernical tests were carried out as indicated below: Portions of the powder were treated as follows:

3.11.1 Test for alkaloids

A small portion (O.2rn1) of the extract was stirred and placed in **I** % acqueous hydrochloric acid (O.5m1) on a steam bath. Then 1 ml of the filtrate was treated with:

(a) Mayer's reagent (3 drops)

(b) Draggendorff's reagent

Mayer's Reagent:

To O.5ml of the extract residues attained above 2-3 drops of mayer's reagent was added.

(Alkaloids were participated from the solution).

(Trease and Evans, 1987)

Draggendorff's Reagents

2-3 drops of Draggendoff's reagent were added to *O.5rn1* of the extract. Formation of a bufl'prccipitatc indicated presence of alkaloids (Trease and Evans 1987).

3.11.2 Test for Tannins

(a) Ferric chloride (Fed3 Test

One milliliter of the plant extract was mixed with 10ml of distilled water and filtered. Ferric chloride (Fecl3) reagent (3 drops) was added to the filtrate. A blue black or green precipitate confirmed the presence of tannins or catechol tannins.

(b) Lead subacetate test

Three (3) grams of the powdered sample was boiled in fifty (50m1) millilitres of water for three (3) minutes on a hot plate. The mixture was filtered while hot and to the tilterate, two to three (2-3) drops of lead sub acetate solution were added. Formation of a coloured precipitate indicated the presence of Tannins.

3.11.3 Test for Steroids

(a) Libermann-burchard test

The acqueous extract of the plant (10ml) was placed in a beaker and evaporated to dryness. The residue was dissolved in acetric anhydride (O.5ml) and chloroform (0.5 ml).

The solution was transferred into a test tube and concentrated sulphuric acid (2m1) was added down the side of the test tube. Brownish red or violet ring at the zone of the intersection with the supernatant and a green or violet coloration indicate the presence of sterols, steroids and triterpenes.

(b) Salkowiski's test:

To the extract of the powdered sample two - three (2-3) drops of concentraed sulphuric acid were added from the side of the test-tube (held at an angle of **450).** An immediate colour change at the interphase of the extract and sulphuric acid, (the colour gradually changed over a period of one hour), indicated the presence of unsaturated sterols.

3.11.4 Test for Saponins

(a) Five milliliter of another extract of the plant was vigorously shaken with I 0m1 of distilled water for 2 minutes. The test tube was allowed to stand (in a vertical position) and observed. The appearance of foam that persists for at least 15 minutes confirmed the presence of saponins.

(b) Haemolysis test:

Water extract of about 2g of the powdered sample was filtered. 2m1 of 1.8% aqueous sodium chloride solution in two (2) separate test tubes were taken. Two (2) mls of the filterate (above) were added to one of the test tubes. To the other test tube two (2) mis of distilled water was added (control). From a syringe, five (5) drops of animal blood were added to each of the test tubes and mixed (by gently inverting the test tubes), NOT **SHAKEN. The** tubes were observed for fifteen (15) to thirty (30) minutes for haemolysis (settling down of the red blood cells. Presence of haemolysis in the tube containing the extract indicated presence of Saponins.

3.11.5 Test for Flavonoids

2g of the powdered drug was detanned with acetone. The acetone extract was decanted. The remaining acetone present in the extract was evaporated on a water bath. The dried extract was mixed in warm water and mixture was filtered, while hot, and allowed to cool. The filtratre was used for the following tests:

(a) Lead acetate Test

To 5m1 of the extract (the water extract above), 2-3 drops of lead acetate solution were added. Formation of a buff coloured precipitate indicated the presence of flovonoids.

(b) Ferric chloride Test

2rn1 of the water extract (above) was diluted in distilled water in a ratio 1:4, and a few drops of 10% ferric chloride (Fed3) solution were added. A green or blue colour formation indicated the presence of phenolic nucleus (i.e. flavonoids).

c. Metallic magnesium and concentrated hydrochloric acid test

A portion of the extract of the plant (2m1) was heated, metallic magnesium and concentrated hydrochloric acid (HCL), *5* drops, were added. A red or orange coloration indicated the presence of flovones aglycones.

3.11.6 Test for reducing sugar (CHO)

(a) Two milliliter of the plant extract was placed in a test tube and 5rn1 mixture of equal volumes of fehling's solution A and B were added and boiled in a water bath for 2 mins. A red or brisk red precipitate indicated presence of reducing compound.

(b) **Molisch's test:**

About 0.5g of the powdered sample was heated with about three (3)mls of water. A few drops of Molisch's reagent were added to the filterate, and a small amount of concentrated sulphuric acid was added from the side of the test tube to form a lower layer. A reddish colour of the interfacial ring indicated presence of carbohydrate.

3.12 Extraction procedures of various classes of constituents

Extraction of the powder sample was carriedout using distilled water. The extract was reduced in volume (by concentration on a water bath), and subsequently air-dried weighed, various concentrations were prepared from the dried extract which were used in carrying out this study.

3.12.1 Alkaloids

5g of crude *of Momordical balsainina* was made into a paste with 1% Sulphuric Acid 14) (H_2S0_4). The mixture was centrifuged and the marc was discarded. The supernatant was with amrnoniasolution and extracted with chloroform. The chloroform Layer lower layer) was retained and concentrated to about five mls. (5ml) (Trease and Evans, 1987).Tests for the presence of alkaloid were conducted using a small portion of the concentrated (chloroform layer) solvent of the plant powdered.

a. Draggendorf's Reagent:

Two-three drops of Draggendorff reagent were added to the extract. A buff precipitate formation indicated presence of Alkaloid.

(b) Mayer's Reagent

About 0.1m of Mayer's reagent was added to a small portion of the above extract (chloroform) of *Momordica balsam ma* powder (above). Precipitate formation indicated the presence of Alkaloids (Trease and Evans, 1987).

3.12.2 Steroids

30 g of crude powder of the plant was extracted with l5Omls of 80%

Ethanol and heated for (3 minutes) on a water bath. The extract was cooled to room temperature and filtered. The *filtrate* was evaporated to dryness and cooled at room temperature. Fifty *(50)* ml of petroleum ether was added to the dried filtrate (above) and stirred for a minute. The petroleum ether portion was decanted (to remove-chlorophyll pigment etc). One hundred 100 mIs of chloroform was added to the residue and thoroughly stirred for five (5) minutes. Anhydrous sodium sulphate was added. The mixture was filtered and the chloroform portion (filtrate) retained.

Luherman-huichard's test was carried out using the sample of steroids (obtained, from the above extraction) to ascertain that the residue contained steroids.

3.12.3 Tannins

Twenty five gram (25 g) of the powder drug was extracted with about (200 ml) of methylated spirit and left on a mechanical shaker for (6) hrs. The mixture was filtered. The marc was re-extracted with about (120) mIs of methylated spirit, once more for another 4 hrs and the filtrates were combined.

The filtrate was concentrated under vacuum till a semi solid mass was obtained. The semi-solid mass was diluted (1:50) with distilled water and kept

The extract was fi1tred and the flocculent precipitate was discarded. The filtrate was extracted with equal volume of ethyl acetate (3X). The three ethyl acetate extracts were combined and concentrated under vacuum to recover the solvent, and finally the tannic acid obtained (3.12.3 above) was dried in a desiccator.

Ferric chloride test for identification of Tannic acid was carried out, using the tannic acid obtained (Trease and Evans, 1987).

a. Ferric Chloride Test

About five *(5)* drops of ferric chloride solution were added to **a** portion of the sample (tannic acid obtained above), the colour of mixture was noted

3.12.4 Extraction of Saponins

Twenty five (25 g) grams of dried powder of *Momordica balsamin*a was weighed and extracted with alcohol. The process was repeated and the extracts combined and evaporated to dryness. The dried residue was dissolved in boiling water and filtered. The filtrate was extracted with di-ethyl ether, then ethyl acetate and finally with n-butanol (saturated with water). n-butanol fraction was concentrated. Saponin was precipitated by

adding diethyl ether to n-butanol fraction.

Frothing test was then carried out with the saponin-sample obtained from the above extraction, (Oripek *et al*., 2004).

3.12.5 Extraction of Flavonoids

10g of the powdered plant was extracted with 80mls (sufficient quantity) of petroleum ether. The marc was dried. The marc was re-extracted with sufficient quantity of acetone, and the extract was concentrated over water bath. The concentrated residue was treated with petroleum ether, Benzene, and chloroform. The residue was treated with hot water and filtered. The filtrate was retained. The insoluble residue was treated with ethanol and dried over water bath (flavonoid Aglycone). The hot water filtrate obtained (above) was partitioned with ethyl acetate. Two separate layers of aqueous (lower) and organic (upper) layers were formed. The organic (ethylacetate) layer was evaporated to obtain the flavonoid glycosides residue. While the aqueous portion contain the sugar (glycone) (Trease and Evans, 1987).

The extracts obtained were used for chromatography.

Diagrams showing the general methods of extraction of the five constituents are shown below (Fig. 3.1 - 3.5).

(Trease and Evans 1987).

