MICROSCOPICAL AND PHARMACOLOGICAL STUDIES

OF

STEM-BARK OF STEGANOTAENIA ARALIACEA HOCHST FAMILY UMBELLIFERAE

AGUNU ABDULKARIM

NOVEMBER, 1997.

MICROSCOPICAL AND PHARMACOLOGICAL STUDIES OP STEM-BARK OF *STEGANOTAENIA ARALIACEA* HOCHST

BY

AGUNU ABDULKARIM
B.Pharm (A.B.U)

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL AHMADU HELLO UNIVERSITY,IN PARTIAL FULFILMENT OF THE REQUIREMENTS OF THE DEGREE OF MASTER OF SCIENCE IN PHARMACOGNOSY.

Department of Pharmacognosy and Drug Development,
Faculty of Pharmaceutical Sciences,
Ah mad u Bello University,
Zaria.

NOVEMBER, 1997.

THESIS APPROVAL

We certify that we have read this study and that in our opinion ,it conforms to acceptable standard of scholarly presentation and it is fully adequate in scope and quality as a thesis for the degree of MASTER OF SCIENCE (Pharmacognosy)

Date 2-4-98

Dr. E.M. ABDURAHMAN
B.Sc.(Pharm), M.Sc., MBA, Ph.D, D.Sc.
Chairman,supervisory committee.
Department of Pharmacognosy and
Drug Development,
Ahmadu Bello University, Zaria.

Date 2.4.98

Professor I. ABDU-AGUYE,
MBBS, M.Sc., FMCP.
Member, Supervisory committee,
Department of Pharmacology and
Clinical Pharmacy,
Ahmadu Bello University, Zaria.

Date 2-4-98

Head,
Department of Pharmacognosy and
Drug Development,
Ahmadu Bello University, Zaria.

Date: 25/9/99

Dean, Postgraduate School,
Ahmadu Bello University, Zaria.

DECLARATION

I hereby certify that this work is carried out by me under the supervision of Dr. E.M. Abdurahman of the Department of Pharmacognosy and Drug Development and Professor I. Abdu-Aguye of the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. The work of other investigators are duly acknowledged and referred to accordingly. No part of the work reported in this thesis has been submitted elsewhere for a degree.

Agunu Abdulkarim
Department of Pharmacognosy and
Drug Development,
Faculty of Pharmaceutical Sciences,
Ahmadu Bello University,
Zaria, Nigeria.

DEDICATION

This thesis is dedicated to my late father,

AGUNU ODION

May Allah pardon and give him paradise (Amin).

ACKNOWLEDGEMENT

I am most grateful to Dr. E.M Abdurahman for his constant help and relentless supervision during the course of this work. May Almighty Allah continue to reward you. My sincere thanks also goes to Professor I. Abdu-Aguye, who despite the numerous responsibilities contributed immensely to the success of this research.

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My special acknowledgement also go to my `special heart', AISHA OMOTI.

ABSTRACT

Steganotaenia araliacea is a small, soft wooded tree up to 2.5 meters high branches more or less erect. It belongs to the family Umbelliferae. The stem-bark of *Steganotaenia araliacea* has characteristic aromatic nature true to typical Umbelliferae. Macroscopical studies reveals stem-bark of 9.0-14.3cm long, 0.2-0.3cm thick, 2.5-3. lcm width, fracture is short and present double-quill shape.

Microscopical examination of the stem-bark shows the presence of starch grains, fibres, calcium oxalate crystals and oil cells. Microscopical measurement of the starch grains, fibres, calcium oxalates were also carried out.

Phytochemical studies revealed the presence of terpenes, saponin glycosides, and carbohydrates but absence of tannins and alkaloids.
Quantitative evaluation shows moisture content of 10.7% w/w, Ash value of 11% w/w, Acid-insoluble ash value of 1.5% w/w, Alcohol-soluble extractive value of 7% w/w and water-soluble extractive value of 35% w/w.

Steganotaerda araliacea stem-bark is commonly used in Zaria, Kaduna State, Nigeria and its immediate neighbours for induction of diuresis. The diuretic property, effects on urine electrolytes and acute toxicity studies are here highlighted.

The Diuretic studies of the water, methanolic and ethanolic extracts using rats was carried out. Frusemide was used as standard while sodium chloride was used as conti The procedure involves intraperitoneally

administration of the sample to weighed rats in metabolic cages, designed that faecal materials do not mix with urine. The urine volumes were measured at 3hly time intervals for 24 hours.

The urine analysis to determine effects on electrolytes revealed that there was increased potassium excretion with the ethanolic extract but no effect with water extract.(This implies that individuals using ethanolic extract stem-bark of *Steganotaenia araliacea* will benefit from potassium supplement).

The toxicity studies using mice shows LD50 of 1.83g/kg and 1.75g/kg for ethanolic extract and water extract respectively, signifying a slightly toxic property of the stem-bark in mice.

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CHAPTER ONE
INTRODUCTION

1.1 TRADITIONAL MEDICINE

Traditional medicine unlike orthodox medicine interwoven with the culture, tradition, religion and mode of life of the people.

It involves the use of plant parts like the leaves, roots, seeds, barks etc. and animal parts and sometimes physical and spiritual means in treatment of various ailments. Up till the present time because of shortage of orthodox medical practitioners, cost, accessibility, acceptability, time wastage etc. the majority of rural people(up to 80%) depend on it for their health and well-being (Sofowora, 1979).

Traditional medical practice despite its ancient nature has proved its worth in provision of medical services. Some of these services are in bone setting, birth attendance and psychiatry . The practice of traditional medicine and its overall contribution towards health care is enormous and hence appreciation of its drawbacks is necessary for the betterment of the majority of our populace.

1.2 MEDICINAL PLANTS

The use of plants for medicinal purposes is well known in several parts of the world. Some plants have be known to synthesize large varieties of chemical substances such as terpenes, steroids, alkaloids, glycosides and a host of other chemicals which do not have any effect on the plant itself but

have prominent effects on the animal systems. These plants commonly referred to as medicinal plants are widely distributed throughout the world.

Nigeria, for example, is blessed with many medicinal plants, some of which are in demand all over the world with an estimated aggregate market ofN 2125 million that is about $25 million (Iwu, 1996). Two examples of these include *Physostigma venenosum* (Calabar beans) from which physostigmine is obtained and *Acacia senegalensis* (Gum Arabic) which is used as a suspending agent in tablet granulation.

Other medicinal plants though not commercially obtained from Nigeria include *Digitalis purpurea* L. A decoction of the leaves is used for the treatment of dropsy. Scientific evaluation leads to isolation of digoxin and digitoxin.

Similarly, the root of *Rauwolfia serpentina* in traditional medicine is used to treat mental illness, insanity and insommia. Reserpine and other related alkaloids have been isolated and found useful as sedatives, tranquilizers and hypotensives.

The bark of Cinchona species are used in traditional medicine for the treatment of malaria. The active antimalarial constituents were discovered to be quinine, Cinchonine and cinchonidine.

The leaves and fruits of senna (*Cassia senna* L. or *Cassia augustifolia*) were used by Arabian physicians long ago as purgatives (Taylor, 1965; Le Strange, 1977). Investigation showed that the purgative properties were due to the anthraquinone d vatives; sennoside A, B, C, and D and primary

glycosides which have higher potency than sennosides A and B (Trease and Evans, 1989).

The leaves of *Thea sinensis* L (The plant) have been discovered to contain caffeine and theophylline. The diuretic activity observed with consumption of tea is due to the caffeine content. The periwinkle *(Catharanthus roseus)* has been found to contain two alkaloids with anticancer properties. These are vincristine and Vinblastine. These alkaloids are clinically in use for treatment of cancer.

The root of *Artemesia annua* L. is used in treatment of malaria in Asia. Phytochemical investigation has revealed artemicillin as the constituent responsible for the antimalaria property. The concentration in the leaves have been discovered to be more than the roots.

The potent analgesic morphine is an alkaloid obtained from *Opium poppy.* This alkaloid is widely used for its narcotic analgesic property.

The Neem tree *Azadirachta indica*, contains Nimbim, Nimbidin, Nimbinin among others. It has been investigated that administration of sodium nimbidate provoke a prompt lasting diuresis compared to urea (Martin , 1988).

The list of scientifically evaluated medicinal plants with proven therapeutic usefulness is large and cannot be presented here. Despite the proven status of some, only few are clinically useful (Ojewole, 1983).

1.3 DIURETICS

A diuretic is a substance or an agent which increase or induce urine production or both. A diuretic can be a natural or a synthetic product and cause diuresis in various ways.

1.3.1 Importance Of Diuretics

Diuretics are important because they are clinically used as follows:

(a) in treatment of hepatic, renal or pulmonary diseases and in congestive heart failure when salt and water retention results in oedema.

(b) alone or in combination with other drugs in management of hypertension.

(c) in elimination of toxemia of pregnancy and in periodic recurrent oedema e.g. premenstrual oedema.

1.3.2 CLASSIFICATION OF DIURETICS

Diuretics can be classified into five groups according to their mechanism of diuresis.

1.3.2.1 Increasing The Effective Glomerular Pressure

These include the digitalis groups which induce diuresis due to increase in heart activity.

Xanthines, which increase renal blood flow with a decreased tubular absorption e.g. theophylline and theobromine (Dasgupta, et al 1969).

Plasma and plasma substitutes similarly acts by shifting fluid form extracellular space to general circulation in hypoproteinaemic conditions.

1.3.2.2 Water And Osmotic Diuretics

These acts by increasing the non-coloidal osmotic pressure because the concentration and types of solute delivered to the renal tubule determine the volume of urine example saline, urea, and sugar.

1.3.2.3 The Loop Or High-Ceiling Diuretics

Acts by inhibiting sodium and chloride reabsorption in the ascending loop of Henle. They produce an intense, dose-dependent diuresis of relatively short duration. Examples are frusemide, ethacrynic acid and bumetamide.

1.3.2.4 Carbonic Anhydrase Inhibitors

They inhibit the enzyme, carbonic anhydrase which accelerates the attainment of equilibrium in the reaction.

$$CO_2 + H_2O \longrightarrow H_2CO_3 \longrightarrow H^+ + HCO_3$$

These inhibitors depresses the reabsorption of bicarbonate in the proximal tubule thereby preventing acidificatin of urine in the distal tubule. This leads to increase bicarbonate excretion which is accompanied by increased excretion of both sodium and potassium with the result that water is retained in the tubule and diuresis results. e.g acetazolamide and dichlorphenamide.

1.3.2.5 Potassium-Sparing Diuretics

(Aldosterone antagonists). They act at distal tubule inbibiting potassium secretion while increasing sodium secretion. E.g. are amiloride, triamterene and spironolactone.

1.4 MEDICINAL PLANTS WITH PROVEN DIURETIC ACTIVITY

Many medicinal plants are used for diuretic purposes. Some have been scientifically evaluated to establish the rational for diuresis while others are yet to be proven.

Such plants with record of diuretic properties include:

(a) ***Alstonia boonei*** is used traditionally for treating impotence, breast pain, dizziness and malaria. Phytochemical screening has revealed alkaloids (echitamine and echitamidine). These alkaloid have diuretic and hypotensive properties.

(b) ***Gardenia erubescens*** is locally used for treating gonorrhoea, impotence and similar ailments. Phytochemical evaluation reveals presence of mannitol in all parts of the plant. Carotenoids, tannins and flavonoids are also reported. The presence of mannitol is reported to be responsible for its mild diuretic activity (Husseini, 1988).

(c) ***Paullinia cupana;*** the flower of this plant have been discovered to contain caffeine and traces of theobromine. These constituents are reported to be responsible for the diuretic activity.

1.5 METHOD OF SCREENING DIURETIC AGENTS IN RATS.

The most common and widely used is that of Kau *et al* (1984).It involves the administration of a known dose of the sample and a known dose of an established diuretic agent. The urine output over a period of time is compared with control. The doses are administered in consideration to the body weight.

Metabolic cages capable of collecting urine free from faecal matter are employed.

1.6 *Umbelliferae*

1.6.1 Chemistry Of the Umbelliferae

All umbellifers are aromatic plants. Members of the family contains petroselinic acid as one of the major fatty acids of their seed fats. The raffinose isomer umbelliferose is characteristic of the family. Mannitol is the chief hexitol of the umbellifers. Triterpenes also occurs widely in umbellifers.

