PHARMACOGNOSTIC AND ANTI-ULCER EVALUATIONS OF *CASSIA SIEBERIANA* D.C. (FABACEAE) ROOT

BY

HADIZA BELLO

DEPARTMENT OF PHARMACOGNOSY AND DRUG DEVELOPMENT
FACULTY OF PHARMACEUTICAL SCIENCES
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA

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BY

Hadiza BELLO (Bsc Biology A.B.U.2006)
(P13PHPD8005)

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FACULTY OF PHARMACEUTICAL SCIENCES
AHMADU BELLO UNIVERSITY,
ZARIA NIGERIA

NOVEMBER, 2015

DECLARATION

I declare that the work in this dissertation entitled 'Pharmacognostic and Anti-ulcer Evaluations of *Cassia sieberiana* D. C. (Fabaceae) Root' has been carried out by me in the Department of Pharmacognosy and Drug Development. The information derived from the literature have been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

BELLO HADIZA		
Student	Signature	Date

CERTIFICATION

This dissertation entitled 'Pharmacognostic and Anti-ulcer Evaluations of *Cassia sieberiana* D.C. (Fabaceae) Root' by Hadiza BELLO (P13PHPD8005), meets the regulations governing the Award of Master Degree of Science in Pharrnacognosy of the Ahmadu Bello University, and is approved for its contribution to knowledge and literacy presentation.

DR. ZAINAB MOHAMMED
Chairman, Supervisory Committee (Signature) Date

DR. UMAR ADAM. KATSAYAL
Member, Supervisory Committee (Signature) Date

DR.GARBA IBRAHIM
Head of Department (Signature) Date

PROF. KABIR BALA
Dean, Postgraduate School (Signature) Date

DEDICATION

I dedicate this work to the entire family of Alhaji Ibrahim Adoke(Late) may your soul continue to rest in perfect peace (Amin).

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ABSTRACT

Cassia sieberiana D.C. (Fabaceae) though widely used in traditional medicine as an analgesic in dysmenorrhoea, ulcer and general body pains, there is no published report on it standardization. Pharmacognostic evaluation of the root will therefore assist in standardization for quality, purity and sample identification of the plant drug. Evaluation of fresh and powdered root of this plant was carried out to determine its pharmacognostical profile, including macroscopic, microscopic, histochemical and quantitative parameters. Chemical profile of the root was also determined using thin layer chromatography (TLC). The safety profile of the root was determined using acute toxicity testing. Whereas the anti-ulcer properties of the ethanol root extract was determined using ethanol induction model in laboratory rats. The root was found to be cylindrical in shape, brown in colour, with characteristics odour and very bitter taste when chewed. When the root was cut transversely it revealed the presence of cork cells, narrow cortex, beneath which is a layer of parenchyma cells, prominent vessels, biserate medullary rays and small pith. Cellulose, hemicellulose and lignified cell wall were present. Calcium oxalate crystals are prisms in shape, measuring 19.29 ± 1.4 µm. Starch grains are oval in shape measuring 10.64 ± 0.82 µm. Moisture content was found to be 6.2 ± 0.3 (%w/w), total ash was 5.8 ± 0.43 (%w/w), acid-insoluble ash was 1.0 ± 0.24 (%w/w), water-soluble ash was 3.5 ± 0.24 (%w/w), alcohol soluble extractive was 12.0 ± 0.47 (%w/w), water soluble extractive was 6.0 ± 0.47 (%w/w), Swelling index was 3.5 ± 0.00 (%w/w),foaming index was less than 100,tannins content, was 39 (%w/w), Bitterness value, was 8400 unit/g. Phytochemical constituents include anthraquinones, flavonoid, saponins, steroid/terpenoids, tannins, and cardiac glycoside.Oral LD_{50} value was above 5000mg/kg and the tested rats did not show any clinical sign of toxicity with oral administration of the extracts. The mean ulcer indices