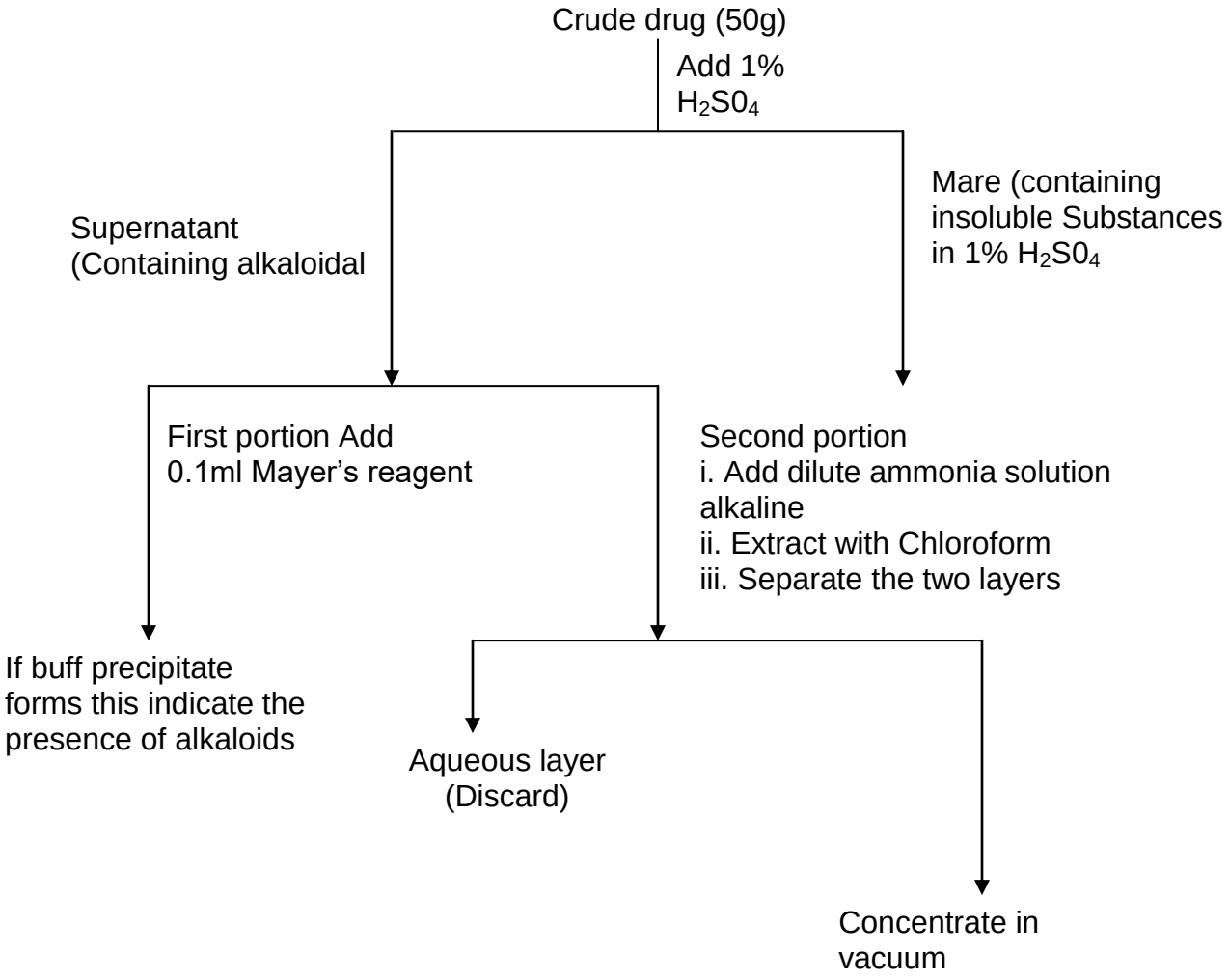


Fig. 3.1: *The* General Method of Alkaloids extraction from plant materials

(Oripek et al., 1998)

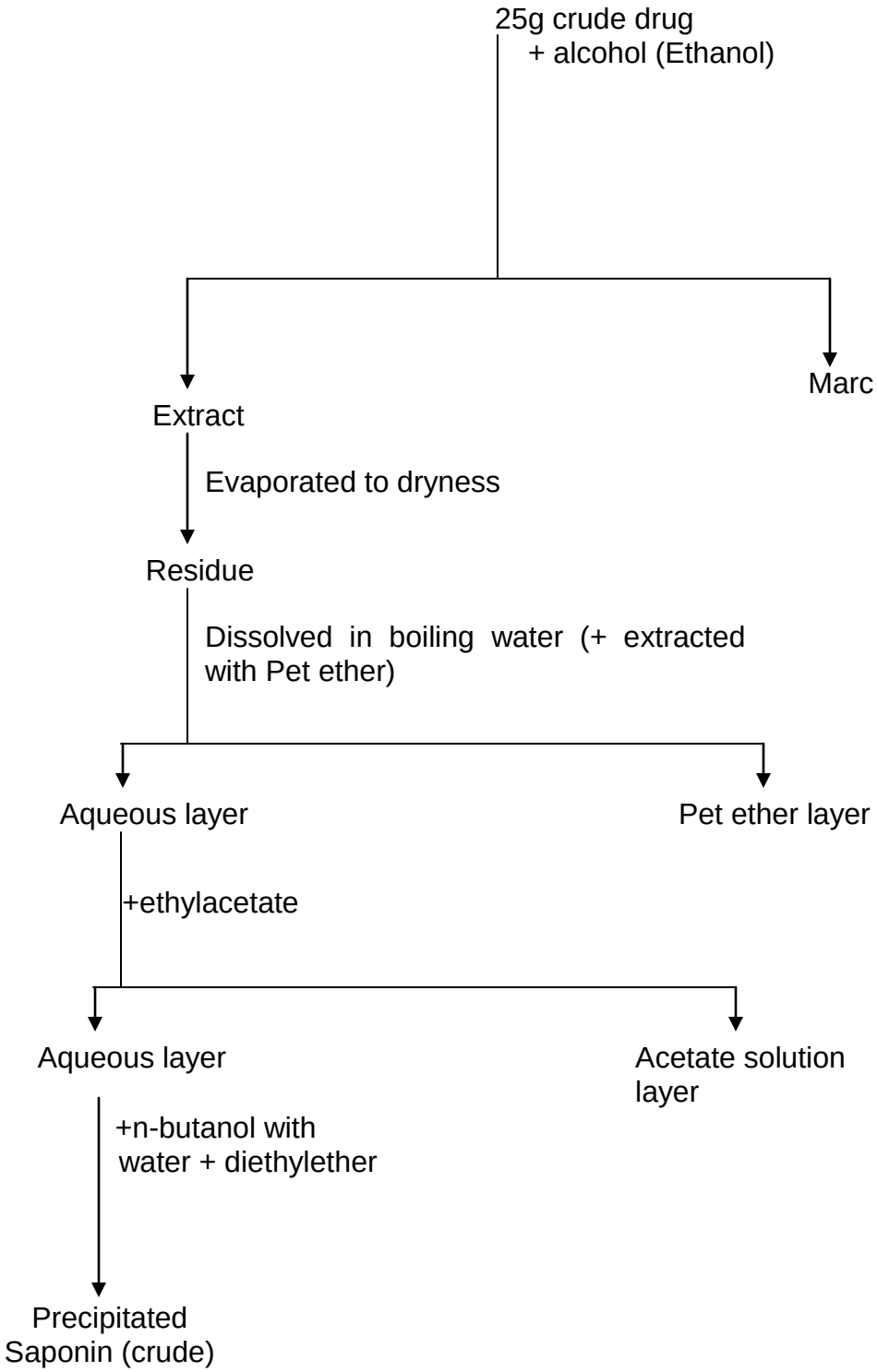


Fig. 3.2 Extraction of Saponins

(Oripek et al., 1998)

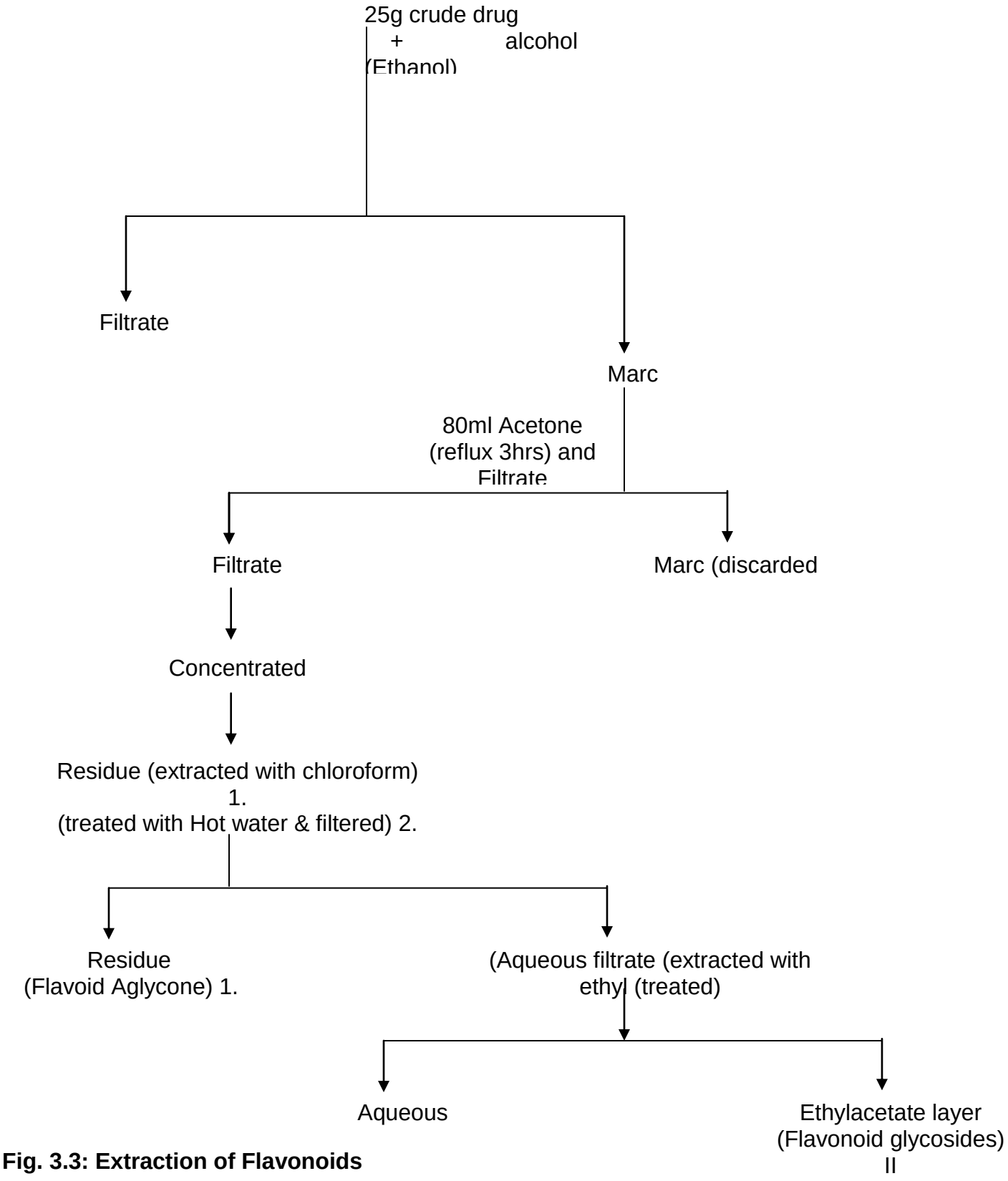


Fig. 3.3: Extraction of Flavonoids

(Kokate, C.K. 1985)

