PHARMACOGNOSTIC AND BIOLOGICAL STUDIES OF *WALTHERIA INDICA* LINN (FAMILY STERCULIACEAE)

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2006

DECLARATION

I hereby certify that the work reported in this thesis was carried out by me in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria. I solemnly declare that no part of this thesis has been accepted in substance or concurrently submitted in candidature for any other degree. The works of other investigators are acknowledged and referred to accordingly.

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CERTIFICATION

This thesis entitled PHARMACOGNOSTIC AND BIOLOGICAL STUDIES OF *WALTHERIA INDICA* LINN (FAMILY STERCULIACEAE) by ZAINAB MOHAMMED meets the regulations governing the award of the degree of Ph.D (Pharmacognosy) of AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to the memory of my loved ones:

My Father, Abdullah, nephew, Musa Usman, brothers, Adamu and Umoru,

sisters, Hadiza and Juma and my grandmother, Aishatu

and to my

darling husband, Mohammed Bako, and our three offsprings,

Farida, Sadiq and Iman.

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ABSTRACT

The pharmacognostic and biological evaluation of the leave, stem and root of Waltheria indica have been carried out.

Waltheria indica Linn, (family Sterculiaceae), is commonly used in Northern Nigeria, in conjunction with other plants, for the treatment of a variety of ailments ranging from diarrhoea, syphilis, sorethroat, pain to some childhood diseases like convulsion.

The microscopical studies revealed the presence of thick-walled polygonal cells on both epidermises. Numerous stellate covering trichomes were observed in all parts of the plant, and this seemed to be the main diagnostic feature of the plant. Anisocytic stomata were present on both epidermises; simple starch grains devoid of hilum and maltese cross, reticulate xylem vessels and calcium oxalate crystals (prisms and clusters) were observed in some cells of the parenchyma tissue.

The phytochemical screening of the different parts of the plant revealed the presence of carbohydrates, tannins, flavonoids (found only in the leaves), saponins, steroids and alkaloids. Fats, oils, resins, anthraquinones and balsams were however absent.

Chemo-microscopy revealed the presence of proteins in the leaves, mucilages in secretory cells of all parts of the plant, cutin in the cell walls of the leaves, suberin in the cork cells of root and stem and lignin in the parenchyma cells and some tissues like the xylem.

The quantitative values obtained were: moisture content; 13.53 ± 0.65%w/w, 8.64 ± 0.65%w/w and 10.00 ± 0.70%w/w for the leaf, stem and root respectively. Ash values were 12.53 ± 0.77%w/w, 7.50 ± 0.65%w/w and 7.50 ± 0.71%w/w for leaf, stem and root respectively; acid insoluble ash values were 2.50 ± 0.74 %w/w, 2.53 ± 0.52%w/w and 4.90±0.71 w/w respectively for the leaf, stem and root; alcohol soluble extractive values were 2.43 ± 0.55%w/w, 1.50± 0.60%w/w and 1.10± 0.63%w/w for leaf, stem and root respectively and the water soluble extractive values were 10.01 ± 0.01%w/w, 4.03 ± 0.05%w/w and 2.30 ± 0.05%w/w respectively for leaf, stem and root.

The analgesic activity studies showed that the water extracts of all the plant parts had some degree of activity. The extracts inhibited the acetic acid- induced writhing in mice dose dependently. The root was found to have the highest analgesic activity, followed by the stem and the leave respectively.

The purgative activity, which was measured by the production of wet feaces, was found to be more in the roots, followed by the leaves and the stems respectively. The purgative activity of the roots was seen at 50mg/kg body weight to have the same percentage senna-activity as senekot (reference purgative drug). The values of 100%, 12% and 26% were obtained as the percentage senna-activity of the root, stem and leaf extracts respectively.

The acute toxicity study was carried out using mice and by intraperitoneal administration of the water extract of the plant parts, showed that the roots were more toxic followed by the stem and root respectively.

The LD_{50} values obtained for the leave, stem and root respectively were 363mg/kg body weight, 141mg/kg body weight and 69mg/kg body weight. Values obtained for the acute toxicity showed that the plant may be considered moderately toxic based on the World Health Organization's classification and this evidence may be a drawback in recommending the plant as an analgesic or purgative agent without some form of detoxification.

However, results obtained could justify the uses of the plant as analgesic and purgatives in traditional medicine practice by traditional medicine practitioners.

TABLE OF CONTENTS

CONTENTS	PAGES
Title Page	i
Declaration	ii
Certification	iii
Copyright Statement	v
Dedication	vi
Acknowledgment	vii
Abstract	ix
Table of Contents	xii
List of Tables	xviii
List of Figures	xix
List of Plates	vx

CHAPTER ONE

1.0	**INTRODUCTION**	1
1.1	Description of the Family Sterculiaceae	4
1.1.2	Taxonomy	6
1.1.3	Phytochemical constituents of the family Sterculiaceae	8
1.1.4	Medicinal and Economic uses of family Sterculiaceae	8
1. 2	Description of the Genus Waltheria	12

1.3 Description of the Specie *Waltheria indica* Linn 12

1.3.1 Distribution and Ecology of *Waltheria indica* 13

1.3.2 Reproduction and Growth of *Waltheria indica* 16

1.4 Ethnobotanical and Economic Uses of the Family Sterculiaceae 16

1.5 Phytochemical Constituents of Waltheria indica 22

1.6 Pharmacognostic Evaluation 28

1.6.1 Morphological and Organoleptic Evaluation 28

1.6.2 Microscopical Evaluation 29

1.6.3 Phytochemistry 30

1.6.4 Quantitative Evaluation of Crude Vegetable Drugs 37

1.6.5 Biological Evaluation 39

1.7 Analgesic Activity 40

1.7.1 Analgesics 40

1.7.2 Pain 41

1.7.3 Kinds of Pain 41

1.7.4 Nature of Pain 42

1.7.5 Pain as a Sensory Experience 44

1.7.6 Pain Pathway 44

1.7.7 Threshold for the Perception of Pain 44

1.7.8 Measurement of Pain Threshold 45

1.7.9 Threshold for Reaction to Pain 46

1.7.10 Drugs used in Pain Relief 46

1.7.11 Screening Methods for Analgesics 53

1.7.12 Primary Screening Methods 54

1.7.13 Secondary Screening Methods 56

1.8 Purgative Activity 58

1.9. Acute Toxicity Testing 60

1.10. Aims and Objectives 62

2.0 **MATERIALS AND METHODS** 64

2.1 Plant Collection and Identification 64

2.2 Materials 64

2.2.1 Solvents and Reagents 70

2.3 Pharmacognostic Studies on Leaf, Stem and Root of *W. indica* 72

2.3.1 Macroscopical Examination of the different parts of *W. indica* 72

2.3.1.1 Root 72

2.3.1.2 Stem 72

2.3.1.3 Leaves 73

2.4. Chemomicroscopical and Microscopical Examination of (Leaf, Stem and Root). 73

2.4.1 Chemomicroscopical Examinations 73

2.4.2 Microscopical Examinations 76

2.5 Quantitative Leaf Microscopy 76

2.6 Phytochemical Screening of Leaf, Stem and Root powder of *W.indica* 78

2.6.1 Test for Carbohydrates 78

2.6.2 Test for Tannins 80

2.6.3 Test for Glycosides 82

2.6.4 Tests for Sterols and Terpenes 85

2.6.5 Test for Flavonoids 86

2.6.6 Test for Resins and Balsams 88

2.6.7 Test for Alkaloids 89

2.7 Quantitative Evaluation of the Crude Vegetable Drug 90

2.7.1 Determination of Moisture Content 90

2.7.2 Determination of Ash Values 91

2.7.3 Determination of Extractive Values 93

2.8 Biological Evaluation 94

2.8.1 Drying and Extraction of Plant Material 94

2.8.2 Experimental Animals 94

2.9 Acute Toxicity Studies 95

2.10. Analgesic Activity Studies 96

2.10.1. Acetic Acid-Induced Writhing Method 86

2.11. Purgative Activity 96

2.11.1. Preparation of Extracts and Animal Studies 96

2.12. Statistical Analysis 97

3.0 RESULTS AND DISCUSSIONS 98

3.1 Results of Macroscopical Examinations of the Leaf ,
Stem and Root of *W. indica* 98

3.1.1 Leaves 98

3.1.2 Stem 98

3.1.3 Root 99

3.2 Results of Microscopical and Chemomicroscpical Examination
of the Leaf, Stem and Root of *W. indica* 100

3.2.1 Microscopical Examination 100

3.2.2 Chemomicroscopical Examinations 120

3.3 Results of Phytochemical Screening of Leaf,
Stem and Root powder of *W.indica* 123

3.3.1 Test for Carbohydrates

3.3.2 Test for Tannins 124

3.3.3 Test for Glycosides 126

3.3.4 Test for Terpenes and Sterols 127

3.3.5 Test for Flavonoids 127

3.3.6 Test for Resins and Balsams 129

3.3.7 Test for Alkaloids 129

3.4 Results of Quantitative Evaluation of Crude Vegetable Drugs 131

3.5 Results of Analgesic Activity Studies 133

3.5.1 Results of Acetic Acid – Induced Writhing Method 133

3.6 Results of Purgative Activity Studies 136

3.7 Results of Acute Toxicity Testing 141

CONCLUSION 145

REFERENCES 148

APPENDIX 160

LIST OF TABLES

TABLE		PAGE
1.	Results of quantitative Leaf microscopy	108
2.	Results of chemomicroscopical examination of the Leaf, Stem and Root of *W. indica*	122
3.	Phytochemical Constituents of Leaf, Stem and Root of *W. indica*	130
4.	Quantitative Evaluation of Crude Vegetable Drugs (powdered leaf, stem and Root of *W. indica*).	131
5.	Effect of Extracts of the Plant on Acetic Acid-Induced Abdominal Writhing in Mice.	135
6.	Percentage Wet Faeces in Rats Produced by Leaf, Stem and Root extracts of *W. indica.*	139
7.	Biological Senna-Equivalent of Leaf, Stem and Root extract of *W. indica.*	141
8.	Percentage Senna – Activity of Leaf, Stem and Root extract of *W. indica.*	141
9.	Result of Acute Toxicity Testing	143

LIST OF FIGURES

FIGURE		PAGE
1.	Picture of *Waltheria indica* Linn showing Leaves and Flowers	14
2.	Picture of *Waltheria indica* showing Leaves, Flowers and Seeds.	15
3.	Structure of the Peptide Alkaloids of *Waltheria indica*	25
4	Upper and lower surfaces of the leaf of *W. indica*	103
4a.	Microscopical features of leaf powder of *W. indica*	104
4b.	Longitudinal section of leaf of *W. indica*	106
4c.	Transverse section of leaf of *W. indica* through midrib and lamina	107
4d.	Transverse section of leaf through lamina	107
5a.	Microscopical Features of powdered stem of *W. indica*	111
5b.	Longitudinal section of stem of *W. indica*	112
5c.	Transverse section of stem of *W. indica*	113
6a.	Microscopical Features of powdered root of *W. indica*	117
6b.	Transverse section of root of *W. indica*	118
6c.	Longitudinal section of root of *W. indica*	119
7.	Results of Extracts 0f *W. indica* on Acetic Acid-induced Abdominal Constriction in Mice	136
8.	Comparison of Purgative Activity of leaves, stem and root of *W. indica*	149

LIST OF PLATES

PLATES	PAGE
1. *Waltheria indica* in its natural habitat	65
2. Fresh leaves and flowers of *W. indica*	66
3. Fresh roots of *W. indica*	67
4. Fresh stem of *W. indica*	68

CHAPTER ONE

1.0. INTRODUCTION

The world over, and especially in developing countries, research on medicinal plants is easily the most popular subject in the chemical and related biological sciences. The Ancient man is known to have utilized plants as drugs and source of food, which is first in the hierarchy of man's basic needs for survival. Man has also been dependent on plants for his clothing, shelter and day -to - day needs of medicaments (Sofowora, 1986, Akerele *et al*, 1992).

The origin of the use of plants is difficult to trace, but the history of traditional medicine can be traced back to a stone aged man who used plants and other natural substances for prayer and rituals to overcome various diseases.

In Africa in particular, there is abundance of plants which are used in traditional medicine practice and these plants have been reputed, through experience handed down from one generation to another, to have useful medicinal activity. A very large number of these plants are, however, yet to be identified. It is anticipated that many of these plants can provide the basis for the pharmaceutical industry. Similarly from plants, new drug agents can be discovered that are useful against diseases for which suitable cures are yet to be available (Akerele *et al,* 1992).This is because, inspite of the tremendous advances in medicine, there are a number of diseases for which modern medicine has no cure

yet (Mohammed, 1998). In such cases it treats only the symptoms to provide relief to patients. These include viral diseases like herpes, cancer, Aids etc. Recent trends have shown that plant drugs have the answer to such cases.

Since indiscriminate use of synthetic drugs and antibiotics have resulted into serious problems all over the world, the demand for plant based raw materials for pharmaceuticals has increased enormously. Moreover, the synthetic drugs and intermediary chemicals are extremely expensive. The World Health Organization, (WHO), has emphasized the utilization of indigenous systems of medicines based on the locally available raw materials, that is, medicinal plants (Mohammed, 1998). Furthermore, approximately one third of all drugs are plant- based, and if bacteria and fungi are also included, nearly sixty per cent of all pharmaceuticals are of plant origin.

The use of medicinal plants is due primarily to the presence of certain chemical constituents contained in them. Within the realm of pharmacology, there are three major ways in which plants may be used: isolated plant constituents may be used directly as therapeutic agents for example, digitoxin and morphine; those used as starting materials for the synthesis of useful drugs, for example steroid hormones, and natural products which serve as model for pharmacologically active compounds in the field of drug synthesis (Akerele *et al*, 1992).

Before a plant can be used for medicinal purposes, it has to conform to certain pharmaceutical standards and for the study of the crude plant

drugs, preliminary examinations will often give indications as to whether they will satisfy the more exacting pharmaceutical and medical standards which may be applied to them. Characteristic features of drugs could be obtained by systematic study of their morphology (Shellard, 1958). However, the evaluation of a drug requires not only that it be identified in both its entire and powdered forms by means of its macromorphological and cytomorphological features, but must also be subjected to evaluation procedures which will indicate their acceptability by criteria other than their morphology (Brain and Turner, 1975).

Recently, a number of plant- based formulations have come to the market for control of some diseases like liver disorders (Mohammed, 1998). There is a considerable scope to screen such plants for their active constituents which may be used in future for treatment of some incurable diseases. The plants used in traditional medicine are known as medicinal plants, because one or more of their organs contain substances that can be used for therapeutic purposes or which are precursors for synthesis of useful drugs.

In Nigeria, herbs, leaves, stem, roots, seeds of plants, and parts of animal metaphysical phenomena have been used in traditional medicine (Tella,1968). Concoctions of these plants are in variety of dosage forms such as liquid, semi-solid, solid or gaseous (Akerele, *et al*, 1992).

No one would seriously challenge the fact that man is still largely dependent on plants in treating his ailments. According to an estimate of the World Health Organization, (WHO), approximately 80% of the people in developing

countries rely chiefly on traditional medicines for their primary health care needs, of which the major portion involves the use of plant extracts or their active principles (Farnsworth *et al*, 1985).

Many research work has been carried out on medicinal plants in Nigeria, and, from the results, many promising plants that could be useful against many ailments have also been discovered (Martin, 2000). Infectious diseases like eye, ear and other systemic diseases due to bacteria, fungi and other parasitic agents have been treated using traditional medicine (Ogunlana *et al*, 1975). The treatment of fungal infections in northern Nigeria by drinking the decoction of *Striga senegalensis* and by rubbing the fresh leaves on the skin (Choudhury *et al* ,1998); use of fallen leaves of *Carica papaya* (pawpaw), for abortion, treatment of boils, wounds and traumatic bleeding using whole plant of *Allium sepa* (onions) and *Newbounda laevis* used to treat scrotal elephantiasis, roundworm infections and wounds (Unaeze and Abariukwu, 1986), are such examples . A very large number of the medicinal plants in Nigeria are yet to be identified and exploited for therapeutic uses.

The locally available plant under investigation here is *Waltheria indica* Linn. (synonyms *W. americana, W. elliptica Cav.*) of the family Sterculiaceae. It is readily available in abundance in the tropics and temperate zones, where it finds wide application in traditional medicine for the treatment of a variety of ailments ranging from childhood diseases of diarrhoea, teething etc. to other uses like pain relief, cough, cancer etc.

1.1. **DESCRIPTION OF THE FAMILY STERCULIACEAE**

The family consists of plants that are either herbs, trees or shrubs of soft wood. They are rarely herbs, mostly with stellate hairs as a common feature. Plants are non-succulent, self supporting, or climbing. The leaves, arranged alternately, petiolate , may be simple or digitate compound leaves. Sometimes palmate. Stipules are usually present. Leaves without a persistent basal meristem. Lamina dissected, or entire.

Leaf anatomy:

Mucilaginous epidermis present. Stomata usually anomocytic, or paracytic. Hairs present, aglandular and glandular, unicellular and multicellular. Complex hairs present, peltate and stellate.

Lamina generally dorsiventral; with secretory cavities, or without secretory cavities. Secretory cavities containing mucilage. The mesophyll contains mucilage cells, or not. Minor leaf veins without phloem cells.

Stem anatomy:

Secretory cavities present (schizogenous and lysigenous), or absent, with mucilage. Cork cambium present. Medullary bundles mostly absent, or present. Internal phloem absent. Secondary thickening develops from a conventional cambial ring. Xylem is without tracheids fibre, with vessels. Vessels without vestured pits, tile pits present (Watson and Dallwitz, 1999).

The flowers are variously arranged, hermaphrodite or unisexual and actinomorphic. They are aggregated in 'inflorescence', sometimes cauliflorous, in cymes.

Flowers usually regular, or somewhat irregular.

The sepals of the flowers are valvate, mostly or partly cannate, or rarely spathaceous.

The petals are usually five in number but maybe absent, and if present, are contorted, imbricate and often hooded. They are usually clawed, or sessile.

The stamens maybe free or cannated into a column, sometimes with staminodes.

The ovary is the superior type, composed of one or more carpels.

The fruits are various and carpels are often separating (Keay *et al*, 1964). They may be fleshy, or non-fleshy, aggregate or not aggregate, dehiscent, or indehiscent, or a schizocarp.

Seeds endospermic, or non-endospermic. Endosperm oily, or not oily. Seeds with starch. Cotyledons 2; flat, or rolled. Embryo is chlorophyllous, straight, or curved.

The family also has scattered tanniferous cells, which are frequent.

Proanthocyanins are produced but without ellagic acid, often accumulating methylxanthine derivatives - purine bases like theobromine and caffeine, and also cyclopropenoid.

1.1.2 TAXONOMY

Sterculiaceae is one of 7 families belonging to the order Malvales, which consists mainly of herbs, shrubs or trees, tropical and temperate (Evans,1996). The common name for the family is Cacao family which is

composed of 60 genera and 700 species Some important families in this order are;

Tiliaceae;

This is made of 50 genera and 350 species. The genera include Corchorus (100 species) and Tilia (50 species). Jute fibre is obtained from *C. capsularis* and *C. olitorius*. The genus Conchorus is reported to contain cardiac glycosides. *Tilia europaea* is a source of phloem fibres used by gardeners (Evans,1996).

Elaeocarpaceae:

This consists of 12 genera and some 350 species of tropical and subtropical trees and shrubs. The main genera are Elaeocarpus (200 species) and Sloanea (120 species). Elaeocarpus contains indolizidine (Evans,1996).

Malvaceae:

The family contains 75 genera and about 1000 species of herbs, shrubs and trees. Some genera include Malva (40 species), Gossypium (20-47 species) which is an important source of cotton seed hair and oil, Thespesia (15 species) and Althea (12 species) whose root is used as a demulcent due to its rich mucilage (Evans, 1996).

Members of this family may be recognized by their flowers which have axillary - positioned inflorescence, usually dense glomerules that contain fragrant, yellow to orange flowers. The capsules of the plant hold an obovoid seed (Howard, 1989).

1.1.3. **PHYTOCHEMICAL CONSTITUENTS OF THE FAMILY STERCULIACEAE**

The phytochemical constituents of the Sterculiaceae, as reported by Watson and Dallwitz (1999), include the flavonoids, kaempferol and quercetin, cyanogenic glycosides, alkaloids, anthocyanodins, cyanidins and ellagic acid. Saponins may be present or absent.

Mucilage is common in the family and tannins may also be present. Purine bases occur in Theobroma and Cola (Evans, 1996). Pyridine alkaloids are contained in *Melochia corchorifolia* and *Helicteres isora* is a source of diosgenin which is important in the manufacture of steroidal contraceptives. *M. pyramidata* is also the source of triterpenoids (Evans,1996). *Waltheria indica* contains peptide alkaloids namely adouetine x, y and z, saponins, mucilages, sugars, flavonoids, steroids and tannins (Watt and Breyer-Brandwijik, 1962, Petrus, 1990, Ogbede *et al*, 1988 Williaman, 1970).

1.1.4. **MEDICINAL AND ECONOMIC USES OF THE FAMILY STERCULIACEAE**

The Sterculiaceae is a family made up of about 60 genera and 700 species. It contains the most important tropical woody plants rich in polypeptides and are used in beverage-making and fooder.They are mainly important as ornamentals whereas some yield beverages and pharmaceuticals. Some of the economically important members of the family are listed below:

Theobroma cacao L: (Cocoa plant)

Also known as cacao butter, coca butter or cocao seeds, is the source of Theobroma oil or cacao butter which is obtained by expression of the ground kernels of the cocoa bean. Cocoa butter oil is a yellowish - white solid with chocolate odour and taste. The chemical constituents of coca butter consists of the glycerides of stearic (34%), palmitic (26%), oleic (37%) and other acids (Mohammed, 1998). The seeds contain 35-50% of a fixed oil, 15% of starch, 15% of protein, 1-4% of the alkaloid theobromine and 0.07-0.36% of caffeine. Cocao-red is formed by the action of ferment on a glyceride and gives red colour to the seeds (Mohammed, 1998). Theobromine is also present in the shell (0.19-2.98%) and kernel (1-1.7%).

Cocao butter is useful as a lubricant in massage creams and base for suppositories, ointments, pharmaceuticals and cosmetics. It is also used in the manufacture of chocolates and cocoa butter, toilet soaps, creams and the popular 'cocao' beverage. Cocao bean is composed mainly of theobromine and cocoa butter but little caffeine (Kokate and Gokhale, 2000). Other uses of the plant are as a diuretic and stimulant. The diuretic properties are due to the presence of theobromine , a lower homologue of caffeine. It has less action on the central nervous system than caffeine, but is more diuretic (Evans, 1996). In its isomer, theophylline, the diuretic effect is even more marked (Evans,1996).

The cocoa waste product cause theobromine poisoning that could be toxic.

Cola acuminata (Kola seeds, kola nut):

Also known as kola nut, 'Bissy' or 'Gooro'(Hausa) seeds, it consists of the dried cotyledons of the seeds of various species of Cola. The colour of the fresh seeds varies, with those of *Cola acuminata* being white or crimson, *C. astrophora* red, *C. alba* white and *C. vera (C. nitida)* either red or white (Evans, 1996). Kola seed contain purine alkaloids namely caffeine and theobromine, and tannins which may be in combination to form kolacatechin (Evans, 1996, Kokate and Gokhale, 2000). It has been suggested that the differences in the stimulatory action between fresh and dry seeds may be due to the formation of a caffeine-catechin complex in the later. The quantity of caffeine and theobromine is 1-3% and 1% respectively. Kola nuts are used as stimulants and in the preparation of aerated beverages. *Cola acuminata* is used to prepare a dye, while the inner bark have tough fibres which are used to produce learning materials and papers.