Furo- and dimethylpyranocoumarins are characteristic metabolites of the umbellifers. They are characterised by a total lack of true ta ns flavonols except myricetin, are present in rather high amount in many species flavonols are often replaced by flavones. They grow in all regions of the world. Flavonoids occurs universally whereas alkaloids for example, are restricted to a single taxon, *Conium maculatum*

1.6.2 Morphology

They are characterised by a little varying floral pattern comprising an inferior bicarpellate ovary, a reduced calyx, five free petals and stamens and a stylopodium supporting styles. They develop ticolporate pollen grains. They also develop collenchyma support, there are secretory ducts and multilacunar nodes. Significant number of umbels have swellings in the root or just above the root e.g. carrot (*Daucus corrota* L.).

1.7 *STEGANOTAENIA ARALIACEA*

1.7.1 Morphology

A small, soft-wooded tree up to 2.5 meters high, over 0.5 meters in girth, bark thick, branches more or less erect, leaves pinnate, leaflets 3-5 pairs with terminal leaflets, leaflets stalked, stalks nearly half inch long, blades ovate-lanceolate, softly pointed around edge and many pairs of lateral nerves, flowers yellowish brown, reddish in bud monoecious, fruits winged.

1.7.2 Traditional Uses

The leaves are odours, yield a scent and are used for scenting garments in Nigeria.

In Uganda, the leaves are much relished by elephants. The plant is specially planted for hedges e.g. in Sierra-Leone or Northern Nigeria, and for fence-poles in Nyasaland. The ground roots, rubbed into an incision on the side of he head are used in Northern Rhodesia for headaches. The bark yield a resin when cut. The scented leaves are used in Sierra-Leone as vermifuge. The leaves are used in Tanganyika for eye trouble. The leaf-juice is given to children in the Gambia for convulsions. The leaf infusion is considered diuretic and deputative while the decoction is used as lotion in Guinea.

1.7.2.1 Medicinal Uses:

Some members of the umbelliferae are used in gastro-intestinal tract problems e.g. fennel used for nausea, ferula for constipation and *Cuminum cyminum* for diarrhoea.

Similarly some members of the umbelliferae have been used for upper respiratory tact complaints e.g. Anise for catarrh, ferula species for cough and coriander for halitosis.

Daucus carota is used for menstrual disturbances. Similarly *Centella asiatica* is used in treatment of syphilis.

Eryngium species are also used for diuretic activities.

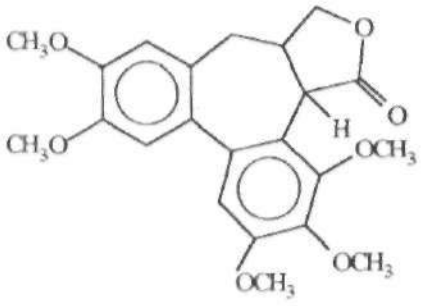
1.7.2.2 Veterinary Medicinal Uses:

Some members of the family are used as carminative, antispasmodic and stimulant. Some are used as spices e.g. Carrots and Caraway. Some members of the family are poisonous e.g. *Conium maculatum.* This poisonous nature enable some to be use in arrow poisons. (Heywood; 1971).

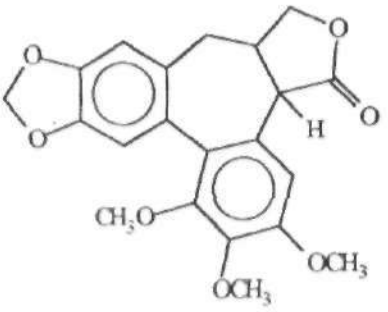
1.7.3. CHEMICAL CONSTITUENTTS

A large number of lignans have been isolated from *Steganotaenia araliacea.* These include Neoisostegane, steganacin, steganangin etc. The antiteukemic activity of the last two have been reported (JAAFROUT, *et._al.* 1983).Structures of some important lignans are shown in figure 1 below.

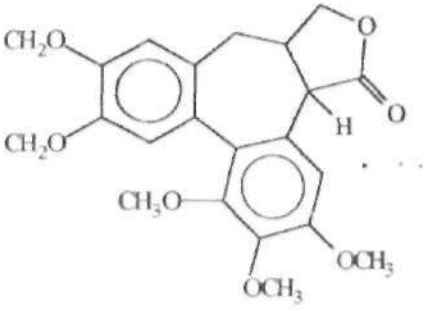
LIGNANS



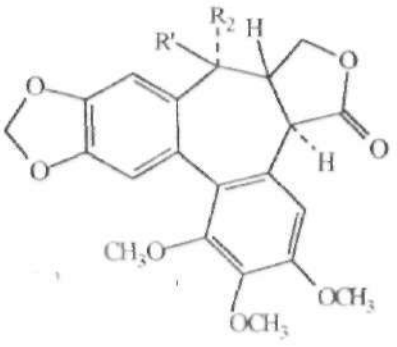
Neoisostegane



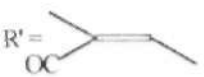
Isostegane



Stegnane



R' = OAc: R_2 = H = Steganacin



R' = , R_2 = H = Steganangin

Figure 1

Lignans are a group of natural compounds with their carbon skeleton derived from two phenylpropane (C_6-C_3) units joined together by at least one C-C bond between the two central positions (β and β¹) of the two C_3 chains they are widely distributed in higher plants (Kupchan, *et al*, 1972).

1.7.3.1 Pharmacological Activities Of Lignans

(i) Antitumur/Cytostatic activity - About thirty-three lignams are known to posses activity. Examples are the podophyllotoxins, which are constituents of the medicinal resins. Others include peltatins and burseran etc.

(ii) Lignans have antimitotic activity.

(iii) They inhibit synthesis of DNA, RNA and protein.

(iv) They have antivial activity. Resin from podophyllim have been used several years against veneral wart. They are also active against measles and herpes simplex type I.

(v) Most lignans have enzyme activity inhibition.

(vi) They have cathartic activity.

(vii) Lignans have cardiovascular effects; causes transient rise followed by a decrease in heart rate.

(viii) Lignans have antimicrobial activity, with activity against Staphylococcus, Streptococcus. *B subtilis*. Antifungal activity have also been reported.

(ix) Lignans are capable of acting as CNS depressants and as antidepressants

1.8 BARKS

1.8.1 *Introduction*

Barks refers to the outer dead tissues of stems and roots which occur outside the active cork-combium and includes cork, epidermis and lenticels. However, pharmacognostical bark includes all tissues outside the woody part of the stem or root separated by the main combium.

The bark of Botany is regarded as the outer bark in pharmacognosy and constitutes the outer part of the pharmacognostical or medicinal bark. The outer bark is often removed from the medicinal bark when it lacks the medicinally active constituents of the drug or contains undesirable amount of tannin or colouring matters.

The bark when viewed from within outwards may present the following in some or all commercial barks; secondary phloem, primary phloem, cortex and periderm, cork and phelloderm. Epidermis is present in very young bark only and not in commercial barks. When present it gives flitening membrane, giving a silvering appearance to the piece of bark. This easily separate and there is a formation of cork beneath it. External tissues of older barks often have a rugged and scaly appearance due to the splitting away or exfoliation of some of the outer tissues. They are often dead phloem alternating with bands of cork frequently from a somewhat massive external covering to a bark. These are referred to as Rhytidoma.The features of a typical bark tissue are shown in figure 2.

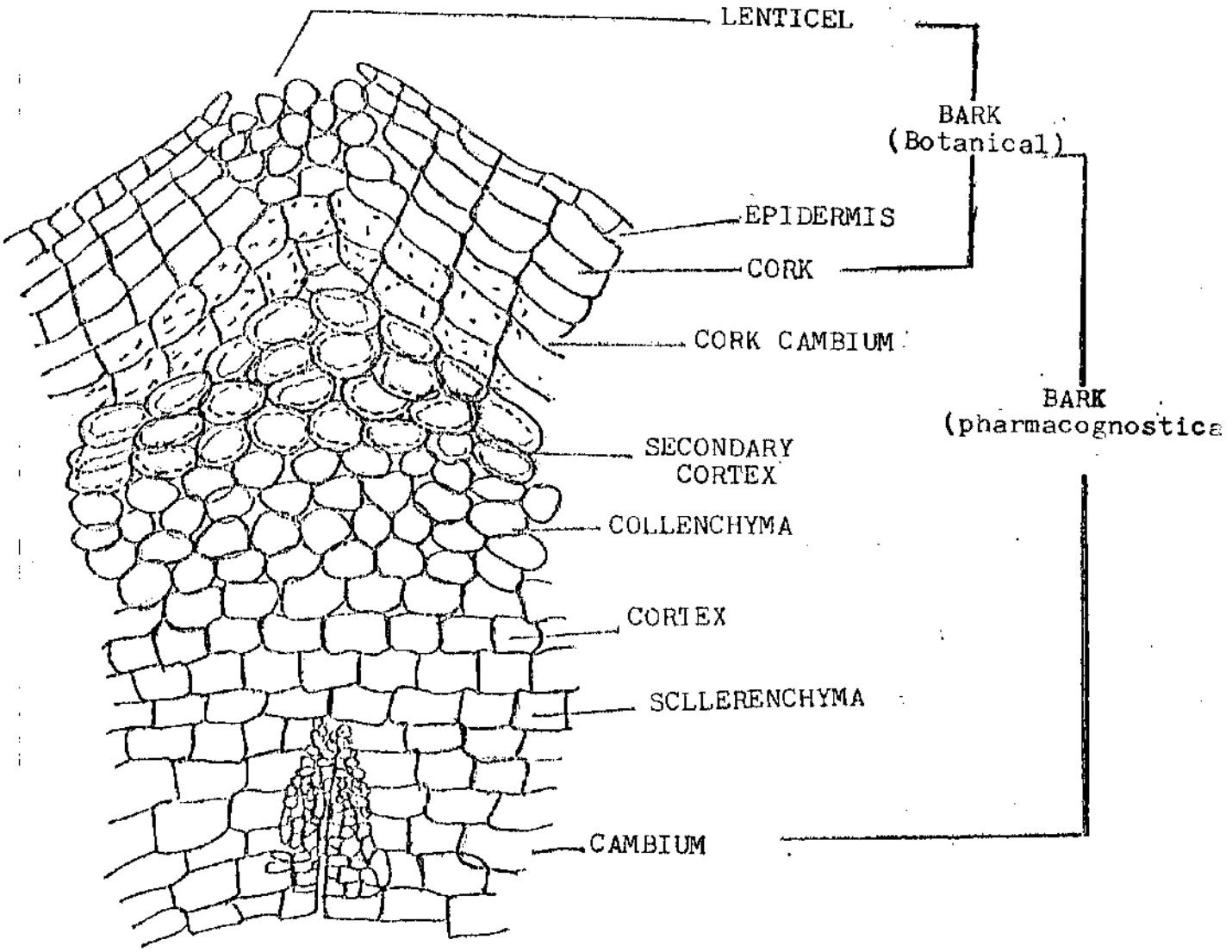


Fig. 2: **Tissues of a Bark**

(Redrawn from "Abdul Ghani 1990")

Cork Or Phellem

It is a protective tegumentary tissue of secondary origin developed from a single secondary menstematic layer, the phellogen or cork cambium, by repeated division. Cork is formed of dead polygonal cells, which are either slightly radially elongated or radially flattened.

Cork cells are usually uniform in shape and arranged in radial rows, each row being a result of the tangential division of a phellogen cell. They are tightly fitted together and except of lenticels, lacking intercellular spaces.

The cell walls are usually thin, strongly refractive colourless or yellowish to brownish. They may be equally or unequally thickened. The wall is usually composed of three layers; an inner (next to lumen) and an outer (primary wall) cellulosic layer and carbohydrate-free Suberin lamella.

The cellulose layers may be lignified as cassia, and cascarrilla. The cork usually appears dark brown in colour due to the presence of ammorphous brown or reddish cell contents, which are rich in tannin and other related substance.

In the cell-walls may be embedded crystals of calcium oxalate as in cascarilla (inner walls). In surface view, cork cells are polygonal and nearly isodiametric. The cork and dead tissue outside it constitute the bark of botanists and outer bark of pharmacognosists.