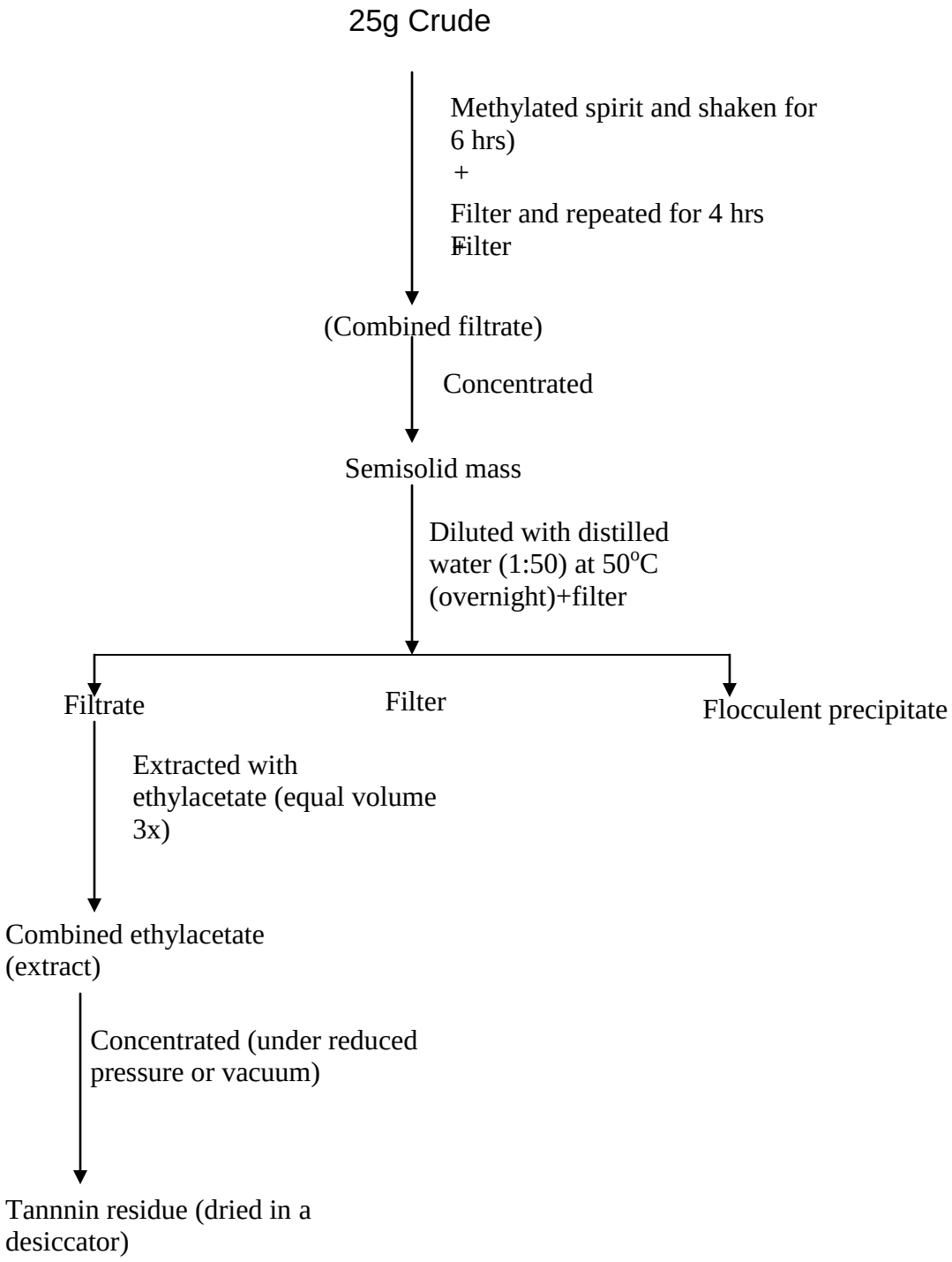


Fig. 3.4: Extraction of Tannins

Trease and Evans, 1987

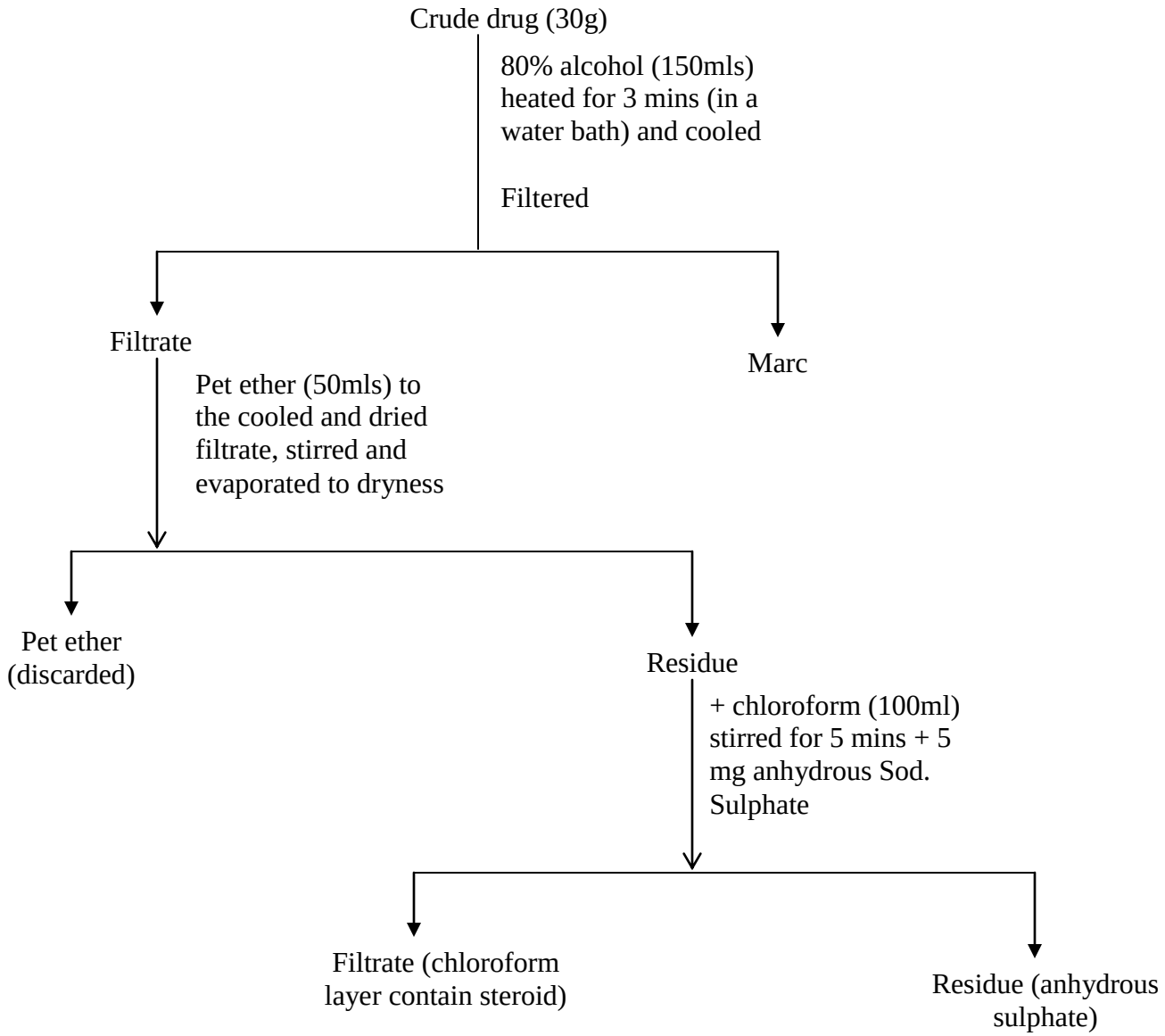


Fig. 3.5: Extraction of steroids

3.13 Chromatography

Thin layer chromatography (TLC) of some extracted chemical constituents of Momordica balsarnina powder.

a. TLC chromatographic analysis of the flavonoid fraction on silica geL The solution of the flavonoid fraction was applied as a spot near one end of the TLC. The plate was deeped in the solvent B.A.W. (n-butanol-acetic acid- water, 4: 1:5). The solvent was allowed to move up the plate until the solvent front has progressed to a point near the opposite edge of the plate, which was then removed and dried. After drying, the paper was examined in ordinary light, under ultra-violet light and sprayed with suitable reagent (ammonia vapour) and re-examined. The positions of the components were marked and the R: values were calculated.

b. The procedure was repeated for the TLC chromatographic analysis of the remaining of fractions (i.e. Tannin, saponin, alkaloid and steroid) of momordica balsamina extract, using suitable solvents, and appropriate reagent for visualization (as below).

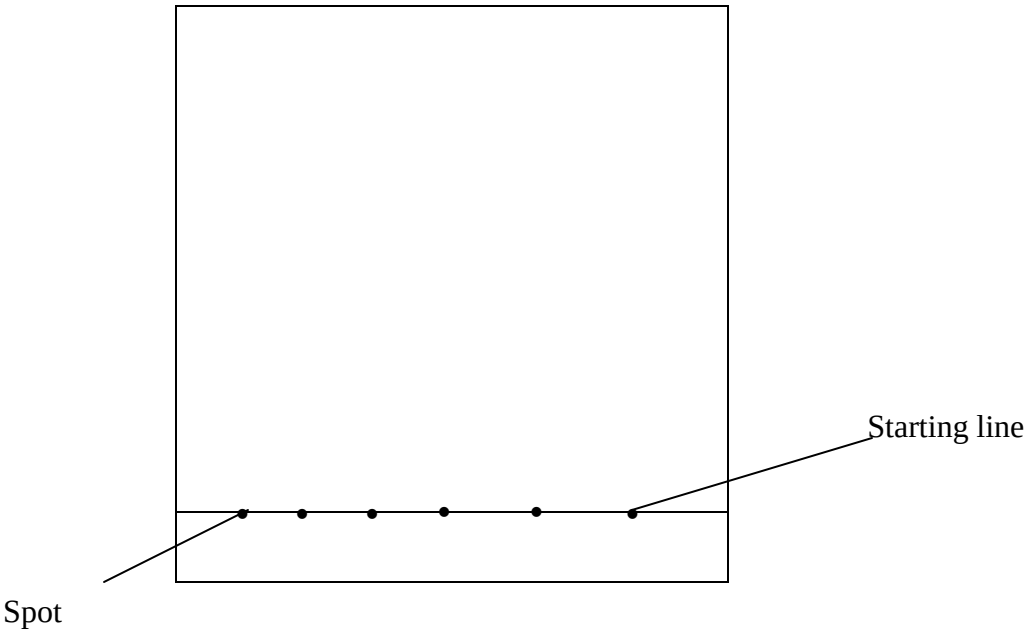
Ascending techniques was used for all the chromatographic processes carried out for this study.

Application of the Sample

Sample application is very important in the technology of TLC. Improperly applied samples result in poor chrornatograms.

Samples were applied as a 0.01-1.00% in the last polar solvent in which they are soluble. The sample were applied near one side of the layer or plate (about 1.5- 2cm from the edge), so that the layer made contact with the mobile phase, but the samples zone were not immersed in the liquid. Capillary tubes were used for the application of the spots.