Both kola and cocoa contain a flavouring substance as well as a drug, theobromine.

Guarana cola contains caffeine, other xanthine derivatives and tannins used in combating fatigue, for slimming and for the treatment of diarrhoea. The grated nut powder is also used as a beverage in South America (Evans, 1996).

Sterculia species:

These consist of *S. tragacanth, S. villosa* Roxburgh, and *S. urens,* which are sources of sterculia or karaya gum, a dried gummy exudate obtained by incisions made to the heartwood of the trees. The pure gum is

colourless, slightly soluble in water, but swells and has the distinct odour of acetic acid (Mohammed, 1998). The gum contains about 8% of acetylated branched hetero - polysaccharide group and more than 37% uronic acid residues comprising of D- galacturonic and D- glucuronic acids. On partial acid hydrolysis, D-galactose, D-galacturonic acid, aldobiuronic acid and an acid trisaccharide are obtained from the gum. Sterculia gum has the ability to swell 60-100 times its initial volume in water. It is neither digested nor absorbed by the body, hence it is considered a good bulk laxative, being second only to psyllium seed in use in this respect (Evans, 1996). It is also used as denture adhesive in dental treatment and in pharmaceuticals as emulsifying, thickening, suspending and stabilizing agents. The powdered gum is used in lozenges, pastes and denture fixative powder. It is also employed in wave set solution, skin lotions, textile and printing industries and in the preparation of composite building materials (Mohammed, 1998). Karaya gum is listed in the Food Chemical Codex as ice pops, cheese spread and grounded meat products (Kokate and Gokhale., 2000).

Sterculia campanulata has a characteristic evil smell and when combined with lead (pb) is used to make paints.

Other species of the Sterculiaceae family that could have medicinal or some economic values include:

Helicteres isora :

It is a new source of diosgenin, a saponin glycoside that could be used for making steroidal contraceptives. Diosgenin , until 1970, was isolated

only from the Mexican and China yams, *Dioscorea* species (Evans, 1996).

Waltheria indica:

It is a very important tropical plant that has various uses. The aerial parts and root of the plant are used as laxative, and the infusion taken orally is a remedy for cough, scabies and fevers. The hot water extract of the entire plant is used in South Africa as a remedy for sterility, abortion and to strengthen infants during teething (Hedberg *et al,* 1983). In Northern Nigeria, the plant extract is used by the Fulanis during their 'flogging' festival of 'sharo' to make the pain of beating bearable.

The flowers are effective in treating skin diseases and the leaves are useful against diarrhoea, convulsion and wound healing. The roots are used to treat epilepsy, internal haemorrhage, syphilis and dysentery.

Some other traditional medicine uses of this plant include treatment of sore throat, arthritis, period pain, jaundice and asthma.

1.2. DESCRIPTION OF THE GENUS WALTHERIA

***Waltheria* Linn. is the genus of a group of trees or shrubs with soft wood, rarely herbs and occur mostly with stellate hairs. They are easily recognized by the presence of bract and calyx which are villous with long simple hairs.**

The calyx is about 4-4.5 mm long, attenuated, about 1mm wide at the base. The plant shows presence of congested cymes; flowers that are usually sessile.

The stem surface and under surface of the leaves are densely tomentosed.

The leaves are ovate to lanceolate in shape with round or subcordate base. The leaf apex may be obtuse or subacute, the length and breadth of the leaves are 14 cm and 6cm respectively.

1.3. DESCRIPTION OF THE SPECIE *WALTHERIA INDICA* LINN.

W. indica (synonyms *W. americana L, W. elliptica Cav.*) is a very common tropical plant, which can be found growing in many parts of the world such as Hawaii, Mexico, Suriname, Polynesia, South Africa and Nigeria.

It is an erect herb which is widely distributed in the tropics. It is usually up to 6 m high, has woody, fibrous hairy stem, with the leaves having length and breadth of up to 14 cm and 6 cm respectively. They are borne alternately and may be simple or digitately compound, often serrated, more or less ovate, grey edges and closely toothed being permanently hairy below. The cyme is usually sessile, terminal and auxiliary. The flowers are yellowish and unscented (Irvine, 1961). The flowers appear in August through October to November. It is a short-lived shrub or sub-shrub. The yellow petals turn orange or brown with age. The only and second Waltheria species is *W. lanceolata*.

1.3.1. **DISTRIBUTION AND ECOLOGYOF *WALTHERIA INDICA***

The plants are very commonly found growing in open places, on old farms and in open savannah. The plant grows well in disturbed dry and well-drained but moist habitat. It colonizes a wide variety of soils in areas with igneous and sedimentary rocks. The species may be found on old fields, construction sites,

roadsides, burned forest, grasslands and stream overflow areas also. The plant is intolerant of shades and will not survive under a closed tree canopy and cannot compete with grass in dense swards (Irvine, 1961). It does not withstand drought, salt spray and mildly salty soils. This widely distributed tropical plant can be found extending from Gambia to Botswana and through out tropical Africa (Irvine, 1961). It also grows in the warmer sub-tropics.

Locality: It is located throughout the Krobo plains, Achimota, Cape coast and Gambaga in Ghana. In Senegal, it is found in Kaolack, in Gambia it is in Geneni and Qualia, Labbezenga and areas of Timbuktu in Mali. Other places where the plant can be located are Freetown, Rokupr and Magbile in Sierra Leone, Cinco in Liberia, Ivory Coast, Guinea Coast, Abra Cape Coast , Damongo and Lome in Togo. In Nigeria, it is found in Jos Plateau, Lokoja, Katsina, Naraguta, Lagos, Abeokuta, Old Oyo, Uguoko and Awka (Irvine, 1961).

Fig.1: ***WALTHERIA INDICA* SHOWING LEAVES AND FLOWERS**

(Reproduced from Agoha,1974)

Fig. 2: ***WALTHERIA INDICA*** **LINN. SHOWING LEAVES FLOWERS AND SEEDS**

(Reproduced from Tropilab Inc. 2003)

Waltheria indica apparently naturalized in Hawaii soon after the arrival of non-native colonists. Howard (1989) indicated that the species is native to Florida and Texas to Brazil. The plant, which is easily available in all parts of Nigeria,

particularly in the northern part of the country, is a popular herbal remedy among the Hausa, Fulani and other peoples of the region. It is known by the following vernacular names:

hankufah or hankubah (Hausa), kafifi (Fulani) (Irvine, 1961), korikodi (Yoruba) (Agoha, 1974), efu-abe (Nupe) (Samson, 2000), icha-okho (Igala and Idoma) (Ochune, 2002).

W. indica is known by different common names also such as sleepy morning, velvet leaf, marsh-mallow, monkey bush, boater bush, leather coat and buff coat.

Its colloquial names include: basora prieta, malvavisco, fufutafu and kafaki (Haselwood and Motter, 1966; Howard, 1989; Liogier, 1994; and Burkill, 2000). In other parts of the world where they are also found, they are known by different names such as 'uha- loa' in Hawaii, 'hierba de soldado' and 'guasimilla' in Suriname. The Natural Products Alert, NAPRALERT, of April 19, 2000 gave the following vernacular names for the plant: Ala'ala- pu- loa, Alwani saika, Bari yari, Hi aloa, Kankane tema, Nallabenda, Numvu, Raichie, Remedio de sanpote, Uha- loa and Wiwi saika.

1.3.2. **REPRODUCTION AND GROWTH OF *WALTHERIA INDICA***

Waltheria indica begins flowering at about 6 months of age and blooms more or less continuously for the rest of its life. Reproduction is by seeds which are dispersed by water, agricultural equipment (Keay *et al*, 1964) and grazing animals. Seedlings are relatively common in disturbed habitat. The plant has a life span of 1 or 2 and occasionally 3 years.

Death usually occurs during the dry season. Perennial growth is more likely in continuously moist habitats. The seeds are sold commercially and the species is cultivated in gardens as medicinal plants.

1.4. ETHNOBOTANICAL AND ECONOMIC USES OF *WALTHERIA INDICA* LINN.

Waltheria indica is widely distributed in the tropics and is well known in African traditional medicine where it has a great reputation as a remedy for a variety of ailments. Being a tropical plant, the plant is also popularly used in other parts of the world such as Hawaii, Tanzania (Chhabra *et al*, 1993, Hope *et al,* 1993), Mexico, Guinea and Jamaica having tropical weather. The uses are diverse and vary from one place to the other. Plant parts (leaves, stem, root and flowers), whole plant or these mixed with other plants are often used in the traditional medicine practice to treat the various ailments (Maiwada, 2000). The uses of the plant can be grouped under appropriate headings as follows:

1. Antibacterial and Antifungal

In many parts of the world where this plant is found, the root is chewed or the hot water extract used in the treatment of veneral diseases because the plant is said to increase immunity. The infusion or decoction of the leaves, dried root, and juice are used for treating syphilis in East Africa (Chhabra *et al,* 1993, Hedberg *et al,* 1983) in the treatment of cough and it is used as eye lotion (Oliver, 1960).

In West Africa, the hot water extract is given orally as children's medicine at birth, for example, during teething and against childhood diarrhea, convulsion

and cough to strengthen infants. For treating convulsions in children, the leaves are boiled and the vapour inhaled.

The roots of *W . indica* are used as chewing stick and as a cough remedy in Togo. A spoonful of the pulverized plant in hot water is taken morning and evening (Dalziel *et al* , 1954).

In Southern Nigeria, particularly among the Yoruba people, the hot water extract of the plant is used as a constituent of the 'agbo mixture' commonly used as a body wash or drink to afford immunity to syphilis.

The ground roots are applied to sores and to treat skin diseases while the paste of the leaf is used as a poultice to treat infected finger. The dried flowers and leaves, said to be mucilaginous, are used externally to treat sores, skin diseases and wounds (Hedberg *et al*, 1983) .

It is also used as a cure for mouth and gum ulcers by the Hausa and Nupe peoples of Northern Nigeria (Samson, 1999). The fresh flowers are used in Tanzania for traditional medicine practice.

The 'Gas' of Gambia apply the ground root externally on sores, while the decoction is used in the Philippines for skin diseases and to facilitate healing of infected fingers.

The stems are used as a chew stick, extract of the plant is used as an eye bath and a remedy for hemoptysis (Agricultural Research Service, 2001).

The root extracts are also used externally to treat wounds and aching eyes.

Other medicinal applications of *W. indica* according to literature include the chewing of the root bark in Hawaii to treat sore throat (Neal, 1965).

Also the Polynesians use the bark of the mature roots either chewed or the juice swallowed for sore throat taken 3 or 4 times until the sore throat is gone.

2. Anti-virals:

In a search for the antiviral properties of some medicinal plants in Panama, the branches of *W. indica*, were observed to moderately inhibit the proteolytic activity of HIV-1-PR (Matsue *et al*, 1997).

Another antiviral use of the plant is as a remedy against the common cold.

3. Analgesic and Anti- inflammation

The plant extract also find use in the treatment of general body ache and rheumatism. It is no wonder then that a decoction of the root is drunk by agriculturalists as a restorative during the labour of harvesting. It is also taken in mixture with potash by the Fulani herdsmen during the 'sharo' or 'shadi' festivals, a pre-requisite for taking a bride, which is characterized by whipping the bare skin of prospective bridegrooms in a public place, as a test of endurance of pain. The aqueous extract of the root is said to increase the ability to bear the pain (Maiwada, 2000).

The root bark is used to treat neuralgia and arthritis. It is also used to treat painful menstruation, characterized by spasmic pain of the lower abdominal area which radiate to the back, and fatigue (Tropilab, 2003).

4. Anti- haemorrhage:

The extract of the root bark is used in South Africa as an astringent to stop internal haemorrhage and for sterility (Irvine, 1961).

5. Purgatives:

The hot water extract of the root is used as a laxative. The dried aerial parts of the plant can also be used (Heinrich *et al*, 1992).

6. Anticancer:

The dried entire plant extract is used as a febrifuge, and externally to treat cancer and swellings, especially bumps on the head (Dimayuga *et al,* 1987, Hedberg *et al,* 1983).

The dried leaves and root are used in Mexico-Baja California for the treatment of cancer (Chhabra *et al,* 1993, Dimayuga *et al,* 1987).

7. Cosmetics:

Waltheria indica is a component of a cosmetics formulation called 'phase visible fading out', which is claimed to be the strongest skin lightener available anywhere in the world (Janssen, 2001). The constituent of the plant that is used in this formulation is called 'Derma white extract' , which reduces melanin synthesis by inhibiting the tyrosinase, which is the key enzyme in the process.

8. Fertility / Aphrodisiac:

The Shangana women of Guinea use the root to treat headache and barrenness in females. This is also used to treat impotence and sterility in men in South Africa. The leaves mixed with those of other plants are used as aphrodisiac by both men and women in some parts of Nigeria, particularly Sokoto (Maiwada, 2000).

9. Abortaficient and Contraceptive:

The entire plant is used as an abortaficient and as contraceptive in Ghana .

10. Other uses:

The dried stem is used in Sudan for treating jaundice. Also the hot water extract of the root bark is used in Hawaii to treat asthma. An infusion of the stem and leaves may also be so used. The roots are used orally to treat epilepsy, fecundity induction and asthma.

The leaves are used to treat giddiness in Northern Nigeria (Irvine,1961).

The roots, leaves, buds and flowers are also used, with other plants, for the treatment of chronic cases of asthma. All the plants are pounded together, the juice pressed out, and the liquid strained. The strained juice is then heated and the liquid taken every morning for 5 days.

The Hawaiians make use of the leaves as a tea, to treat giddiness, and the leaf juice is used in the treatment of rabies. The entire plant is used by the Igala and Idoma communities for the treatment of 'jedi-jedi' or pile.

When the leaves are dried and used as tea, they resemble, in taste the, flowers of verbascum and both have the same action (Hepper, 1976). In the Turks and Caicos Island, the plant is used to make herb tea.

From the literature available (NAPRALERT, 2000), the water extracts of the plant parts, either by maceration, decoction, concoction or infusion, are the forms of preparations of the medicaments used for the treatment of the various diseases. The plant parts, leaves, stem, roots and flowers, are all used in the various treatments.

The above mentioned uses of the plant, *Waltheria indica,* can be evaluated under the following headings:

1. Analgesic
2. Anti- inflammatory

3. purgative
4. Abortaficient
5. Antibacterial
6. Antifungal

The non-pharmaceutical uses of the plant is seen among the people of Hawaii who use the powdered leaves for filling the seams and cracks of their canoes (Holland, 1922) and the leaves for washing dishes. The plant produces a fibre that was formerly used for making cords, sacking, padding and sandals (Guzman, 1975). The plant is browsed by all types of livestock, especially when young (Keay *et al,* 1964) and it is a host for a number of insects harmful to agricultural crops.

1.5. PHYTOCHEMICAL CONSTITUENTS OF *WALTHERIA INDICA*

Waltheria indica Linn., when first studied for its phytochemical groups, was found to contain mucilages, tannins and sugars but lacked alkaloids. But the work of Loustalot and Pagan (1947), who tested several plants for their total alkaloidal and quinine sulphate content used locally in Puerto Rico for the cure of fevers, revealed that the plant, among others, tested positive for alkaloids according to Dragendorff, Mayer and Wagner's tests. Further testing of the alcoholic extract of the dried ground bark and leaves of the plant gave strongly positive results with Wagner and Dragendorff's reagents but only weakly with Mayers (Loustalot and Pagan, 1947). It was revealed that *W. indica* contained no quinine or quinoline but alkaloids. On extraction of the alkaloid with benzene(C_6H_6), solubilising with sulfamic acid and separating by chromatography, alkaloidal peptides known as adouetine X,Y, Y' and Z were detected (Pais *et al*, 1968).

Being peptides in nature, the alkaloids of *W. indica* are composed of amino acid moieties and belong to the group of alkaloids called the cyclopeptide alkaloids. Their amino acid moieties are as outlined below (Pais *et al,* 1968):

1. Adouetine X is composed of leucine, 3-hydroxyleucine and isoleucine
2. Adouetine Y – isoleucine, phenylalanine and 3- hydroxyleucine
3. Adouetine Z - 3-hydroxyphenyl alanyl, phenylalanyl-prolyl, phenylalanine and 3-hydroxyleucine.

Their molecular structure and other characteristics are also given below:

1.Adouetine X :

Molecular formula: $C_{28}H_{44}N_4O_4$

Melting Point: 277oc-279 oc

Molecular weight: 500.69

2. Adouetine Y:

Molecular formula: $C_{34}H_{40}N_4O_4$

Melting point: 272oc-274oc

Molecular weight: 568.72

3. Adouetine Z (a tetra peptide).

Molecular formula: $C_{42}H_{45}N_5O_5$

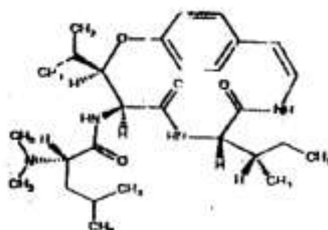
Melting point: 140oc-145oc

Molecular weight: 699.86

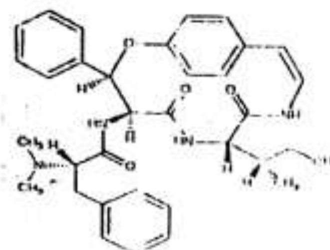
The structures of the alkaloidal constituents of *W. indica* are presented in fig.3 (Harbone and Baxter,1993).

Fig.3 Structures of Peptide Alkaloids of *Waltheria indica*

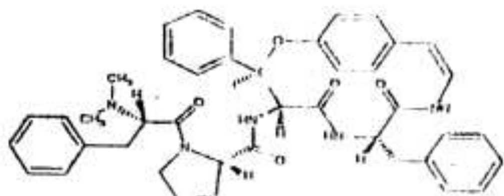
Adouetine X



Adouetine Y



· **Adouetine Z**



Adouetine Z is a tetra peptide and is more soluble in water than the others.

It is also the most complex of the three peptide alkaloids. It has been used as sedative, hypotensive and as a febrifuge in Ivory Coast (Harborne and Baxter, 1993). The presence of the alkaloids of this plant could be responsible for the physiological activity of *W. indica* and this in turn could justify the use of the plant in traditional medicine.

Although drugs have been used in various forms for centuries, it is only recently that peptides have become serious candidates as potential drugs (Namikhosi and Rinehart, 1996). it was concluded from animal studies that some tripeptides such as the thyrotropine- releasing hormone (TRH) show antidepressant effects.

Peptides may also have anti- viral effects (Namikoshi and Rinehart, 1996). The arguments against the application of peptide drugs are the easy susceptibility to enzyme attack in the gut.

Examples of some important polypeptides are the hormones oxytocin, a hormone secreted by the posterior pituitary gland. They cause the contraction of uterine muscles, induce labour in pregnant women and stop haemorrhage after child birth. Vasopressin is used in the treatment of intestinal paralysis and diabetes and insulin is also employed in the treatment of diabetes (Mohammed, 1998).

Examples of other peptide alkaloid- containing plants are *Rhamnus frangula* (family rhamnaceae) which contains franguanine and frangufoline(Tschesche, 1971) , Z*iziphus jujuba* and *Z. oenoplea* (zizyphine A, C and D) in the stembark, root and bark ((Maurya et a*l*, 1995).

Other plants are *Claviceps purpurea* (ergogorine, ergononorine and ergorine) (Cvak *et al*, 1994), *Discaria americana* (discarine B, C and D) in the root bark and bark and *Melochia corchorifolia* (melofoline and corchorifoline) from the aerial part.

The peptide alkaloids namely adouetine x, y and z are contained in all parts of *W. indica* namely the leaves, stem, root and other aerial parts of the plant (Petrus, 1990; Loustalot and Pagan, 1947 and Deeni and Hussain, 1991).

Other constituents of the plant are flavonoids namely apigeninidin glucoside, pelargonidin glucoside and cyanidin glucoside (Ogbede *et al,* 1986). The flavonoids are mainly concentrated on the petals and aerial parts of the plant (Ogbede *et al,* 1986). The flavonoids which occur in the free state and as glycosides, are the largest group of naturally occurring phenols. Of the different types of flavonoid compounds like anthocyanins and coumarins, the flavones and flavonols are contained in *W. indica*. The flavonols consist of trifolin, kaempferol, herbacetin and herbacetin-8-o-beta-d-glucuronide, which are also common on the aerial parts of the plant. The flavones consists of vitexin, 2- o- beta-d- glucosyl. A phenylpropanoid, caffeic acid, is contained in the petals of the plant also (Petrus, 1990). Phytochemical screening of the plant has revealed the presence of mucilages, tannins, fibres, sugars and steriodal derivatives (Burkill, 2000). These constituents are probably responsible for the biological action of the plant. The forage of the plant in the Mozambique valley during the rainy season is known to contain 6.4% crude protein, 0.12% phosphorous and 0.15% calcium.

1.6. PHARMACOGNOSTIC EVALUATION

The evaluation of a drug refers to the confirmation of its identity and determination of its quality, purity and detection of the nature of adulteration. The evaluation includes the method of estimating the active constituents present in the crude drug in addition to its morphological and microscopic analysis. For this purpose, actual collection of the drug is done from identified plant and the characteristics of the unknown sample compared to those of authentic monographs written in the pharmacopoeia. The high quality of the drugs is maintained by collection of the drug from a correct natural source at the proper time, preparation of the samples at the proper time by proper cleaning, drying and preservation of the cleaned, dried and pure drug. The evaluation of a drug is done by studying its organoleptic, microscopic, chemical and physical properties.

Some of the different techniques involved in the pharmacognostic standardization of crude drugs are as follows:

1.6.1. MORPHOLOGICAL AND ORGANOLEPTIC EVALUATION

This refers to the evaluation of a drug on the basis of colour, odour, taste, size, shape and other special features like touch, texture, etc. It is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. The conclusions drawn from studies based on impressions on organs of senses is referred to as organoleptic or sensory evaluation, while the study of the form of the crude drug is referred to as morphology. The fractured surface of cinchona, quillaia and cascara barks are examples of this as they form important diagnostic characteristics of these

plant parts. The pungent taste of ginger, brown colour of cinnamon, the odour and taste of spice-like drugs like nutmeg, black pepper and cumin are important diagnostic organoleptic characteristics.

1.6.2. **MICROSCOPICAL EVALUATION**

The microscope was first used for the examination of drugs by Schleiden in 1847 (Mohammed, 1998). It is one of the most important optical instruments that has the property of magnifying small objects, which cannot be seen with the naked eye. This magnification is necessary to allow the study of details of the structural constitution and inclusions of such objects. It is also used to study details of the internal structure and contents of larger objects, plant parts and animal tissues with the aid of certain chemicals or stains.

There are many types of microscopes, the most commonly used being the compound or simple microscope and the electron microscope.

The most commonly used in the laboratory is the compound microscope, which consists of lenses of short focal lengths for producing high magnifications.

The microscope is also one of the most commonly used optical instruments in the study of crude plant drugs. Qualitatively, minute morphological and histological structures which are most frequently used to identify most natural drugs and to detect adulterants in them, can only be studied under the microscope in powders and sections, as such structures very often possess very few macroscopic characters other than colour, odour and taste.