Phellogen

It's a secondary laterai moristem developed in the activation of parenchyma of primary, rarely of secondary origin. It produces rows of cork cells to the outside and unsuberised cells, phelloderm, to the inside.

The phellogen and is products of division i.e. cork and phelloderm are generally spoken of as periderm in which the only immature cells are those of the phellogen.

Phellogen arises in different positions in the different plants. Most commonly it originates in the subepidermal layer as in cascarra, cinnamon, quiillaia, but rarely arise in the ›idermis as in *Salis solanum*, dulcamara, viburnum.

In few roots e.g. valeriana, the phellogen arises in the piliferous layer. When phellogen keeps peace with the increase in circumference, it gives rise to an even, smooth outer bark, but if it fails to increase in diameter cracks or fissures, are produced owing to lack of elasticity of the dead tissues. In most cases, as in quillaia, the first formed phellogen, is replaced by new phellogens produced successively in the phelloderm, then in the cortex and lastly even in the secondary phloem.

In this case, if the later phellogens form cotinuous zones, the cork may show successive annual rings like those of the wood, but if they form a senes of discontinuous area, the cork always flakes off in pieces or exfoliate and thus becoming scaly and rough as in Oak.

Phellodern (Secondary Cortex)

It's developed from phellogen on its inner surface. Made up of unsuberised cells in one or two layers frequently in several layers in rows and are usually devoid of colouring matter but may sometimes contain chloroplasts and starch granules. It could sometimes be wholly parrenchymaton or wholly sclerenchymatous. When it constitute the whole cortical part of the bark as in root barks, they are called secondary cortex e.g. calisaya.

Cortex Or Primary Cortex

This is composed of parenchymatous tissue the cells of which are tangentially elongated with well marked intercellular spaces. Its outer larger maybe collenchymatous while the rest is being formed of parenchyma with more or less marked intercellular spaces. Near the cork, it often differentiates into strands of collenchyma. It often contains sclerenchymatous fibres either isolated or in groups.

Secretory tissues of various kinds such as oil cells, oil glands etc. maybe present and contribute to the identification of the drug. Chlorophyll, calcium oxalate and starch grains are often observed.

Endodermis

It's seldom found in many barks or sometimes indistinguishable by the growth of other tissue. The term "middle bark" is used by Pharmacognosists to indicate primary cortex alone or sometimes including both primary and secondary cortex.

Pericycle

Varies in extent and may be one to many cells thick. It may be parenchymatous and thus indistinguishable from the cortex as in Cinchona, Canella, Cascarilla or Sclerenchymatous, formed frequently of either interrupted as in Cinnamon or Continous band of Sclereid cells as in with-hazel. In such casse, the Sclerenchyma ring is generally accompanied, on the outside by pericyclic fibres, either isolated or in groups, appearing as yellow lines on the outer surface when the bark is decorticated as in Cinnamon.

Bast Or Phloem

In barks, the primary phloem is usually collapsed and even becoming indistinguishable or totally absorbed. Its composed of secondary phloem which constitute the inner bark of pharmacognosists. It is transversed by medullary rays and there is presence of sieve tubes which may be accompanied by companion cells and phloem parenchyma.

The phloem may also contain bast fibres and sclereids cells. The absence of such sclerencymatous elements from the phloem is characerteristics of some barks e.g. Canella, Pomegranate, Euonymus. Phloem may also show oil cells as in Canella, mucillage cells as in Cassia and other secretory structures as in Euonymus. Parenchyma usually contains starch granules and calcium oxalate e.g. Quillaia, Canella, the innermost i.e. the youngest portion of the phloem is formed of unformly much softer tissue than the remainder which dries up to form an extremely hard layer which gives the bark smooth inner surface.

Medullary Rays

The phloem medullary rays are homogenous in almost all dicots. Cells are parenchymatous and usually not pitted, but in some woody plants some of the cells particularly in the periphery may become in age, lignified and pitted as in Quillaia, Cascara etc. Medullary rays frequently contain starch granules and calcium oxalate crystals. They are usually indicaed by small depression on the inner surface of many barks. They are normally of uniform thickness except in the case of the primary rays which gradually widen towards the outside. They vary both in width and height being from one cell, uniseriate to many cells thick viz multiseriate as in Cascara and ranging from 3 to 4 cells.

Sieve Tubes

They are the fundamental elements of the phloem and may constitute the major part of the tissues. They are living cells with thin protoplasmic linings that include leucoplasts with minute starch granules but no nuclei.

The cells are cellulosic, thin or thick, refractive and non-lignified. Cells are arranged end to end showing distinct and or crosswalls and are connected together by pores which occurs in groups restricted to area called Sieve plates. Three main types of sieve tubes are recognised according to the characters and disposition of the end-wall.

(a) Distinct end-walls transversely set or at right angle to the side walls i.e. horizontal and perforated with distinct pores, forming one sieve plate.

(b) End wall is distinct, but obliquely set. Here, several sieve areas are recognised on the end walls forming a compound sieve plate.

(c) Sieve tubes with long bluntly tapering ends and no distinct end-walls are apparent. Sieve tubes usually have no definite arrangement. At certain age, the sieve tubes may cease to function and in this case, the sieve place is blocked on either side by a highly refractive pad called callus or callus plate.

Companion Cells

These are elongated, narrow, thin-walled cells with abundant protoplasmic c .ents and a prominent nucleons. Companion cells lies along the sieve tubes extending in some cases even to the full length of the corresponding sieve tube segment or in other words, the end walls of the companion cell coincide with the ends of the sieve tube segment. Companion cells is connected by pits with the sieve tube but its other walls are not pitted.

Companion cells are present in almost all angiosperms except a few plants e.g. cascara but lacking in pteridophytes and in Gymnosperms were their function is taken by the albuminous cells of Medullary rays.

Phloem Parenchyma

They posses thin cellulosic walls and contain small starch granules. With exception of few families e.g. Ranunculaceae, they are found in the secondary phloem of all dicot plants to a variable extent. They usually appears smaller than sieve tubes but bigger than companion cells.

Bast Fibres

These are dead polygonal cells usually with tapering pointed ends. Walls are generally thick, lignified and pitted, rarely cellulosic or only slightly lignfied. They contain calcium oxalate crystals and are of common occurrence in most commercial barks. They vary much in their sizes and their dimension are characteristic.

Sclereid Or Stone Cells

They have thick lignified walls with small to wide lumen. The cells vary in shape from rounded to irregularly branched sometimes elongated but the length is never as many times as the breadth.

Sclereid cells are usually devoid of contents but in some cases contain starch grains as in Cinnamon or crystals of calcium oxalate as in Calumba. They are useful diagnostic characters e.g. absence of stone cells from fragula differentiates this from cascara bark which contain sclereids

1.8.2 TIME OF COLLECTION

Barks are usually collected in Spring or early Summer when the sap is rising in the stem and the cambium is active and therefore more easily torn. If there is raining season it is then that the bark is more easily torn than in any other seasons. Example is Cinnamon bark. Occasionally a bark is collected at some other time as is done with wild cherry bark from *Prunus serotina* which is removed in autumn because at this season, amount of active principle is greatest.

1.8.3 REMOVAL OF BARKS

Longitudinal incisions are made at intervals around the circumference of the stem and the bark is stripped off in long pieces as in *Cascara sagrada* or longitudinal incisions are made and also horizontal ones at intervals of 30cm and pieces removed as in Cinnamon. Sometimes bark is whittled off with a knife producing small pieces with adherent wood on the inner surface.

1.8.4 DRYING OF BARKS

This is nearly always effected by the sun's heat in open air, or sometimes after a preliminary drying in open air. It's completed by some kind of artificial heat.

1.8.5 SHAPES OF BARKS

This depends on the type of incisions made in removing it and also upon the extent and nature of the shrinkage which occurs during drying.

Very large heavy pieces of barks from old tree trunks are often dried under pressure and often appears as flats e.g. Cinchona and quillaia barks. More usually the commercial barks are obtained from smaller branches and becomes curve during drying owing to the unequal shrinkage of its constituent parts.

When only slightly curved on the inside pieces are termed curved e.g. wild cherry. When concavity is on the outside, as occur in rare instances, its recurved e.g. Pomeranate root bark.

When the curvature is so great as to form a deep trough, the piece is said to be channelled e.g. Cassia, Hammamelis. And when the curvature is

present that one edge overlaps the other, a Quill is formed e.g. Cascara, Cinchona, Cascarilla and Cassia. And if each edge is rolled independently into a quill, the piece is called double quill. e.g. Frangula and Calisaya. When quills are packed one inside the other, as is done with Cinnamon and Sometimes with Cinchona, compound quill results.

1.8.6 COLOUR AND CONDITIONS OF THE OUTER SURFACES OF BARK

The outer surfaces of barks may bear certain epiphytes such as lichens with dark fructifications e.g. Cascara, Cinchona, Liverworts as in Cascara or Moss e.g. Cinchona. These epiphytes differentiate stem bark from root bark.

When cork is evenly developed, a smooth surface results and this is frequently marked by lenticels which are commonly elongated. The presence of rhytidoma gives scaly appearance.

Cracks and fissures of characteristic types occur in outer surfaces owing to the lack of elasticity in the dead tissues and the continued increase in girth of the tree. Cracks and fissures are characteristics. E.g. clean cut edges in *Cinchona succirubra* but thickened, recurved edges in *Cinchona officinalis*. On older barks, small circular dusty patches frequently develop in the cork, these are called corky wartz. e.g. old bark of *Cinchona succirubra*.

The inner surface of piece of bark also show features of colar and condition which are of diagnostic value. The shrinkage results in the production of parallel longitudinal ridges which are sometimes very fine and in others coarse. These are termed striations. Occasionally also, as in Cascara

longitudinal shrinkages produces parallel transverse wrinkles known as conjugations.

The behaviour of bark when broken across transversely and the appearance of the exposed surfaces are known as fractures and provide useful diagnostic characters.

When the fractured surfaces are smooth, the fracture is described as short; if the surface exhibit small rounded prominences, it is granular, if jagged projecting points are formed, the fracture is splintery, if fine fibrous thread extend from broken surfaces, it is fibrous and if the fractured region breaks into tangentially arranged layers as in quillaia, it is laminated.

The arrangement of tissues in bark is largely responsible for the type of fracture and the arrangement can be best seen in the smoothed transversely cut surfaces, this offers valuable diagnostic character for most barks.

The constituents of which barks owe their action are very various. Nearly all barks contains tannins, well-known exceptions being Canella, euonymus and Cotton-root barks. Other widely distributed constituents include volatile oils, alkaloids, glycosides, mucilages, starch and Calcium oxalates.

The following tissues are normally examined in Commercial barks:- the secondary phloem, the Periderm and the dead tissues outside the Periderm, the primary phloem, the Cortex and the Cork.

The epidermis is present in very young barks only and not in commercial barks. Similarly the endodermis is seldom to be found in many barks.

1.9 TOXICITY

1.9.1 INTRODUCTION

Whether a substance is poisonous depends on the type of organism exposed, the amount of substance and the route of exposure. In cases of human poisoning, the degree of harm done depends on whether the exposure is to the skin, by inhalation or through ingestion (Iyaniwura , 1996).

Toxicological studies has develo[illegible] at an increasing pace with advances in the basic biological, chemical and biochemical sciences. These has enable measuring of chemical poisons and their metabolites at very low levels and with remarkable sensitivity.

The medium lethal dose (LD_{50}) is often used in assessing the degree of toxicity. Mortality, however is usually not a good parameter for toxicity measurement. Moreover, it is important to distinguish acute toxicity which has an effect soon after exposure and chronic toxicity which has a long latency period.

1.9.2 FACTORS INFLUENCING TOXICITY

1.9.2.1 Toxic Substance and its Matrix:

A toxic substance can be a gas, solid or liquid. The substance which a toxicant is associated with is called the matrix and may have strong effect on the toxicity of the toxicant.

The pH of toxic substances can greatly influence its absorption and therefore its toxicity.

Many toxic substances exhibit acid-base behaviour and pH is a factor in their uptake. Solubility is an obvious factor in determining the toxicity of systemic poisons. These must be soluble in body fluids or converted to a soluble form in the organ or system through the gastrointestinal tract without doing harm, whereas they would be quite toxic if they could dissolve in body fluids.