calculated were found to be 15.2 ± 0.68, 4.6± 0.21, 0.0±0.0 for ethyl acetate fraction and 10.27 ± 0.46, 1.4 ± 0.06 for butanol at 200, 400 and 800 mg/kg respectively when compared with 53. 8 ± 2.40 mg/kg in ethanol treated rats. At 800 mg/kg the preventive index% for ethyl acetate fraction was 100% and butanol fraction was 97%, these were found to be higher than cimetidine standard drugs with 88% preventive index. The roots of *C.sieberiana* produced significantly and dose dependently anti-ulcer activity. Therefore *C. sieberiana root* could bedeveloped as anti- ulcer drugs and can also be incorporated into the second editions of the Nigeria Herbal Pharmacopoeia (NHP) and the West African Herbal Pharmacopoeia (WAHP).

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LIST OF ABBREVIATION

CSE- *Cassia sieberiana* extract

P.I- Preventive index

U.I- Ulcer index

EAA – Ethylacetate

CHL – Chloroform

MeOH –Methanol

TLC –Thin layer chromatography

T S –Transverse section

SEM –Standard error of mean

EAF-Ethylacetate fraction

NBF-Butanol fraction

RQF-Residual aqueous fraction

CHAPTER ONE

1.0 INTRODUCTION

1.1 Peptic Ulcer

Peptic ulcer, also known as *ulcus pepticum* is a conglomerate of heterogeneous disorders which manifest itself as a break in the lining of the gastro intestinal mucosa that is usually acidic and thus extremely painful(Cullen *et al.*, 1997).

The aetiology of gastric ulceration is multi-factorial and not clearly defined, but some predisposing factors have been implicated. These include duration of starvation, nature of food ingested, bile reflux (Gerald, 1981), lessened mucosal resistance (Cho and Ogle 1992), alteration of gastric mucosal blood flow (Guidobono *et al.*, 1997), over dosage and or prolonged administration of Non-Steroidal Anti-inflammatory Drugs (NSAIDS), (Tanaka *et al.*, 1983).

As many as 80% of ulcers are associated with *Helicobacter pylori*, a spiral-shaped bacterium, that lives in the acidic environment of the stomach. This organism weakens the protective coating of the stomach and first part of the intestine and allows damaging digestive juice to eat away at the sensitive lining below (Burger *et al.*, 2002.).

The typical symptom of ulcer is pain about 2 hours after eating. Digestive acids acting on a meal irritate the ulcer after most of the meal has moved to the jejunum area of the small intestine. The primary risk associated with an ulcer is the possibility that it will erode (perforate) entirely through the stomach or intestine wall. The gastro-intestinal contents could then spill into the body cavities causing a massive infection, called peritonitis. In addition, an ulcer may erode a blood vessel, leading to massive blood loss (heamorrhage) (Fox *et al.*, 2007).

1.2 Mortality and Morbidity in Peptic Ulcer Disease.

Peptic ulcer is one of the most common, chronic gastrointestinal disorder in modern era. Now it has become a common global health problem affecting a large number of people worldwide and also still a major cause of morbidity and mortality **(**Chan and Leung, 2002).

About 6,000 people die of ulcer related complications in United State (Sandler *et al* 2002). It has been shown that the incidence of peptic ulcer disease has generally declined, and is approximately 1 death per 100,000 cases. Its prevalence has been estimated as 5 – 10% in the adult population in different countries (Bernersen*et al.,* 1990). There is decline at peptic ulcer over the past decade with decrease being more marked for gastric than duodenal ulcers which are four times more common in men than gastric ulcer, but both are becoming common in the elderly (Brewer *et al*., 1994). Peptic ulcer causes significant morbidity which is mainly related to pain and hospitalization for complication (Chan and Leung 2002).

However, the number of patients affected by bleeding and perforation has not changed significantly (Jordan*et al.,* 1988), such that perforated ulceration affect up to 20% of peptic ulcer disease patients (Ashley *et al.,* 1999). The incidence of complicated ulcer disease especially of duodenal ulcer significantly decreased in some populations (Hosseini*et al.,* 2008).Frequency of peptic ulcer disease shows the following.