Quantitatively, the various microscopic structures such as trichomes, calcium oxalate crystals, starch grains etc., can be measured under the microscope

with the help of microscopic accessories like the micrometers. Physical constants like the palisade ratio, stomatal number, stomatal index, vein islet number and vein termination number of leaf drugs and also micro analysis of admixed or adulterated powdered drugs can be determined by quantitative microscopy. The quantitative parameters are valuable in the identification and purity determination of crude plant drugs. The drawing of internal structures of plant or animal organs in their exact shape and arrangement can be done under the microscope also. For a more effective result in confirming cellular constitution or details of internal structures of plant drugs, various reagents or stains are often used on small quantities of the drug in powdered form or added to histological sections of the drug. This is referred to as chemo-microscopy.

1.6.3. **PHYTOCHEMISTRY**

Phyto - chemistry is the study of chemical constituents that are present in plants. The plant constituents' are usually classified into two groups namely, the primary metabolites such as carbohydrates, proteins and lipids and related compounds and secondary metabolites such as glycosides, alkaloids, tannins, terpenes, steroids, resins and balsams. The primary metabolites do not normally have pharmacological activity but may be of pharmaceutical necessity while the secondary metabolites may have pharmacological and therapeutic activity. For proper evaluation of crude drugs, plant materials must be subjected to phytochemical screening in order to detect the various plant constituents. Some important phytochemical groups are discussed below:

Carbohydrates:

Formerly, the carbohydrates were defined as compounds consisting of carbon, hydrogen and oxygen, with the later two elements being usually present in the same proportions as in water. They are among the most abundant constituents of plants. Many, such as the sugars and starches, are important food reserves for the plant and as foodstuffs. Cellulose and other polysaccharides are constituents of cell walls of plants. Of special pharmaceutical importance is the fact that sugars unite with a wide variety of other components to form glycosides.

The low molecular weight carbohydrates are generally water-soluble, have a sweet taste and are crystalline while the more complex ones are tasteless, amorphous and relatively less soluble in water e.g. starch, cellulose and gums. The solubility decreases with increase in complexity. Examples of simple sugars are glucose, fructose and sucrose. The carbohydrates are usually classified into the monosaccharides, polysaccharides and oligosaccharides.

The monosaccharides are made up of one sugar molecule of 3-9 carbon atoms. Those of five and six carbon atoms (pentoses and hexoses respectively) are the most abundant in plants. The oligosaccharides are made up of five to nine sugar molecules. The polysaccharides consist of more than nine sugar molecules. (Trease and Evans, 1989)

Tannins

The term 'tannin' denotes substances present in plant extracts which are able to combine with proteins of animal hides and skins, prevent their putrefaction

and convert them into leather. From the above definition, tannin is a substance which is detected qualitatively by a tanning test (the Goldbeater's Skin test) and is determined quantitatively by its absorption on standard hide powder. This definition excludes similar phenolic substances, often present with tannins, such as gallic acid, catechins and chlorogenic acid, although under certain conditions they may give precipitates with gelatin and be partly retained by hide powder. Such substances of relatively low molecular weight are called 'pseudotannins'.

Tannins are widely distributed in the plant kingdom and are present in many crude drugs in high concentration and localized in specific parts. A number of tannin- containing drugs were and are still used in folk medicine (Ribereau-Gayon, 1972).

Most true tannins have molecular weights of from 1000 to 5000. These true tannins are classified into hydrolysable tannins and condensed tannins (Trease and Evans, 1989). Hydrolysable tannins are readily hydrolysed by chemicals or enzymes e.g. tannase. Condensed tannins are formed by condensation of catechins and are resistant to hydrolysis (Ribereau-Gayon, 1972).

Glycosides

Glycosides are complex compounds which yield upon hydrolysis one or more sugars (glycone) and one or more of other products (aglycone or genin) (Miller, 1957). Glycosides have many functions. In plants they serve as sugar reserves, as waste products of metabolism, as a means of detoxification, to

regulate osmosis and to stabilize labile substances of importance in metabolism (Mcllory, 1951).

The glycosides are normally linked between the glycone and aglycone through oxygen. These form of glycosides are known as O-glycosides. They are easily hydrolysed to the parent sugars and the aglycone by either enzymes or acids. The sugar moiety can exist either in α and or β forms, thereby yielding both α and β glycosides. If the sugar is linked to a thiol, S-glycosides, are obtained. Present in plants also are N-glycosides which involve linkage to an amino group such as the nucleosides from ribose and purines. Several glycosides are known in which the sugar moiety is not a true sugar, but a sugar derivative such as uronic acid.

Included in the consideration of glycosides is a group of compounds known as C-glycosides, which resist normal acid hydrolysis but have infrared spectra, and yield alkaline-degradation products which indicate the presence of a sugar-link chain (Miller, 1973). Glycosides are used for the treatment of various illnesses e.g. digitalis contains cardiac glycosides and are used as cardiac stimulants. The pharmacological classification is dependent on their activities.

The different types of glycosides include:

- Cyanogenic glycosides
- Anthraquinones.
- Flavonoids
- Saponins
- Cardiac glycosides

Cyanogenic glycosides are those glycosides that liberate hydrocyanic acid among the products of hydrolysis. Many of these glycosides, but not all, are derived from the nitrile of mandelic acid. Although they contain nitrogen, their structure is that of 0- and not N-glycosides (Trease and Evans, 1989)

This group is represented by amygdalin which is the most widely distributed in nature. Amygdalin is the major component of bitter almonds, apricots, cherries and peaches. Drugs containing cyanogenic glycosides are widely used as flavouring and anticancer agents (Mohammed, 1998).

Anthraquinones are glycosides containing anthracene or its derivatives; as the aglycone. Borntrager's test is usually used for their identification (Trease and Evans, 1989). Their hydrolysis yield aglycones which are di-, tri- or tetra-hydroxyanthraquinones.

This group of glycosides are found in drugs like senna, aloe, cascara, rhubarb, cochineal etc. They are usually orange-red compounds, soluble in water or dilute alcohol.

The anthraquinone aglycones, in the free state, exhibit little therapeutic activity but the anthraquinone and related glycosides act as stimulant cathartics and increase the tone of the smooth muscle in the wall of the large intestine. Glycosides of anthranols and anthrones exhibit more drastic action than the related anthraquinone glycosides (Mohammed, 1998).

Flavonoids which occur in both the free state and as glycosides, are the largest group of naturally occurring phenols. The flavonoids have a basic

nucleus of the chromone (benzo-γ-pyrone). They may occur in different forms such as the flavones, isoflavones, flavonols, flavanones, chalcones or xanthones. Most are 0-glycosides and may occur as dimeric compounds (biflavonyls). Flavonoids dissolve in alkalis giving yellow solutions which, on the addition of acid, became colourless (Trease and Evans, 1989). The flavonoids are important to man not only because they contribute to plant colour but because many members are physiologically active (Harborne *et al,* 1975). Although the therapeutic usefulness of flavonoids have not been fully realized, it is probable that a number of herbal remedies whose constituents are as yet unknown, will be shown to contain active flavonoids (Trease and Evans, 1989). Many flavonoid-containing plants have been shown to have anti-inflammatory, anti-allergic, anti-diuretic, anti-tumor, anti-fungal and anti-bacterial properties (Trease and Evans, 1989).

Saponins are highly complex glycosides which are widely distributed in the higher plants, and are characterized by their property of producing a frothing aqueous solution. They have the property of causing haemolysis of red blood corpuscles, even at great dilution (Mohammed, 1998). The fact that a plant contains haemolytic substances is not a proof that it contains saponins. Most of the saponins are highly toxic when injected into the body, but when taken by mouth they are comparatively harmless. They are also very toxic to fish.
Saponins have a high molecular weight and their isolation in a state of purity presents some difficulties. As glycosides they are hydrolysed by acids to given an aglycone (sapogenin) and various sugars and related uronic acids.

According to the structure of the aglycone, two types of saponins are recognized namely the steroidal and triterpenoidal.

Steroidal saponins are less widely distributed in nature than the triterpenoidal, but they are of great importance because of their relationship with such compounds as the sex hormones, cortisone, diuretic steroids and cardiac glycosides. Some are used as starting materials for the synthesis of these compounds.

Cardiac glycosides are those glycosides which therapeutically affect a diseased heart. The heart- arresting properties of these glycosides also render them most effective as arrow poisons. The glycosides increase the force of systolic contraction and decrease the heart rate. Their therapeutic efficiency depends both on the structure of the genin and the type and number of sugar units to which it is attached. There are two types of cardiac glycosides depending on whether the genin has a five or six numbered lactone ring. These types are known as cardenolides and bufanolides or bufadienolides (Trease and Evans, 1989).

Generally, the glycosides are soluble in aqueous solvent while the aglycones are soluble in non-polar solvents.

Alkaloids

Alkaloids (alkali-like) are a group of naturally occurring compounds derived from plant sources. There is no clear cut boundary between them and naturally occurring complex amines. They form water soluble crystalline salts with acids. The free alkaloids are insoluble or slightly soluble in water, but are

soluble in non-polar solvents like ether. They are generally colourless and bitter in taste.

Alkaloids are basic in nature and contain one or more nitrogen atoms (usually in a heterocyclic ring) and have a marked physiological action on man or other animals (Trease and Evans, 1989). In plants, alkaloids act as poisonous and stimulating agents

Resins and Balsams

The term resin is applied to some amorphous substances which are more or less solid, of complex mixtures of resin acids, resin alcohols (resinols), resin phenols (resinotannols), esters and chemically inert compounds called resenes. Resins are also often associated with volatile oils and gums (oleo-resins). They are plant exudates formed in schizogenous or schizolysigenous ducts or cavities and are formed as a result of injury to a plant. Resin-containing drugs possess purgative, sedative, expectorant and antihelminthic properties.

Balsams are resinous substances which contain a high proportion of aromatic balsamic acids, chiefly benzoic and cinnamic acid (Trease and Evans, 1989).

1.6.4. QUANTITATIVE EVALUATION OF CRUDE VEGETABLE DRUGS

The evaluation of a crude drug is important because of the requirement that it should be identified in both its entire and powdered form by means of its morphological and cytomorphological characters. The quantitative evaluation of crude drugs helps to ascertain their purity, which will indicate their acceptability by criteria other than their morphology (Brain and Turner, 1975).

Some of the different parameters for the quantitative analysis are as follows:

Determination of Moisture Content:

The percentage of active chemical constituents in crude drugs is defined on air-dried basis. Hence, the moisture content of a drug should be determined and should also be controlled. The determination of moisture content of a crude drug is important because the presence of moisture can lead to the following:

1. It decreases the percentage quantity of the drug by increasing the weight.

2. It causes the activation of enzymes which may lead to hydrolysis of the constituents.

3, It causes chemical reactions and encourages the growth of micro-organisms and insects. It is therefore necessary to keep the moisture content of all drugs reasonably low (Shellard, 1958).

Determination of Extractives:

The determination of extractives is a method designed to measure the amount of constituents which are extractable by a solvent under specified conditions. It is applied to those vegetable drugs for which there is no suitable method of chemical or biological assay for their active constituents (AP,1986) . The evaluation is important to guard against adulteration or substitution with drugs which have already been extracted (Shellard, 1958).

Determination of Total Ash Value:

The determination of total ash is a method of measuring the amount of residual substances not volatilized when the drug is ignited (AP,1986). The total ash represents the 'physiological ash' i.e. the ash that comes from the

plant tissue itself. Physiological ash consists of carbonates, sulphates, nitrates, phosphates, chlorides and silicates of the various metals that are present in the plant which it takes up during growth. Elements taken up from the soil by plants include sodium, calcium, carbon, sulphur, oxygen, nitrogen and phosphorous .

1.6.5. **BIOLOGICAL EVALUATION**

This form of evaluation involves the estimation of the potency of a crude drug or its preparation by means of its effect on living organisms like bacteria, fungi or animal tissue or entire animal. It is also known as bioassay. This method is generally called for when standardization cannot be adequately done by chemical or physical means alone and also for conformity of therapeutic activity of raw material and finished products. It enables the determination of the quality of the sample being tested that is capable of producing biological effect equivalent to that of the standard preparation. Bioassay measures the actual biological activity of a given sample. In any one test, the animals of only one strain are used (Mohammed, 1998).

Biological assay methods are mainly of 3 types:

1) toxicity
2) symptomatic and
3) tissue methods.

In toxic and symptomatic techniques, animals are used and in tissue method the effect of a drug is observed on isolated organ or tissue. In the evaluation

of herbal crude drugs, the assessment of the biological efficacy is found to be the most popularly used method.

Some of the biological activities that are commonly evaluated are:

1) Analgesic
2) Anti- inflammatory
3) purgative activity
4) Anti-microbial and anti fungal evaluation
5) Acute Toxicity

studies are carried out on whole animals and isolated animal tissues to determine the different activities, with the exception of (4) above which is carried out on standard micro organisms and clinical isolates.

There are some disadvantages of bioassays. Quantitative accuracy is usually less than observed with most chemical analysis, and the techniques and interpretations involved may vary with different operators. Also, the effect measured in the test animals may differ from that observed in treating patients (Mohammed, 1998).

1.7. **ANALGESIC ACTIVITY**

1.7.1 **ANALGESICS**

An analgesic is a drug that alleviates or removes pain. An account of what pain is, types of pain, the nature, mechanism and physiology and the drugs used in the treatment of pain would be useful.

1.7.2. **PAIN**

One of the greatest stumbling blocks in understanding pain is the lack of a single suitable definition for it. Scientists from different disciplines have investigated pain and each group has used a definition suitable for that field. Such terms used include pain, discomfort, suffering and the sense that damage is being done in some area of the body or the sense of something noxious. Also, pain is often listed as a sensation (Benjamin and Gorsky, 1981). Pain can also be defined as a more or less localized sensation of discomfort , distress or agony resulting from the stimulation of specialized nerve endings. It serves as a protective mechanism in so far as it induces the sufferer to remove or withdraw from the source. Also pain aids in the diagnosis of an underlying illness.

1.7.3. **KINDS OF PAIN**

There are different types of pains and some of them are listed below:

1. Bearing down pain:

This is the pain accompanying uterine contraction during the second stage of labour.

2. **False pain:**

This is an ineffective uterine contraction preceeding and sometimes resembling true labour, but distinguished from it by lack of progressive effacement and dilation of the cervix.

3. **Gas pain:**

This is the pain caused by distention of the stomach or intestine by the accumulation of air or other gasses, occurring as a result of ingestion of gas forming foods.

4. **Hunger pain**:

This type of pain is associated with the time of feeling of hunger for the next meal. It is often a symptom of gastric disorder.

5. **Boring pain:**

This is often a sensation of being pierced with a long, slender, twisting object. This is also called terebrant pain.

6. **growing pains**:

This type of pain ,which are aching pains, are frequently felt at night in limbs of growing children. It is attributed variously to growth, rheumatic state, faulty posture, fatigue or ill-defined psychic causes.

7. **Rest pain:**

It occurs in the extremities during rest in the sitting or lying position.

The adequate stimulus for pain is tissue damage, slight and altogether reversible, perhaps, but nevertheless damage. The role of pain in the bodily economy is to warn of danger. If the tissue damage is allowed to increase, this might ultimately be irreversible.

1.7.4. Nature of Pain

There are two types of pains namely acute and chronic pains. The acute pain usually gives the warning that something is wrong and that the part of the body suffering pain has being damaged or is suffering. Since pain can be

localized quite specifically, it often points to a call for attention. Such a localized pain is called referred pain. This is the pain perceived as coming from a situation remote from its actual origin e.g. arm, elbow or wrist pain felt in *angina pectoris* and in appendicitis where there is referred pain around the umbilicus(navel). The painful sensation spreads from structures deep within the body and are often felt in other areas than the site of the stimulation. It is often dependent on the development of an excitatory state in the spinal cord at the level of the incoming noxious impulses. Depending on the intensity of the impulses, the excitatory state spreads by way of neurons of the dorsal horn of the spinal cord and association pathway to the segment adjacent to the ones into which impulses are originally conducted. In turn, fresh impulses travel to higher levels in the nervous system where neural activity is ultimately felt as pain.

Chronic pain however, exists for years, sometimes for decades, and is usually not associated with nociception. There is a functional difference and also some apparent differences in associated mood between acute and chronic pain . In acute pain, there is fear of what the pain represents, if it will become worse or whether the pain will eventually be cured or not. On the other hand, the chronic pain sufferer is constantly depressed and because of his anxiety , he is left with chronic depression. He is thus always sad because of the continuous existence of the pain and of the changes that have occurred in his life due to the pain (Encyclopedia Britannica, 1973).

1.7.5. **PAIN AS A SENSORY EXPERIENCE**

Pain on the surface of the body has a pricking or burning quality. Deeper tissue pain is felt as an ache. The variation in the aching quality depends on the timing, location and intensity. Aching pain is often throbbing, wavelike which may be steady or intermittent. Also pain may radiate from one spot to the other, and its experience differs from other common sensations like sight, hearing, smell or touch in that the perception may be modified by analgesic agents (Encyclopedia Britannica, 1973).

1.7.6. **PAIN PATHWAY**

Neural impulses from noxious stimulation may be conveyed by nerve fibres of many sizes that follow a rich variety of peripheral nerve channels to the dorsal root of the ganglion. Here, cell bodies send projections into the dorsal horn of the spinal cord and from here, secondary neurons carry the impulses across the anterior commissure to the opposite side, through the spinothalamic tract to the brain. Here the sensation is registered, interpreted and reacted to. The fibres involved in aching and burning sensations are anatomically and physiologically different from those involved in pricking pain. The later are phylogenetically more recent and are myelinated, are larger and conduct impulses more rapidly (Encyclopedia Britannica, 1973).

1.7.7. **Threshold For the Perception Of Pain**

Pain of low intensity is barely perceptible and is called threshold pain. It is, however, not established whether nerve-endings have to be injured by noxious stimulation or that they need to be stimulated directly. It appears rather that nerve endings may be stimulated by products liberated as a result

of injury to other tissues. A polypeptide, neurokinin, appears to be involved in the pathophysiology of pain and hyperalgesia. It has some common properties with histamine, serotonin and bradykinin, but is distinct from them because of its ability of lowering pain threshold when injected into normal skin (Encyclopedia Britannica, 1973). Neurokinin has been shown to be the product of a local action of proteolytic enzyme in response to local tissue damage or stimulation of nerve roots. It is, however, not known if neurokinin acts as a neurohumour mediating all pain phenomenon.

Pain threshold is measured quantitatively with an instrument that induces pain without causing major tissue damage. The evidence accumulated from such measurements indicates that all persons with healthy bodily structures have approximately the same capacity for perceiving pain.

1.7.8. **Measurement of Pain Threshold**

Three general methods are used for assaying the effectiveness of an analgesic agent:

1. testing the effects of the agent on the reactions of an animal to the noxious stimulation. This can be done using thermal, electrical, chemical or mechanical stimuli.
2. measuring the pain-reducing effects in normal human subjects with experimentally induced pain.
3. measuring the pain–reducing effects in patients experiencing pain spontaneously.

1.7.9. **Threshold For Reaction to Pain**

Certain automatic responses to noxious stimuli that occur without awareness of pain are mediated through reflex arcs in the spinal cord and brain stem. The reactions are characteristically inhibited and variously modified by impulses from higher centers set in motion due to perception and interpretation of the painful experience. Since most pains are interpreted as unpleasant, they give rise to a reaction of aversion on the part of the affected subject. What he thinks, feels or does about it constitutes his reaction to the pain. Thus, threshold for pain reaction unlike the threshold for the perception, varies within wide limits for individuals and for the same person under different circumstances.

The ability for a person to perceive pain depends on the intactness of relatively simple and primitive nerve connections. On the other hand, reactions are modified by highest cognitive functions and depends on what the sensation means to the person in the light of his past experience.

1.7.10. **DRUGS USED IN PAIN RELIEF**

1. **ANALGESICS**

There are certain drugs which can alleviate pain by reducing the source of the pain. An example is the use of amyl nitrite for treating the pain in myocardial ischaemia. It may dilate the coronary arteries and reverse the chain of events which has caused the noxious stimuli. In distinction, the analgesics prevent, without producing unconsciousness, the nervous integration of noxious stimuli

which would otherwise be interpreted as pain by the central nervous system. These drugs may be considered in two main groups:

a. **Antipyretic Analgesics:**

Aspirin, (acetyl salicylic acid, ASA), is the typical example of this group. They are usually weak analgesics and their effect is peripheral in the nervous system so that behavioural changes do not usually occur. Aspirin produces analgesia by blocking the effects of bradykinin, an important mediator of pain. It also interferes with the synthesis of prostaglandins which sensitize the nerve endings to bradykinin and this effect will also contribute to the analgesia. The antipyrexial and anti-inflammatory effects of ASA are probably the results of its interference with the synthesis of prostaglandins since these play a key role in febrile and inflammatory processes.

The initial members of this group were produced as synthetic substitutes for quinine and despite their lack of antimalarial activity, they have proved useful as antipyretic, analgesic and anti-inflammatory agents. Broadly, this group now includes derivatives of these chemical compounds; salicylic acid e.g. ASA, aniline e.g. phenacetin given in a compound tablet with ASA or ASA and codeine (A.P.C.), pyrazole e.g. phenazone, indole e.g. indomethacin and anthranillic acid e.g. mefenamic acid.

b. **Narcotic Analgesics:**

Opium, the dried juice obtained from the unripe poppy of *Paperver somniferum,* has been used from ancient times. It contains over twenty alkaloids but its analgesic effect is mostly due to the morphine constituent, which has remained one of the most popular, successful and potent

analgesics available. It is the standard by which the analgesic activities of new and potent synthetic analgesics are judged. Also, it is the standard by which addiction is assessed depending on the chemical relationship of the narcotic analgesics to morphine, pethidine or methadone. They can be divided into three groups, namely the morphine, pethidine and methadone groups. Unlike the other groups already mentioned, the morphine group is comprised of both natural derivatives and synthetic agents.

Morphine Group- Natural Derivatives:

This is made up of the opium alkaloids namely morphine, codeine, papaverine and noscapine. These are rarely used except, for instance, in the treatment of diarrhea, due to their ability to inhibit the increased intestinal peristalsis. In Britain, papaveretum B.P., an injectable aqueous extract of opium, is very popular among anaesthetists for premedication and postoperative analgesia (Hannington-Kiff, 1974). It is thought that papaveretum causes less postoperative respiratory depression and alimentary complications.

Morphine is the most potent analgesic. Pharmacologically, it produces a combination of depressant and excitatory actions in the central nervous system. Its depressant actions promote analgesia, tranquillization, sleep, respiratory depression and cough suppression. Its excitatory action can cause vomiting, anxiety and restlessness.

The Morphine Group- Synthetic Agents:

These were synthesized to produce morphine- like compounds with potent analgesia unaccompanied by stupor and addiction. The most useful clinically

are diamorphine hydrochloride (heroin), levorphanol tartrate, phenazocine hydrobromide, pentazocine, dihydrocodeine tartrate and oxycodone pectinate.

Pethidine Group

These consist of - pethidine hydrochloride, phenoperidine hydrochloride, fentanyl citrate, and ethoheptazine citrate.

The Methadone Group:

The group is comprised of methadone hydrochloride (used to wean morphine addicts), dextromoramide tartrate, dipipanone hydrochloride and dextropropoxyphene hydrochloride

2. **Other Analgesics:**

These include:

1. Carbamazepine: an antidepressant and potent epileptic agent. It has no specific analgesic activity, but it has been used successfully to relieve headache of migraine. Its side effects are aplastic anaemia, leucopenia and liver damage.