For laboratory studies of toxicity, several vehicles are commonly used. Among the most commonly used are water and aqeous saline solution. Lipid soluble substances may be dissolved in vegetable oils. The two major classes used for insoluble substances are the natural gums (examples like Acacia and Tragacanth) and synthetic colloids like methyl cellulose and carboxymethylcellulose.

Some drug formulations contain excipients that have been added to give a desired consistency or form. These excipients have influence upon toxicity. These excipients acts as either binders, lubricants, diluents, preservatives etc.

1.9.2.2 Circumstances of Exposure:

The most important factor of consideration on exposure is the dose. That is whether the toxicant concentration is from pure substance (100%) or a very dilute solution of a highly potent poison. The duration and frequency of

exposure are also important factor. Also important are the exposure site and route of exposure.

1.9.2.3 The Subject and its Environment:

The most important factor under this subject is the species and strains. Included in this are the body mass, sex, age, and degree of maturity. Immunological status as well as the psychological state all affect toxicity observations.

The environmental factors include temperature, pressure and humidity as well as composition of the atmosphere pollutants like ozone or carbon monoxide.

1.9.3 ACUTE TOXICITY

Acute toxicity signifies the toxic effects produced by a single dose or multiple dose of a compound given during a 24 hour period. The effects are usually observed for a period of two weeks after exposure. The route of administration is dependent on the intended use of the chemical. Median lethal dose (LD_{50}) is usually determined in acute toxicity studies which are carried out in the course of safety evaluation of chemicals intended for use as household products, food, additives, cosmetics, drugs etc.

1.9.4 CALCULATION OF LD_{50}

LD_{50} can be calculated using different methods.

1.9.4.1 Graphic Calculation of Miller and Tainter:

In this method, the data (between 10 and 90% effect) are plotted on logarithmic percentage - probability graph paper.

If a straight line fits plotted point satisfactory, the LD_{50} is represented by the dosage value at 50% (probit 5) and is read directly from the plot.

If the percent scale is absent, the probit corresponding to a percent value must be found in table of probits.

1.9.4.2 Arithmetic Method of Karber:

In this method, the difference between 2 consecutive doses and the mean of the number of animals dying at 2 dose levels are calculated and entered into a table.

The sum of the products of the mean dead and the dose difference is divided by the number of animals in e . group. The resulting quotient is subtracted from the least dose that kills all the animals in order to obtain the LD_{50}.

1.9.4.2 Arithmetic Method of Reed and Meunch:

This method employs cumulative values. It is assumed that an animal killed by a certain dose would have died with any higher dose, and that an animal surviving a given dose would have survived any lower dose. Thus, the cumulative dead are recorded by adding successive entries downwards; and the cumulative survivors are recorded by adding successive entries upward.

1.10 AIMS AND OBJECTIVES OF THE STUDY

The literature reviewed showed no pharmacognostic work on the plant. However isolation of lignans with proven antitumor properties from the stem-bark have been reported.

In this work the pharmacognostic studies of the stem-bark as well as evaluation of the diuretic and acute toxicity study of the stem-bark have been examined to find out the rationale of using the plant in traditional medicine as a diuretic. Urine analysis have been carried out to determine the effect on the electrolytes.

CHAPTER TWO

MATERIALS AND METHODS

MATERIALS

(a) Reagents

The reagents used were all of laboratory grades. In all cases requiring use of water, distilled water was used. The reagents used were:-

- Acetic anhydride
- Acetone
- Aniline phthalate
- Alcohol
- Anisaldehyde
- Ammonia solution
- Barfoed's reagent
- Chlor-zinc-iodine
- Chloroform water
- Copper acetate
- 3,5 Dinitrobenzoic acid in 95% alcohol
- Dragendorff's reagent (Bismuth potassium iodine)
- Fehling's solution Iand II
- Ferric Chloride
- Formaldehyde
- Glacial acetic acid
- Hagner's reagent

- Hydrochloric acid
- N/50 iodine
- Lead acetate
- Lime water
- Normal saline
- Mayer's reagent
- Magnesium fillings
- Molisch's reagent (10% solution of alpha-naphthol in alcohol)
- Phloroglucinol
- Ruthenium red
- Sodium acetate
- Frusemide (obtained from lifepharm Ltd.)

(b) Apparatus:

- Pestle and mortar
- Compound Microscope (Wild 11 Switzerland)
- Slides and Cover Slips
- 250ml Beakers
- Measuring Cylinder
- Funnel
- Oven
- Air blower
- Fume Cupboard
- Water bath
- Thermometer

- Syringes and Needles
- Mechanical Shaker
- Plastic Metabolic Cages

(c) Animals

- Mice (16 -27 gm bodyweight)
- Rats (115 -185 gm bodyweight)

METHODS

2.1 PLANT COLLECTION AND IDENTIFICATION

The stem-bark of *Steganotaenia araliacea* was collected in December, 1994 from the University dam area within the main campus of Ahmadu Bello University, Zaria (Nigeria). The plant was collected from its natural habitat.

The plant was identified in the field with the assistant of a traditional medical practitioner (Mal. U. Maiwada) and later by direct comparison with the original herbarium specimen placed in the herbarium of Biological Sciences department, Ahmadu Bello University, Zaria (Nigeria).

2.2 PREPARATION OF HERBARIUM SPECIMEN

The plant parts were arranged between old pieces of newspapers or blotting papers and inserted between more sheets of papers placed on flat board. Another board was placed on top and weighed down with two building blocks. The wet outer newspapers were changed on alternate days until the specimens were completely dry. The dried samples were removed gently off the drying papers and placed on thick plain white mounting sheets cut to

herbarium size. The dried samples were fastened to these fresh sheets with cellotape.

An information card indicating locality, botanical and vernacular names, habitat and date of specimen collection was affixed on each herbarium sheet. These herbarium sheets were later placed in folded cardboard papers and were occasionally placed in the sun to prevent mould growth (Walkers 1963).

2.3 DRYING

The bark was stripped off from the stem, by cutting. The stripped fresh bark were dried under the sun. Quantities of the dried stem-bark were used for this study.

2.4 MACROSCOPICAL EXAMINATIONS OF THE STEM-BARK

The following bark features were used for the characterisation of whole-bark samples.

- Size
- Surface
- Fracture
- Fractured Surface Colour
- Odour and taste of powdered bark (Trease and Evans, 1983) .

2.5 MICROSCOPICAL AND CHEMOMICROSCOPICAL EXAMINATION OF THE STEM-BARK

2.5.1 Microscopical Examination

The powdered bark, transverse, tangential longitudinal, and radial longitudinal sections of the fresh bark were used for this study (Trease and Evans; 1983, Iyengar and Nayak, 1975).

A small quantity f the powdered stem-bark or a thin anatomical section was placed on a clean slide. A few drops of chloral hydrate solution was added and covered with a clean cover slip. The slide was heated for few seconds followed by the addition of few drops of dilute glycerol and the slide observed under a compound Microscope.

2.5.2 Microscopical Measurement Of Some Features Of The Powdered Stem-Bark

Measurement of calcium oxalate, starch grains and fibres of the stem-bark of *Steganotaenia araliacea* were done using micrometry technique, chloral hydrate clarified specimens were used. Staining for lignin was made with phloroglucinol and concentrated hydrochloric acid and iodine solution was used for starch. Twenty-five different readings were taken and the average calculated.

METHOD

This involves calibration of the eyepiece micrometer with the stage micrometer. The stage micrometer is then replaced with a chloral hydrate

clarified specimen. Measurement is then carried out, at magnification x40,and drawn with assistance of camera lucida.

2.6 CHEMO-MICROSCOPICAL EXAMINATION

The anatomical sections of the stem-bark were treated separately with appropriate chemical reagents on microscope slides and observed under the microscope for the presence of chemical substances such as cellulose, gums and mucilages, tannins, oils, starch and calcium oxalate crystals.

2.6.1 Test For Lignin

The powdered bark was mounted in a few drops of phloroglucinol and concentrated hydrochloric acid. A red colour indicates the presence of lignin. The intensity of the red colouration indicates the extent of lignification (Trease and Evans 1983).

2.6.2 Test For Tannins

The powdered bark was mounted in ferric chloride solution. A bluish-black or greenish colour indicates the presence of tannins (Trease and Evans, 1983).

2.6.3 Test For Starch

The powdered stem-bark was, mounted in N/50 iodine. A blue colour indicates the presence of starch (Trease and Evans, 1983).

2.6.4 Test For Calcium Oxalate Crystals

The powdered stem-bark was mounted in chloral hydrate solution. Calcium oxalate crystals were seen as bright structures. After addition of a few drops of concentrated sulphuric acid (80%) and reviewed under the

microscope, the disappearance (dissolution) of calcium oxalate crystals confirms their presence. (Trease and Evans, 1983).

2.6.5 TEST FOR FIXED OILS

The powdered stem-bark and anatomical sections were mounted separately in Sudan IV reagent. A pink colour in any of the structure indicates the presence of oils.

2.6.6 TEST FOR CELLULOSE

The powdered sample was mounted in chlor-zinc-iodine. A blue colour indicates the presence of cellulose (Trease and Evans, 1983).

2.6.7 TEST FOR MUCILAGE

The powdered bark was mounted separately in Ruthenium red. A red or dark-pink colour indicates the presence of mucilages.(Trease and Evans,1983).

2.6.8 TEST FOR SUBERIN

Powdered stem-bark of *S. araliacea* was treated by warming with sudan 1V (in alcohol).Presence of red colour around the periderm and cork cells is indicative of presence of suberin. Suberin is a polysaccharide found mainly in cork cells of barks (Wallis,1967).

2.7 PHYTOCHEMICAL SCREENING OF THE POWDERED STEM-BARK

2.7.1 TEST FOR CARBOHYDRATES

3g of the powdered stem-bark was boiled in 50ml of distilled water on a hot plate for three minutes. The mixture was filtered while hot and the resulting filtrate cooled and used for the following tests for carbohydrates.

2.7.1.1 *General Test for carbohydrates - Molisch's Test*

A few drops of Molisch's reagent was added to 2ml of the water extract as obtained above, then a small volume of conc. sulphuric acid was allowed to run down the test tube to form a lower layer. A purple colour at the interface of the liquids indicates the presence of carbohydrates.

The mixture was then diluted with 5ml of water, shaken and then allowed to stand for 2 mins. A dull violet precipitate also indicates the presence of carbohydrates.

2.7.1.2 *Tests For monosaccharides*

2.7.1.2.1 *Barfoed's Test - (General Test for Monosaccharides)*

1ml of dilute solution of the water extract and 1ml of Barfoed's reagent were taken in a test tube and heated in a water bath. A red precipitate indicates the presence of monosacchorides (Trease and Evans, 1983).

2.7.1.2.2 *Fehling's Test: (Standard Test for Free Reducing Sugars)*

To 2ml of the water extract was added 5ml of a mixture of equal volumes of Fehling's solutions I(A) and II(B) and boiled on a water bath for 2 min. A brick red colour indicates the presence of free reducing sugars.

2.7.1.2.3 *Standard Test for Combined Reducing Sugars*

1ml of the water extract was hydrolysed by boiling with 5ml of dilute hydrochloric acid. This was neutralised with sodium hydroxide solution. The Fehling's test was then repeated. A brick red colour indicate the presence of combined reducing sugars (Trease and Evans, 1983i).

2.7.1.2.4 *Resorcinol or Selivanoff's Test (Test for Ketoses)*

To a small volume of the water extract was added a few crystals of resorcinol and an equal volume of concentrated hydrochloric acid then heated on a water bath. A rose colour indicates the presence of ketoses (e.g. fructose) (Trease and Evans, 1983).

2.7.1.2.5 *Test for Pentoses*

To a small portion of the water extract was added an equal volume of hydrochloric acid containing little phloroglucinol and heated in a water bath. A rose colour indicates the presence of Pentoses (e.g. xyloses) (Trease and Evans, 1983).

2.8 TEST FOR GLYCOSIDES

2.8.1 TEST FOR CYANOGENIC GLYCOSIDES

A small amount of the powdered bark was put in a test tube and sufficient distilled water was added to cover the bark. A prepared moist sodium picrate paper was suspended in the neck of the tube by means of a cork. The tube was placed in a water bath for one hour. A brick-red colour on the paper indicate the presence of cyanogenetic glycosides (Trease and Evans, 1983).