- One year point prevalence is 1.8%
- Life time prevalence is approximately 10%
- Peptic ulcer disease (PUD) affects approximately 4.5 million people annually.
- Lifetime prevalence is approximately 11 – 14% for men.
- Lifetime prevalence is approximately 8 - 11% for women (Sung *et al* 2010).

Several studies shows that age had a major role for prediction of mortality in patients with ulcers. The mortality rate following surgery in elderly patients with perforated peptic ulcer is higher than in younger patients (Kocer *et al*., 2007). The duration of symptoms is the most important factors in the elderly that influences the prognosis after surgery and is responsible for the high mortality rate (Uccheddu *et al*., 2003).

Perforated peptic ulcer disease carries considerable mid-term mortality that can be affected by age, treatment delay, size of ulcer and admission to intensive care unit (ICU) further-more, it is accompanied by a high frequency of post-operative complications that should be controlled by improving surgical skills, wound care and administrative regulations (Sung *et al.*, 2010).

1.2 Treatment of Peptic Ulcers

The majority of patients with peptic ulcer disease(PUD) are treated successfully, medically with cure of *H pylori* infection and/or avoidance of NSAIDs.Along with appropriate use of anti-secretory therapy.Surgery has a very limited role in the management of PUD.(Wolfe Mmetal, 1999).

Younger patients with ulcer-like symptoms are often treated with antacids or H_2 antagonists before an esophagogastroduodenoscopy(EGD) is undertaken. Bismuth compound may actually reduce or even clear organisms, though the warning labels of some bismuth subsalicylate products indicate that the product should not be used by someone with an ulcer (Najm, 2011).

Patients who are taking non-steroidal anti-inflammatories (NSAIDs) may also be prescribed a prostaglandin analogue (Misoprostol) in order to help prevent peptic ulcers, which may be a side-effect of the NSAIDs (Harley 2013).

When *H. pylori* infection is present, the most effective treatment are combinations of 2 antibiotics (e.g. Clarithromycin, Amoxicillin, Tetracycline, Metronidazole) and 1 proton pump inhibitor (PPI), sometimes together with a bismuth compound. In complicated treatment-resistant cases, 3 antibiotics (e.g. amoxicillin plus clarithromycin plus metronidazole) may be used together with a PPI and sometimes with bismuth compound. An effective first-line therapy for uncomplicated cases would be Amoxicillin plus metronidazole plus pantoprazole (PPI). In the absence of *H.pylori*, long-term higher dose PPIs are often used (Sheila, 2011).

Treatment of H. pylori usually leads to clearing of infection, relief of symptoms and eventual healing of ulcers. Recurrence of infection can occur and retreatment may be required, if necessary with other antibiotics. Since the widespread use of PPI's in the 1990s, surgical procedures (like"highly selective vagotomy") for uncomplicated peptic ulcers became obsolete (Lassen *et al.,* 2006).

Perforated peptic ulcer is a surgical emergency and requires surgical repair of the perforation. Most bleeding ulcers require endoscopy urgently to stop bleeding with cautery, injection, or clipping (Sheila, 2011).

1.4 Statement of Research Problem

C. sieberiana plant has been used extensively without standardization or concerns as to its level of toxicity.Peptic ulcer disease is an important cause of morbidity and mortality throughout the world affecting the lives of millions of people in their everyday life. In the United States, approximately four million people have peptic ulcers (duodenal and gastric), and 350, 000 new cases are diagnosed each year. Around 180,000 patients are hospitalized yearly (Bardhan, 2008). The burden of peptic ulcer

disease is much greater in Nigeria and is a major cause of morbidity and significant cause of mortality (Mbagwu and Adeyemi 2006).

1.4 Justification

Scientific evaluation of *C. sieberiana* is important to the discovery of novel drugs and also helps to assess it safety and efficacy associated with the use of either herbal preparation or conventional drugs of plant origin. However, a systematic standardization including pharmacognostical and physio-chemical study is still lacking. The present investigation on root of *C. sieberiana* is therefore necessary to establish certain pharmacognostic standards which would help in its identification, as well as in checking for adulteration, quality and purity control of the drug,this study will greatly help in quality assurance of finished products of herbal drugs.