2. General anaesthetic: They are inhalation agents like nitrous oxide, trichloroethlene and methoxyflurane.

3. **Psychotropic Drugs**

There are instances in some patients where there is heightened anxiety, and pain can be easily relieved by decreasing the emotional attribute with tranquillizers. Where there is depression associated with chronic pain, changing the emotional attribute towards euphoria with an anti-depressant or psycho stimulant may be useful in easing suffering. The psychotropic drugs

can modify the experience of pain because of their central nervous effects yet are not analgesics. The drugs in this group are divided into:

1. **Major Tranquillizers**

The drugs in this group have profound effects on the central nervous system, including its autonomic responses. Because of this effect, psychotic patients can be effectively managed and even brought into remission. In this group are:

Rauwolfia alkaloids: The principal alkaloids are reserpine and deserpidine which are used nowadays more for their antihypertensive than psychotropic effects.

Phenothiazines: The most commonly used is chlorpromazine hydrochloride (largactil).

Butyrophenones: Droperidol, haloperidol and oxypertine are the examples in this group.

2. **Minor Tranquillizers**

These have limited effects in the central nervous system compared with the major tranquillizers and are used to treat neurotic states and anxiety reactions in every-day stresses. Included in this group are the benzodiazepines and carbamates.

Benzodiazepines: These drugs are currently very popular and the most common are chlordiazepoxide (librium), diazepam (valium), medazepam (nobrium) and oxazepam (serepax), but the most popular is diazepam. It produces muscular relaxation by a central action and can directly relieve the pain of musculo-skeletal disorders in which muscle spasm is prominent.

Nitrazepam (mogadon) is a valuable hypnotic related to diazepam, but the effects of an overdose are far less serious than with barbiturates which is an important consideration in potentially suicidal patients.

Carbamates: The most common example is meprobamate whose serious disadvantage is addiction with insomnia, vomiting, tremors, anorexia, ataxia and even epilepsy on withdrawal. This is in contrast to diazepam which is valuable in the treatment of epileptic seizures. Meprobamate is contra-indicated in patients with epilepsy.

4. **Antidepressant Drugs**

These drugs used in the treatment of pathological depressive states are in two groups:

Monoamine Oxidase inhibitors (MOI): They cause the accumulation of catecholamines in the central nervous system together with others like noradrenaline, dopamine and 5-hydroxytryptamine, which all have stimulant properties. They have been found to be clinically useful in cases of reactive depression, where there is a recognizable precipitating factor such as recent bereavement or prolonged pain. The administration of monoamine oxidase with certain drugs like the sympathomimetic agents, pethidine or other narcotic analgesics, often cause serious complications due to wide spread inhibition of the listed group of drugs. Also, certain common foods like cheese and other milk products contain active amines and patients on mono amine oxidase inhibitors need to be cautioned. These drugs have lost much of their initial popularity and cannot be used in patients who require narcotic analgesics for pain relief.

Tricyclic (Dibenzazepine} Antidepressants: They are indicated in endogenenous depression, which is characterized by inactivity. They prevent the uptake of catecholamines at nerve endings, but the mechanism of their pain alleviation is not known. It may take as long as 2 to 3 weeks before the symptoms of depression are relieved (Hannington-Kiff, 1974).

Unlike with MOIs, side effects with tricyclic antidepressants are not usually serious.

5. **Psychostimulants**

Included in this group are caffeine, amphetamine and cocaine which can stimulate the cerebral cortex and affect the mental state.

Caffeine: In the amount contained in a strong cup of tea or coffee (150mg), caffeine has the ability of alerting the cortex and producing a mild degree of euphoria. It is a common constituent of compound analgesic tablets such as the popular APC tablets that contain aspirin (225mg), phenacetin (150mg) and caffeine (50mg).

Amphetamine: The use of this and related drugs were relegated due to the discovery of more appropriate drugs. It enhances the effect of antipyretic and narcotic analgesics, but lacks the respiratory depression effect of the narcotic analgesics.

Cocaine: Cortical stimulation is the first effect of cocaine on the central nervous system, which results in mental alertness and euphoria. It causes indifference to pain, fatigue and hunger due to the great sense of well-being which it promotes. In high doses, convulsions can occur and death may result from depression of the medullary centers after their initial stimulation. Only

psychic dependence results from the use of cocaine. The great pain and general anguish of terminal malignancy can be greatly eased by cocktails of cocaine, diamorphine and morphine in sweetened spirits. The cocktails produce excellent euphoria and avoid the need for injection to produce potent analgesia.

6. **The Barbiturates**

These are the most commonly prescribed hypnotics and they are also used as minor tranquillizes. Barbiturates cause restlessness in the presence of pain and is an important consideration in prolonged pain which can cause suicidal tendencies.

7. **The Placebo Effect**

When inert medicines are administered to patients, about 30% of them may be expected to report symptomatic relief in any illness. The placebo effect is more evident in the case of pain and serves to emphasize the power of mind over body. Other forms of treatment, including surgery, have a high level of placebo effect. When a placebo intentionally produces an improvement, the physician need not feel guilty, but only regret that most of the improvements are temporary.

1.7.11. **Screening Methods for Analgesics**

In general, it may be said that animal testing serves as a rough assay of analgesia. Test on human subjects offer a more precise assay of strength of analgesia, and measurements in patients with pain evaluate the effectiveness of the agents under circumstances most relevant to clinical use (Balla and Bhargava, 1980; Dipalma, 1971). However, the characteristics of clinical pain

can be reproduced in part in animal models and thus the common methods that are mostly used nowadays, has provided a firmer foundation for predicting efficacy in man.

Many methods have been devised to determine the ability of a substance to produce analgesia, either primarily for screening purposes or to determine clinical usefulness. However, two methods are now most often used for centrally acting analgesics (D'Amour and Smith, 1941;). The procedures give results that are of good predictive value for man, except for narcotic antagonists. Generally, the most commonly used methods for the investigation of new analgesic and narcotic agents are:

1. Hot- plate test for analgesia (mouse).
2. Mouse writhing test for analgesia.
3. Mouse narcotic dependence model.
4. Rat self – administration model.
5. Monkey self administration model.
6. Rat narcotic dependence model.

1.7.12. **Primary Screening Methods:**

1. **Thermal Method (Hot plate method).**

The method is used to detect narcotic agonist and antagonist activity of unknown compounds by their effect on the reaction time to a thermal stimulus. In each case, groups of 10 mice of about 18-25g are tested 3 times at 20 minute interval to determine the time in seconds that will be required to respond to being placed individually in a restraining cylinder kept on a hot plate. The response is the observation of either licking of a foot or jumping.

Mice taking longer than 15 seconds to respond to the stimulus are discarded. The median of the three pretreated response time is used for later comparisons in all the three versions of the tests described below:

1. In the initial screening method, saline or the compound of interest is administered interperitoneally (i.p) immediately after the 3rd pretrial. After a period of 30 minutes, the mice are retested on the hot plate to determine the inherent analgesic activity of the test compound. The trial is then followed by the administration of morphine sulfate at a standard dose of 40mg/kg i.p. This is further followed by the hot plate trials at 15, 30, 60 and 90 minutes later. Any failure of a mouse to respond within 30 seconds after being placed on the hot plate, should terminate the trial and the analgesic effect considered maximal.
2. To evaluate the analgesic activity, the standard hot-plate method is used. Following the 3rd hot plate trial, saline or the test compound (analgesic) is administered i.p and each mouse is then retested at 15, 30, 60, 90 and 120 minutes following the drug administration.
3. To evaluate narcotic antagonist, morphine sulfate at a standard dose of 40mg/kg i.p is administered after the 3rd hot plate trial. After a period of 15 minutes, the mice are retested on the hot plate to determine the new response time for each mouse. Saline or the test compound is then administered I.p at a dose of 25mg/kg and the mice are again retested on the hot plate at 15, 30, 60,and 90 minutes post injection.

   The crucial point in analgesic testing is the determination of the potency or efficacy in the relief of clinical pain. The potency of an analgesic is

usually expressed as the ED_{50}, which is the dose in mg/kg of body weight that produces a significant effect in 50% of test animals.

2. **Electrical Method**

This method makes use of the flinch-jump test using rats. It involves the use of groups of 6- 10 albino mice kept in a test chamber consisting of a compartment containing a grid floor through which shocks of increasing intensity are delivered every 8 seconds to the feet. This is done with the aid of a timer and grid scrambler with a 1.5-megohm resistor. The current is increased progressively from 143v until a threshold of response is obtained or a maximum of 385v is applied. The threshold is designated as the minimum voltage which produces tonic extension of the hind limbs and jumping in response to three successive shocks. Morphine administered subcutaneously at 5mg base/kg raises the control threshold from a mean of 220 to 317v.

1.7.13. **<u>Secondary Screening.</u>**

1. **Mechanical Methods:**

This procedure involves the use of groups of 6-10 male albino rats, weighing 60-100gm. They are tested for responsiveness to pressure applied to a fixed point upon the non inflamed plantar surface of the foot. The pressure is applied by means of a calibrated syringe-plunger system, and animals responding to discomfort by vocalization or struggle to 60mm Hg or less are selected. The cut off for analgesia is taken as the mean threshold (pretreated) response for the group. All determinations are made 30 minutes after

administration of the test material. This technique easily detects the analgesic effects of the antagonists, nalorphine and pentazocine.

2. **Chemical Method.**

This involves the use of a chemical substance, bradykinin, which evokes responses characteristic of reaction to pain when injected intra arterially into a dog. Bradykinin (1-5µg) is administered through an indwelling catheter inserted through the abdomen of male and female dogs. Within 15 seconds, a reaction occurs consisting of howling, barking, biting and violent body movements. Vocalization, which is the most consistent response, is used as the primary criterion. The dogs responding satisfactorily are selected for the After the threshold dose of bradykinin is established for the individual dogs, each is administered the test compound by a route of choice. The test with bradykinin is then carried out at 5-15 minutes intervals thereafter until the animal vocalizes.

Another chemical method, the acetic acid-induced writhing, using acetic acid may be used also. It has the ability of causing abdominal constriction in animals upon administration intraperitoneally. The ability of the test substance to inhibit the constriction is a measure of the analgesic property of the substance.

1.8. PURGATIVE (LAXATIVE) ACTIVITY

1.8.1 PURGATIVES

The term purgative, cathartic, laxative, aparient and evacuate are synonymous. The substances termed purgatives or laxatives facilitate the passage and elimination of a soft formed stool from the colon and rectum. The cathartics implies a more fluid evacuation. There are few recognized medicinal indication for the use of purgatives, but many people misuse them to alleviate what they consider to be constipation. The term constipation means different things to different patients, however, it is generally defined as a decrease in the frequency of feaces elimination characterized by difficulty of passing hard and dry stool. It usually results from abnormally slow movement of feaces through the colon with resultant accumulation in the descending colon. The ideal laxative should be non-irritant and non-toxic. It should act only on the descending colon and produce a normally formed stool within a few hours, thereafter it should resume normal bowel movement. These drugs have been classified according to the site of action, intensity of action, chemical structure or mechanism of action. The most meaningful classification is, however, that based on the mechanism of action whereby they may be classified as:

1. **Bulk Forming purgatives**

Because they approximate most closely the physiological mechanism in promoting evacuation, bulk-forming products are the recommended choice as initial therapy for constipation. These purgatives are natural

and semi-synthetic polysaccharides and cellulose derivatives that dissolve or swell in the intestinal fluid, forming emollient gel that facilitates the passage of the intestinal content and stimulate peristalsis. They are usually effective in 12-24 hours and may require as many as 3 days in some individuals. These agents are recommended for certain hospitalized patients and in patients in whom it is desired that the stool be maintained soft, to avoid straining at the rectal part. Examples are agar, psyllium and bran.

2. **Stimulant Purgatives**

This group of purgatives increases the propulsive peristaltic activity of the intestine by local irritation of the mucosa, or by a more selective action on the intramural nerve plexus of the smooth muscles of the intestine. The site of action is normally the small intestine and sometimes in the large intestine or both.

All stimulant purgatives produce gripping, increased mucus secretion and in some people, excessive evacuation of fluid. Examples in this class are the anthraquinone purgative agents like *Cascara sagrada*, rhubarb, senna and frangula.

3. **Emollient Purgatives**

These are agents which, when administered orally, increase the wetting efficiency of intestinal fluid. They also facilitate the admixture of aqueous and fatty substances to soften the feacal mass. Examples are the sulfosuccinates and mineral oil. They promote defecation by modest

softening of the feaces. Consequently, their clinical usefulness is only limited to situations in which it is desired that the feaces be kept soft.

4. **Lubricant Purgatives**

This group of compounds soften feacal content by coating them and thus preventing coloric absorption of feacal water. Examples are liquid paraffin and certain digestible oils like olive oil.

5. **Saline Purgatives**

The active constituents of saline purgatives are relatively non-absorbable cations and anions such as magnesium and sulphate ions. Sulphate salts are considered to be most potent of this category of laxatives. The walls of the small intestine acting as a semi permeable membrane to magnesium sulphates, phosphate and citrate ions retain highly osmotic ions in the gut. The presence of these ions draws water into the gut causing an increase in intestinal motility. Saline laxatives are indicated for use only in acute evacuation of the bowel.

The latency and intensity of the effect vary with the salt and the dosage. Full doses of the saline cathartics (15gm of magnesium sulphate or its equivalent) produce a semi fluid or watery evacuation in 3 to 6 hours or less. Therefore, in testing for purgative or laxative activity of substances, the production of wet feaces is taken as the measure of the purgative activity (Elujoba *et al*, 1988; Akunyili, 1992)).

1.9. ACUTE TOXICITY TESTING

It is often stated that medicinal products from plant sources are devoid of any harmful or toxic effects. However, it is a well known fact that

some herbal drugs from natural sources, such as plants, are capable of producing adverse side effects when taking at certain concentrations.

The acute toxicity assessment of a substance is the first step in the toxicological investigation of an unknown substance. The index of the acute toxicity is the LD_{50}, which is the lethal dose needed to kill 50% of test animals. The method of acute toxicity testing by Lorke (1983) gives a simple means by which the poisonous effects of a chemical substance can easily be determined, whether toxic, less toxic or very toxic. It can determine whether the toxic effects of a substance are insignificant for dealing with the substance in practice or not. A figure, which expresses the toxic effect, indicates the amount of the substance that is injurious after a mode of intake is sought. Thus, an objective criterion namely death, as a means of determining the acute toxicity of substances was used. The LD_{50} was recognized and justified as being the best parameter. However, the LD_{50} should not be regarded as a biological constant since different results are obtained on repetition when determinations are done in different laboratories (Hunter *et al,* 1979). In traditional medicine, the traditional medicine practitioners do not exactly quantify the amount of medication they instruct their patients to take. In fact, they do not know the amount and nature of the active constituents in the concoctions they prescribe and in such ‘blind practice of prescription’; the probability of administering toxic level of the medication is very high.

1.10. **AIMS AND OBJECTIVES**

The plant *Waltheria indica*, Linn. grows widely in Nigeria. It is recognized throughout the West African sub region for its various medicinal uses, and it is commonly used in all parts of the country, especially Northern Nigeria, for the treatment of a variety of ailments. Due to the poor state of the socio-economic condition of the country, more people have resorted to the use of herbal remedies which is also part of the life of the people. It is cheap, effective, safe, readily available and more easily acceptable to the populace. Although the plant is used in traditional medicine for the treatment of various ailments, there is no report on the evaluation of its activities. Hence, this project aims at evaluating the plant to justify the acclaimed uses, set pharmacopoeial standards and to document data for its possible inclusion in the African Pharmacopoeia.

Specifically, the aims and objectives of this project therefore are as stated below:

- To carry out the macroscopical studies of the different parts of the plant that are commonly used in traditional medicine practice by herbalists for the various treatments namely the leaves, stem and roots.
- To carry out microscopical and chemomicroscopical studies of the various parts of the plant for observation of diagnostic characters which can aid in their identification.

- To carry out Phytochemical studies of the various parts of the plant for the detection of different constituent groups such as alkaloids, tannins, saponins, flavonoids etc. and to correlate the different constituents to the ethnomedical uses of the plant.
- To establish pharmacopoeial standards such as moisture content, extractive and ash values for the leaves, stem and root.
- To carry out the Biological evaluation of the water extracts of the different parts (leaves, stem and root) of the plant so as to establish the scientific basis for the traditional uses, and also to correlate the scientific investigation with the uses. The following activities will be evaluated:
- Analgesic properties of the different plant parts using the acetic acid-induced writhing in mice.
- Purgative activity of the leave, stem and root of the plant.
- Toxicity studies of the different plant extracts in order to establish the acute toxicity of the plant parts, and to establish the LD_{50}.

2.0. MATERIALS AND METHODS

2.1. Plants Collection and Identification

The plant *Waltheria indica* was collected in August, 2001 from the bushes around the A.B.U. dam, which is situated on the main campus of the Ahmadu Bello University, Zaria.

The samples were identified in the field using keys and descriptions in the 'Woody Plants' of Ghana (Irvine, 1961); 'Medicinal Plants in Nigeria' (Oliver, 1960) and 'Flora of West Tropical Africa' (Hutchinson and Dalziel. 1958). The sample was further authenticated by comparison with original herbarium specimen, placed in the Herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, and had the voucher number 2718. A herbarium specimen of the plant has also been kept in the Department of Pharmacognosy and Drug Development of the University.

2.2. Materials

The materials used to carry out the different experiments are listed below:

- Pestle and mortar
- Sieve, mesh size no. 20
- Compound microscope (Wild 11 Switzerland)
- Slides and cover slips
- 250ml Beakers
- Measuring cylinder
- Bunsen Burner
- 250ml Flat bottom flask
- 250ml conical flasks
- Nickel and porcelain crucibles
- filter paper

Plate 1: *Waltheria indica* in its natural habitat

Plate 2: Flesh leaves and flower and *Waltheria indica*

Plate 3: fresh roots of *W. Indica*

Plate 4: fresh stem of *W. Indica*

-- Ashless filter paper

- Separatory funnel
- Oven (Gallenkamp)
- Water bath
- Razor blade
- Photographic Camera (Canon, Japan)
- Mechanical shaker (Gallenkamp)
- Erlenmeyer flask
- Refrigerator
- Oven
- Weighing balance
- Cages
- Rats
- Mice
- Needles and syringes
- Wristwatch
- Stirring rod
- Aluminium foil
- Aspirin
- Thermometer
- Normal saline
- Universal bottles
- Extracts of the different plant parts
- Drinkers

- Senokot tablets
- Tissue paper
- Stage micrometer
- Eyepiece micrometer
- Hot plate
- Descicators
- Sand

Magnesium fillings

2.2.1. **Solvents and Reagents**

The reagents used were all of laboratory grade and include: -

- Concentrated sulphuric acid
- Concentrated hydrochloric acid
- Chloroform
- 95% Ethanol
- Acetone
- Petroleum ether ($60\text{-}80^0$)
- Methanol
- Lead acetate (10% w/v)
- Lead sub-acetate
- Ferric chloride
- Ferric ammonium citrate
- Acetic acid
- Formaldehyde
- Sodium nitrate

- Glacial acetic acid
- Acetic anhydride
- Fehling's solution A and B
- 10%Tannic acid
- Wagner's (solution of iodine in pot. Iodine).
- Hager's (saturated solution of picric acid).
- Mayer's (potassium mercuric iodide solution)
- Dragendorff's (Potassium bismuth iodine solution)
- Molisch's reagent (10% solution of Naphthol in alcohol)
- Ruthenium red
- N/50 Iodine
- Limewater
- Barfoed's Reagent
- Chlor-zinc-iodine
- Phloroglucinol
- Copper acetate solution
- Conc. Ammonia solution
- Resorcinol crystals
- Chloral hydrate
- Dilute glycerol
- Sudan IV solution
- Millon's reagent
- Ferrous sulphate
- Sodium picrate paper

- Sodium chloride

- 3,5-dinitrobenzoic acid

- Sodium nitropruside

- pyridine.

- Amyl alcohol

- Normal saline

2.3. PHARMACOGNOSTIC STUDIES OF THE LEAF, STEM AND ROOT OF WALTHERIA INDICA

2.3.1 Macroscopical Examinations of the Leaf, Stem and Root of *Waltheria indica*

2.3.1. 1 Root

The general macroscopical features and organoleptic properties were noted for the leaf, stem and root using standard procedures (Herbert and Ellery, 1948, and Trease and Evans, 1989).

- Origin and preparation
- Size
- Surface
- Colour
- Fracture
- Fractured surface
- Colour and taste (of powdered bark)

2.3.1.2. **Stem**

_The features used in the characterization of whole bark as applied to the root samples were employed for the stem.

2.3.1.3. **Leaves**

- The following features were used for the macroscopical identification of the leaves (Trease and Evans, 1989):
- duration
- Leaf base
- Petiole
- Lamina
- Incision
- Shape
- Venation
- Margin
- Apex
- Base
- Surface
- Texture
- Colour
- Odour and taste (powdered sample)

2.4. CHEMO-MICROSCOPICAL AND MICROSCOPICAL EXAMINATIONS OF THE LEAF, STEM AND ROOT OF *WALTHERIA INDICA*

2.4.1. Chemo-microscopical Examinations

The presence of substances such as cellulose, tannins, lignin, fats and oils, starch, protein, gums and mucillages, cutin, suberin, callose, calcium oxalate crystals and calcium carbonate in the various anatomical sections as well as the leaf, stem and root powders were treated with appropriate chemical reagents on microscope slides and observed under the compound microscope (Iyenger and Nayak, 1975).

1. **Test for Lignin**

   The powdered samples and their anatomical sections were mounted in a mixture of phloroglucinol and conc. hydrochloric acid (1:1 v/v). Tissues like xylem and sclerenchyma because of lignification would take up red colouration. The intensity of the red colour would indicate the extent of lignification (Trease and Evans, 1989).

2. **Test for Mucilages**

   The various sections and powdered samples of the root, leaf and stem were treated separately with Ruthenium red. Mucilage in tissues would stain pink or red (Trease and Evans, 1989).

3. **Test for cellulose**

   The various powdered sample were mounted in Chlor-zinc-iodine, or N/50 iodine followed by 66% sulphuric acid. A blue colour would indicate the presence of cellulose (Trease and Evans, 1989).

4. **Test for starch**

The different powdered samples as well as the anatomical sections were mounted separately on a slide with N/50 iodine solution. Presence of starch is indicated by blue or blue-black colouration (Iyenger and Nayak, 1975 and Trease and Evans, 1989).

5. **Test for oils**

The samples were separately mounted in Sudan IV reagent. Presence of oils in any of the tissues is indicated by pink colour (in the parenchyma and epidermal cells)(Trease and Evans, 1989).

6. **Tests for Tannins**

Each of the various samples was mounted in Ferric chloride solution. A blue-black or greenish colour would indicate the presence of tannins (Ramstad, 1959).

7. **Test for Calcium Oxalate Crystals**

Warming with Chloral hydrate solution separately cleared the powdered samples as well as the anatomical sections. The calcium oxalate crystals would appear as bright structures of definite shapes and sizes. On addition of a few drops of concentrated sulphuric acid (80%), the crystals slowly dissolve (Brain and Turner, 1975) and disappear when viewed under the microscope. The disappearance confirms their presence (Trease and Evans, 1989).

8. **Test for Calcium Carbonate**

The powdered samples on a white tile were treated with dilute sulphuric acid. Presence of calcium carbonate was indicated by effervescence with rapid evolution of gas (Brain and Turner, 1975).

9. **Test for Cutin and Suberin**

When the different samples are stained with tincture of alkanna, a red colour would indicate the presence of cutin and suberin.

10. **Test for Callose**

The different samples were stained with corallin soda. A red colour would indicate the presence of callose.