2.8.2 TEST FOR ANTHRAQUINONE GLYCOSIDES

2.8.2.1 Borntrager's Test (To show the presence of free Anthraquinones).

0.5g of the powdered bark was taken in dry test-tube and 10ml of chloroform was added and shaken for five minutes. Extract was filtered while hot and allowed to cool. To the filtrate was added equal volume of 10%

ammonium solution and shaken. A bright pink colour in the upper aqueous layer indicates the presence of free anthraquinones (Trease and Evans, 1983).

2.8.2.2 *Test for Presence of Free and/or Combined Anthraquinones*

0.5g of the powdered bark was boiled with 10ml of 10% hydrochloric acid for about 2-3mins. The extract was filtered while hot and allowed to cool. To this extract was added equal volume of chloroform. The tube was inverted a couple of times to avoid vigorous shaking. The solution was transferred into a separating funnel and the two layers allowed to separate. The lower chloroform layer was poured into a clean test tube and 10% ammonium 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, 1983).

2.8.3. TEST FOR SAPONIN GLYCOSIDES

2.8.3.1 Froth test.

To a small quantity of the powdered bark was added 95% ethanol and boiled. The mixture was filtered while hot and cooled. 2.5ml of the ethanol extract was dissolved in 0ml of distilled water in a test-tube. The test tube was corked and shaken vigorously for about 30 sec. then it was allowed to stand for over half an hour. A honey-comb froth is indicative of the presence of saponin glycosides. (Sofowora, 1982).

2.8.3.2 Haemolytic test.

0.2g of the powdered material was extracted with 10ml warm water, filtered and filtrate retained. 2ml of 1.8% sodium chloride solution was added to two test tubes. To one of the test tube (t^1), 2ml of distilled water was added

and the other (t_2) 2ml of extract 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 the finger-nail and blood was drawn into a small pipette. Five drops of the blood was added to each of the test tube (t_1 and t_2). The tubes were stoppered and inverted gently to mix the contents. Haemolysis in test tube containing extract (t_2) and not in the control (t_1) indicates presence of saponins (Brain and Turner, 1975).

2.8.4 TEST FOR CARDIAC GLYCOSIDES

Extraction

0.5g of the powdered bark was boiled with 10ml of 95% alcohol for 2 mins. The mixture was filtered and cooled. The filtrate was diluted with water and three drops of a strong solution of lead sub-acetate was added. This was mixed thoroughly and filtered. The filtrate was kept for the test (2.8.4.2) 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.

2.8.4.1 Keller-Killani Test

One of the chloroform residue from above was dissolved in 1ml of glacial acetic acid containing a trace of ferric chloride solution.

The solution was carefully poured on the surface of 1ml sulphuric acid already contained in a test-tube to form a separate layer. A blue colour in the upper acid layer and a red colour at the interface of the two layers indicates the presence of digitoxose (Trease and Evans, 1983).

2.8.4.2 *Kedde Test (For Free or Combined Cardenolide Aglycone)*

The reserved filtrate from the extraction 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 indicates the presence of free or combined Cardenolide aglycone (Sofowora, 1982).

2.8.4.3 *Legal Test (for Cardenolide Aglycone)*

The second reserved residue from the extraction process 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 solution was added. A deep red colour indicates the presence of cardenolide aglycone (Sofowora, 1982).

2.9 TEST FOR TERPENES AND STEROLS

Extration

5g of the powdered stem-bark was extracted by maceration with 50ml of ethyl alcohol (95%), filtered and the filtrate was evaporated to dryness. The residue was dissolved in 10ml of anhydrous chloroform and then filtered. The filtrate was divided into two equal portions and the following tests were carried out.

2.9.1 *LIEBERMAM-BURCHAND TEST*

A portion of the chloroform solution was mixed with 1ml acetic anhydride followed by the addition of 1ml of conc. sulphuric acid down the wall of the test tube to form a layer underneath. The formation of a reddish violet colour at the junction of the two liquids and a green colour in the chloroform solution indicates the presence of terpenes (Sofowora, 1982).

2.9.2 SALKOWSKIS TEST

A second portion of the solution was mixed with 2ml of concentrated sulphuric acid carefully so that the sulphuric acid formed a lower layer. A reddish-brown colour at the interface indicates the presence of a steroidal ring (Sofowora, 1982).

2.10 TEST FOR FLAVONOIDS

2g of the powdered bark was completely detanned (Segelman *et al.*, 1971) with acetone. The residue was extracted in warm water after evaporating the acetone on a water bath. The mixture was filtered while hot. The filtrate was cooled and used for the following tests.

2.10.1 Lead Acetate Test

To 5ml of the detanned water extract was added 10% lead acetate solution. A heavy buffe precipitate indicates the presence of flavonoids.

2.10.2 Ferric Chloride Test

To 2ml of the detanned water extract was diluted with distilled water in a ratio of 1:4 and a few drops of 10% ferric chloride solution was added. A green or blue solution indicates the presence of phenolic nucleus.

2.10.3 Shinoda Test

0.5g of the powdered bark was extracted in ethanol by boiling in a water bath for 5min, filtered and cooled. To the extract was added four pieces of Magnesium filling followed by few drops of concentrated hydrochloric acid. A pink or red colour indicates the presence of flavonoids (Geissman, 1961).

2.11 TEST FOR ALKALOIDS

10g of the powdered stem-bark was taken in a small beaker and a strong ammonia solution was added in a quantity sufficient to moisten it and allowed to stand for about 10 mins after thorough mixing of the contents. Sufficient quantity of a mixture of chloroform and ethanol solution (1:1) was added just to soak the powder. The mixture was allowed to stand for 20min. with occasional stirring with a glass rod.

The mixture was filtered through a plug of cotton wool. The marc was washed twice with 2ml of chloroform and the washings combined with the filtrate. The bulk filtrate was concentrated to dryness on water-bath. The residue was cooled and dissolved in 5ml of chloroform .

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, the lower chloroform layer was drained off and discarded. 3ml of chloroform was further added and shaken, drained off and discarded until upper acid layer was colourless.

The acid layer was made completely alkaline with strong ammonia. The extraction with 3ml of chloroform extract 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 neutralising with dilute sulphuric acid.

2.11.1 GENERAL TESTS

To each little amount of the ethanolic residue obtained above was added few drops of the following reagents in separate test tubes;

Mayer's reagents — (Potassium mercuric iodide solution).

Dragendorff's reagent — (Potassium bismuth iodide solution).

Wagner's reagents — (Solution of iodine in potassium iodide).

Hagner's reagent — (Saturated solution of picric acid).

10% tannic acid solution.

The presence of precipitate in most or all of the above reagents indicates the presence of alkaloids (Trease and Evans, 1983).That is :-

Mayer's reagent - white or creamy white precipitate

Drangendorff's reagent - orange or orange-yellow precipitate

Wagner's reagent - light brown or brownish precipitate

Hagner's reagent - yellow precipitate

10% tannic acid solution - buff coloured precipitate.

2.12 QUANTITATIVE EVALUATION OF CRUDE POWDERED STEM-BARK

2.12.1 DETERMINATION OF MOISTURE CONTENT OF THE POWDERED STEM-BARK BY THE "LOSS ON DRYING METHOD"

An evaporating dish was heated to a constant weight in an oven at 105°C and its constant weight was noted. 3g of the powdered stem-bark was accurately weighed into the dish. The evaporating dish with its content was heated to 105°C in the oven. The weight was taken periodically at 30min interval until such a time that two consecutive weight readings is a constant.

The total loss in weight (i.e. the weight of the moisture) was determined by subtracting the constant weight of the dish and powdered stem-bark after heating, from the weight of the dish and content before heating.

The percentage of the moisture content with reference to the initial weight of the powdered stem-bark used was then calculated. This was done by dividing the weight of the moisture by the weight of the drug multiplied by hundred. Three different determinations was done and the average of these was taken as the moisture content of the drug (Trease and Evans, 1983).

2.12.2 DETERMINATION OF TOTAL ASH

A nickel crucible was heated at 105°C to a constant weight and its accurate weight was noted after cooling in the desiccator. 2g of the powdered stem-bark was accurately weighed into the crucible. The crucible and its content were gently heated over a micro-burnsen flame until it was moisture free and completely charred. The micro-burnsen flame was increased until only 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 crucible from the final weight of the crucible and ash (B. P., 1980).

2.12.3 DETERMINATION OF ACID INSOLUBLE ASH VALUE

The crucible with the ash from above was transferred into a beaker containing 25ml of dilute hydrochloric acid. The beaker and its content were boiled for 5min and filtered through an ashless filter paper. The crucible and

the beaker were washed with distilled water and the washings passed through the filter paper. The washings were repeated three times, everytime the washings were passed through the filter paper in a manner as to allow the collection of the residue at the tip of the cone of the filter paper.

The funnel along with the filter paper were dried in the oven at 105°C. The weight of a clean and heated nickel crucible was accurately determined using a sensitive balance. The filter paper with its residual was folded into a small cone and transferred into a crucible. The crucible with the filter paper was heated until the filter paper was completely ashed. Then cooled, weighed and the final weight was noted. T' weight of the residue (ash) was then calculated. This was done by subtracting the weight of the crucible from the constant weight of the crucible and the ash. The acid-insoluble ash value with reference to the initial weight of the powered stem-bark was calculated and expressed in percentage.

The weight of the ash divided by the initial weight of the drug multiply by hundred was taken as the acid-insoluble ash value (B. P. 1980).

2.12.4 DETERMINATION OF EXTRACTIVES

2.12.4.1 Alcohol-Soluble Extractive Value

5g of the powdered stem-bark was accurately weighed into a 250ml stoppered 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 evaporated to dryness. The residue was dried to a constant weight at 105°C 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 from the total weight of evaporating dish and 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).

2.12.4.2 Water-Soluble Extractive Value

The above experiment (2.12.4.1) was repeated using 0.25% chloroform in water instead of 90% ethanol as the extracting solvent. Chloroform added acts as fungustatic. The water soluble extractive value was calculated similarly as in the previous experiment (Brain and Turner, 1975).

2.13 PHARMACOLOGICAL TESTS

2.13.1 Diuretic Activity

2.13.1.1 Test Animals

Adult male and female albino rats (Wistar strain) weighing between 115g - 185g were obtained from the animal house of the Department of Pharmacology and Clinical Pharmacy of Ahmadu Bello University Zaria, they were fed on mouse pellets - which is a concentrate with a high protein content. Water was supplied to the animals in standard drinking bottles.

2.13.1.2 Standardization of frusemide.

In each metabolic cage was placed four albino rats. The rats were observed for 2 days to obtain the mean basal value of urine output. Varying doses of frusemide (10mg/kg, 15mg/kg, 20mg/kg and 25mg/kg) were administered interperitonially to the rats in their separate cages. The urine output over a period of 24 hours was recorded. The most consistent result corresponding to the dose was used as the standard dose to which the extract will be compared.

2.13.1.3 Test for Diuretic Activity

Five metabolic cages were used. In each metabolic cage was placed four albino rats. The rats were separated into metabolic cages capable of collecting uncontaminated urine (void of feacal matter). Normal saline was administered to each group intraperitoneally (IP). Volumes of urine output was measured (in mls) at 3hrs interval for 24 hours.

After 24 hours, one group of four rats were injected with Normal Saline as control, three groups of four rats each were injected with water extract (20mg/kg) methanolic extract (20mg/kg) and ethanolic extract (20mg/kg) respectively, while the fifth group was administered with frusemide, (20mg/kg) used as a standard (Johnson 1992).

The results were tabulated and graphically illustrated as cumulative urine output versus excretion time. Estimation of diuretic activity was based on a modification of the method described by Kau *et. al* (1984).

2.13.2. *EFFECT OF EXTRACT ON URINE ELECTROLYTES*

The urine output of the ethanolic and water extracts was analysed at the Chemical Pathology Department of Ahmadu Bello University Teaching Hospital, Zaria, Nigeria and compared to those of the control and standard.