Starting line



The following are the reagents used for visualization of different chemical constituents that were in the chromatography of extracted chemical components of *Momordica balsam ma* powder.

I. Alkaloids

Reagent for visualization	Draggendorff spray reagent
Procedure	to be sprayed

2. Flavonoids

Reagent for visualization	Ammonia vapour
Procedure	to be exposed to ammonia vapours and viewed under ultra-violet light.

3. Steroids

(a) Reagent for visualization	Iodine
Procedures:	Plates were to be placed in a covered tank containing a few cryptal of Idoine that have evaporated.

4. Tannins

Reagent for visualization	Iodine crystals
Procedure	to be sprayed.

5. Saponins

Reagent for visualization	Ferric chloride
Procedure	to be sprayed with 10% solution of ferric chloride

The solvents (mobile phase) used in the chromatographic process of identified - constituents *of Momordica balsamina* extract were as follows:

1. Alkaloids

Solvents: Benzene - ethanol (9:1)

2. Flavonoids

Solvents: Petroleum ether - ethyl acetate (2:1)

3. Tannins

Solvents: Benzen

4. Steroids

Solvents: Benzene - ethylacetate (2:1)

5. Saponins

Solvents: Isopropylether - acetone (5:2)

3.14 Acute toxicity test of the Extract of *Momordica balsamina* Linn

The different groups of compounds identified in the plant extracts were: alkaloids, saponins, tannins, steroids and flavonoids.

In this study of Acute Toxicity Test *of Momordica balsam ma,* we intended to determine the acute toxicity or otherwise of the water extract of the plant, using two different routes of administrations (i.e. the Oral route and the intraperitoneal, route).

(a) Acute Toxicity

(i) The toxicity study of aqueous extract *of Momordica balsamina* Linn. on rats through oral route of administration.

(ii) The toxicity study aqueous extract of the plant on rats, through intraperitoneal route of administration.

The effect of aqueous extract of the plant on rats was carried out. Three groups of rats (three in each group) were used for the study. Three different doses of the extract were administered (using two different routes of administration i.e. IP-intraperitoneal and Oral) to each group of the animals. The animals were observed for a period of 24 hours (for acute toxicity signs i.e. death of the animals). The LD50was calculated using Lorke method. A control group of three (3s), two groups, received distilled water only.

Different doses of the extract (10, 100, 1000 mg kg1) body weights were injected into the rats, IP, in the groups A. B. C. respectively.

The other two groups (D & E) were given Oral administration of the extract. Higher doses upto 5000mgkg body weight were administered to these groups (i.e. (D & E), the animals were observed for 24 hours, after each doses.

CHAPTER FOUR
RESULTS

4.0 Preparation of Plant Sample

4.1 Collection of Plant Materials

Collection of plant materials of medicinal value for its pharrnacognostic screening is known to be affected by the following factors:

* Seasons (Annual, Biannual or perennial)

* Plant parts (flowers, flowering tops, roots, stem leaves, seeds, barks (root or stem), fruits or whole plant).

* Time of the day (especially, when materials are flowers)

All the above factors were put into consideration in the collection of plant samples of *Momordica balsam ma,* for the study carried out. The plant was collected from Zaria - City, where it was found (in its natural habitat) growing over the fences and gardens of local inhabitants. Initial identification of the plant was carried out in the field before certification by the herbarium attendants in the Department of Biological Science A.B.U. Zaria and further confirmed by the Taxonomist in the Biological Sciences Department. The plant was collected in October and was given a herbarium No. 2718 by the herbarium attendants in Department of Biolgoical Sciences. A herbarium specimen was prepared in accordance with standard procedures. (University of Floria March, 2007).

4.2 Drying and Pulverization of plant sample

The plant sample was dried under room temperature and in the shade, in the Department of Pharmacognosy and Drug Development of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

The dried sample was pulverized into fine powder before it was used for the Pharmacognostic screening.

4.3 Macroscopical - Investigations of the Plant Sample

Momordica balsamina is a climbing annual plant having ornamental fruits, the fruit is diverse in sizes (depending on the soil and climatic condition of where it was grown).

The macrospical investigations showed that the leaves were alternate and usually palmately, five lobed or divided, stipules were absent in the leaves. The petiole was not grooved, flattened or winged. It was pubescent and 9 - 12cm long. The Larnina - composition was simple, its venation was pinnate with Acute apex and cordate base. The colour of the Lamina was green and the odour was characteristic. The margin of the leaves was acicular ordendate, while the surface had no presence of hairs or oil glands. The leaf was generally ovate in shape tough and bitter in taste.

The macrospical investigations revealed that flowers are actinormorphic and nearly always unicellular. Usually yellow and six petaled, fleshy and oval shaped. The perianth has a short to prolonged epizygous zone, that bears a calyx of three to six (3- 6) segments and most frequently three to six (3 -6) sympetalous corolla. Calyx is about 2 3 cm long while the corolla in about six (6) centimeters long. The androecium is highly variable consisting of basically five distinct to completely connate stamens that frequently are twisted, folded or reduced in number. The gymnecium consists of a single compound-pistil of two to five *(2—5)* carpels, generally with one style and as many style branches or major stigma lobes as carpels and an inferior ovary with one loculla and usually numerous ovules on two to five (2- *5)* parietal placenta (or three locules with numerous ovules on axile placenta.

The macroscopical investigations of the fruits show that they (fruits) are about seven (7cm) centimeter long, ovoid or ellipsoid tapering at both ends, covered with ridged irregular, protruberings, bursting at maturity. It is succulent, greenish, and bitter (before maturity) but yellow — green and sweet, with orange-red pulp (at maturity), bursting to reveal the embedded seeds, usually four in number.

The macrospical investigations carried out also showed that the seeds were u embedded in an orange - red pulp. They were usually four (4) in number. They dark or black in colour, small, smooth (or shiny) and hard. They were about two to (2 - 3) millimeters long, ovate in shape, and possess no hairs.

4.4 Microscopial - investigations of the plant

The following features of the plant were seen under the microscope. The epidermal of the leaves were seen as interlocking wavy cells joined together by single cell wall stomata present in-between the cells (see Fig 4.1 - 48).