Even though the plant has been used extensively, little is known about the possible side effects associated with its use.Owing to the persistent problem of reoccurring ulcers after treatment and the occurrence of several adverse reactions with the anti-ulcer agent, new approach is constantly being pursued. Different therapeutic agents especially plant extracts are currently undertaken in various studies primarily to evaluate its anti- ulcerogenic potentials.

C. sieberiana roothas been used in folk medicine for the treatment of gastric ulcers (Sam *et al.,* 2011). It is against this background that this study was designed to provide scientific information on the efficacy and use of the root extract in rats,and also to find justification for its traditional uses in Nigeria.

1.5 Hypothesis

- *C. sieberiana* have certain pharmacognostic characters that need to be standardised.

- *C. sieberiana* root extracts has a dose-dependant anti-ulcer activity.

1.6 Aim and Objective of theStudy

- The present investigation is aimed at establishing certain pharmacognostic standards and antiulcer effect of *C. sieberiana* root on ethanol –induced gastric ulcers in wistar rat.

The study is designed to determine the following specific aims,

- To evaluate the pharmacognostic characters of *C. sieberiana*root.
- To evaluate the chemical parameters of *C. sieberiana* extracts.
- To study its anti-ulcer properties using ethanol gastric ulcer induction model in rat.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of the Genus Cassia

Cassia is a genus of Fabaceae in the subfamily Caesalpinioideae. "Cassia'' is also the English name of *Cinnamomum aromaticum* in the Laureceae (from which the spicy cassia bark is derived) and some other species of Cinnamomum. In addition, the genus *Cassia* was for long ill-delimited with regards to the related cassiinae – especially senna-, (ILDIS 2005). About 600 species are mostly distributed in tropics and subtropics (Burkill 1995, Ghledhill 1991).

Many references to some sort of "Cassia" are less than clear. Usually it is hard or even impossible to determine if a species of the present genus, of Senna, or of Cinnamomum is meant. "Cassia gum" for example is not made from Cassia in the present sense, but from Chinese Senna (Sicklepod, *Senna obtusifolia*), formerly known as *Cassia obtusifolia*, *C. toroides* and several other taxa in the present genus(Burkill, 1995).

Cassia is not in frequently encountered in texts on herbalism and alternative medicine that is usually Senna. However, while both genera contain plants with medical properties, those of Senna seem to be more pronounced (or are simply better studied). Still, Golden shower tree (*C. fistula*) is unequivocally indentified and considered very potent in Ayuredic medicine, where it is called aragvadha ("disease killer,").It contains elevated quantities of anthraquinones and consequently is mainly useful against gastro-intestinal conditions (e.g. constipation or acid reflux) and to stop bleeding (Kerharo,1974).

Ecologically, Cassia tolerates a wide range of climates and temperature though it tends towards loving warmth.*Cassia*are mostly trees, shrubs, herbs, leaf paripinnate or rarely

reduced to phyllode, stipules of various shapes, petiole glands often present. Inflorescence are axillary or terminal raceme, terminal panicle or flowers subsolitary axillary, bracts and bracteoles variable. Calyx are 5, imbricate tube short, Petal are 5, imbricate, subequal or lower most larger, stamen 10, all perfect or sub equal or the upper ones smaller or imperfect or only 5 stamen, anthers, uniform or upper ones smaller dehiscing by an apical pore or short slit,ovary sessile or stipitate, ovules numerous, style with terminal stigma. Fruit or woody, often two valved, sometimes indehiscent rarely winged lengthwise seeds are mostly compressed endosperm (Arbonnier, 2004).

TAXONOMY .

Kingdom:Plantae

Subkingdom:Varidae Plantae

Phylum: Euphyllophytina

Infraphylum: Radiatopses

Class: Magnoliopsida

Subclass:Rosidae

Superorder:Fabanae

Order: Fabales

Family:Fabaceae

Subfamily:Caesalpinioideae

Tribe:Cassiae

Genus: *Cassia*

Species: *sieberiana*

Binomial: *Cassia sieberiana*D.C.(ILDIS, 2005).