2.14 ACUTE TOXICITY STUDIES OF THE STEM-BARK OF *S. ARALIACEA*.

2.14.1 *PILOT TOXICITY STUDIES*

A pilot study was conducted to determine the maximum dose of the ethanolic extract which did not cause death (0.5g/kg) and the minimum dose that caused 100% death (2.5g/kg). Other doses 1.0g/kg, 1.5g/kg and 2.0g/kg where selected within the dose range of 0.5g/kg - 2.5g/kg and administered intraperitonially (Johnson Patricia *et. al* 1992).

2.14.2 *ACUTE TOXICITY STUDY*

Male mice (16-27g) were fasted overnight but allowed water. The mice were grouped into 6 mice per cage. The first group of six mice were administered ethanolic extract dose of 0.5g/kg, the second group received dose of 1.0g/kg, the third group were administered 1.5g/kg while the fourth and fifth group were administered 2.0g/kg and 2.5g/kg respectively. The sixth group were administered normal saline to serve as control. Time of onset of signs of toxicity in the mice were recorded using the control group as reference. The dose that killed 50% of the population LD50 were calculated by arithmetic method of Karber, as modified by Aliu and Nwude (1982).

The same procedure was used for the water extract.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 Plant Collection And Identification

The plant material were collected at the University dam area of Ahmadu Bello University, Samaru, Zaria (Nigeria) and fitted the descriptive features of *Steganotaenia araliacea* Hochst (Irvine, 1961; Hutchinson and Dalziel, 1958). The identity of the plant was further confirm by comparison with the herbarium specimen at the Department of Biological Sciences, Ahmadu Bello University, Zaria (Nigeria) where a voucher specimen has been deposited.

3.1.2 PREPARATION OF HERBARIUM SPECIMEN

The specimen were prepared as described. A voucher specimen has been deposited in the herbarium specimen of the Department of Biological Sciences Ahmadu Bello University, Zaria (Nigeria) and another at the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria .

3.1.4 MACROSCOPICAL EXAMINATION OF THE STEM-BARK

Origin and Preparation:

The bark of *S. araliacea* was obtained from the stem as whole bark, that is, consisting of all parts outside the cambium.

Size: 9.0-14.3cm long.

0.2-0.3cm thick.

2.5-3.1cm width.

Shape: Double quill.

Fracture: Short.

Surface: (i) Inner surface :- light brown with striations.

(ii) Outer surface:- dark brown with furrows.

Odour: Pleasant.

Taste: Slightly salty.



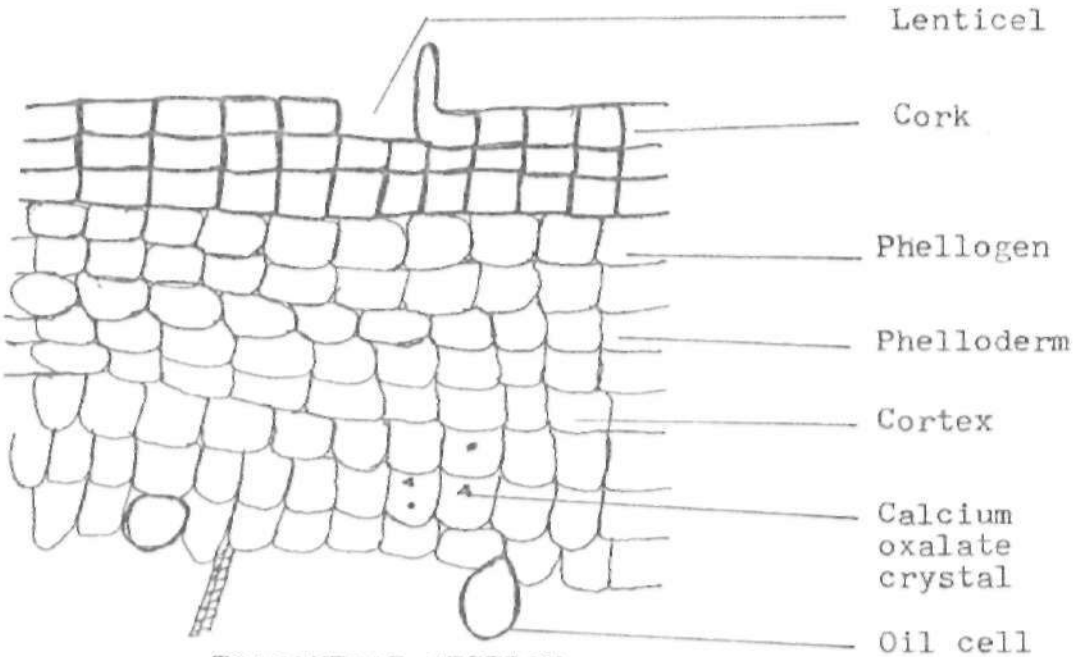
Plate 1: Samples of Stem-bark of *Steganotaenia araliacea*

3.1.5 MICROSCOPICAL AND CHEMOMICROSCOPICAL EXAMINATIONS OF THE STEM-BARK

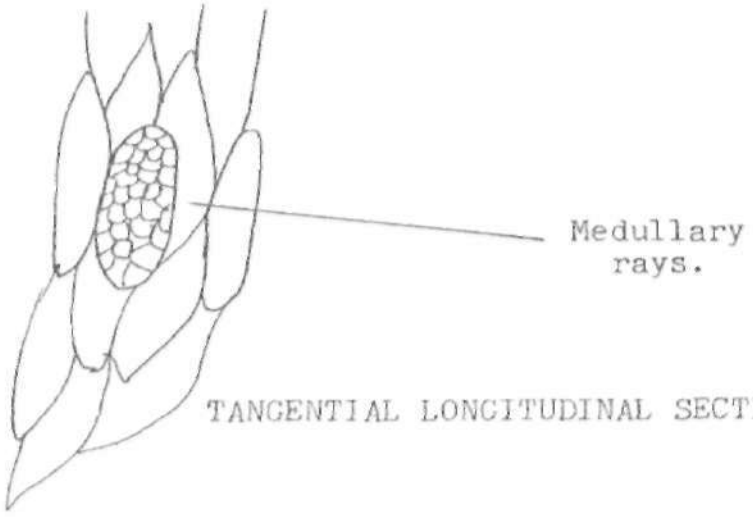
3.1.5.1 Microscopical Examination

The transverse section of the fresh bark is as shown in fig. 3A. The arrangement of various tissues of the bark like the lenticel cork, phellogen and cortex are shown. The cortex area reveal the presence of calcium oxalates and oil cell. The tangential longitudinal section shows medullary ray, Fig. 3B. The Radial longitudinal section (fig 4) showing the arrangement of the medullary ray, cortex and oil cell.

FIGURE 3

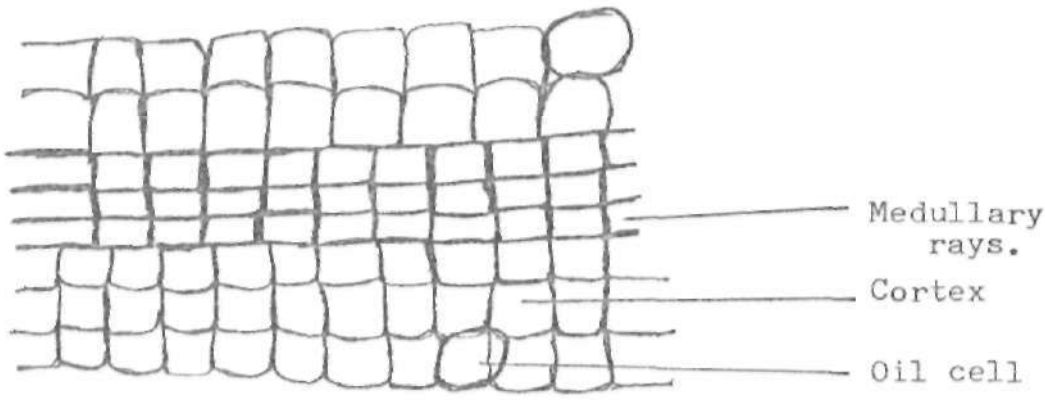


A TRANSVERSE SECTION



B TANGENTIAL LONGITUDINAL SECTION

FIGURE 4



RADIAL LONGITUDINAL SECTION

The microscopical examination of the powdered stem-bark revealed the following features: Starch grains, calcium oxalate crystals, cork cells, secondary cortex and phloem fibres.

Sieve tubes, xylem tissues and aleurone grains were not observed in the samples of the stem-bark examined.

The various microscopical features of the powdered stem-bark are illustrated below:

3.1.5.2 Microscopical Measurement of Some Features of Powdered Stem-Bark

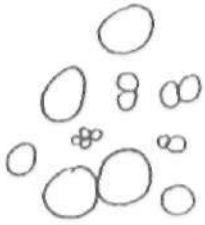
The starch granules were of different shapes and sizes. Occurring singly or in clusters and numerous (fig. 5A). 18.8μ-45.2μ-75.2μ.

The calcium oxalate were in different forms and sizes. They are not many. Some occurs as prisms, clusters or sandy crystals (fig. 5B).

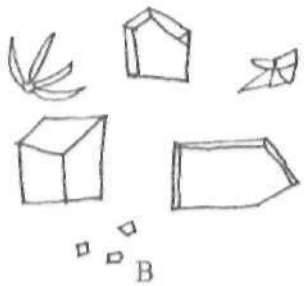
9.4μ-19.7μ-28.3μ

The fibres are fairly numerous with tapering apex. They are nonlignified. There is conspicuous lumen. Some occur singly while others occurs in group (fig. 5 E). 4.0μ-9.9μ-30.0μ

FIGURE 5



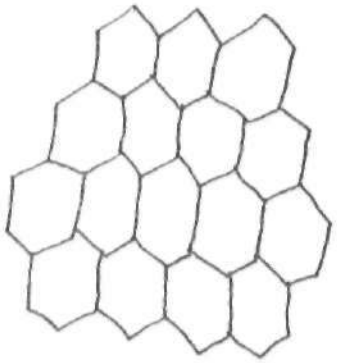
A
STARCH
GRAINS



B
CALCIUM
OXALATES



C
OIL CELLS



D
CORK CELLS
(surface view)



E
FIBRES

3.1.6 CHEMO-MICROSCOPICAL EXAMINATION

3.1.6.1 Test For Lignin

The fibres observed in the transverse section, radial longitudinal section and the powdered stem-bark tested negative for lignin.

3.1.6.2 Test For Tannins

The powdered bark mounted in ferric chloride solution tested negative to tannins. That is there was absence of bluish-black or greenish colouration.

3.1.6.3 Test For Starch

The powdered bark treated with N/50 iodine give blue colouration which indicates presence of starch.

3.1.6.4 TEST FOR CALCIUM OXALATE CRYSTALS

The powdered stem-bark mounted in chloral hydrate solution reveals presence of calcium oxalate crystal which disappears on addition of few drops of concentrated sulphuric acid. Thus, calcium oxalate crystals are present.

3.1.6.5 TEST FOR FIXED OILS

The powdered stem-bark and anatomical sections treated with sudan IV reagent give pink colour structures. Thus oils are present. This account for the odour observed with the bark as its the volatile oils that give the odour to plants.

3.1.6.6 Test For Cellulose

The powdered sample mounted in chlor-zinc-iodine produce blue colour which indicates the presence of cellulose .

3.1.6.7 Test For Mucilage

The powdered bark mounted separately in Ruthenium red did not produce red or dark pink colouration. This indicates absence of mucilage .

3.1.6.8 Test For Suberin

Powdered stem-bark of *S. araliacea* treated by warming with Sudan IV (in alcohol) did not produce red colour around the periderm and cork cells. This indicates absence of suberin .

3.1.7 PHYTOCHEMICAL SCREENING OF THE POWDERED STEM-BARK.

3.1.7.1 CARBOHYDRATE TEST

3.1.7.1.1 General Test - Molisch's Test:

On addition of Molisch's reagent to 2ml of the water extract followed by small volume of concentrated sulphuric acid along side of test-tube, a purple colour at the interface of the liquid indicates the presence of carbohydrates.

3.1.7.1.2 Test for monosaccharides.

3.1.7.1.2.1 *Barfoed Test-General Test for Monosacharrides.*

When 1ml of the dilute solution of the water extract was heated with 1ml of Barfoed's reagent in a water bath, no red precipitate was observe hence

monosaccharides are absent.