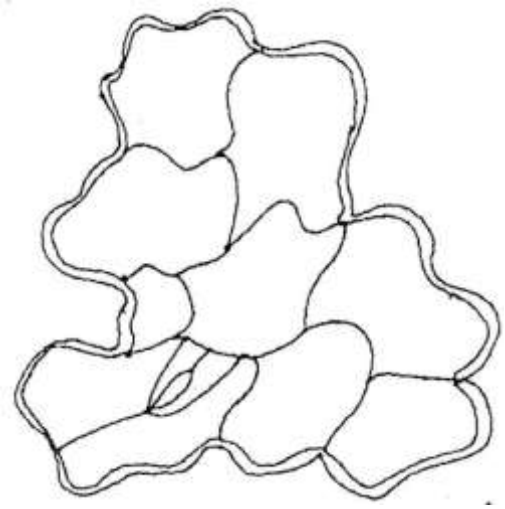


Fig. 4.1: Upper epidermal cells of the plant leaf x 400

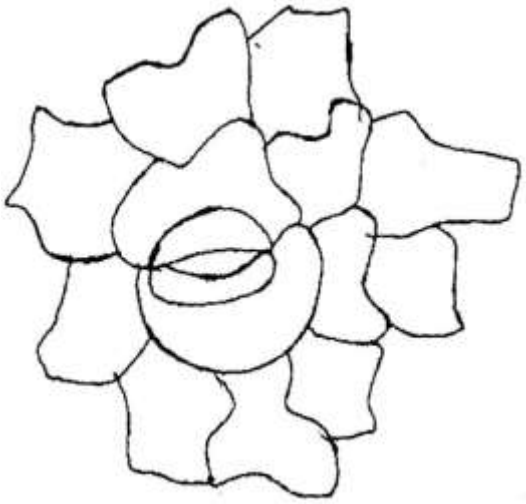


Fig. 4.2: Paracytic stoma in the lower epidermis of the plant Leaf x 400

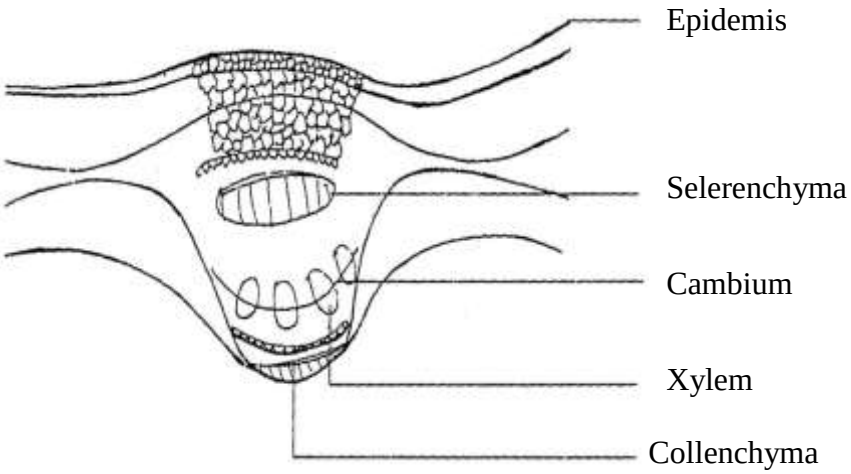


Fig. 4.3: Transverse Section of Leaf (through the midrib) of the plant x400

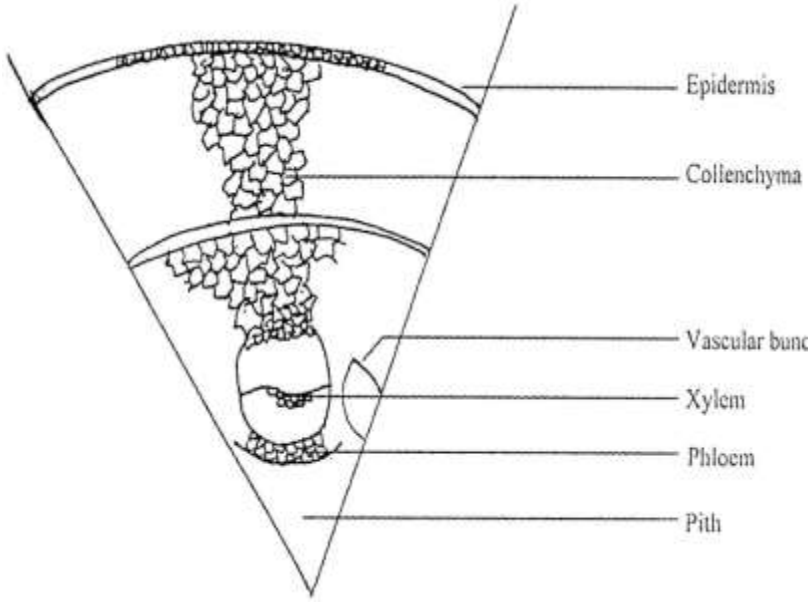


Fig. 4.4: Transverse Section of the stem of the plant x 400

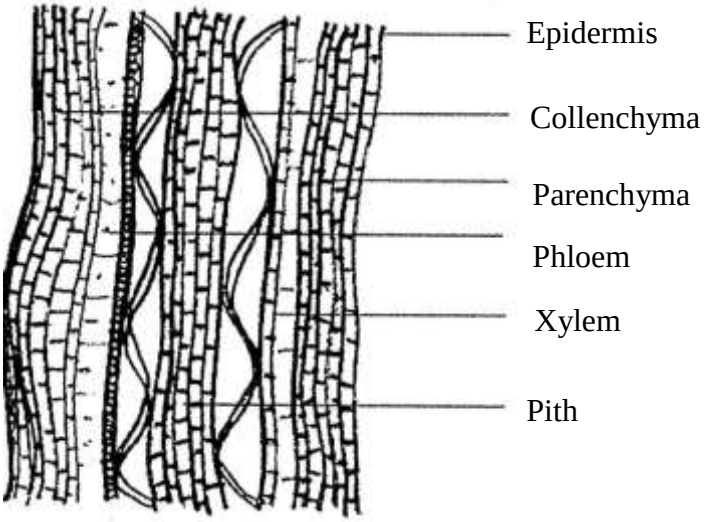


Fig. 4.5: Longitudinal Section of a portion of stem of the plant x400

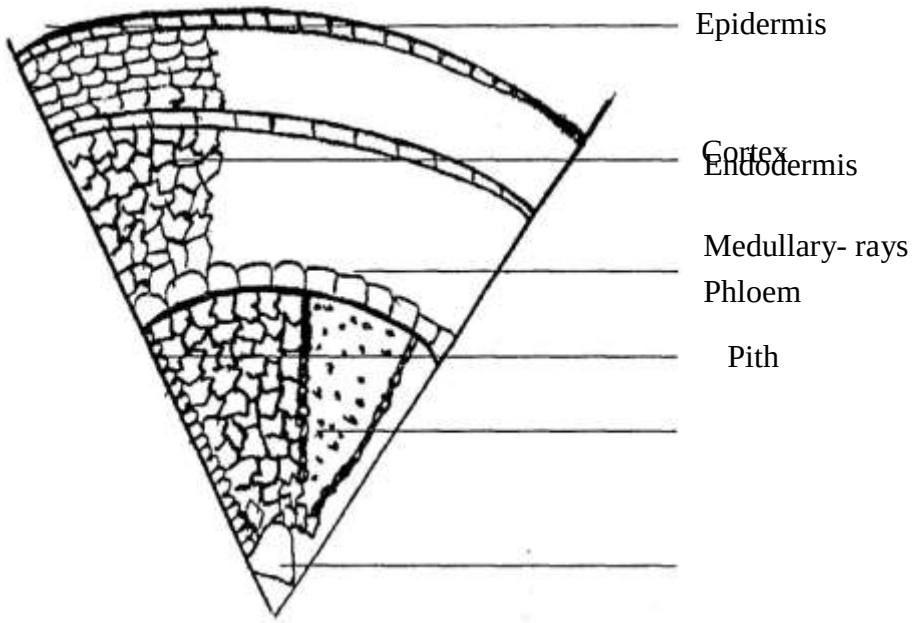
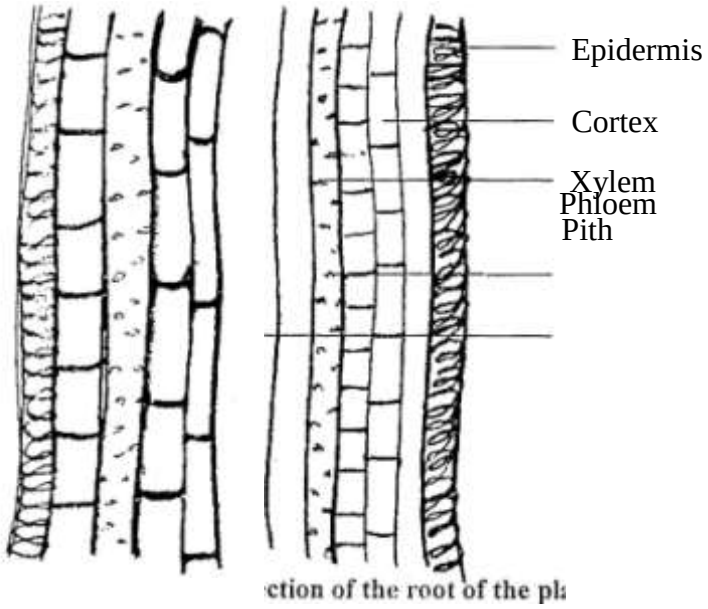


Fig. 4.6: Transverse Section of the root of the plant



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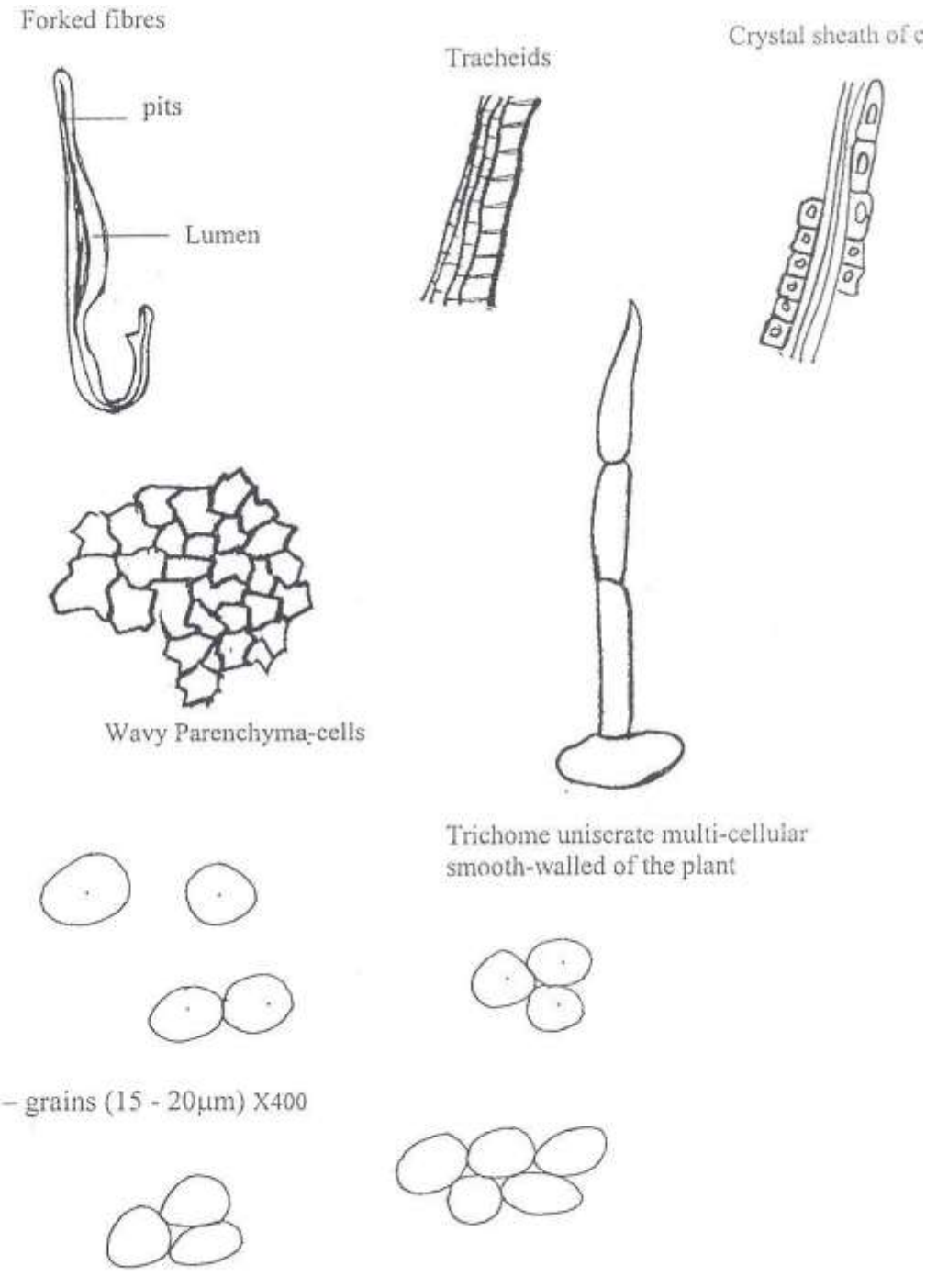

Fig. 4.8: Microscopical features of the plant powdered sample X 400

The sample of the powder of *momordica balsamina* was mounted in general reagent and examined under high power magnification of the microscope to observe the constituents and component of the plant. Physical characteristics of the powdered sample were initially observed and noted or recorded.

a. Physical characteristics of the Powder

- colour - greenish
- odour- characteristic
- Taste- bitter

b. Microscopical investigations of the **powdered plant sample**

Parenchyma

Cell - epidermal

Shape - polygonal

Thickening - cellulose

Stomata

Type - paracytic

Frequency - few

Location - upper and lower epidermis

Trichome

Type uniserate, multicellar

Frequency - few

Wall - smooth

Xylem

Type - vessels

Frequency - numerous

Apex - none

Thickening - annular, spiral, pitted and reticulate

Fiber

Type - forked

Lumen - small

Starch grains

Shape - oval

Size l5-20um

Hiilum - central

Striations - absent

Aggregation - single, dual, multiple

Frequency - numerous

Calcium oxalates

Type - prisms

Location - free

Frequency - few

Ca- oxalate crystals were observed in the powdered sample of *Momordica*

balsamina. They were seen as reflective substances (structures or objects) under the microscope.

Starch grains were seen in clusters of three, four or fives. They were seen as ovoid shaped single walled structures under the microscope. Some were seen as single grains.

Vascular bundles, many cells of the epidermis and trichornes were all noted under the high power magnification of the compound microscope. The trichornes were uniserate but multicultural and smooth - walled. Stomata were also seen. The stomata - type observed were paracytic.