11. **Test for Proteins**

1% picric acid and Millon's reagent were used. When the different samples are stained separately with picric acid, a yellow stain on the microscopic structures would indicate the presence of protein. A red stain with Millon's reagent would indicate the presence of protein.

2.4.2 **Microscopical Examination**

Both fresh and powdered leaf, stem and root were used for the qualitative and quantitative studies using standard methods (Evans, 1996). A thin anatomical section or small quantity of the powdered sample, was placed on a clean slide and few drops of chloral hydrate solution added and covered with a clean cover slip. The slide was slightly heated on a flame for 30 seconds. Over heating was avoided so as to guard against false observation as a result of chloral hydrate crystal formation. When the section was sufficiently cleaned up, air

bubbles, if present, were removed. After a few drops of dilute glycerol was added, the prepared slide was observed under a compound microscope (Iyenger and Nayak, 1975).

2.5. Quantitative Leaf Microscopy

The following methods of quantitative microscopy as suggested by Trease and Evans (1989) were carried out:

1. **Palisade Ratio**

Pieces of leaf about 2mm square were cleared by boiling with chloral hydrate solution, mounted in dilute glycerol and examined with a 4mm (x40) objective. A camera lucida was used to focus the epidermal cells and the palisade cells lying below them. A number of groups of each of four epidermal cells were used and the number of palisade cells lying beneath each group was then counted. The palisade cells that were more than half covered by the epidermal cells were included in the count. The figure obtained divided by four gives the palisade ratio of that group. A range of a number of groups from different specimen was recorded.

2. **Stomatal Number**

Fragments of leaf from the middle of the lamina were cleared with chloral hydrate solution. Using a camera lucida and a stage micrometer, the paper was divided into squares of 1mm using a 16mm(x10) objective. The stage micrometer was then removed and replaced by the cleared preparation of the leaf sample. The number of

stomata was counted by indicating on the paper small crosses. The total number of stomata in square of 1mm x 1mm was determined.

3. **Stomatal Index**

Pieces of leaf other than the extreme margin or midrib were suitably cleared with chloral hydrate, mounted in glycerol and viewed using a 4mm (x40) objective. Counts were made of the number of epidermal cells and of stomata within the square grid. Successive adjacent fields were examined until and counted. The stomatal index was calculated as follows (Trease and Evans, 1989):

$$\text{Stomatal Index, I} = \frac{S \times 100}{E+S}$$

S = number of stomata per unit area.

E = number of ordinary epidermal cells in same unit area.

4. **Vein Islet Number**

Pieces of leaf were cleared with chloralhydrate solution and mounted in glycerol then viewed using a 16mm (x 10) objective. Using a camera lucida, the veins were traced in four contiguous squares in a square of 1mm x 1mm. The vein-islet number was counted excluding those that were incomplete or cut by the top and left hand sides of the square.

5. **Vein Termination Number**

Using the same method as for the vein-islet number, the number of vein-let termination per mm of the leaf surface was determined.

2.6. PHYTOCHEMICAL SCREENING OF POWDERED ROOT, STEM AND LEAF OF *W. INDICA*

2. 6.1. Test for Carbohydrates

Extraction

23 grams of the powdered samples were boiled separately in 50ml of distilled water on a hot plate for 3 minutes. Each mixture was filtered while hot and the resulting filtrate cooled and used for the following carbohydrate tests:

1. **Molisch's test - General test**

To 2ml of each of the water extract obtained from above in a test tube, was added few drops of Molisch's reagent, then a small quantity of conc. sulphuric acid was allowed to run down the side of the tube at an angle of 45^0 to form a lower layer. A purple to violet colour at the interface would indicate presence of carbohydrates. The mixture was then shaken gently and allowed to stand for two minutes and then diluted with 5ml water. A dull violet precipitate would further indicate presence of carbohydrates (Trease and Evans, 1989).

2. **Test for Sugars (Barfoed's test for monosaccharides)**

To 1ml of dilute solution of each sample in a test tube was added 1ml of Barfoed's reagent and the mixture heated in a water bath for 2 minutes. A red precipitate would indicate the presence of monosaccharides (Trease and Evans, 1989).

3. **Fehling's Test (Standard Test for Free Reducing Sugars).**

To 2ml of the water extract, 5ml of a mixture (1:1) of Fehling's solution I (A) and Fehling's solution II (B) was added and mixture boiled in a water bath for five minutes. A brick red precipitate would indicate the presence of free reducing sugars.

4. **Standard Test for Combined Reducing Sugars**

Boiling with 5ml of dilute hydrochloric acid hydrolyzed 1 ml of each of the water extract. This was neutralized with 10% dilute sodium hydroxide solution. The Fehling's test was repeated (as in above). A brick red colour would indicate the presence of combined reducing sugars (Trease and Evans, 1989).

5**.** **Salivanorff's Test (Standard test for ketoses)**

To a small volume of the water extract was added few crystals of resorcinol and an equal volume of concentrated hydrochloric acid, and then heated in a water bath. A rose colour would indicate the presence of ketoses e.g. fructose (Trease and Evans, 1989).

6**.** **Test for pentoses**

To a small portion of the water extract, was added an equal volume of dilute hydrochloric acid containing little phloroglucinol. The mixture was heated in a water bath. A red colour indicates the presence of pentoses e.g. xyloses (Trease and Evans, 1989).

2.6.2. **Test for Tannins**

Extraction

3g of each of the powdered samples were boiled in 50ml. of distilled water for 3 minutes on a hot plate. The mixture was filtered and the resulting filtrate was used to carry out the following tests for tannins:

1. **Ferric Chloride Test**

A portion of each of the water extracts was diluted with distilled water in a ratio of 1:4 and a few drops of 10% ferric chloride solution was added. A blue or green colour indicates the presence of tannins (Trease and Evans, 1989).

2. **Ferric ammonium citrate Test**

To 1ml. of each of the water extract was added 0.25% ferric ammonium citrate solution. To the mixture was added sufficient solid sodium acetate to adjust the solution to P^H7 using P^H indicator paper. The mixture was boiled in a water bath and filtered. A coloured precipitate would indicate the presence of tannins (Trease and Evans, 1989).

3. **Bromine Water Test**

A few drops of bromine was added to 1ml. of each of the water extract. A precipitate indicates the presence of condensed tannins.

4. **Formaldehyde Test**

To a small portion of each of the water extract was added a drop of formaldehyde solution and 3 drops of 10% hydrochloric acid. A precipitate indicates the presence of

hydrolysable tannins.

5. .**Test for Chlorogenic Acid**

To 1ml. of each of the water extracts was added two drops of 10% ammonia solution. The mixture was heated over a flame and then exposed to air. A green colour indicates the presence of chlorogenic acid (Trease and Evans, 1989).

6. **Lime water Test.**

To a small portion of each of the water extract was added 1ml of lime-water. A precipitate indicates the presence of pseudotannins.

7. **Goldbeater Skin Test**

A small piece of a membrane from intestine of an ox was soaked in 2% hydrochloric acid and then rinsed with distilled water. The membrane was soaked in the water extract for five minutes, washed with distilled water and then transferred to 1% solution of ferrous sulphate. A brown or black colour on the membrane denotes presence of tannins (Trease and Evans, 1996).

2.6.3 **Test for Glycosides**

1. **Test for Cyanogenetic Glycosides**

A small amount of each of the powdered roots, stem and leaves was taken in a test tube and distilled water was added just to cover the powder. A prepared moist sodium picrate paper was suspended at the neck of the test tube by means of a cork. The tube was placed in a water bath for one hour. A brick-red colour on the picrate paper indicates the presence of cyanogenetic glycosides (Trease and Evans, 1989).

2. **Test for Anthraquinone Derivatives**

i. **Borntrager's Test (for the presence of free anthraquinones).**

0.5g of powdered samples were taken in separate dry test tubes. To this, 10ml of chloroform was added, shaken and heated for about five minutes; the extract was allowed to cool and then filtered. To the filtrate was added equal volume of 10% ammonia solution and shaken. A bright pink colour in the upper aqueous layer indicates the presence of free anthraquinones (Sofowora, 1982).

ii. **Test for Presence of Free and/or Combined anthraquinones**

0.5g each of the powdered samples was boiled with 10ml. of 10% hydrochloric acid for 2 to 3 minutes. The extract was filtered while hot and allowed to cool. To the filtrate, an equal volume of chloroform was added. The tube was inverted a couple of times; taken care to avoid vigorous shaking. The solution was transferred to a seperatory funnel and the two layers allowed to separate. The lower chloroform layer was drained into a clean test tube and 10% ammonia solution added and shaken. The two layers were again allowed to separate. A bright pink colour in the upper aqueous layer indicates the presence of free and/or combined anthraquinone glycosides (Trease and Evans, 1989).

c. **Test for Saponin Glycosides**

i. **Froth Test**

To a small quantity of each of the powdered samples was added 95% ethanol, and boiled. The mixture was filtered and about 2.5ml of the

filtrate was added to 10ml. of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 seconds then it was allowed to stand for over half an hour. A honey- comb froth is indicative of the presence of saponins (Sofowora, 1982)

ii. **Haemolytic Test for Saponins (Steroidal and Triterpenoidal)**

0.2g of the powdered material was extracted with 10ml warm water, filtered and the filtrate retained. 2ml of 1.8% sodium chloride solution was added to four test tubes (t, t1, t2 and t3). To one of the test tubes (t), 2ml of distilled water was added and to the others (t1, t2 and t3), 2ml of each of the extracts was added. The concentration of sodium chloride in each case is now isotonic with blood- serum. Blood was obtained by pricking the thumb at the base of nail and blood was drawn into a small pipette. Five drops of the blood was added to each of the test tubes (t, t1 – t3). The tubes were stopped and inverted gently to mix the contents. Haemolysis in test tubes containing extracts (t1- t3) and not in the control (t) would indicate presence of saponins (Brain and Turner, 1975).

d. **Test for Cardiac Glycosides**

i. Extraction

0.5g of each of the powdered samples were boiled with 10ml of 95 alcohol for 2 min .The resulting mixture was filtered and cooled .The filtrate was diluted with water and 3 drops of a strong solution of lead sub–acetate was added. This was mixed thoroughly and filtered. The

filtrate was divided into two portions. One portion of the filtrate was kept for the test (iii) described below. The other portion of the filtrate was extracted with 5ml chloroform in a separating funnel. The lower chloroform layer was divided into two small evaporating dishes and evaporated to dryness.

ii . **Keller - Killiani Test for 2-Deoxy sugars**

One of the chloroform residues from (d i) above was dissolved in 1ml of glacial acetic acid containing a trace of ferric chloride solution. This solution was carefully poured on the surface of 1ml sulphuric acid already contained in a test tube to form a separate layer. A reddish- brown colour at the interfaces of the liquids would indicate the presence of 2-deoxy sugars (Trease and Evans, 1989).

iii. **Keddes Test (for free or combined cardenolide aglycones).**

The reserved filtrate from d i above) was treated with 1ml of 2 % solution of 3, 5-dinitrobenzoic acid in 95% alcohol. The solution was made alkaline with 5% sodium hydroxide. A purple- blue colour is an indication of the presence of free of combined cardenolide aglycone. (Sofowora, 1982)

iv**. Legal Test (for Cardenolide aglycones).**

The second reserved residue (from d i) was dissolved in a few drops of pyridine and a few drops of 2% sodium nitroprusside was added then , a drop of 20% sodium hydroxide was added . A deep red

colour indicates the presence of a cardenolide aglycone (Sofowora, 1982).

2.6.4. **Test for Sterols and Terpenes**

Extraction

5g of each of the powered samples were extracted by maceration with 50ml of 95% alcohol. The extract was filtered and the filtrate was evaporated to dryness. The residue was dissolved in 10ml of chloroform (anhydrous) and then re-filtered. The filtrate was divided into two equal portions that were used for the following tests:

i. **Salkowski's Test (for steroidal ring).**

The first portion of the chloroform solution (from above) was taken in a test tube and 2ml of concentrated sulphuric acid was added carefully to form a lower layer. A reddish brown ring at the interface indicates the presence of a steroidal ring (Sofowora, 1982).

ii. **Lieberman-Burchard's Test (for Terpenes)**

To the second portion of the filtrate from above was added about 1ml of acetic anhydride followed by 1ml. of conc. sulphuric acid down the side of the test tube to form a layer underneath. The formation of a reddish violet colour at the interface of the two liquids and a green colour in the chloroform layer indicates presence of terpenes.

2.6.5. **<u>Test for Flavonoids</u>**

Extraction

5g of the powdered roots were completely detanned with acetone and the acetone was then decanted. The remaining acetone was completely evaporated on a water bath. The residue was mixed in warm water and mixture was filtered while hot then allowed to cool. The filtrate was used for the following tests: (Brain and Turner, 1975).

I. **Lead Acetate test**

To 5ml of the detanned water extract, was added 2-3 drops of lead acetate solution. A buff coloured precipitate would indicate the presence of flavonoids. (Trease and Evans, 1989).

2. **Sodium Hydroxide test**

5ml of the detanned water extract was taken in a test tube and an equal volume of 10% sodium hydroxide was then added. A yellow solution indicates the presence of flavonoids. On addition of dilute hydrochloric acid, the solution would become colourless.

3. **Ferric Chloride Test**

2ml of the detanned water extract was diluted with distilled water in a ratio of 1:4 and few drops of 10% ferric chloride solution was added. A green or blue colour would indicate the presence of phenolic nucleus (Brain and Turner, 1975).

4. **Shinoda's Test**

0.5g of each of the powdered samples were extracted by boiling in ethanol in a test tube on a water bath for 5 minutes, filtered and cooled. Four pieces of magnesium fillings were added to the filtrate followed by

few drops of concentrated hydrochloric acid. A pink or red colour indicates the presence of flavonoids (Geissman, 1962).

5. **Amyl Alcohol Tests**

3g of each of the powdered samples were macerated in 50ml.of 1% hydrochloric acid and filtered.

I. 4ml. of the filtrate was shaken with 5ml. of amyl alcohol and the colour produced was observed. Production of a yellow colour indicates the presence of free flavonoid aglycones.

ii. 10ml. of the filtrate was shaken with about 7ml. of amyl alcohol and the mixture transferred to a separating funnel. The amyl alcohol layer was discarded and the aqueous layer boiled with 10ml. of 10% hydrochloric acid for 2 minutes. The acidic solution was cooled and divided into two portions.

The first portion was shaken with amyl alcohol. Production of a yellow colour indicates the presence of combined flavonoids.

To the second portion magnesium fillings were added and the colour of the solution observed (Abdurahman, 1986; Hilal, 1976). Production of a red colour indicates the presence of flavanone and flavonol glycosides.

2.6.6. **Tests for Resins and Balsams.**

1 **Tests for Resins.**

i. 15ml of petroleum ether extract was made from 0.1g, of each of the powdered samples (roots, stem, leaves) and filtered into a test tube.

An equal volume of copper acetate solution was added and shaken vigorously then allowed to separate. A green colour indicates the presence of resins.

ii. 0.5g.of each of the powdered samples was dissolved in acetic anhydride and 1 drop of concentrated sulphuric acid was added. A purple or violet colour indicates the presence of resins.

2. **Test for Oleo-Gum-Resins.**

0.1g of each of the powdered samples was triturated in 0.5g of sand. The mixture was transferred into a test tube containing 3ml.of ether and shaken. This was filtered into an evaporating dish and the filtrate allowed to evaporate to a thin film. In the fume cupboard bromine vapour was passed over the residue. A violet colour indicates the presence of oleo - gum- resins.

3. **Test for Balsams.**

2 drops of alcoholic ferric chloride solution was added to 5ml. of 90% ethanol extract of each of the powdered samples, (Roots, stem, leaves). A dark green colour indicates the presence of balsams.

2..6.7 **Tests For Alkaloids**

Extraction (General Method).

10g.of each of the powdered samples was taken in a small beaker and a strong solution of ammonia solution was added in a quantity sufficient to just moisten it and allowed to stand for 10 minutes after thorough mixing of the contents.

Sufficient quantity of a mixture of chloroform and ethanol solution (1:1) was added just to soak and suspend the powder. The mixture was allowed to stand for 20 minutes with occasional stirring with a rod. The mixture was filtered through a plug of cotton wool. The marc was washed twice with 2ml. of chloroform and the washings were combined with the filtrate. The bulk of the filtrate was concentrated to dryness without overheating. The residue was cooled and dissolved in 5ml. of chloroform only. The chloroform solution was transferred to a small separating funnel and shaken with 3ml. of dilute sulphuric acid. The two layers were allowed to separate and the chloroform layer drained off and discarded. 3ml. of chloroform was further added and shaken, drained off and discarded until the upper acid layer was colourless. The acid layer was made completely alkaline with strong ammonia solution (tested with indicator paper). The extracts with 3ml. of chloroform were retained and evaporated to dryness (Brain and Turner, 1975). The residue was dissolved in 3ml. of ethanol and the following tests were carried out after neutralizing with dilute sulphuric acid (Trease and Evans, 1989):

i. **General Tests for alkaloids**

A set of three test tubes containing the various samples were taken and to each, a little ethanolic solution (0.5ml.) obtained above, was added 2-3 drops of the following reagents:

Mayer's reagent

Wagner's reagent

Dragendorff's reagent

Hager's reagent

10% Tannic acid solution.

The presence of precipitate in at least three or all of the above reagents indicates the presence of alkaloids (Trease and Evans, 1989).

2.7. QUANTITATIVE EVALUATION OF CRUDE VEGETABLE DRUGS (POWDERED ROOT, STEM AND LEAF)

2.7.1. Determination of Moisture Content by the " Loss on Drying Method"

An evaporating dish was heated to a constant weight in an oven at 105^{oc} and its constant weight noted. 3g of the powdered leaf sample was accurately weighed into the dish. The evaporating dish with its content was put in the oven at 105^{oc} and the content dried at 10 minutes intervals after an initial drying of one hour to a constant weight. Two consecutive similar weights confirm a constant weight. The total loss in weight (the weight of the moisture) was determined by subtracting the constant weight of the dish and powdered sample after heating from the weight of the dish and content before heating. The percentage of the initial weight of the powdered sample used was then calculated. This was done by dividing the weight of moisture by the weight of the drug taken multiplied by a hundred. Three different determinations were done and the average of these taken as the moisture content of the drug (Trease and Evans, 1989). This was carried out for each of the different powdered samples.

The calculation below was used:

$$\frac{C - a}{3} \times 100$$

C= weight of crucible and sample and a is weight of crucible

2.7.2. Determination Of Ash Value

a. Determination of Total Ash

A nickel crucible was heated at 105^{oc} to a constant weight and its accurate weight noted after cooling in the desiccator. 2g of the powdered sample was accurately weighed into the crucible. The crucible and its content were then gently heated over a bunsen flame until it was moisture free and completely charred. The Bunsen flame was slightly increased until most of the carbon had volatilized and the inorganic ash could be seen. The ash was cooled, weighed and the weight noted. The heating and cooling was continued until the weight of the ash was constant. The weight of the ash was calculated by subtracting the weight of the empty crucible from the final weight of the crucible and ash residue (B.P, 1980.). This was done to each of the different samples thus.

b – a = weight of residue, a = weight of empty nickel crucible,

b = weight of crucible and residue.

The ash value (in percentage) of the samples was calculated with reference to the initial weight of the powdered samples. Four determinations were done for each sample and the mean of this was used for each calculation.

Ash value = $\underline{(b – a)\ 100}$

3

b. **Determination of Acid Insoluble Ash Value**

The crucible with the ash from the above experiment (a) was transferred into a beaker containing 2ml. of 10% hydrochloric acid. The beaker and its content were boiled for about 5 minutes and filtered through an ash less filter paper. The crucible and the beaker were washed with distilled water and the washing repeated three times, with the washings being passed through the filter paper each time in a manner that would allow the collection of the residue at the tip of the cone of the filter paper. The funnel along with the filter paper was dried in the oven at 105^{oc}. The weight of a clean and heated nickel crucible was accurately determined using a sensitive balance. The filter paper with its residue was folded into a small cone and transferred into a crucible. The crucible was gently heated until the filter paper was completely ashed, then heated strongly for some few minutes. The crucible and its content were cooled, weighed and the final weight was noted. The weight of the residue (ash) was then calculated by subtracting the constant weight of the crucible from the constant weight of the crucible and its content (ash). Also, the acid insoluble ash value with reference to the initial weight of the powdered sample was calculated and expressed in percentage. The weight of ash divided by the initial weight of the drug multiplied by hundred was taken as the

acid insoluble ash value (B.P., 1980). The value was determined for each of the different samples separately.

Weight of ash = b – a,

Acid- insoluble ash value = $\frac{(b - a)\ 100}{2}$

a = weight of crucible, b = weight of crucible and residue.

2.7.3. **Determination of Extractive Values.**

a. **Alcohol - soluble Extractive Value**

5g of each of the powdered samples were accurately weighed into a 250 ml stopped conical flask. 100ml of 90% ethanol was added and the stopper was replaced firmly. The flask was shaken on a mechanical shaker for six hours and then allowed to stand for eighteen hours. The extract was filtered by suction filtration. The weight of a heated, cooled flat bottom-evaporating dish was accurately determined. 20ml of the filtrate was taken into the weighed evaporating dish. The filtrate was dried to a constant weight at 105oc in an oven and the final weight was noted. The weight of the residue obtained from 20ml extract was determined by subtracting the weight of the evaporating dish from the total weight of evaporating dish and the residue. The alcohol extractive value was then calculated with reference to the initial weight of the powdered drug and expressed in percentage (Brain and Turner, 1975).

Alcohol extractive value = $\frac{c - b \times 100}{3}$

a = weight of powder, b = weight of evaporating dish,

c = weight of evaporating dish and residue.

b. **Water – Soluble Extractive Value.**

The method (a) above was repeated using 0.25% chloroform water instead of 90% ethanol.

2.8. BIOLOGICAL EVALUATION

2.8.1 Drying and Extraction of Plant Material

The different parts of the plant namely root, stem and leaves, were removed from the whole plant and air-dried separately for about 7 days. The different parts of the plant were dried under controlled condition in order to prevent chemical changes or degradation occurring due to enzymatic and microbial activity during storage. The dried parts were coarsely powdered by pulverizing with a mortar and pestle separately. About 50g of each powdered material were boiled with 500ml distilled water for 30 minutes. The liquid obtained by filtration using a filter paper, was then evaporated to dryness on a water bath to yield a solid residue (extract). Appropriate concentrations of the extracts were made in distilled water when required for use in the experiments (analgesic activity and acute toxicity studies). This method of extraction was adopted to mimi traditional medicine practice (Heinrich *et al*, 1992; Dalziel *et al*, 1954; Chhabra *et al*, 1993).

2.8.2 Experimental Animals

Swiss albino mice weighing 18- 25g of either sex were obtained from the animal house, Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria. Animals were maintained in well-ventilated area and fed on standard feeds (Growers mash, Excel Feeds Plc. Kaduna) and water *ad libitum.*

2.9 ACUTE TOXICITY STUDIES

The LD_{50} was determined using the method of Lorke (1983). In the initial phase, mice were divided into 9 groups of three mice each. Each group was treated with the aqueous extracts of the leaves, stem and root at doses of 10, 100 and 1000mg/kg intraperitoneally (i.p) and observed for signs and symptoms of toxicity for 24 hours. In the final phase, mice were divided into 12 groups of 1 mouse each and the various extracts administered at specific doses ranging from 20 – 800 mg/kg body weight i.p and the LD_{50} calculated using the geometric mean of doses for 0/1 and1/1.