3.1.7.1.2.2 *Fehlings Test: Standard Test for Free Reducing Sugars*

Brick red colour was observed when 2ml solution of the water extract was heated with 5ml mixtures of Fehling's I(A) and II(B) and boiled in a water bath for 2 mins.

3.1.7.1.2.3 *Standard Test for Combined Reducing Sugars*

When 1ml of water extract was treated with dilute hydrochloric acid, neutralised with sodium hydroxide solution before the addition of Fehling's reagent, no brick red colour was observed showing the absence of combined reducing sugars.

3.1.7.1.2.4 *Resorcinol or Salivanoff's Test (Standard Test for Ketoses)*

On addition of few crystals of resorcinol and an equal volume of concentrated hydrochloric acid and heated on a water both, no rose colour was observed. This shows absence of ketoses.

3.1.7.1.5 Test for Pentoses

Treatment of small portion of water extract with equal volume of hydrochloric acid with little phloroglucinol did not produce rose colour. Hence pentoses e.g. xyloses are absent.

3.1.8 TEST FOR GLYCOSIDES

3.1.8.1 Test For Cyanogenic Glycosides

The suspended sodium picrate paper did not produce any brick-red

colour. Thus indicating the absence of cyanogenetic glycosides.

3.1.8.2 TEST FOR ANTHRAQUINONE GLYCOSIDES

3.1.8.2.1 Borntrager's Test (Test for free anthraquinones)

Filtrate treated with equal volume of 10% ammonium solution did not produce bright pink colour hence absence of free anthraquinones.

3.1.8.2.2 Test for Presence of Free And/or Combined Antraquinones

Result was negative for free and or combined antraquinones.

3.1.8.3 TEST FOR SAPONIN GLYCOSIDES

3.1.8.3.1 Froth test

After shaken the powdered stem-bark with water for 30 sec. a honey-comb forth was observed and persisted after half an hour. Thus saponin glycosides are present.

3.1.8.3.2 Haemolytic test

The test tube containing the extract (t2) showed haemolysis while the test tube containing distilled water did not produce any haemolysis indicating the presence of saponins.

3.1.8.4 TEST FOR CARDIAC GLYCOSIDE

3.1.8.4.1 Keller-Kiliani Test

There is absence of digitoxose due to negative result of the test.

3.1.8.4.2 Keddle Test (for free or combined cardenolide aglycone)

A brownish colour was observed which indicates the absence of free or

combined cardenolide aglycone.

3.1.8.4.3 Legal Test (for Cardenolide aglycone)

A deep red colour which indicates presence of cardenolide aglycone was observed.

3.1.9 TEST FOR TERPENES AND STEROLS

3.1.9.1 Liebermam-Burchard Test

A reddish violet colour at the junctions of the two liquids and a green colour in the chloroform solution was observed. Thus terpenes are present. This support the fact that terpenes occurs universally within the family umbelliferae.

3.1.9.2 Salkowski's Test

No reddish-brown colour at the interface was observed thus there is absence of steroidal ring.

3.1.10 TEST FOR FLAVONOIDS

3.1.10.1 Lead Acetate Test

On addition of 10% lead acetate solution to the detanned water extract, no coloured precipitate was observed. This shows the absence of flavonoids.

3.1.10.2 Ferric Chloride Test

On addition of ferric chloride to a detanned solution of the extract, no green or blue solution was observed. Thus shows the absence of phenolic nucleus.

3.1.10.3 Shinoda Test

On addition of four pieces of magnesium fillings and few drops of concentrated hydrochloric acid, no pink or red colour was observed. Thus flavonoids are absent in material tested.

3.1.11 TEST FOR ALKALOIDS

3.1.11.1 General Tests

No precipitate was observed on treatment with all the reagents used for alkaloidal test. Thus indicates the absence of alkaloid.

3.1.12 QUANTITATIVE EVALUATION OF CRUDE POWDERED STEM-BARK

3.1.12.1 *Determination Of Moisture Content*

The moisture content of *S. araliacea* is 10.7% w/w (Appendix No 1).

3.1.12.2 Determination Of Ash Value

The total ash value observed for *S. araliacea* stem-bark was found to be 11% w/w.(Appendix No 2).

3.1.12.3 Determination Of Acid-Insoluble Ash Value

It measures the amount of silica especially sand and saliceous earth present in drug. The acid insoluble ash was found to be 1.5% w/w. (Appendix No. 3).

3.1.12.4 DETERMINATION OF EXTRACTIVES

3.1.12.4.1 Alcohol-Soluble Extractive Value

The alcohol-soluble extractive value was found to be 7% w/w (Appendix No. 4).

3.1.12.4.2 Water-Soluble Extractive Value

The water-soluble extractive value was found to be 35% w/w (Refer Appendix No. 5).

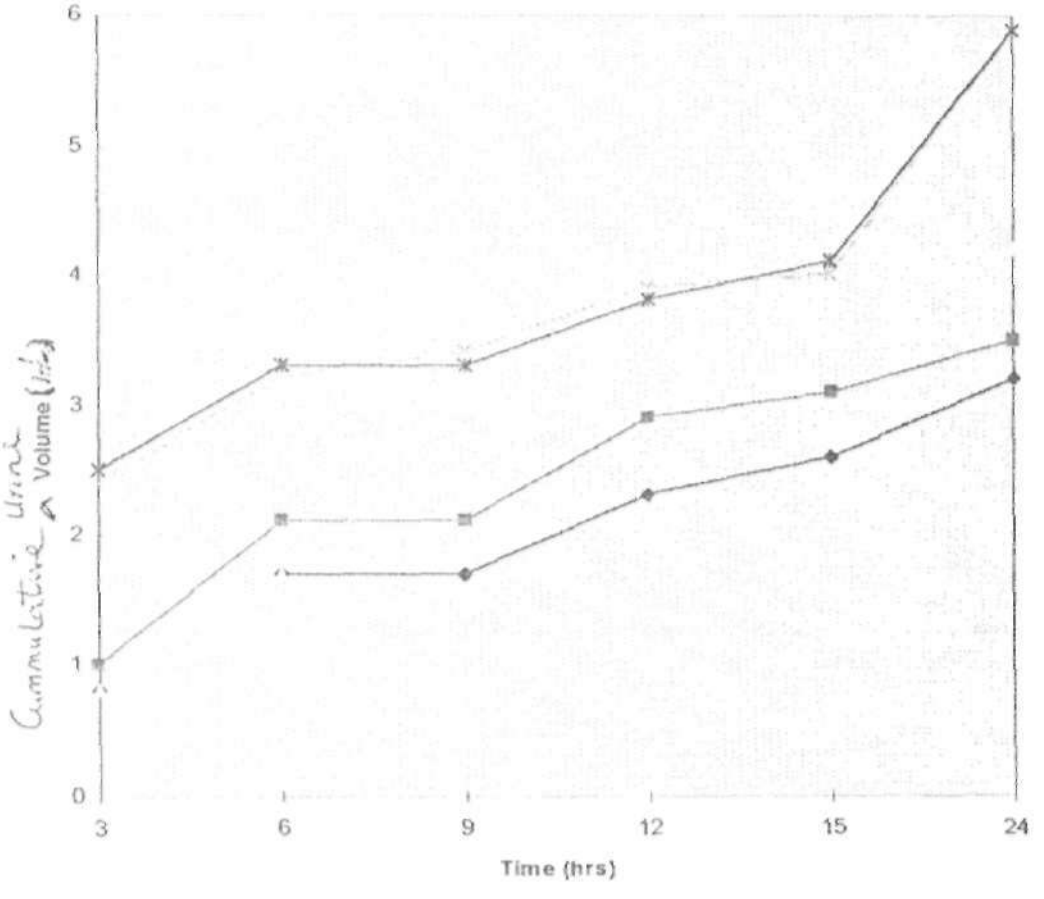
3.1.13 PHARMACOLOGICAL TESTS

3.1.13.2 Frusemide Standardization

Table I: **Cummulative Urine Output against time (hrs) of various doses of frusemide compared with normal saline.**

Time (hrs)	Control	FRUSEMIDE			
	A	10mg/kg B	15mg/kg C	20mg/kg D	25mg/kg E
3	0.8	1.0	0.8	2.4	2.5
6	1.7	2.1	1.7	3.2	3.3
9	1.7	2.1	2.8	3.4	3.3
12	2.3	2.9	3.7	3.9	3.8
15	2.6	3.1	3.9	4.0	4.1
24	3.2	3.5	4.2	5.9	5.9

: Cummulative Urine Volume (ml) against Time (hrs) of Various Doses of Frusemide Compared with Control



Key

a = Control
b = 10 mg/Kg
c = 15 mg/Kg
d = 20 mg/Kg
e = 25 mg/Kg

3.1.13.3 DIURETIC ACTIVITY

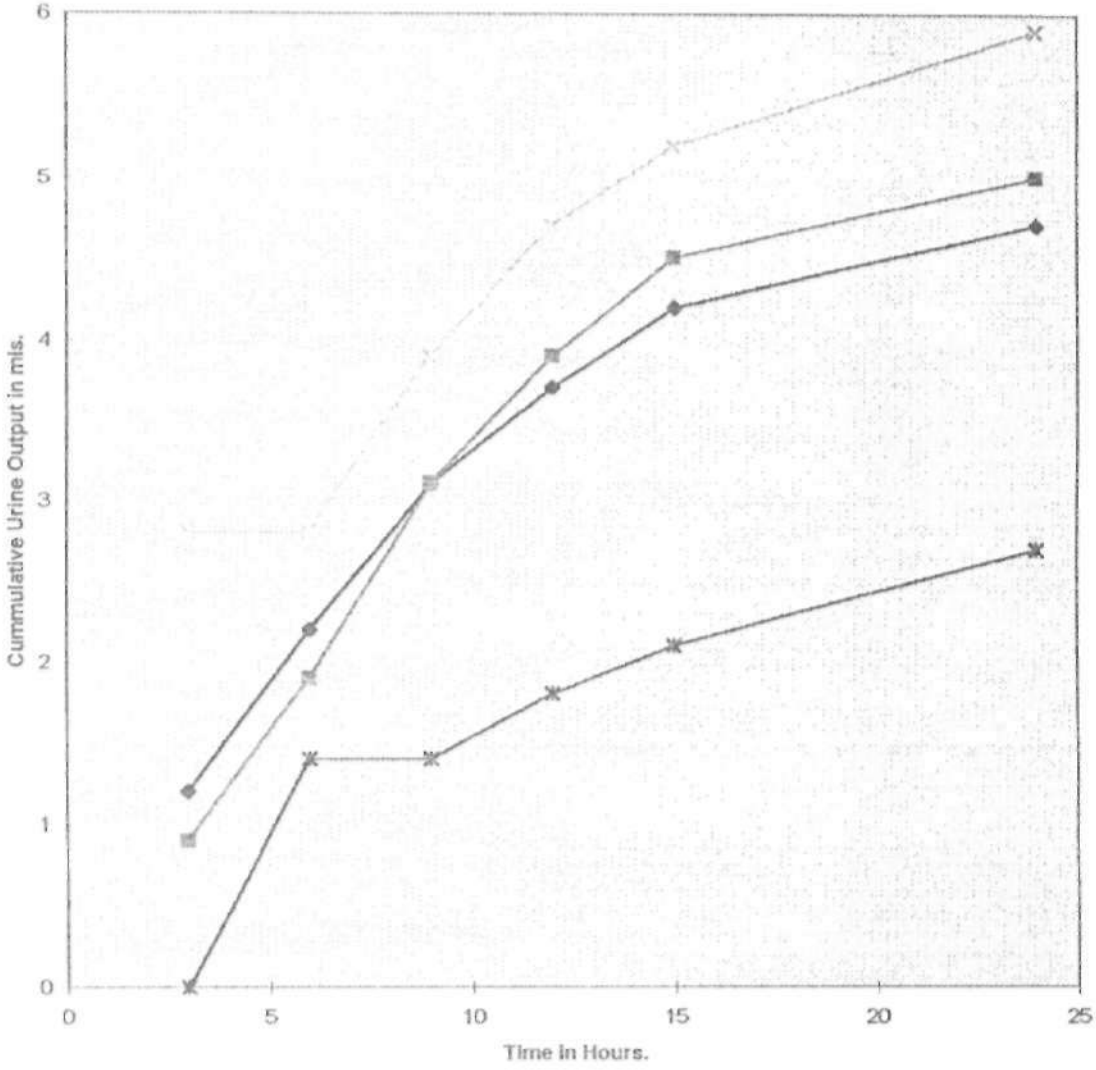
Table II:

DIURETIC ACTIVITIES OF 20MG/KG OF VARIOUS EXTRACTS OF STEM-BARK OF *STEGANOTAENIA ARALIACEA* AND FRUSEMIDE.