Table 4.1: Quantitative Leaf Microscopy

Momordica balsamina	**Upper surface**	**Lower surface**
Palisade ratio	15 17.5—20	12—10—11
Stornatal number	20—27.5—35	26—28—30
Stornatal index	30 — 32.5 — 35	36 — 37 — 38
vein islet number	6 —7.5 —9	9 — 10 — 11
Veinletterminalnumber	1.2—1.6-2	2—2.2—2.5

c. Chemomicroscopy of the dried powder

The followings are the features observed (using different reagents) as seen under the microscope: Momordic

Table 4.2: Chemomicroscopical examination of the powdered sample of *Momordica balsamina.* Linn

Test	Observation	Inference
Sample + N/50 Iodine	Blue black coloration (15-20um) size indicates presence of starch	+ve
Sample + Alcoholic Picric acid solution	Yellow coloration was observed indicates presence of protein	+ve
Sample + Chloral hydrate	Reflective, prism shaped objects 25-30um	+ve
Sample + N/50 Iodine + H,S0 (80%w/v)	No coloration observed. Indicates absence of cellulose	-ve
Sample + Hydrochloric acid	Effervescence produced indicates the presence of calcim carbonate	+ve
Sample + Ferric chloride solution	Brownish black precipitate was seen. Indicate the presence of hydrolysable Tannins	+ve
Sample + Phloroglucinol (in alcohol + concentrated hydrochloric-acid)	Red coloration (not permanent) was observed. Indicates the presence of lignin	+ve

Key:

+ve = positive

-ve = negative

Table 4.3: Quantitative Evaluation of Powdered Plant Sample

Quantitative Evaluation parameters	Average value % *w/*
Moisture content	12.33
Ashvalue	17.61
Acid Insoluble ash value	2.33
Alcohol soluble extractive value	5.00
Water soluble extractive value	10.00

4.5 Phytochemistry of the powdered plant sample

In the phytochernical investigations of powdered plant sample attention was paid to the detection and extraction of its chemical constituents. Using standard and established methods, the phytochcmical screening was carried out in three stages.

a. Preliminary identification tests for detection of chemical constituents of the powder sample.

b. Extraction of chemical constituents, using standard and documented producers.

c. Chromatography of the isolated or extracted chemical constituents.

a. Preliminary identification -test

Small portion of finely powdered crude of the plant was treated with certain and specific reagents for identification of the types of chemical constituents (compounds) that is contained in it, thus:

Table 4.4: Phytochemical screening of the powdered plant Sample

Observation	**Possible Constituents**
A buff precipitate positive with 1. Draggendorff's 2 Mayer's reagent	Alkaloids
1. Positive (Libermann-burchard's reagent) bluish-green-colouration that changed, over a period of time; to reddish-ring 2. Positive (Salkowiski's test)	Steroids
1. (Positive Ferric-chloride test) Browish –black precipitate 2. Metallic magnesium + Conc HCl. Redor organge colour	Hydrolysable tannins flavonoids
1. (Positive Frothing test) Honey comb froth that persisted for more than fifteen (15) minutes 2. Positive Haemolysis test No colour change with moist yellow Sodium picrate paper	Saponin No anthraquinones Carbohydrates
No pink coloration with Borntrager's test 1. A purple to violet colour with Molisch's test 2. Fehling solution A and B + ve	No anthraquinones Carbohydrates

Key:

+ve = positive

-ve = negative

4.6 Extraction of chemical constituents of the powdered plant sample

After the preliminary identification tests standard procedures for the extraction of chemical constituents, identified to be present in the powdered crude of the plant, were carried out. The constituents were differently extracted and tested. The chemical - constituents were confirmed to be the same as those identified earlier. These were:

- Alkaloids
- Saponnins
- Tannins
- Stereoids
- Flavonoids

4.6.1 Chromatographic Analysis

'Chromatographic analysis of the extracted chemical constituents of the plant'

Chromatographic analysis conducted on chemical constituents of the plant (i.e. Alkaloids, flavonoids, Tannins, Steroids, saponins), using specific and appropriate solvents and reagent (for development of the chromatogram) further confirmed the presence of the chemical constituents.

Table 4.5: Results of Chromatographic processes of the plant powder

Constituents	**Solvents**	**Reagents used**	**Result**
Alkaloids	Benzene-ethanol (9:1)	Draggendorf's	(+ve)
Flavonoids	Petroleum ether – ethylacetate (2:1)	Ammonia vapour	(+ve) dark-brownish coloration of sport
Tannins	Benzene	Ferric chloride	(+ve) bluish-black-colouration)
Steroids	Benzene-ethylacetate (2:1)	Libermann-burchard	Yellowish-brown spot on a yellow (+ve) background
Saponins	Benzene	Vanilla + sulphuric acid	(+ve) brownish spot a faint yellow background

Key:

(+ve) = positive

(-ve) = negative

Reference:

C.F. Poole, *et al* (1988)

G. Guiochon *et al* (1980)

E. Tyikak *et al* (1988)

S. Nyiredy *et al* (1989)

F. Geiss *et al* (1987)

4.7 Acute Toxicity Study of extracts of the plant powder

4.7.1 Acute Toxicity Tests

The toxicity tests carried out were divided into two parts.

(a) Toxicity of the aqueous extract of the powdered plant sample through the Oral route of administration.

(b) Toxicity of the aqueous extract of the powdered plant sample through the intra-peritoneal route of administration.

The acute toxicity of the methanolic extract was not carried out because traditionally, only the aqueous extract of the plant sample is used, in the treatment of patients.

Table 4.6: Effect of water extract of the plant on rats through IP route

Wt(g)	**Doses (mgkg')**	**No of deaths**		**GROUPS**
214.40				
198.60	10.00	None	(0)	A
157.20				
205.60				
124.30	100.00	One	(1)	B
207.40				
154.30				
188.20	1000.00	Two	(2)	C
123.20				

Table 4.7: Acute toxicity Studies of effect of Water extract of the plant on rats through the Oral route of administration

Wt.(g)	Doses $mgkg^{-1}$	No. of deaths	Groups
153.4	1600.00	-	
124.1	800.00	-	D
174.5	400.00	-	
138.0	200.00	-	E
160.4	1800.00	-	
180.3	3600.00	-	F
170.5	5200.00	-	
165.4	2600.00	-	G
160.2	1600.00	-	
180.5	200.00	-	Control (distilled
172.3	400.00	-	water was administered)

Table 4.7 shows the effect of different doses of aqueous extract of the plant on rats, through Oral route of administration. Increasing doses of the extract (upto about $5000mgkg^{-1}$ produced no fatal results, in the three (3) groups of three's.

CHAPTER FIVE

5.0 DISCUSSION

5.1 The Plant Collections

The plant was collected from Zaria - City (in Zaria) in the Northern part of Nigeria. Since constituents and chemical component of the plant could depend on the type of soil on which it is grown further studies or comparative studies, on type of constituents of the plant collected from different climate conditions, need to be carried out. This is necessary to enable pharmacognostists and scientists, in general, make a general statement on the use of the plant obtained from different types of climatic conditions.

The constituents, identified in this study, when compared with the constituents identified in the study carried out in Maiduguri (Bukhari and Ahmed, 1995) are more numerous and, thus, the biological actions of the plant extract used in this study are more in number than that obtained from Maiduguri, Borno State of Nigeria. This may be due to the differences in the soil-types of the two areas, or due to other factors that are needed to be identified.

Momordica balsainina, one of the most popular African herbs has alterante leaves and usually palmately five lobed (divided) in nature, which is a

distinguished feature from other species.

Another feature that is characteristic of this species (i.e. *balsamina)* is the fruit (Ann Bot, 2006). This is used for identification and differentiating the plant species. The fruit is about seven (7) cm long, ovoid or ellipsoid (i.e. tapering at both ends) in shape, with ridged or irregular protruberings. *Momordica* herb (plant) is at times classified on the basis of the shape of its fruit.

Other pharmacogostic parameters that help in the detection of the adulteration of the powdered sample of *Momordica balsarnina,* are the evaluative pararneteis (i.e. moisture content, ash value, acid - insoluble - ash - value, alcohol - insoluble - ash - value, etc). These parameters are constant (i.e. physical constants) and are therefore specific (+ value) for specific powder (i.e. powdered drug sample). Evaluation of a drug requires that it will be identified in its entire and powdered form by means of its morphological and cytomorphological characters. These diagnostic features enable the nature and origin of the material to be ascertained. Therefore, drugs are submittive to evaluation procedures which will indicate their acceptability by criteria other than their morphology (Brain and Turner, 1975).

The following evaluative parameters were carried out on the plant using specific standard procedures:

Moisture content: This helps to evaluate the quantity of moisture present in the crude drug. The quantity of moisture in a crude drug

determines its stability as well as its safety. If the quantity of moisture is high, degradation of the crude drug (on storage) will be faster. Bacteria activities will be higher, and the safety of the drug, for consumption, will be reduced. Further more the quantity of the active constituent present in the crude drug will be lower if the moisture content is high, and by extension the efficacy of the crude drug with high moisture content will be lower.

Similarly high acid-insoluble-ash value is an indication of lesser efficacy or quality of the crude drug when compared with crude drug with lower acid-insoluble-ash value.

On the other hand the higher the extractive value (of both alcohol and water) of a crude drug the better its quality and thus its efficacy. Moisture content of the crude powdered plant *(Momordica balsam ma)* of 12.33 % is on the high side. However, this could be reduced and brought to acceptable range by drying. Its ash value of 17.61 % is a little high too. This could be compared with the crude plant obtained from different environment (from the one obtained in Zaria City) that could have higher quantity and better quality of chemical constituents. The same thing could be said about acid-insoluble-ash value of 2.3%. Extractive value showed that the water extract of the crude powdered plant will be more potent (efficacious) than the methanolic extract. Thus, the use of water extract in the treatment of ailment, by the herbalist, is justified. The water extractive value of the plant powder is higher than that of the alcohol extractive value.

Importance of evaluative parameters

Determination of moisture content present in a crude powdered drug is important because:

The quantity of moisture present in the drug decreases the percentage quantity of the drug by increasing the weight. Moisture causes activation of enzymes (present in the drug powder) which will lead to hydrolysis and consequently deterioration of the drug. Moisture presence in the drug may cause chemical reaction. The presence of moisture may create a good medium for the growth of micro-organisms and insects.

Due to these and many other factors, moisture - content of all drugs should be kept reasonably low (Shellard, *1958).*

Determination of ash-value is the measurement of the amount of residual substance, not volatized when the drug is ignited (African, pharmacopoeia, 1986). The total ash represent the 'physiological ash' (i.e. the ash that is obtainable from the plant itself) and non- physiological ash (i.e. the ash obtained from other extraneous matter) (African Pharmacopoeia 1986).

Determination of acid-insoluble ash-value is intended to measure the amount of silica (or siliceous earth) present in the drug (African Pharmacopoeia 1986). In the determination ofAcid - insoluble Ash-value, it is the physiologcial ash that is dissolved in the dilute acid (Shellard, 1958).