2.10 ANALGESIC ACTIVITY STUDIES

2.10.1 Acetic Acid-Induced Writhing Method

The method of Koster *et al* (1959) was used to determine the analgesic activity of the extracts. Swiss albino mice of either sex were divided into 5 groups of 6 mice each and each animal placed in individual cage. The first group served as the control (distilled water 0.2mls), group 2 received extracts at doses of 10 and 20mg/kg, group 3 received 25 and 50mg/kg and group 4 were given 50 and 100mg/kg body weight intraperitoneally (i.p). Group 5 was administered aspirin, the reference drug, i.p at a dose of 150mg/kg body weight. 30 minutes later, when all the animals had been treated with the extracts and aspirin respectively, the animals in the groups were then treated

with the acetic acid (0.6% v/v) solution in water at dose of 10ml/kg. The numbers of writhing or abdominal contractions that occurred were counted for 15 minutes. The percentage inhibition of writhing was obtained (Koster *et al,* 1959):

Inhibition % =:

$$\frac{\text{Mean no. of writhing in control} - \text{Mean no of writhing in treated animals X100}}{\text{Mean no. of writhing in control}}$$

2.11 PURGATIVE ACTIVITY STUDIES

2.11.1 Preparation of extracts and Animal Studies

The methods of Elujoba *et al* (1999) and Akunyili (1992) were used for the study. Hot infusions were prepared by adding 50ml of boiled, distilled water to 5g of each powdered plant part (leaf, stem and root). The resultant mixture was filtered, cooled and made up to a stock solution of 100mg/ml. Concentrations required were then prepared from the stock solution with distilled water. The animals, chosen randomly, were kept in individual cages lined with white blotting papers for five days, during which time they were fed with standard food pellets and water *ad libitum* until 6 hours before the experiment. Any mice producing wet feaces at this stage were eliminated from the experiment (Akunyili, 1992). Appropriate doses of 10, 20 30 and 50mg/kg body weight of the leaf, stem and root extracts, were administered orally in duplicate to the mice that were divided into 5 groups of eight animals each. Groups represented control (5ml distilled water), leaf, stem, root extracts and reference drug, senokot tablet of commerce respectively. The feaces (dry and wet) produced in 12 hrs by each extract, were examined hourly for wetness using the ‘wire-adhesion’ method. Thereafter, the production of wet feaces by

the mice was used to evaluate the purgative activity of *W. indica* in this study. The purgative activity was assessed by reference to the percentage wet-feaces against the total feaces and then with reference to senokot tablets. The percentage wet-faeces as a function of the dose as well as the percentage senna- activity as an index of comparison was calculated from the results (Elujoba *et al,* 1988). The onset and duration of purgation of the extracts was also recorded. The reference drug, senokot, was manufactured by Reckitt and Colman, Hull, United Kingdom.

2.12 STATISTICAL ANALYSIS

The results of the studies were expressed as Mean ± S.E.M, standard error of mean. Statistical analysis was carried out using the Students' t-test with level of probability (P) calculated versus the control group. $P<0.05$ implies significance

3.0 **RESULTS AND DISCUSSIONS**

3.1. RESULTS OF MACROSCOPICAL EXAMINATION OF THE LEAF, STEM AND ROOT OF *WALTHERIA INDICA*

3.1.1. Leaf:

The following features observed for the leaves are described below:

Arrangement - alternate

Petiole - petiolate (1.2-2.1-3.0cm length)

Dimension of leaves - length: 2.8- 5.6- 8.5cm

Width: 1.2- 3.2 – 5.2cm

Lamina:

I. Composition - simple
II. Incision - none
III. Shape - ovate
IV. Venation - reticulate
V. Margin - serrate to crenate
VI. Apex - round to acute
VII. Leaf base - rounded to subcordate
VIII. Surface - upper surface: spongy and pubescent, light green
- Lower surface: spongy but less pubescent, dark green in colour.

Organoleptic characters:

Texture: soft and hairy
Odour: characteristic
Taste: slightly bitter.
Colour: green

3.1.2 **Stem**

The following features were observed:

Size- length: 4.5- 8.0 – 14.2cm

Width: 1.1- 2.2cm

Fracture- splintery

Fractured surface- rough

Surface- outer surface: brown

Inner surface: cream

Organoleptic characters:

Colour- dark brown

Taste- slightly bitter

Odour- characteristic

3.1.3. **Root**

The following features were observed:

Size- length: 10.5- 16.5- 25.5cm

Thickness: 0.1- 0.54- 0.9cm

Shape- curved

Surface- inner surface: cream, outer surface: brown, no striations.

Fracture- splintery

Fractured surface- rough

Organoleptic characters-

Colour: brown

Odour: characteristic

Taste: slightly bitter

3.2. RESULTS OF MICROSCOPICAL AND CHEMOMICROSCOPICAL EXAMINATIONS OF THE LEAF, STEM AND ROOT OF *WALTHERIA INDICA*

3.2.1 Microscopical Examinations

a. Leaves:

The microscopical features of the powdered leaves observed were as follows: Polygonal epidermal cells with projecting unicellular and multicellular stellate trichomes, anisocytic stomata, prismatic calcium oxalate crystals, reticulate xylem vessels and phloem fibres. The details shown in figs.4a to 4d are described as follows:

Stomata:

Type - anisocytic

Size - 100- 115μm

Frequency - few

Calcium oxalate crystals:

Type (a) - prism

Size - 55 - 100μm

Frequency - few

Type (b) - clusters

Size - 15μm

Frequency - numerous

Trichome:

Types - multicellular stellate covering trichomes

- unicellular covering trichomes

Size - 250-425μm

Frequency - numerous

Xylem vessels:

Type - reticulate

Size - 30μm

Frequency - few

Phloem fibres:

Size - 30-45μm

Frequency - few

Apex -tapering

Secretory cells:

Type - mucilaginous

Shape- roundish

Size - 10μ

Frequency - very few

The various transverse sections of the lamina and midrib (fig.4c and 4d.) revealed the exact positions and arrangement of the different tissues found in the leaf of *W. indica*. The leaf was observed to be dorsiventral. This leaf had its palisade cells below the upper epidermis only. This is in conformity with the report of Watson and Dallwitz (1999) that the Sterculiaceae family has leaves that are dorsiventral.

The upper epidermis of *W. indica* consisted of a row of epidermal cells with fairly large polygonal cells that were thickened. They were compactly arranged with a layer of cuticle on top of it. Also, some

secretory cells or cavities were observed. They probably contain mucilages since this is a characteristic feature of the family Sterculiaceae (Burkill, 2000, Watson and Dalliwitz, 1999, Hedberg *et al,* 1983). Numerous calcium oxalate crystals and clusters (Fig.4a) were observed in the mesophyll. Anisocytic stomata were also present on both epidermal layers, with more on the lower layer. Projecting stellate covering trichomes, comprising of the unicellular and multicellular types, were found to be abundantly present on both epidermal layers.

The transverse section of the leaf through the midrib and lamina (Fig.4c and 4d) showed a clear differentiation of a cuticular upper epidermal layer followed by a layer of perpendicularly arranged palisade cells. This was followed by the spongy mesophyll consisting of irregular shaped cells and some secretory cells, roundish in shape. The parenchyma cells were made up of 3-4 rows, with intercellular spaces. The vascular bundles comprising phloem and xylem were also observed on the lower epidermal layer collenchymas cells and stomatal opening, were also observed.

The powdered leaf sample revealed the fragments of leaf lamina with anisocytic stomata and abundance of prisms of calcium oxalate, reticulate xylem vessels and phloem fibres.

Fig 4. Upper and lower surfaces of the leaf of *Waltheria indica*

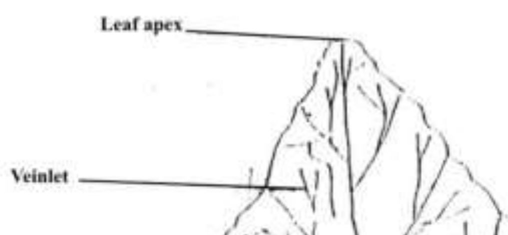
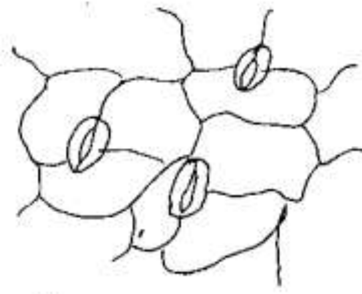
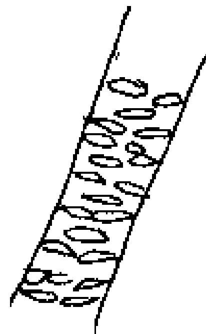


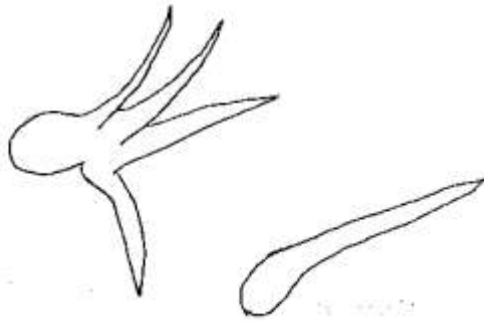
Fig. 4a: **Microscopical Features of the Powdered Leaf of *Waltheria indica***



Anisocytic stomata



Reticulate xylem vessel



Stellate trichomes

Fig. 4a. Continue

Fig 4b. Longitudinal section of the Leaf of *Waltheria indica*

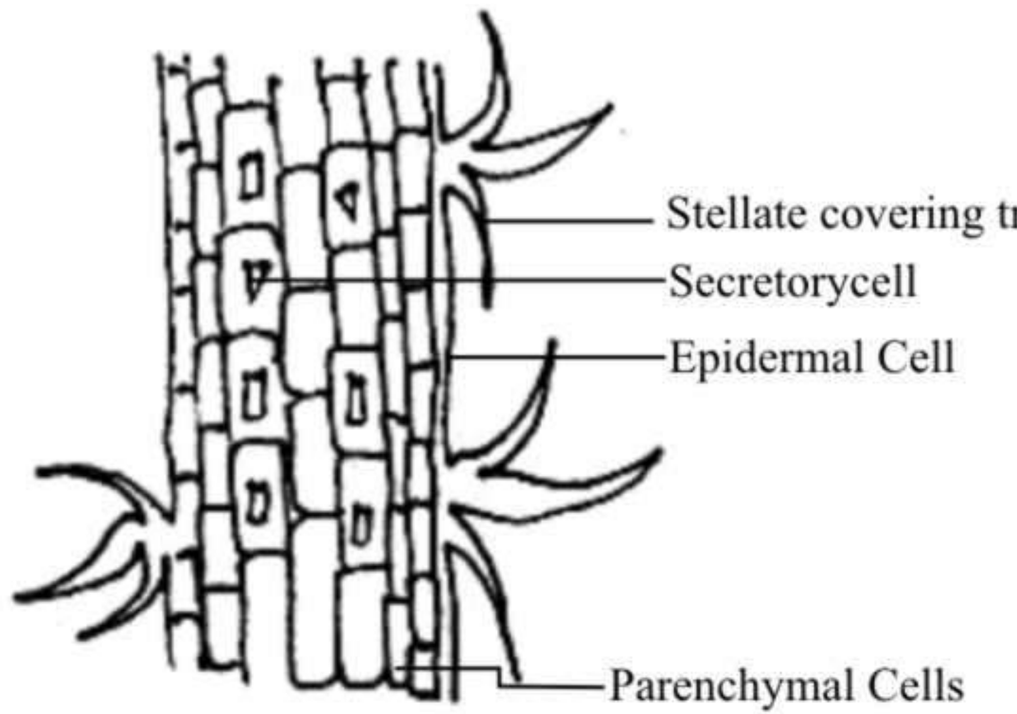
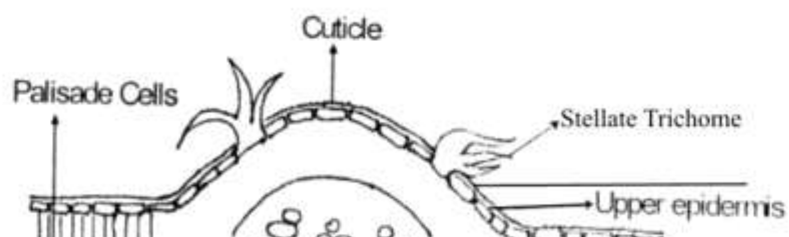


Fig. 4c. Transverse section of leaf of *Waltheria indica* showing midrib and lamina



The most important distinguishing features which can be attributed to the leaves and other samples of *W. indica was* the presence of numerous stellate unicellular and multicellular covering trichomes in groups of 4-5 that were

found in abundance in all parts of the plant. The stiff arms of the trichomes all tend to point in one direction in most cases, with the arms bent at an angle. Stellate hairs are characteristic of the family Sterculiaceae (Watson and Dallwitz, 1999). Some secretory cavities that could contain mucillages were observed.

Anisocytic stomata was observed in the leaf sample, but the anomocytic and paracytic types are usually present in the Sterculiaceae (Watson and Dallwitz, 1999).

The results of the quantitative leaf microscopy of *W. indica* are given in Table 1.The results were obtained from the mean of 4 readings. The values obtained for the quantitative microscopical evaluation could serve as a diagnostic and distinguishing features of the plant, *W. indica.*

Table 1. **Results of Quantitative Leaf Microscopy**

Parameters	Values	
	Range	Mean
Palisade ratio	3.75 – 4.75	4.25
Stomatal number (upper epidermis).	5 – 7	6.0
Stomatal number (lower epidermis.)	2.2 – 3.0	2.6
Vein – 1slet number	8.75 – 10.5	9.62
Vein termination number	6.75 – 22.0	14.37
Stomatal index (upper epidermis)	3.6 – 4.8	4.2%
Stomatal index. (lower epidermis)	12.5 – 13.9	13.2%

b. **Stem**

The microscopical features of the powdered stem were as follows:

Calcium oxalate crystals, stellate, unicellular and multicellular covering trichomes, lignified parenchyma cells, simple starch grains without a hilum or maltese cross, cork cells and phloem fibres. The various microscopical features of the powdered stem are illustrated in figs.5a, 5b and 5c and can be summarized as shown below:

Calcium oxalate

Type-prism

Size - 45-70µm

Frequency - frequent

Trichomes-

Type- unicellular covering trichomes

- multicellular covering trichomes

Size- 160-220µm

Frequency- numerous

Starch grains:

Shape- oval

Size- 20-35µm

Frequency- few

Maltese cross - absent

Cork cells:

Shape- polygonal

Anticlinal walls- straight

Thickening- present

Phloem fibres:

Size- 16-24μm

Frequency- very few

Apex- tapering

Xylem vessels:

Type - reticulate, thickened

Size - length, 80-150μ

Width, 40-80μ

Frequency - few

The various features observed in the powdered stem were typical of powdered samples containing the bark and was very much similar to the features observed in the root sample, except for differences in the frequency or size.

The transverse section (fig.5c.) was seen to consist of an outer epidermal cells. This was composed of polygonal cells of a single layer. An inner cortex consisting of the collenchyma and parenchyma cells and an inner endodermis followed it. The vascular bundles are arranged in a ring and are composed of the xylem and phloem. The pith occupies the major portion of the stem and extends from below the vascular bundles to the center. It has conspicuous intercellular spaces that appeared to be bigger than that of the root.

Calcium oxalate crystals in the parenchyma cells and stellate covering trichomes on the epidermis were observed in the longitudinal section.

Fig.5a Microscopical features of the powdered stem of *Waltheria indica*

Fig. 5b: **Longitudinal section of the stem of *Waltheria indica***

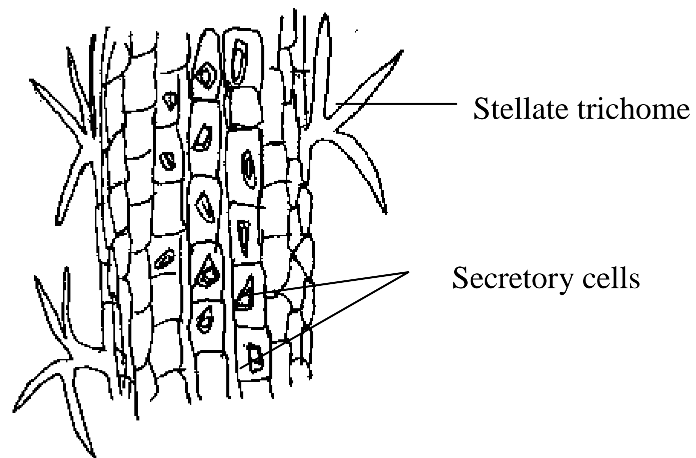
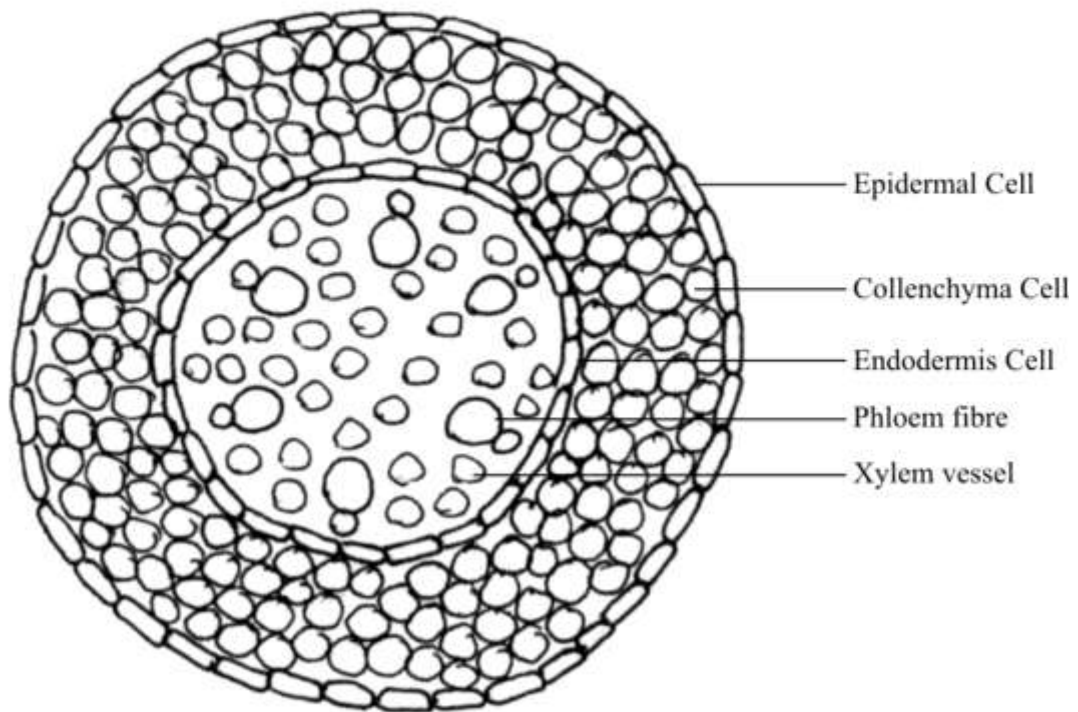


Fig. 5c: **Transverse section of the stem of *Waltheria indica***


Epidermal Cell
Collenchyma Cell
Endodermis Cell
Phloem fibre
Xylem vessel

C. Root:

The microscopical examinations of the powdered root revealed the following features: stellate unicellular trichomes, phloem fibres, calcium oxalate crystals, cork cells, lignified parenchyma and starch grains both simple and compound without a hilum and they can be described as follows and their microscopical features are presented in figs. 6a, 6b and 6c:

Cork cells:

Shape - polygonal

Thickening - present

Anticlinal walls - straight

Phloem fibres:

Size- 35 – 50μm

Frequency - moderate

Apex - tapering

Trichomes:

Type – unicellular and multicellular

Size – 450 - 700μm

Frequency - numerous

Starch grains:

Shape - oval

Size - 23 - 56μm

Hilum - absent

Aggregation - simple and compound, in chains

Frequency - moderate

Maltese cross - absent

Calcium oxalate crystals:

Type - prism

Size – 105 - 160μm

Frequency - numerous

The transverse section of the stem and root revealed the arrangements and features of the different tissues seen in the powdered samples. Other features normally seen in sections of samples containing the bark, such as cambium and pericyclic fibres, were however not observed.

The section showed a single layer of an outer epidermal layer without a cuticle. This was followed by a cortex of irregular sizes of parenchyma cells with air spaces.

The endodermis consists of a single layer of closely packed cells without intercellular spaces. The protoxylem was contained in this layer.

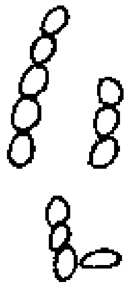
The vascular bundles, consisting of the phloem and xylem, are arranged radially. The last layer was the pith, which was much smaller than that of the stem. It consisted of circular parenchyma cells with air spaces in between.

The longitudinal section revealed starch grains and calcium oxalate crystals in the parenchymatous cells. Also, the plant is obviously composed of simple tissues, which is the parenchyma. It is of universal

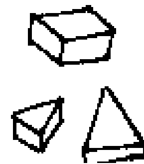
occurrence only in soft parts of plants (Dutta, 1970). Sterculiaceae family is composed of soft wood and there is absence of medullary bundles (Watson and Dalliwitz, 1999).

There are no specific characteristic microscopical features that can be attributed to the leave, stem and root samples except the numerous stellate unicellular covering trichomes, found in all parts of the plant. They had some of their projecting arms pointing towards the same direction in some cases. There was also presence of simple starch grains in the leaves and stem and both simple and compound in chains in the root. Starch grains lacked hilum or maltese cross. The secretory cavities observed could contain mucillages. The various features observed could aid in the identification of the different parts of the plant studied, which also have great reputation in the treatment of various diseases and ailments by traditional medicine practitioners.