Time (Hrs)	Cummulative Urine Output (in mls)				
	Control	Water extract	Methanolic extract	Ethanolic extract	Frusemide
3	NIL	1.2	0.9	1.4	2.8
6	1.4	2.2	1.9	2.5	2.8
9	1.4	3.1	3.1	3.4	3.9
12	1.8	3.7	3.9	4.3	4.7
15	2.1	4.2	4.5	4.8	5.2
24	2.7	4.7	5.0	5.3	5.9

FIGURE 7

Cummulative Urine Output Vs. Time of various Solvent Extract of *Steganotaenia araliaceae* Stem-bark.



KEY

Control,

Water extract,

Methanolic extract

Ethanolic extract

Frusemide

Table III:

ELECTROLYTE CONTENTS OF URINE EXCRETED BY RATS TREATED WITH ETHANOLIC AND WATER EXTRACTS OF THE STEM-BARK OF *STEGANOTEANIA ARALIACEA* AGAINST CONTROL AND STANDARD

Sample	Na^+ mMol/L	K^+ mMol/L	Cl^- mMol/L
Control	117	96	12
Ethanolic Extract	72	127	12
Water Extract	102	99	12
Frusemide	98	90	16

Dose = 20mg/kg.

3.1.14 ACUTE TOXICITY STUDIES OF THE STEM-BARK OF *STEGANOTAENIA ARALIACEA*

Ethanolic Extract

Table IV:
SIGNS OF ACUTE TOXICITY OF ETHANOLIC EXTRACT OF STEM-BARK OF *STEGANOTAENIA ARALIACEA*

Signs		Approximate time (in hrs) of appearance of signs of toxicity after injection of toxic dose 2.0mg/kg
1.	Micturition and Defeacation	½
2.	Prostration	½
3.	Respiratory Distress	2
4.	Sedation	2½
5.	Coma	3½-10
6.	Death	5-18

Table V:

CALCULATION OF LD_{50} USING ARITHMETICAL METHOD OF KARBER

Group	Dose g/kg	Dose Difference	Dead	Mean Dead	Product of dose difference and mean dead
1	0.5	0.5	0	0.0	0.0
2	1.0	0.5	0	0.5	0.25
3	1.5	0.5	1	2.5	1.25
4	2.0	0.5	4	5.0	2.50
5	2.5		6		4.00

$$LD_{50} = 2.5 - \frac{4.00}{6}$$

$$= 2.5 - 0.667$$

$$= 1.83 g/kg$$

Water Extract

Table VI:

SIGNS OF ACUTE TOXICITY OF WATER EXTRACT OF STEM-BARK OF *STEGANOTAENIA ARALIACEA*

Signs		Approximate time (in hrs) of appearance of signs of toxicity after injection of toxic dose 2.0mg/kg
1.	Micturition and Defeacation	½
2.	Prostration	½
3.	Respiratory Distress	1½
4.	Sedation	2
5.	Coma	5½-10
6.	Death	4-16

Table VII:

CALCULATION OF LD_{50} USING ARITHMETICAL METHOD OF KARBER

Group	Dose g/kg	Dose Difference	Dead	Mean Dead	Product of dose difference and mean dead
1	0.5	0.5	0	0.0	0.0
2	1.0	0.5	0	1.0	0.5
3	1.5	0.5	2	3.0	1.5
4	2.0	0.5	4	5.0	2.5
5	2.5		6		4.5

$$LD_{50} = 2.5 - \frac{4.5}{6}$$

$$= 2.5 - 0.75$$

$$= 1.75 g/kg$$

3.2 DISCUSSION

The values of microscopical measurement of some of the features of the powdered bark as shown above are not distinctly characteristic and therefore cannot be singly used for identification. For example, the values of starch grain is 8.8μ-45.2μ-75.2μ

There is a wide difference between 18.8μ, which is the smallest grain, as compared to 75.2μ measured or the largest starch grains.

Similarly, the values for the fibre is 4.0μ-9.9μ-30.0μ

The difference between the smallest fibre measured 4.0μ and the largest fibre 30.0μ is significantly large.

Phytochemical studies of the powdered stem-bark reveals the presence of carbohydrate, saponin and terpenes. However, test for tannins, flavonoids, anthraquinones and alkaloids are negative.

The family Umbelliferae do not have alkaloid as a common constituent as it is only a single taxon, *Conium maculatum* that is reported to contain alkaloid (Heywood 1971).

The distribution of terpenes and mannitol is well documented in the family umbellifferae.

Quantitative evaluation of a drug requires that it be identified in both its entire and powdered form by means of its morphological and cytomorphological characters. These features enable the nature and origin of the drug to be ascertained. However, evaluation procedures which will indicate their acceptability by criteria other than their morphology are also

used (Brian and Turner, 1975).

The following evaluative parameters were determined for the stem-bark of *Steganotaenia araliacea*. The values are average of three separate determinations.

Table VIII:

PHYSICAL PROPERTIES OF THE POWDERED STEM-BARK OF *STEGANOTAENIA ARALIACEA*

Test	% w/w
Moisture Content	10.7
Ash Value	11.0
Acid-insoluble Ash Value	1.5
Alcohol-Soluble Extractive Value	7.0
Water-Soluble Extractive Value	35.0

Determination of moisture content is important because the presence of high amount of moisture encourages micro-organisms that interfered with the drug, activation of enzymes, causes chemical reactions and causes decrease in quantity of the drug by increasing the weight of the drug. For these reasons, the moisture content of drugs is recommended to be kept reasonably low (Shellard, 1958).

The moisture content of 10.7% w/w as obtained for *S. araliacea* is within acceptable limit (B.P. 1980).

The ash value determination is important because ash is obtained from both physiological source, from the plant growth itself, and the non-physiological source which arise from extraneous matter. In controlling ash value, it is the non-physiologcal source that must be regulated.

Extractive value determination is designed to measure amounts of constituents which are present in a solvent under given condition and applied to vegetable drugs for which there is no suitable method of chemical or biological assay (African Pharmacopoeia, 1986). The alcohol and water soluble extractives signify the presence of fair amounts of constituents that are leached into alcohol and water respectively.

Diuretics are normally viewed in two ways: one refers to increase in urine volume per se and the other refers to the net loss of solute and water (De Stevens, 1963, Irwin, 1990).

These two processes results in suppression of renal tubular reabsorption of electrolytes, water and low molecular weight organic compound into the blood stream, consequently promoting the formation of urine. (De-stevens, 1963; Milton et al, 1970).

The diuretic action of the ethanolic , methanolic and water extracts were investigated in comparison to the action of frusemide. The choice of ethanol and methanol along with water extract is to find out the possible advantage any commonly used solvent will have in the constituent(s)

responsible for diuretic activity. The result as shown in Table II indicates that water extract is less active as compared to methanolic and ethanolic extracts. The latter showing more activity than methanolic extract. Frusemide acts by inhibiting electrolyte reabsorption in thick ascending limb of the loop of henle. It induces a brisk and significant diuresis within a short time of administration. If increases chloride (Cl) excretion with relatively no effect on potassium ion (k^+). However, the mechanism of diuresis in either of the extract have not be ascertained from this investigation.

The electrolyte analysis Table III shows an increased potassium ion excretion with the ethanolic extract. While the water extract shows no effect on the electrolytes when compared to control. This implies individuals using ethanolic extract of *S. araliacea* will benefit from potassium ion supplement.

The signs of acute toxicity as shown for ethanolic extract and water extract, Tables IV and VI respectively shows earlier toxicity signs with water extract than ethanolic extract. Examination of the varying doses of extract shows increase in toxicity with increase in dose. Consequently , the toxicity of both extract are dose-related.

The LD_{50} for the ethanolic extract and water extract is 1.83g/kg and 1.75g/kg respectively. This means that water extract is more toxic than ethanolic extract. However, these values falls within the WHO (Dubois and Geiling, 1959) of moderately toxic range (0.5-5.0g/kg). In traditional medical practice, only a small quantity of the water extract, 35-0ml is administered twice daily, morning and afternoon (Mal. Maiwada personal communication).

This may probably explain why there has not been recorded cases of severe poisoning in literature.

Toxicity evaluation is being carried out to identify the safety of the desired and undesired constituents present in extracts often administered.

CHAPTER FOUR

CONCLUSION

This research has given further detailed knowledge on the Pharmacognostic characters of the stem-bark of *S. araliacea*.

The phytochemical studies reveals the presence of terpenes, saponins and carbohydrates. Alkaloids, tannins, flavonoids, cardiac glycosides are however absent. Diuretic activity of the stem-bark reveals that water extract to be less diuretic than methanolic extract which is also less than ethanolic extract. The urine analysis revealed increase potassium ion excretion with ethanolic extract which is not the case with water extract. The LD_{50} of water extract is less than ethanolic extract, 1.75g/kg and 1.83g/kg respectively. This implies that water extract is more toxic than ethanolic extract.

Finally, the results and data in this research are useful in preparation of monograph of *S. araliacae*. The data presented in this thesis support the use of this plant as a diuretic agent by traditional medical practitioners.

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APPENDIX

EVALUATION OF THE POWDERED STEM-BARK OF *STEGNOTAENIA ARALIACEA*

1. **Determination of Moisture Content**

3g of powdered stem-bark was used.

(a) Constant weight of crucible (g) = 29.0g

(b) Weight of crucible and the powdered stem-bark before heating (g) = 32.0g

(c) Constant weight of crucible and the powdered stem-bark after heating (g) = 31.68g.

$$\text{Moisture Content (\%)} = \frac{b - c}{\text{Initial wt of drug}} \times 100$$

$$= \frac{32 - 31.68}{3} \times 100$$

$$= 10.7\% \text{ w/w}$$

2. **Determination of Ash Value**

2g of the powdered stem-bark was used.

(a) Constant weight of crucible (g) = 31.85g

(b) Weight of crucible and powdered stem-bark before heating to ash (g) = 33.85g

(c) Constant weight of crucible and ash (g) = 32.07g

$$\text{Ash Value (\%)} = \frac{c - a}{2} \times 100$$

$$= \frac{32.07 - 31.85}{2} \times 100$$

$$= \frac{0.22}{2} \times 100$$

$$= 11\% \text{ w/w}$$

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

(a) Constant weight of crucible = 31.85g

(b) Weight of crucible and ash after acid treatment = 31.88g

Acid insoluble ash Value

$$= \frac{\text{Step (b) - Step (a)}}{\text{Weight of Drug}} \times 100$$

$$= \frac{31.88 - 31.85}{2} \times 100$$

$$= \frac{0.03}{2} \times 100$$

$$= 0.015 \times 100$$

$$= 1.5\% \text{ w/w}$$

DETERMINATION OF EXTRACTIVES

4. Alcohol-Soluble Extrative Values

5g of the powdered stem-bark was used in 100ml of 90% ethanol.

(a) Constant weight of crucible (g) = 38.75g

(b) Weight of crucible and Content before heating (g) = 43.75g

(c) Constants weight of crucible and content after heating = 38.82

Alcohol Extractive Value

$$= \frac{c - a}{5} \times 5 \times 100$$

$$= \frac{38.82 - 38.75}{5} \times 5 \times 100$$

$$= \frac{0.07}{5} \times 5 \times 100$$

$$= 7\% \text{ w/w}$$

5. **Water-Soluble Extractive Values**

5g of the powdered stem-bark was used in 100ml of 0.25% chloroform in water.

(a) Constant weight of crucible (g) = 40.63

(b) Weight of crucible and content before heating (g) = 45.65

(c) Weight of crucible and Content after heating (g) = 40.98

Water-Soluble Extractive Value

$$= \frac{c - a}{5} \times 5 \times 100$$

$$= \frac{40.98 - 40.63}{5} \times 5 \times 100$$

$$= \frac{0.35}{5} \times 5 \times 100$$

$$= 35\% \text{ w/w}$$