While determination of extractives helps to determine the amount of

constituents that are extractable by the solvent, under specified conditions. The value of extractives help to determine the extent of adulteration or otherwise of the drug - powder.

Microscopic examination is a technique used for study and observation of minute structures, which can only be measured in micros or nanometers. It is one of the important techniques employed in Pharmacognosy for the study and identification of crude drug.

The microscope is the principal instrument which is used to magnify smaller objects (particularly those that are invisible to the naked eyes) in order to study the detail of their structures, constituents and inclusions. Diagnostic features of drugs are the microscopic characters that are examined and observed under microscope with or without the aid of chemical reagents. These characters are needed for the identification of powdered drugs. Microscopical methods of analysis, not only yield qualitative result but can also be used to obtain reliable quantitative figures for the percentage composition of mixed powder of the vegetable origin (where there is adulteration), such as compounded medicated powder and for the estimation of adulterant in a powdered vegetable drug. Such quantitative results are usually difficult to obtain by other methods of analysis. High power magnification of the microscope was used to

examine various morphological features of the plant *(Momordica balsamina).* Those features of *Momordica balsamina* were seen under the microscope (See results).

The microscopic examination of the powdered sample of *Momordica balsamina* helps the scientists to identify the powder of the plant (among group or a number of powders) for pharmaceutical use. Furthermore it assists the pharmacist in the detection of adulteration (if any) present in the powdered drug — sample that is to be used for pharmaceutical purposes and also to know the extent of the adulteration.

The average number of palisade cells beneath each upper epidermal cell is termed the Palisade ratio.

The average number of stomata per square millimeter of epidermis is termed the stomata — number.

Stomata number are usually useless for distinguishing between closely allied species, however, in certain cases the ratio between the number of stomata on the two surfaces may be of diagnostic importance- Accordingly the range, as well as the average value should be recorded for each surface of the leaf and the ratio of values for the two surfaces.

Stomatal Index is the percentage proportion of the ultimate divisions of the epidermis of a leaf which have been converted into stomata. While stomatal number varies with the age of the leaf, stomatal index is highly constant for a given species and may be determined on either entire or powdered samples.

The powder of *Momordica balsamina* contains calcium- oxalate, starch vascular bundles and trichomes. Protein presence and the presence of cellulose and calcium-carbonate were also established in the chemomicroscopic study of the powder. There were Tannins and Lignin as well. All these are microscopic features of the powder that guides one towards the identification of the powder sample, when its needed. Furthermore, the microscopic examination of fresh parts of *Momordica ba/sam/na* showed that it has a wavy epidermal cells having paracytic type of stomata. The plant is a dicotyledonous plant with a uniserate - multi — cellular smooth — walled type of trichomes.

The study of the biological actions of *Momordica balsam/na* cannot be complete, without initially, determining the chemical constituents contained in it. The determination and identification of the chemical — constituents contained in the powdered sample of *Momordica balsamina* help to explain

the biological activity exhibited by the plant and in addition, help to elucidate on the future, possible use to which the plant could be put (employed). Thus, modification and substitution (of chemical radicals) into the parent - compound of any of the constituent (of medicinal or pharmaceutical interest) could result into formation of a more potent (or a more useful) drug of medicinal or pharmaceutical uses (J Herb, 2005).

Momordica balsamna has been shown to have, among others. The following chemical constituents:

- Flavonoids
- Saponins
- Tannins
- Steroids
- Alkaloids

Flavonoids:- Interest in the physiological action of flavonoid compounds and in their possible application to clinical therapy has long existed. The well-known ability ofplorizin to induce glucosuria has made it a widely - used compound in experimental physiology and pharmacology.

The early studies of Szent - Gyorgyi (Armentano et al., 1936) on the so-called “Vitamin P” have led in recent years to extensive invetigations on the distribution isolation and physiological investigation of flavone and

flavonone ghycosides. Rutin, to which “vitamin P” action has been attributed and to which the greatest attention has been paid since the description of its therapeutic properties by Griffith, et al (1952), has been found in the leaves and blossom of a great many families and species of higher plants, as well as in a mutant strain of the green algae chlamydomonas.

Therefore, there is a need for further work on the isolation and structural elucidation of the flavonoids identified in *Momordica balsamina.Structural* determination that could lead to possibility of synthesis of the compound, for large scale production, for the benefit of humanity is needed to be carried out as well.

Saponin, tannins, steroids and alkaloids identified to be present in the crude drug should be clarified through further researches with regards to their various biological action that was exhibited by them. Correlation of the actions of these compounds to the traditional use of the plant by herbal practitioners, may lead to discovery of knew drugs that could be of importance in the field of medicine.

It has also been established that the extract *of Momordica-balsamina* Linn is non-toxic and therefore safe for human consumption.

The results of the toxicity study of extract of powdered *Momordica balsam ma* have shown that the water extract *of Momordica balsamina* produces no fatal consequences (i.e. non-toxic) or adverse effects when administered orally and is therefore safe, whereas the intraperitoneal administration of the aqueous extract of the plant showed fatal (toxic) effect at doses of l000mgkg' and above. However between the doses lO-l000mgkg-' body weight, the extract could be said to be safe. This observation is an indication that the Oral administration of extract of *Momordica balsamina* (Balsam apple) utilized by the Traditional herbal practitioners (and other Herbal Professionals) in the treatment of various ailment and disorders is, safe and non-toxic.

The effects of different doses of the plant extract on rats are shown on (Table 4.6). The intraperitoneal administration of the extract at various doses (1 Omgkg- 1-1 000rngkg-') caused an increase in the number of deaths in the animal (compared to the control, that recorded no death at all). At the dose of 10 mgkg1 (body weight) there was no death. - However, at the dose of 100 mgkg' (body weight) there was one (1) out of three (3) deaths, while at the dose of 1000 mgkg1, there were two (2) deaths out of three (3) in the group. The toxicity was therefore, dose - dependent, when administered through the I intraperitoneal route.

After the study on *Momordica balsamina* pharmacognostically the plant could be said to be locally and readily available, relatively safe, non-toxic and of very useful medicinal purpose.

CHAPER SIX

6.0 CONCLUSION

Pharmacognostic studies of *Momordica balsam ma* (‘balsam apple’) is essential for proper identification and authentication of the plant sample in **both** fresh and powdered form.

Macroscopical studies of the plant sample, the microscopical investigations of its roots, leaves, stem etc help to establish the nature of the plant in its fresh form, while the physical characteristics of the powdered drug sample, in addition to its microscopical investigations and chemornicroscopical investigation established the type of structures and features of its leaves, stem, root etc, in the powdered stale. Furthermore, the type of chemical food substituents and components present in the plant were also revealed, through the phytochemical screening of the powder. Moisture-content, Ash—value arid other evaluative parameters further elucidated into the identification of the plant in its powder form, and could also be used to detect presence or otherwise of adulteration. Phytochemical studies also revealed the type of chemical constituents embedded in the plant drug sample as well.

All these studies help the Scientists, in the proper and accurate identification of the plant n its fresh and powdered forms.

Finally, the safety of the plant (orally and parenterally) was also established through the Acute toxicity tests conducted, on the drug sample.

REFERENCES

African Pharmacopoeia (1986), O.A.U/STRC, Publication No. 2, Vol. 1 pp. 86-88.

Akinniyi, J.A., Sultambawa, M.U.A. and Manawaka D. (1986) Ethnobotany and Ethnopharmacology of Nigerian Medicinal Plant in: The State of Medicinal Plant Research in Nigeria. University Press, Ibadan, Nigeria pp. 154-164.

Ann Bot, (2006). The Cucurbit Images (1515-1518) of the Villa Farnesina, Rome, PubMed. London, UK (2) 76-165.

Andreoli, Thomas E. Charles C.J. Carpenter, Robert C. Griggs, and Joseph Loscalzo, CECIL. Essentials of Medicine, 6th edition Saunders, 2004.

Armentano, P.L., Bentsath, A., Beres, T., Rusznybk, S. and Szent-Gyorgyi, A. (1936). Meber den Einfluss von substanzen der flavongruppe anf die permeabilitat der kapillaren Dentsche Med. Woehschr. 62, 1326-1328.

Bate-Smith, E.C. and Westall, R.G. (1950). Biochem Biophys. Acta. 4, 427-440.

Bate-Smith E.C. and Swain, T. Flavonoid Compounds. Comparative Biochemistry (a) Chem and Lad. 1953, 376; (b) J. Chem. Soc. 1953 -2185-7.

Bate-Smith, E.C. (1950). Nature (London) 161,8353, (1948); Partition Chromatography; Biochem. Soc. Symp. No. 3 62-71.

Bate-Smith's (1948). Studies on the protein of fish skeletal muscle. Biochem. Journal 48. P. 271.

Beesely Thomas E. and R.P.W. Scott, (1998). Chiral Chromatography, John Wiley and Sons. Chichester New York.

Blank F., and Suter, R. (1948). Experintia 4, 72-73.
Bosch, C.H., (2004). *Momordica balsamina L.* Record from Protabase.

Grubben, G.J.H. & Denton, O.A. (Editors). PROTA (Plant Resources of Tropical Africa/Resources vegetables de l'Afrique tropicale), Wageningen, Nethrlands.

Boulter D. Ranshow, J.A. Thomson, E.W. Richardson M and Broun P.H. (1972). New phytologist Proc. R. Soc. Land. B. 181, 441.

Brain K.R. and Turner T.D. (1975). The Practical Evaluation of Phytopharmaceutical, Wright-Scientica, Bristol U.K. pp. 5-9.

Bukhari, M.H. and M.S.C.H. Ahmed (1980). Food Plants in Borno State, Nigeria. Gulani Publishers, Lahore, India.

CfPoole, J. (1988). Planar Chromatogrpahy 1,373.

Consden, R. Gordon A.H., and Martin A.J.P. (1894). Qualitative analysis of proteins, Biochem Journal Vol. 38 pp. 230-244.

Dalziel J.M. (1956). Useful Plants of West Tropical Africa. Crown. Agency for Overseas Government. London U.K. pp. 540-460.

Dence, J.B. (1980). Steroids and Peptides: Wiley Inter Science New York U.S.A. p. 50.

Discovery Nature at James Cook University (J.S.U. DEV. 011669).

E. Tyikak and E. Mincsovics (1988), J. Planner Chromatography, 1,6.

F. Geiss (1987), Fundamentals of Thin Layer Chromatography, Huethig, Heidelberg.

Finar I.L. (1991), Organic Chemistry (5th edition), Longman, Robertstewart Caserio.

Frank Moewus (1951). Toxicosis of Soil and Biological Factors causing it.