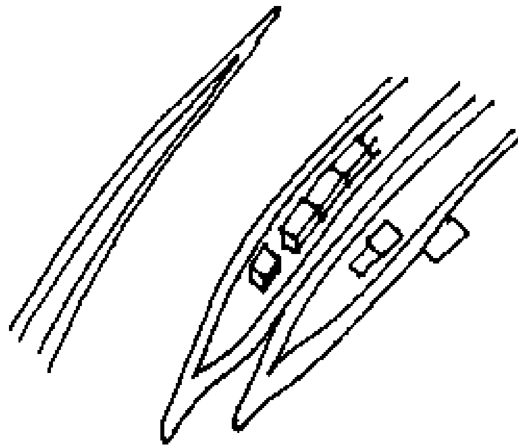
Fig 6a. Microscopical features of the powdered root of *Waltheria indica*



Groups of starch grains in chains



Calcium oxalate prisms



Groups of phloem fibres

Fig. 6b: **Transverse section of the Root of *Waltheria indica***

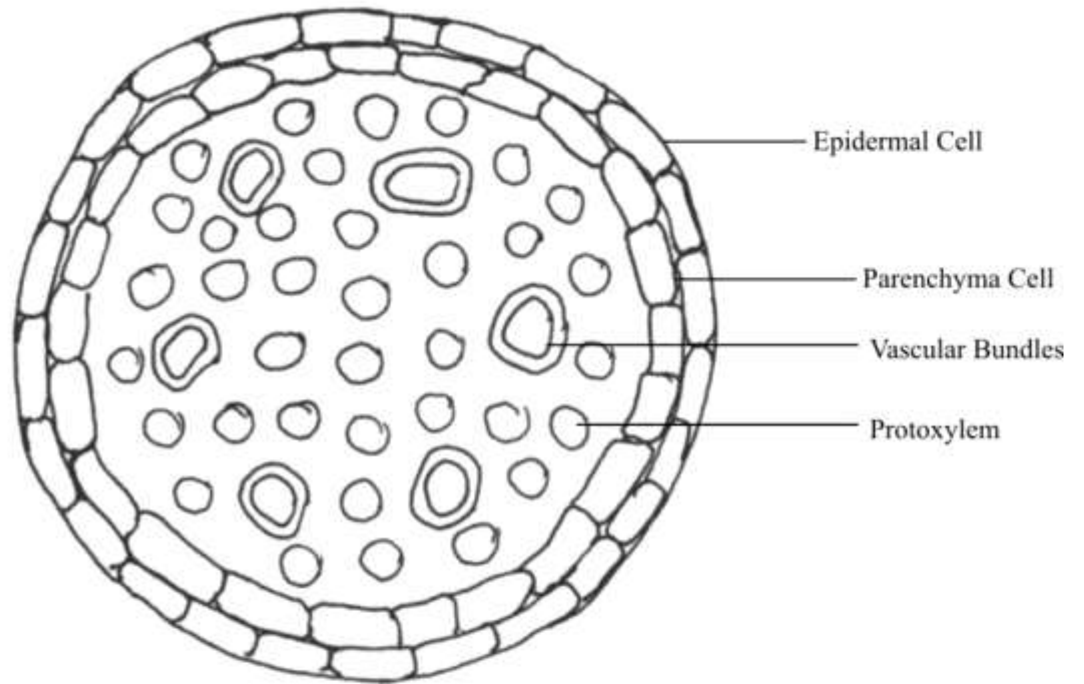
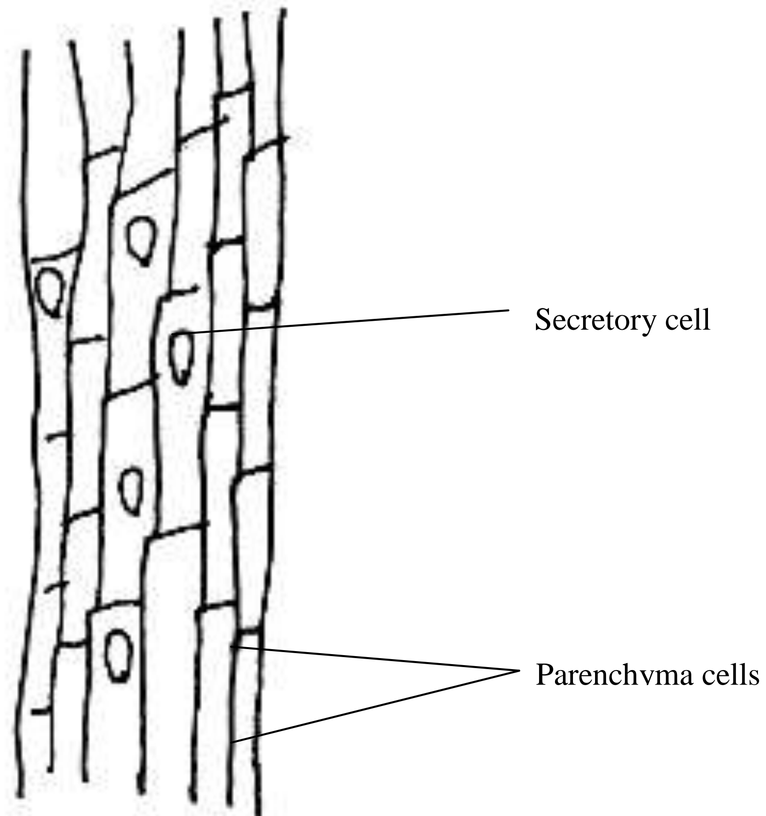


Fig 6c:. **Longitudinal section of the Root of *Waltheria indica* (x100).**



3.2.2. RESULTS OF CHEMO--MICROSCOPICAL EXAMINATIONS

1. Test For Tannins

Some cells like the cork cells, showed the presence of brown or yellowish – brown contents, which might indicate the presence of tannins. This was further confirmed by the presence of greenish black or green colour when mounted in ferric chloride solution. Both anatomical and powdered samples of the plant parts were used. Concentration of colour was in the cork of the stem and roots. This is indicative of the presence of condensed tannins in the plant (Trease and Evans, 1989).

2. Test for Starch

Blue-black coloured grains were observed with N/50 iodine when the different samples were mounted in it, indicating presence of starch (Trease and Evans, 1989). Very few starch grains were observed in the stem and leaves. Moderate amount occurred in the root. The occurrence was mainly in the parenchyma cells.

3. Test for Mucilages

The cell walls of the stem, leaves and root gave positive results (a red to pink colour) when the different samples were mounted in Ruthenium red, thus indicating the presence of mucillages in the cell walls of the plant (Trease and Evans, 1989).

4. Test for Cellulose

Cellulose was found to be present as blue to black colour was observed with chlor-zinc-iodine or N/50 followed by 66% sulphuric

acid (Trease and Evans, 1989). The presence of cellulose was indicated in the parenchyma and epidermal cells.

5. Test for Calcium Oxalate Crystals

The samples were separately cleared in chloral hydrate solution. Any calcium oxalate crystals present were seen as bright structures of definite shapes and sizes. They were mostly prismatic in shape. On addition of a few drops of conc. sulphuric acid (80%), followed by observation under the microscope, the disappearance of the bright structures (calcium oxalate crystals, confirmed their presence (Trease and Evans, 1989). The structures were observed in all the samples.

6. Test for Fats and Oils

The presences of these were negative as indicated by the absence of a red stain with Sudan IV. This was observed in all samples of the plant.

7. Test for Lignin

The powdered samples and anatomical sections of the leaves, stem and roots were mounted in phloroglucinol and conc. hydrochloric acid. A red colour was observed in certain parts of the stem and roots like the fibres, xylem vessels and some parenchyma cells indicating the presence of lignin. Xylem vessels and some parenchyma cells were found to be highly lignified. The intensity of the red colour is an indication of the extent of lignification (Trease and Evans, 1989)

8. Test for Cutin and Suberin

The cork cells in the root and stem tested positive to suberin indicated by a red colouration with tincture of alkanna and the cell wall of the leaves were stained red which is an indication of the presence of cutin.

9. Test for Proteins

When the different samples were stained separately with picric acid and Millon's reagent, a red stain obtained with Millon's reagent and yellow stains with picric acid is indicative of the presence of protein. This was observed in the leaves only, but it was absent in the stem and root of the plant. Available literature reveals the amount of protein in the leaves of *W. indica* in the Mozambique valley during the rainy season to be 6.4% (Faftine *et al,* 2001).

The summary of results of the chemomicroscopical examination is given in Table 2 below:

Table 2: Summary of Results of the Chemomicroscopical Examination of the Leaf, Stem and Root of *W. indica*

Constituents	**Leaf**	**Stem**	**Root**
Starch	+	+	+
Protein	+	-	-
Cellulose	+	+	+

Mucilage	+	+	+
Calcium oxalate crystals	+	+	+
Fats and oils	-	-	-
Tannins	+	+	+
Lignin	-	+	+
Cutin	+	-	-
Suberin	-	+	+

Key: (+) – Present, (-) – Absent

3.3 RESULTS OF PHYTOCHEMICAL SCREENING OF LEAF, STEM AND ROOT POWDERS OF *WALTHERIA INDICA*

The results obtained from the various phytochemical tests are described below:

3.3.1. Test for Carbohydrates

Extraction

Most carbohydrates are wholly or partially soluble in water. The water extract was therefore used for conducting the various tests for the different classes of the carbohydrates (monosaccharides, polysaccharides and oligosaccharides).

1. Molisch's Test

It is a general test for carbohydrates. A purple ring at the interphase of the liquids (the reagent and water extract of samples) and a dull violet precipitate when the mixture of the liquids was shaken together indicated presence of carbohydrates. The leaves, stem and roots gave positive results

for carbohydrates; carbohydrates are normally present in all plant parts.

2. Test for Sugars

The presence of sugars (a brick red precipitate) with Barfoed's reagent was obtained with the leaf sample of the plant. Thus the presence of monosaccharides is indicated in this part of the plant.

3. Fehling's Test for free Reducing sugars

A brick red precipitate was observed for all parts of the plant indicating the presence of free reducing sugars. The presence of oligosaccharides in the stem and roots is indicated, as the monosaccharide tests were negative.

4. Fehling's Test for combined Reducing sugars

Test was positive for the stem and root samples, but negative for the leaf sample. This is indicative of the presence of polysaccharides and oligosaccharides in these parts of the plant, which gave positive result after hydrolysis.

3.3.2 Test for Tannins

Water extracts of the different parts of the plant was used for the different tannin tests as follows:

1. Ferric Chloride

Blue or greenish coloured precipitates with ferric chloride solutions revealed the presence of tannins (Trease and Evans, 1989). The water extracts of the different plant samples gave green to greenish – black colour indicating that condensed tannins are present.

2. Bromine Water Test

All the different samples gave a precipitate with this reagent, indicating the presence of condensed tannins.

3. Lead Sub-acetate Test

A brown precipitate was observed for the different samples indicating presenence of tannins. Tannins are easily precipitated by heavy metals, the metal here is lead.

4. Ferric Ammonium Citrate Test

The water extract of each of the different samples gave a precipitate with this reagent, indicating that tannin, which was precipitated by the heavy metal, iron, could be present.

5. Formaldehyde Test

There was no precipitate with any of the samples when this reagent was used. This indicated the absence of hydrolysable tannins.

6. Test for Chlorogenic Acid

A negative result (absence of a green colour) was obtained with all the different samples of *W. indica*, which may indicate absence of chlorogenic acid.

7. Lime-Water Test

Precipitate was observed in each of the different water extracts of the parts of *W. indica,* which indicating the presence of pseudotannins. The results obtained is indicative of the presence of condensed and pseudo-tannins in *W. indica*. Tannins are known to have antiseptic properties and they are also used in the

laboratories to detect the presence of gelatin, proteins and alkaloids (Tyler et al, 1981). Tannins have antiseptic properties and its presence in the plant could be responsible for the uses in treating dysentery, syphilis, as a general tonic, energy restorer, cough syrup and skin infections (Irvine, 1961; Nwude,1986; Sokomba *et al,* 1983).

3.3.3. Test for Glycosides

1. Test for Cyanogenic Glycosides

No faint red colour was observed on picrate paper for any of the samples indicating the absence of cyanogenic glycosides in the plant.

2. Test for Anthraquinone Derivatives (Borntrager's Test).

No bright pink colour was observed in any of the samples. This indicated the absence of anthraquinones in W*. indica*.

3. Test for Saponins

1. Froth Test

Frothing was observed in all the different samples of the plant, which lasted for more than 30 minutes. This indicated the presence of saponins in the leaves, stem and roots of *W. indica*.

2. Haemolytic Test

Haemolytic test was positive as indicated by the presence of precipitates with the leaf, stem and root samples. This indicated the presence of saponins in the different parts of the plant.

The haemolytic property of *W. indica* and presence of saponins could be responsible for the use as a remedy for internal haemorrhage and purgation (Dalziel *et al*, 1954; Iwu, 1982; Gilman and Goodman,1975).

4. Test for Cardiac Glycosides

Negative results were obtained for the different parts of the plant with Keller-Killiani's, Kedde's and legal's tests. This indicated the absence of cardiac glycosides in the plant.

3.3.4 Test for Terpenes and Sterols

Negative result was obtained for the Liebermann – Burchard's test indicating the absence of terpenes in all the samples. A positive result (a reddish brown or brownish ring) was obtained with Salkowski's test indicating the presence of sterols in the different samples of *W. indica.* The presence of steroidal saponins is indicated. The presence of steroidal derivatives in *W. indica* has been detected by Burkill (2000).

Steroidal saponins are closely related to the sex hormones (Evans, 1992) and this could explain the uses of *W. indica* as a contraceptive and abortaficient in Ghana (Dalziel et al, 1954, Hedberg *et al*, 1983 Watt and Breyer-Brandwijk, l962).

3.3.5 Test for Flavonoids

The result of various tests carried out for these classes of compounds were as outlined below:

1. Lead Acetate Test

Precipitate was observed in the leaf sample, thus indicating the presence of flavonoids in this part of the plant.

2. Sodium Hydroxide Test

A yellow colour was observed indicating the presence of flavonoids. This was observed with the leaf sample only. Trease and Evans (1989) reported that flavonoids dissolve in alkalis to give a yellow solution, which, on the addition of acid, becomes colourless. The reaction observed here, gave a yellow solution, which on addition of acid became colourless. Thus flavonoids are indicated.

3. **Ferric Chloride Test**

A greenish colour was obtained with the leaf, stem and roots samples, indicating the presence of phenolic nucleus. The test is applicable for both tannins and flavonoids.

4. **Shinoda's Test**

The leaf sample gave positive result, which is a red colour, thus the presence of flavonoids is confirmed.

5. **Amyl Alcohol Test**

The test was positive for the leaf sample. Yellow colour was observed indicating the presence of flavonoids. The colour intensity for flavonoids tests were observed in the leaves rather than in the stem and roots, which

is indication that the flavonoids were only present in this part of the plant. The presence of flavonoids has been detected in *W.indica* found in Nigeria by (Ogbede *et al,* 1986; Deeni and Hussain, 1991). The flavonoids are found mostly in the petals and aerial parts of the plant. This is in conformity with the observations made in this work where the intense colour reactions were observed in the leaf and not the stem and root samples.

The positive reaction of the stem and root samples observed with Ferric chloride is indicate of the presence of other phenolic compounds, such as tannins, but not flavonoids.

The anti- inflammatory, antithrombotic, inhibition of tumour, antibacterial and antifungal properties of the plant, *W. indica,* could be due to the presence of flavonoids (Evans, 1996).

3.3.6: **Test for resins and Balsams**

Negative results were obtained for these classes of compounds. Thus, *W. indica* does not contain resins and balsams.

3.3.7: **Test for Alkaloids**

The various tests for alkaloids were carried out and positive results (precipitates) were obtained in all cases for all the samples, with the precipitate being more in the root sample. This is an indication of the presence of alkaloids in *W. indica.* Available literature shows that the plant contains alkaloids of the adouetine group that perhaps make them physiologically active (Burkill, 2000, Petrus, 1990, Loustalot and Pagan, 1947; Deeni and Hussain, 1991). The results of all the phytochemical tests are given in Table 3.

Table 3: **Summary of results of phytochemical evaluation of leaf, stem and root of *Waltheria indica***

Phytochemical Groups	Leaf	Stem	Roots

Carbohydrates			
-Monosaccharides	+	+	+
-Free reducing sugars	+	+	+
-Combined reducing sugars	-	+	+
-Ketoses	+	-	-
-Pentoses	+	-	-
Tannins			
-Condensed	+	+	+
-Hydrolysable	-	-	-
-Psuedotannins	+	+	+
Glycosides			
-Cyanogenic	-	-	-
-Anthraquinone	-	-	-
-Saponin	+	+	+
-Cardiac	-	-	-
Terpene	-	-	-
Sterols	+	+	+
Flavonoids	+	-	-
Resins/Balsams	-	-	-

Key: (+) = positive, (-) = negative.

3.4. **RESULTS OF QUANTITATIVE EVALUATION OF CRUDE VEGETABLE DRUGS (LEAF, STEM AND ROOT POWDERS)**

The following evaluative parameters (Table.5) were determined for the powdered leaves, stem and roots. All the values represent the mean of 3 separate determinations.

Table 4. **Results of Quantitative Evaluation of Crude Vegetable Drugs**

Evaluation Parameters	Leaf	Stem	Root
Moisture content (%w/w}	13.53±0.65	8.64 ± 0.65	10.00±0.70
Ash value {%w/w}	12.53±0.77	7.50 ± 0.65	7.50±0.71
Acid insoluble ash value {%w/w}	2.50 ±0.74	2.53 ± 0.52	4.90±0.71
Alcohol soluble extractive Value {%w/w}	2.43 ± 0.55	1.50 ± 0.60	1.10±0.63
Water soluble extractive value {%w/w}	10.01±0.01	4.03 ± 0.05	2.30±0.05

Determination of Moisture Content

The moisture content of the leaves was the highest followed by the roots and lastly the stem.

The moisture content of some drugs from literature is as follows: Digitalis leaf 6%, Acacia 15% (B.P, 1980). The moisture content is not high (13.5%) and this indicates less chances of microbial degradation. The general requirement for moisture content in crude drugs is that, it should not be more than14% (B.P, 1980). The value obtained in this work is within the acceptable range.

Determination of Ash Value

The leaf was found to have a higher value of total ash, while the stem and root had the same value. The root, being an underground structure, was expected to have the highest value instead of the leaves. This was however not the case in this work.

The total ash value of some official drugs includes: cardamom seeds 6.0% and wild cherry bark4.0%. The result shows that the ash value is variable and diagnostic.

Determination of Acid Insoluble Ash Value.

The acid insoluble ash is a method intended to measure the amount of silica, especially sand and siliceous earth present in the drug (African Pharmacopoeia). In this determination, it is the ‘physiological ash’ that gets dissolved in the dilute acid (Shellard, 1958).

From the results, the extraneous matter taken from the soil was higher in the root. But the total ash of the leaf was more than that of the root, indicating that the ‘physiological ash’ is highest in the leaf.

Some reported official acid insoluble ash values include: cardamon seeds 3.5%, senna leaves 2%, caraway fruits 2.5%, Alexandria senna fruit (pod) 6% and digitalis leaf 5.0%. The result obtained for *W. indica* can thus also serve as a differentiating and identifying characteristic.

Determination of Extractives

Results show that the alcohol and water extractive values of the leaves were more than the values for the stem and roots. Generally, the extractive values were quite low and a mixture of the water and alcohol (aqueous alcohol) may

be better as the extraction solvent for *W. indica*. However, results show that water is a better extraction solvent for the plant than the alcohol. Traditional medicine practitioners however, use the aqueous extracts of the plant mostly in treating such ailments as cough, diarrhoea, sore throat and pain.

The water extractive value of Wild cherry bark is not more than 10%w/w (B.P, 1980). The quantitative evaluation is an important parameter in setting standards for the purity and quality of crude drugs.

3.5. **RESULTS OF ANALGESIC ACTIVITY STUDIES**

3.5.1. **Results of Acetic Acid Writhing Method**

The result of the effect of the plant extracts on the acetic acid-induced abdominal constriction or writhing in mice is presented in Table 5 and Fig.7. The result indicated that the analgesic activity of the extracts was dose dependent.

The leaf, stem and root extracts produced statistical significance ($P < 0.05$) reduction in writhing induced by acetic acid when compared to control.

The root extract at 20mg/kg inhibited the abdominal constriction of the mice induced by acetic acid more than any of the other extracts (86.3%). The stem extract showed significant degree of inhibition, the highest value being 56.7% at 50mg/kg body weight. The analgesic activity of the plant could reside at higher doses. The leaf extract had the least activity; the highest value for inhibition being 36.5% at dose of 100mg/kg body weight. The control showed no analgesic activity, indicated by the absence of inhibition (table. 5).

This could suggest that the root contained more of the constituent(s) responsible for the analgesic activity.

The method employed in this work, also called abdominal constriction response, is very sensitive and able to detect analgesic effects of compounds at dose levels that may be inactive in other methods like the tail- flick test (Collier *et al,* 1968; Bentley *et al*, 1981). The abdominal constriction response is postulated to partly involve local peritoneal receptors (Bentley *et al*, 1983).

The analgesic activity exhibited by the extracts when compared to asp

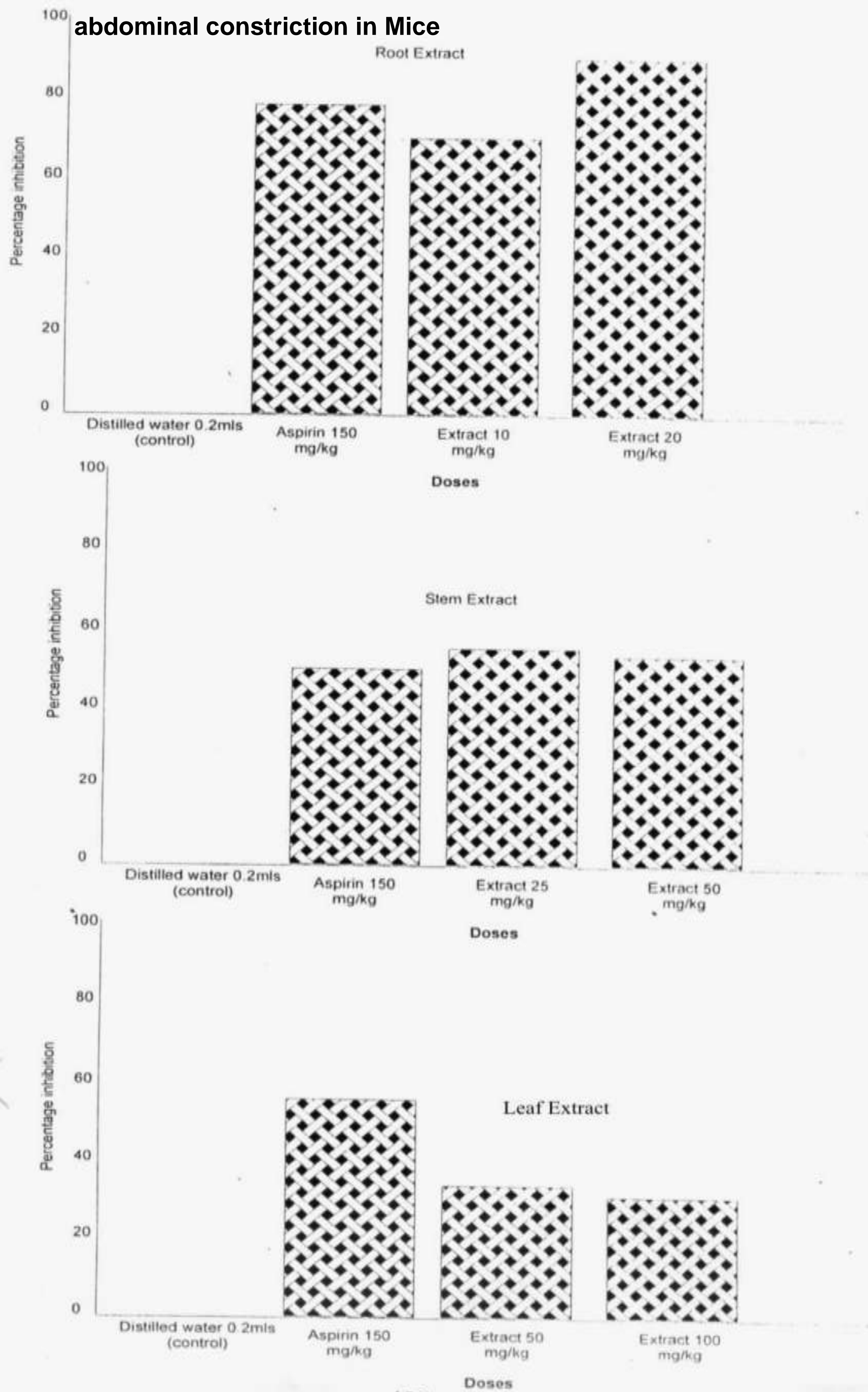
irin, may indicate that they act in a similar fashion as the Non Steroidal Anti-inflammatory Drugs (NSAIDS). The mechanism of action of NSAIDS is prostaglandin synthesis inhibition. The NSAIDS possess anti-inflammatory, antipyretic and analgesic activities. The chemical constituent(s) which could be responsible for the analgesic activity of the plant was however not determined. But analgesic effects have been reported with flavonoids as well as tannins (Ahmadiani *et al*. 1998). Since results from this study showed all the extracts to be tannin – rich, it is therefore pertinent to say that *W. indica* maybe a good source of analgesics. The analgesic activity exhibited by the extracts of *W. indica* justifies the use of the plant by traditional medicine practitioners for treating ailments associated with aches and pain.