2.2 Description of *Cassia sieberiana*

The vernacular names of *C. sieberiana*include West African Laburnum, African Laburnum, drumstick tree (English). Its vernacular names in Nigeria include "Marga" in Hausa, the Fulani people called it "margaje,"in Kanuri it is called "Kiskatigrai" and in Yoruba it is called "Ifo" or 'Aridan tooro".

The plant is widely distributed in the Southern Sahel and Sudan Savanna from Senegal to Cameroon, Gambia East to Democratic Republic of Congo and Uganda. (Micheal, 2004, Von Maydell, 1990).*C. sieberiana* are mostly shrub or small tree and grows up to 15 – 20m high with dropping branches bole short, twisted, bark fissured, grey to brown, with blackish stripes, young branches densely shortly hairy, the leaves are arranged spirally, paripinnately compound with 5 – 14 pairs of leaflets, stipules narrowly triangular, 1.2mm long, caducous leaflets elliptical to ovate, 3.5-10cm x 2-5cm apex rounded to acute, shortly hairy(Von Maydell, 1990).

Inflorescence an axillary pendulous raceme up to 35-45cm long, bracts soon falling, flowers bisexual, slightly zygomorphic, 5 – merous, sepals elliptical, 5-8mm long, slightly hairy, petals oblong to almost circular, 2-3.5cm long, bright yellow; stamen 10, free, ovary superior, sessile ,style slender, much longer than the petals, fruit 9, cylindrical pod 40-60 (-90)cmx c 1.5cm, transversely partitioned, dehiscent by 2 valves, black many seeded with seeds embedded in yellow pulp seeds ellipsoid,8-9mm

long, rusty to dark browns, glabrous. Seedling with epigeal germination (Brenan, 1967; Burkill, 1995).

The plant was described by Gledhill (1991) as an open Savannah tree found in dried area of the forest and thickets. In West Africa *C. sieberiana* flowers in March – April, just before the rainy season when new leaves have formed. The fruits ripen in August-October in West Africa and in September – February in Uganda *C. sieberiana* does not form root nodules (Arbonnier, 2004).

Ecologically, it occurs in tree or shrub Savanna with less than 800mm annual rainfall. Acid sandy soil is preferred. Propagationis mainly by seed. Ripe fresh seeds have nearly 100% viability. One kg of seeds contains 7,000 – 16,500 seeds. Treatment with sulphuric acid is recommended before sowing older seeds. Passage of seeds through cattle intestines also hastens germination and enhances the distribution of seeds in grazed areas. Marcotting and side – grafting are feasible for vegetative multiplication (Arbonnier, 2004).

C. sieberiana is a host of the groundnut bruchid (*Caryedon serratus*), a major storage pest of groundnut, and of the bean flower thrips (*Megalurothrips sjostedti*) a pest of several pulse crops (Burkill, 1995).

Pod of *C. sieberiana* are harvested by hand and the seeds are extracted manually as well. For harvesting the roots, the plant has to be dug up (Elujoba *et al*., 1999).

C. sieberiana is rather common and does not seem to be endangered. However, uprooting is detrimental for the population and is reason for concern in Burkina Faso(Nacro and Millogo-Rasolodimbi, 1993).

However in Nigeria, it is found in the North-West like Zamfara, Zurumi and forest reserve near Sokoto. It is found widely distributed in Borno, Yobe, Bauchi and some part of Adamawa State in the North Eastern part of Nigeria. It is mostly found in Agodi in Ibadan and Awka near Onitsha in the South – West and South – East respectively (Keay *et al.*,1964).



Plate I: *Cassia sieberiana* plant showing the leaves.
Source: Natural habitat during field survey 2014.



Plate II: Cassia sieberiana root.
Source: Natural habitat during field survey 2014.