Griffith J.Q. JR, Couch, J.F. and Lindaver, M.A. (1952). Proc. Indian Acad. Sci. 35, 242.

G. Guiochon, G. Korosi, and A. Siouffi, (1980) J. Chromatographic Sci. 18, 1324.

Hacker N.F., Moore J.G. Morrison B.W., Daniels S.E., Kotey P. (2004) Paediateric Adolescence Gynaeciology 17, 75-9, 16

Hassan L.G. and Umar, K. J. (2006) “Nutritonal Value of Balsam Apple *(‘Monio4rdica balsamina L.)* Leaves: Department of Pure and Applied Chemistry, Usman Danfodiyo University, P.M.B. 2346, Sokoto, Nigeria; Pakistan Journal of Nutrition, 5 (6)522-529, 2006 ISSN 1680-5194 C Asian Network for Scientific Information, 2006522.

Herbal Africa (2007), Ethnomedicine Practitioners, Association of South Africa herbal Africa. Co. 29/Products/Digestive/Tonic.htm.

Herb Pharmacother, J. *(2005).* Screening of some medicinal plants used in South-West Nigerian Traditional Medicine for Antisalmonella Typhi activity. Pubmed (1) 45 60. Akinyemi K. 0. Mewndie U.E., Smith S.T. Ayofolu A.O. and Coker A. O.

Hutchings, A. Scott, A.H. Lewis, G. & Cunningham A.B. 1996. Zulu Medicinal Plants, and Inventory, University of Natal Press, Pieterrnantzburg.

Jeffrey, Co. (1967), Flora of Tropical East Africa. Cucurbitaceae 17-40.

John Kartesz, (2000) Biota of North American Project (BONAP) University of Carolina, National Plant Data Centre, NRCS, USADA Baton Ruege, USA.

Jack, L. H. (2005). Picture of *Momordica balsarnina* (Linn. Okeechobee. Ifas. UFL edu. ../balsam%2OApple.htm.university of Florida.

Jun E. (2004) “Effects of SP-6 Acupressure on Dysmenorrheal, Skin Temperature in the College Students. Taehan Kanho Hakkoe Chi 34(7): 1343-50 PMD 15687775.

Karumi Y., P. Onyeyili and 0. V. Ogogbuaja (2003), Anti inflarnatory and Anti noceptic (Analgesic) Properties of *Momordica balsamina* Linn (Balsam Apple) leaves in Rats. Pakistani Journal Biol Sci 1515 1517.

Kokate C. K. (1985). Practical Pharmacognosy Vallabh. Prakash. pp. 144. Krichner M. Canale, Bistarini, S. and Merler, M. (1951). Chromatography by Silica gel Chromatobars. Prospects of its applicaUon in the field of forensic toxicology. University of Genovoa. Italy.

Kuhn R., Low, J. and Moewus, F. (1942). Naturwiss. Studieson Pollen germination of certain cucurbitacea 130, 374.

Linstedt, G. Actachem. Scando 4:448-55 (1950).

Lowry, A. (1978). Biochem Journal. Chloroperoxidase - catalyzed oxidation of 4 - chioroaniline to 4-chloronitrosobenzene pp. 30.

Mittal, GC. Aguwa, C. N. Exaru, V. U. andAkubu, P.1. (1981). Preliminary Pharmacological Studies on anti-venon action of diodia semider leaves. The Nigerian J. Pharmacy, 12, 432 - 436.

Moewus 1957, Obligate Phototrophy in Chlamydomonas eugametos, Scientific Article No. A, 646, Contribution No. 2833 of the Maryland Agricultural Equipment station.

Olaniyi A. Ajibola A. (2nd edition 2000), Essential Medicinal Chemistry, 14, 406, *15,* 428-9.

Oripek, M. (1995) Saponin of Veron, Department of Pharmacognosy, University of Ankra. Turkey pp.40.

Paeck, K. Tracey, M. U. Springer - Verlag Berlin (1955), Modern Methods of Plant Analysis Vol. III (Deutter Band).

Pakistani Journal of Nutrition (2006).

Resznyak, S. and Szent - Gyorgyi, A. (1936). Nature (London flavonols as vitamions 138, 27.

Roberts V. Brooks, W. Klyne & Miller Biochem J. (1953), *54,* 212-217, Great Britain. Roodt, V. 1998. The Sheel field guide series: Part IT. Common wild flowers of the Okavango Delta, medicinal uses and nutritional value. Russel friedman Books, Halfway House, South Africa.

Roy R. Reeves, DO Ph.D., Terrel L. Algood, RPh, and P. Melonee Wise RPh. (2005) The Journal of The American Botanical Council (Continuing Education) Questions for Physicians and Pharmacists P & T 2005, 30 (9); 518-524. Topic: Skeletal Mussels Relaxants and Associated Medications for non specific Acute back pain.

Scafforth,. C. E. Ada, C. P., and Sylvester, U. (1980). A guide to the medicinal plants of Trinidad and Tobago. Commonwealth Secretariat, London pp. 101 - 105.

Schraufstatter, E., and Dzentsch, 5. (1949). 2 Naturrforschg. (1948); 3b, 163 - 71, 4b, 276 - 80.

Shellard E. J. *91958)* Exercise in the evaluation of drugs and surgical dressings 1St Edition. Pitman Medical Publishing Company Limited, London pp. 1-16.

S. Nyeiredy, L. Botz, and 0. Sticher, J. Plannar Chromatography 2, 53 (1989).

Sofowora E. A. (1982). Medicinal Plants and Traditonal Medicine in Africa. African-Wiley, New York. Pp. 144-146, Pp. 142-146.

Thomson, R. H. (1926). Naturally Occurring Quinones Academic Press, Lodon. Pp 45 - 70.

Trease G. and Evans W. C. (1987). Textbook of Pharmacognosy 12th Ed. Bellaire Tindall, London pp. 343 - 383.

Tswett E. Mikhail Semyonovich (1872-1919). Dictionary of Scientific Biography, American Council of Learned Societeis, Charles Scribner Sons, New York 13, 486 - 488 (1976).

University of Florida (March 2007) - Preparation of Plant specimens for Deposit as herbarium Vouchers, University of Florida Herbarium Copyrigh©.

Van Wyk, B.E, Gericke, N.. 2000. Peoles Plants, a guide to useful plants of South Africa. P. 105. Briza. Pretoria.

WHO 1992. The Promotion and Development of Traditonal Medicine Technical Research Series, 143 Geneva pp 50 - 72.

WHO 2003 - United Nations WHO Fact Sheet No. 134, revised May 2003 Traditional Medicine.

Wright, Jason and Solange Wyath (2003). The Washington manual Obstetrics and Gynaecology Survival Guide. Lippincott Williams and Wilkins.

(Joint) WHO/FAO/UNU (1985) Expert Consultation on Energy and Protein Requirements(9).

APPENDIX

Determination of moisture content

Wt of crucible	34.90g	34.90g	34.90g
Wt of Power	3.0g	3.0g	3.0g
Final wt of the crucible contents	37.41g	37.41g	37.44g
Wt of the residue (after dryness)	2.51g	2.54g	2.54g
Wt. of the moisture	0.49g	0.46g	0.46g
Moisture content	$\frac{0.49}{3} \times \frac{100}{1}$ = 16.34	$\frac{0.16}{3} \times \frac{100}{1}$ = 15.34	$\frac{0.46}{3} \times \frac{100}{1}$ = 15.31

Average moisture content = 15.67% $^{w}/_{w}$ $\pm$ 0.33

Determination of Ash – value

Wt of crucible	34.90g	34.90g	34.90g
Wt of Power	3.03g	3.00g	3.0g
Final wt of the crucible contents	35.43g	35.45g	35.41g
Wt of Ash	35.43 34.90 0.53g	35.45 34.90 0.55g	35.11 34.90 0.51g

% total Ash/Air-dried Sample

$$= \frac{0.53}{3.03} \times \frac{100}{1} = 17.50 \qquad \frac{0.55}{3.0} \times \frac{100}{1} = 18.33 \qquad \frac{0.51}{3.0} \times \frac{100}{1} = 17.00$$

Average Ash value = 17.61% $^{w}/_{w}$ $\pm$ 0.39

Determination of Acid – insoluble ash – value

Wt of crucible	34.90g	34.90g	34.90g
Wt of crucible + Ash	35.43g	35.45g	35.41g
Wt of Ash	0.53g	0.55g	0.51g
Boiled Ash + 25mlHcl)Residue Obtained			
Wt. of residue + crucible	34.97g	34.99g	34.95g
Wt of residue	0.07g	0.09g	0.05g
Acid insoluble Ash of Momordica balsamina			
Air dried power	0.07 3.03	0.09 3.0	0.05 3.0
% acid insoluble ash value	$\frac{0.07}{3.03} \times \frac{100}{1}$ 2.31%	$\frac{0.09}{3.0} \times \frac{100}{1}$ 3%	$\frac{0.05}{3.0} \times \frac{100}{1}$ 1.67%

Average acid insoluble ash value = 2.33% $^{w}/_{w}$ $\pm$ 0.38

Determination of Extractives

i. Alcohol – soluble extractive – value

Wt of evaporating dish	564.75g	564.75g	564.75g
Final wt of evaporating dish + residue	564.80g	564.81g	564.79g
Wt of residue	0.05g	0.06g	0.04g
Initial wt of powdered drug	5.0g	5.0g	5.0g

5g of the powder was extracted (mixed) with 100mls of ethanol

20mls was dried (after filtration)

20mls of the extract (taken) contents – 1g of the powder

Alcohol soluble extractive value

$$\frac{0.05}{1} \times \frac{100}{1} \qquad \frac{0.06}{1} \times \frac{100}{1} \qquad \frac{0.01}{1} \times \frac{100}{1}$$

5.00% 6.00% 4.00%

Average alcohol soluble extractive value = 5.00% $^{w}/_{w}$ $\pm$ 0.58

ii. Alcohol – soluble extractive – value

Wt of evaporating dish	564.75g	564.75g	564.75g
Initial wt of powder	5.0g	5.0g	5.0g

5g of the powder was extracted (mixed) with 100mls of chloroform water

20mls of the extract was air dried

20mls of the extract (taken) contains – 1g of the powder

Final wt of evaporating dish + residue	564.85g	564.85g	564.85g
W t of residue	0.10g	0.10g	0.10g

Water soluble extractive (with reference to the air dried powder)

$$\frac{0.10}{1} \times \frac{100}{1} \qquad \frac{0.10}{1} \times \frac{100}{1} \qquad \frac{0.10}{1} \times \frac{100}{1}$$

10.00% 10.00% 10.00%

Average water soluble extractive value = 10.00% $^{w}/_{w}$ $\pm$ 0.00