Table 5. Effect of Extracts of *Waltheria indica* on Acetic Acid-Induced Abdominal Constrictions in Mice.

Group	Dose (Mg/kg)	Mean no. of writhing /10min±S.E	% Inhibition.
1.Root			
Control distilled water (0.2mls)		24.0 ± 5.5	-
Aspirin	150	6.3 ± 2.6*	74.8
Extract	10	7.0 ± 2.2*	72.8
Extract	20	4.3 ± 2.6*	86.3
2. Stem			
Control distilled water		38.0 ± 7.6	-
Aspirin	150	18.0 ± 3.9	55.3
Extract	25	17.0 ± 2.9	56.4
Extract	50	17.2 ± 3.0	56.7
3. Leaves			
Control distilled water		36.0 ± 4.3	-
Aspirin	150	15.0 ± 3.4	58.3
Extract	50	24.0 ± 4.8	36.3
Extract	100	23.2 ± 2. 4	36.5

* $P < 0.05$ Students' t- test, n=5

Fig. 7. **Effect of extracts of *Waltheria. indica* on acetic acid-induced abdominal constriction in Mice**



3.6. RESULTS OF PURGATIVE ACTIVITY STUDIES OF THE LEAF, STEM AND ROOT OF *WALTHERIA INDICA.*

Purgative activity is often indicated by the elimination of soft stool. The results obtained for the investigation of purgative potencies of the leaves, stem and roots of W *.indica* at varying doses are presented in Tables 6-8. The graph (fig. 8) shows the purgative potencies of the extracts. The onset of purgative action of the extracts was 2 hours and the duration was 8 hours. Purging was indicated by the excretion of wet faeces in considerable numbers and recognized by their rounded, irregular shape, soft consistency and presence of brown stains on white paper while the normal, dry faeces are elongated, regular in shape, hard and dry with no stains on the filter paper (Elujoba and Iweibo, 1988).

Also, production of wet faeces in rats or mice after oral administration of a drug is a positive test for purgative activity (Fairbairn and Moss, 1970, Akunyili, 1992).

Table 7 revealed that the roots contained more purgative activity than the leaves and the least activity was contained in the stem. This was indicated by the percentage wet feaces produced by each extract at different doses. Fig. 8 also confirms this observation. The purgative activity was shown to be dose dependent in the root. The purgative activity of the root may also be comparable to the reference standard, senokot, also referred to as senna in this work. The roots and reference drug produced the same percentage of wet faeces at 50mg/kg body

weight (91%). Both Tables 7 and 8 that contain results for the biological senna – equivalent (B.S.E) and the percentage senna-activity (P.S.A) confirmed this profile. Thus at 50mg/kg, the P.S.A of the root and senokot were both 100% (Table 8).

The biological senna- equivalent at any given dose of the leaves, stem and roots sample is defined as the quotient of the percentage wet faeces produced by any of the samples and that of the reference sample produced within 12 hours of oral administration into mice under the same experimental procedures. This served as an index of comparison between the samples and reference drug. Thus at 50mg/kg, the root sample gave biological senna – equivalent of 1.0 (i.e. activity is equal to that of senokot at the given dose). Similarly, the percentage senna- activity (senokot activity) at a particular dose of the samples is defined as the relative quantity of senna - activity possessed by the sample when assayed at the same dose under the same experimental conditions. Hence in Table 8, the roots of *W. indica* possessed 93% and 100% of senokot activity at 10mg/kg and 50mg/kg respectively.

In general, it was noted that the stem had the least purgative activity while the roots had comparable activity to that of the reference sample and even had the same purgative activity at 50mg/kg. The control did not produce any wet feaces.

The phytochemical tests showed that the leaf, stem and root extracts contained saponins, alkaloids, tannins and flavonoids. Saponins are

natural detergents, which possess emollient principles that have purgative properties (Gilman and Goodman, 1975). They have also been shown to be the active chemical constituents contained in some herbs used as purgatives (Iwu, 1982).

Available literature revealed that the infusions of the entire plant are used in India and the decoction of the dried roots of this plant used in northern Nigeria as purgatives (Petrus, 1990, Heinrich *et al*, 1992).

Table 6**. Percentage Wet Faeces Produced in Mice by the Leaf, Stem and Root extracts of *Waltheria indica* at different doses.**

	Dose (mg/kg) /% wet faeces			
Extracts	10	20	30	50
Leaf	18	16	12	20
Stem	8	12	6	14
Root	75	80	82	91
Senokot	76	82	91	91

Control (5mls of distilled water): No wet feaces.

Fig. 8: **Comparison of purgative activity of the Leaf, Stem and Root of *W. indica* with senokot tablets.**

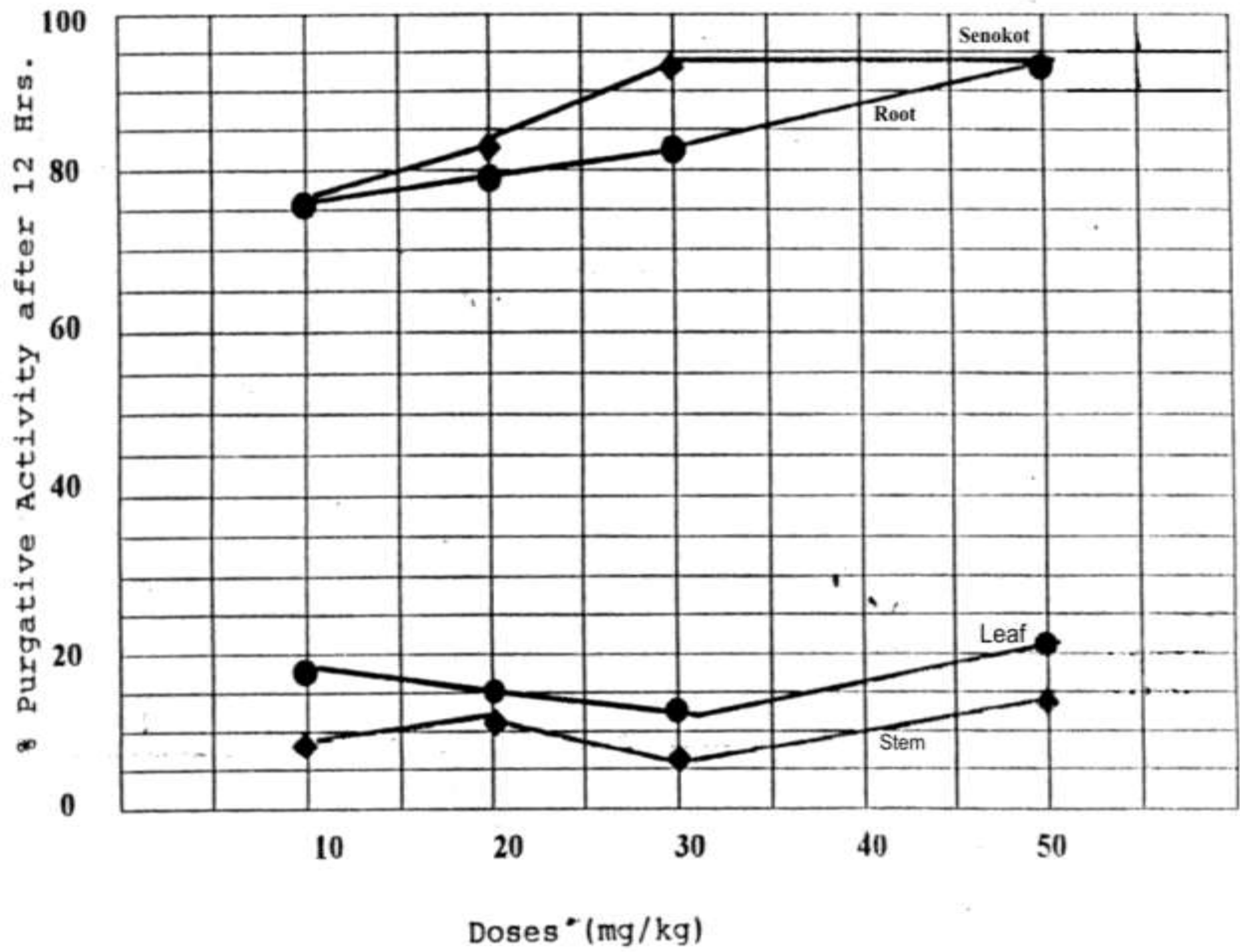


Table 7**: <u>Biological Senna Equivalent of the Leaf, Stem and Root extracts of *waltheria indica* at Different Doses</u>**

Extracts	Dose (mg/kg)/Senna Equivalent			
	10	20	30	50
Leaf	0.24	0.19	0.13	0.26
Stem	0.11	0.08	0.03	0.12
Root	0.93	0.94	0.90	1.00
Senokot	1.00	1.00	1.00	1.00

Table 8**: <u>Percentage Senna Activity of the Leaf, Stem and Root extracts of *Waltheria indica* at Different Doses</u>**

Samples	Dose (mg/kg) / Senna Activity			
	10	20	30	50
Leaves	24	19	13	26
Stem	11	8	3	12
Roots	93	94	90	100
Senokot	100	100	100	100

Therefore, based on its relative potency, the powdered root of *W. indica* could be readily developed as tablet or tea- bag dosage forms for use as a purgative. It is expected therefore, that the root of the plant obtained locally in Nigeria could replace or substitute for senna

products, which are sold as ‘senokot tablets and Herb Tea of Commerce’. These are imported into Nigeria via the United Kingdom.

This use could however be possible, after some form of detoxification of the root might have been done, due to the moderate acute toxicity (Table 9).

3.7 RESULTS OF ACUTE TOXICITY TESTING

Results of the acute toxicity studies for the leaves, stem and roots are presented in Table 9.

The LD_{50} was calculated using the method of Lorke (1983). The value obtained for the leaves, stem and roots were 363mg/kg, 141mg/kg and 69mg/kg body weight respectively. The results indicated that the extracts produced dose- dependent death of the animals with the water extract of the roots being more toxic, followed by the stem and then the leaves. The extracts may be considered toxic from values obtained .The established LD_{50} of one of the peptide alkaloids of *W. indica,* Adouetine z, in mice is 52.5mg/kg per intraperitoneal route (Pais *et al,* 1963). According to WHO (Dubois and Geiling, 1959), these LD_{50} values obtained for the extracts, fall outside the ‘moderately toxic’ range (0.5-5.0g/kg). Therefore the possibility of acute toxicity is an important consideration in the use of the plant.

Table 9. **Results of Acute Toxicity Testing.**

Extracts	first part of investigation (Pilot studies)		second part of investigation	
	Doses (mg/kg)	Mortality	Doses (mg/kg)	Mortality
Leaves	10	0/3	200	0/1
	100	0/3	400	1/1
	1000	3/3	600	0/1
			800	1/1
			LD_{50}= 363mg/kg	
Stem	10	0/3	50	0/1
	100	1/3	100	0/1
	1000	3/3	200	1/1
			400	1/1
			LD_{50}= 141mg/kg	
Root	10	1/3	20	0/1
	100	3/3	40	0/1
	1000	3/3	60	0/1
			80	1/1
			LD_{50}= 69mg/kg	

Lorke (1983) indicated that the use of many animals, particularly in the second investigation, has no practical advantage over the use of one animal and that results from the one group animal are reasonable and of practical purpose. The method is used for a wide range of substances like drugs, agricultural and industrial chemicals. This method may also be used for all routes of administration.

The results obtained showed that the extracts of *W.indica* might not be safe for consumption as is done in traditional medicine practice, without some form of detoxification, because of the acute toxicity. This is particularly so in case of the root, which is the most, commonly used part of the plant (Dalziel *et al,* 1954; Chhabra *et al,* 1993; Hedberg *et al*, 1983), and this should be an important consideration despite the uses as analgesics and purgatives as shown by results from the biological evaluation.

CONCLUSIONS

The pharmacognostic and biological studies of the plant *W. indica* was carried out. The macroscopical and microscopical studies showed some of the characteristics of the family Sterculiaceae, the most distinguishing feature being the stellate trichomes found in abundance in all parts of the plant. This could serve as a distinguishing feature from other plants. The phytochemical constituents of the plant included carbohydrates, tannins, flavonoids, saponins, alkaloids and flavonoids. Cardiac and anthraquinone glycosides, resins and balsams were however absent. The plant was revealed to compose of soft tissue as evidenced by the presence of parenchyma cells. This is a characteristic of the family Sterculiaceae which is composed of soft wood.

The analgesic studies showed the plant to possess some degree of analgesic activity, with the roots having more activity at a dose of 20mg/kg followed by the stem and lastly the leaves. The root and stem extracts significantly ($P<0.05$) reduced the number of abdominal constriction induced by acetic acid, while the leave extract did not show any significance. The activity was dose dependent. The constituents responsible for the analgesic activity were however not established. But tannins are known to exhibit analgesic activities and this was detected in all parts of the plant.

Thus, the use of the plant for the treatment of aches and pains by traditional medicine practitioners in traditional medicine is justified.

The plant extracts also had purgative activity and the root showed comparable activity to the standard drug, senokot, at a dose of 50mg/kg

where the percentage-wet feaces (100%) was the same for both. The purgative activity was shown to be more in the root than the other parts of the plant. The purgative activity of the root could be attributed to the presence of one or more of the constituents like tannins or saponins detected in *W. indica* in this work.

Also, the acute toxicity of the extracts was determined and results showed that the plant, *W. indica*, might not be safe for short time use in traditional medicine practice because of the acute toxicity. The root was found to be more toxic, followed by the stem and then the leaf. The toxic constituent could be more concentrated in the roots than the other parts of the plant, hence the higher toxicity in this part of the plant. The chronic toxicity test may need to be carried out to ascertain the long-term safety of the plant.

Even though there is compelling evidence of analgesic and purgative activities of the extracts of *W. indica*, the toxicity effects observed may be a set back to its recommendation for the said uses, except after some form of detoxification. This is particularly true in the case of the root extract, which is the most commonly used part of the plant by traditional medicine practitioners for traditional medicine according to literature. But it is also the part that possessed more toxicity but more of the activities.

In conclusion, *W. indica* is a very useful tropical plant, which is available in abundance and which could be useful in treating many of the ailments that have been discussed. The results of the qualitative and quantitative microscopical and phytochemical evaluations, could aid in the identification and preparation of a monograph on the plant. Presently, *W. indica* is not

included in the African Pharmacopoeia. The pharmacological activities (purgative, analgesic and acute toxicity) have been shown to be more concentrated in the roots than the other parts of the plant, and could be therapeutically useful after detoxification. The results of the biological evaluations of the extracts, may justify the uses of the plant, particularly the roots, by traditional medicine practitioners for the various uses. Plants are still sources of potent therapeutic agents and their uses are popular due to their effectiveness, easy availability, low cost and comparative absence of serious toxic effects. In view of the harsh economic situation and increased level of poverty in Nigeria presently, the use of herbal remedies is expected to increase.

RECOMMENDATIONS

Some herbal drugs, like *Waltheria indica*, have been shown to exhibit the respective pharmacological actions evaluated and further work on the plant is therefore recommended. Further work could be carried out on the plant such as isolation and identification or characterization of the different constituents of the plant responsible for the various activities by chromatographic and spectroscopic means, and possibly, formulation of pharmaceuticals and herbal remedies from the plant. Also, elucidating the mechanism of actions exhibited by the plant extracts for the various biological activities would be recommended. An appropriate method of detoxification before the plant can be therapeutically used, is also recommended.

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APPENDIX 1

QUANTITATIVE EVALUATION OF CRUDE VEGETABLE DRUGS

A. Determination of Moisture Content

(a) POWDERED ROOT SAMPLE

3g of the powdered roots were used

	Readings		
	1	2	3
a. Constant weight of crucible (g)	38.63	66.30	58.17
b. Weight of Crucible and sample before heating (g)	41.63	69.30	61.17
c. Weight of crucible and sample after heating(g)	41.45	69.15	60.90
	41.32	69.00	60.87
	41.32	69.00	60.87
d. Moisture content(%) = b-c/3 x100 =	10.31	10.00	10.00

e. Mean $= \frac{10.31 + 10.00 + 10.00}{3} \times 100 = \frac{30.31}{3}$

= 10.11% w/w.

f. Example of calculations:

$\text{Moisture content} = \frac{\text{step b} - \text{step c}}{\text{initial weight of drug}} \times 100$

$\frac{41.63 - 41.32}{3} = \frac{0.31}{3} \times 100 = 10.10\%$

These methods were used to obtain values for the other readings and also to determine values for the stem and leave samples.

b. Determination of Ash Value

2g of powdered Stem sample were used

	Readings		
	1	2	3
a. constant weight of crucible(g)	26.58	28.65	24.61
b. weight of crucible and sample(g)	28.58	28.65	26.61
c. weight of crucible and ash(g)	26.85	28.80	24.77
	26.73	28.80	24.76
	26.73	28.80	24.76
d. Weight of Ash(g) (c-a)	0.15	0.15	0.15
e. Ash value (%)	7.5	7.5	7.5

Mean ash value $= \frac{7.5 \times 3}{3} = 7.5$

Example of Calculations:

Weight of Ash $= \frac{\text{step c} - \text{step a}}{\text{initial weight of drug}} \times 100$

$= \frac{26.73 - 26.58 \times 100}{2} = \frac{0.15 \times 100.}{2}$

Ash value = 7.5% w/w

Same methods were used to calculate values for the other samples

a. Determination of Acid Insoluble Ash

2g of the powdered roots were used

	Readings		
	1	2	3
a. Constant weight of crucible(g)	34.52	37.39	
48.89			
b. weight of crucible and ash(g)	34.59	37.44	47.96
after acid treatment	34.57	37.44	47.94
	34.57	47.94	48.84
c. weight of ash(g) after acid treatment(b-a)	0.05	0.05	0.05
d. Acid insoluble ash value (% w/w)	2.50	2.50	2.50

Mean value = $\frac{2.5 + 2.5 + 2.5}{3}$ = 2.50

e. Example of calculations:

Weight of ash = step b- step a

= 34.57 – 34.52

= 0.05

Acid insoluble ash value = $\frac{\text{weight of ash}}{\text{weight of drug}}$ x 100 = $\frac{0.05}{2}$ x 100 = 2.50% w/

Method was used for the other powdered samples.

e. Determination of Extractive Values

(i). Alcohol- Soluble Extractive Value

5g of the powdered leaves were used in 100ml of 90% ethanol

	Readings		
	1	2	3
a. Constant weight of crucible(g)	24.54	27.52	38.00
b. Weight of crucible and ash(g)	24.60	27.46	37.94
After acid treatment.	24.56	27.54	38.03
	24.56	27.54	38.03
c. Weight of ash(g) after acid treatment	0.02	0. 02	0. 03
d. Alcohol- soluble extractive value (% w/w)	2	2	3

Mean Alcohol- soluble extractive value $= \frac{2 + 2 + 3}{3} = \frac{7}{3} =$ 2.33 % w/w

d. Example of calculations using leaves:

From above, weight of ash = step b – step a

= 24.56 – 24.54

= 0.02g

Alcohol - soluble extractive value $= \frac{\text{weight of ash}}{\text{Weight of drug}} \times 5 \times 100$

$= \frac{0.02}{5} \times 5 \times 100 = 2.0\%$

The method was used to calculate Acid insoluble ash values for the leaves and root.

e (ii). **Water – Soluble Extractive Value**

5g of the powdered leaves were used in 100mls of 0.25% chloroform water.

	Readings		
	1	2	3
a. Constant weight of crucible	25.92	30.22	26.05
b. Weight of crucible and content	25.97	30.39	26.12
after heating	25.93	30. 24	26.07
	25. 93	30. 24	26.07
Weight of ash (g)	0.01	0.02	0.02
c. Water soluble extractive value(% w/w)	1.00	2.00	2.00

$$\text{Mean water soluble extractive value} = \frac{1.00 + 2.00 + 2.00}{3}$$

$$= 1.50\%w/w$$

Calculations was as in the Alcohol Extractive Value.

APPENDIX II

TEST FOR PURGATIVE ACTIVITY

1. Purgative Activity of the Leaves, Stem and Root Water Extracts of

W. indica at 20mg/kg body weight.

Sample	mean number of dry feaces	mean number of wet feaces	total number of feaces	% wet feaces
1. Leaves	57	11	68	16
2. Stem	15	2	17	12
3. Root	13	63	76	82
4. Senokot	14	63	77	82

Example of calculations for leaves sample:

Percentage wet feaces = mean number of wet feaces / total number of feaces x 100

= 11/ 68 x100

= 0.16 x 100

= 16%.

The same procedure was used to calculate the values for other samples.

2. Purgative Activity of the Leaves, Stem and Root Water Extracts of *Waltheria indica* at different Doses

a. Purgative Activity of the Root Water Extract at different doses:

Doses (mg/kg)	Mean dry feaces	Mean wet feaces	Total feaces	% wet feaces
10	4	12	16	75
20	4	16	20	80
30	3	14	17	82
50	2	21	23	91

b. Purgative Activity of the Leaves Water Extract at different doses

10	14	3	17	18
20	16	3	19	16
30	15	2	17	12
50	12	3	15	20

c. Purgative Activity of Senokot at different doses:

10	5	16	21	76
20	2	18	20	82
30	1	20	21	91
30	3	29	32	91

Calculation Example for the root at Dose of 300mg/kg:

% feaces = mean wet feaces / total number of Feaces x100

= 16 / 20

= 0.8 x100 = 80 %.

The procedure was used for calculating the values of the other samples.

3. Percentage Senna – Activity of the Water Extract of the Leaves, Stem and Roots of *Waltheria indica.*

Calculation Examples using the root and leaves sample at 200mg/kg:

(a). % Senna – Activity of the root:

% Senna- Activity = Biological Senna- Equivalent x 100

= 0.93 x100

= 93%

(b) % Senna – Activity of the leaves:

= 0.26 x100

= 26%

4. Biological Senna Equivalent of the extracts at different doses:

The Biological Senna- Equivalent is defined as the quotient of the percentage wet feaces produced by the samples and that of the reference sample, senokot tablets, under the same experimental conditions. Thus using the examples of the root and leaf samples in steps 2 a and b above:

Calculation Examples:

At 200mg/kg, % wet feaces of root = 75

% wet feaces of senokot = 76

Biological Senna- Equivalent of the root = 75 /76 = 0.93

APPENDIX III

ACUTE TOXICITY TESTING

Sample	Doses (mg/kg)	Mortality
1. Leaves	200	0/1
	400	1/1
	600	0/1
	800	1/1
		LD_{50} = **363**
2. Stem	50	0/1
	100	0/1
	200	1/1
	400	1/1
		LD_{50} = **141**
3. Root	20	0/1
	40	0/1
	60	0/1
	80	1/1
		LD_{50} = **69**

Lorke's method for calculating LD_{50} is given by the formula below:

GM = $n\sqrt{X1 \ldots Xn}$, GM = geometric mean of doses for which 0/1 and 1/1 were found

n = number of values that produced death and no death.

X = product of values that produced death and no death.

Calculation example using the stem extract:

Value for no death = 100mg/kg, value for death = 200mg/kg

Therefore number of value = 2

GM =$2\sqrt{100 \times 200}$

= 141mg/kg.

ii. Calculation of LD_{50} for the leaves:

using above formula:

GM = $3\sqrt{200 \times 400 \times 600}$

= 363mg/kg.