2.3 Ethnomedicinal Uses of *Cassia sieberiana*

C. sieberiana is commonly used for the treatment of many illnesses especially in the Tropics where the leaves, roots and pods are widely used in traditional medicine. The entire plant is purgative and diuretic. For instance, the aqueous extracts of the roots, stem bark and the fruit pulp have been used traditionally in North-East Nigeria for the treatment of inflammatory conditions, tiredness and joint pains (Modusolumuo *et al*., 1999). The extracts are used to treat fever, malaria, diarrhea, leprosy, bilharzias, stomach pain and as a diureticand dewormer (Dalziel, 1956; Gledhill, 1991; Tamboura *et al*., 2005). Other uses include improvement of lactation after child birth and treatment of rheumatic condition. Herdsmen in some countries including Nigeria, use the stem bark in the treatment of jaundice (Modusolumuo *et al*., 1999).It is also used as a vermifuge, laxative or for wound dressing (Aubreville, 1950; Shahina, 1989).

In Senegal an infusion of the entire plant is given against all children's diseases. The aqueous root extract is also used traditionally to treat pain and inflammation (Kerharo,1974).

In Uganda, powder of different parts of the plant is used to cure toothache, and when mixed with butter it is used to treat skin diseases(Neuwinger, 2000).

In Burkina Faso a steam bath of leafy twigs boiled in water is prescribed to help against malaria attacks and fever, the liquid is also taken. An infusion of the leaves sweetened with honey is taken against stomach-ache, ulcers and diarrhoea. Boiled and squeezed fresh leaves are applied as poultice in pleurisy or burns. Gonorrhea in women is treated by taking leaf powder with food. Capsules made from root bark are prescribed against (HIV/AIDS). The yellow pulp around seed and infusion of the pods is taken as a laxative. Crushed roots are rubbed on the temples to treat headache and debarked roots

are boiled with bark of *Terminalia macroptera* Guill & Perr to combat eczema. Also in Burkina Faso a pinch of powdered dried decorticated roots taken at the end of each meal is said to prevent malaria. The root wood is used as chewsticks (Ra'ed, 1999).

In Benin the twigs are used to treat sleeping sickness. The roots, boiled in water, are used to treat haemorrhoids, bilharzia, leprosy, dropsy and bloody dysentery (Kerharo,1974).

In Coted'Ivoire, the decoction is taken in large doses to treat intestinal worms including tapeworms. An infusion of the root bark is employed against veneral disease, sterility and dysmenorrhoea. After soaking the roots in water, the liquid is used for bath against tiredness and for body massage. A decoction of the roots is considered an aphrodisiac, while the roots and seeds are used as fish poison in Coted' ivoire and Nigeria(Adjanohoun and Aké Assi, 1979)

2.4 Biological Activity of *Cassia sieberiana*

C. sieberiana is savannah tree widely used for the treatment and prevention of various diseases. The phytochemical analysis of the root showed the presence of flavonoids, anthracene derivates and non- hydrolysable tannins (Kerharo, 1974).

Phytochemical analysis on the root and fruit pulp of the plant has been reported to show the presence of three active principles; tannins, saponins and alkaloid, which are of varying concentrations(Modusolomuo *et al.*,1999). Anti- microbial propertiesof the plant has been reported to be due to the presence of triterpenoids and saponins (Mahato *et al.*,1988). It is known that phytochemicals confirm pharmacological relevance on plants generally (Ojo *et al.*, 2008).

Previous studies showed that ethanolic root extract of *C. sieberiana* had an anti-parasitic effect, myorelaxant and anti- spasmodic activity (Fall *et al.,* 2005). It has been reported that *C. sieberiana* extracts had anti- microbial activity against *Neisseria gonorrhoeal, Herpes simplex* virus type 1 and African swine fever virus (Silva *et al*, 1997a).

Analgesic and anti-inflammatory activities of aqueous root extract of *C. sieberiana* were also repoted by Guato Yoro *et al.*,(2009). The flavones from the leaf extractcaused diueresis and have antibacterial and anti-inflammatory activity. In-vitro test only showed a low activity of the extracts against trypanosomes.Leaf extracts were found to be active against *Staphylococcus lutea, Mycobacterium phlei, Bacillus subtilis* and *Proteus sp*. but not against *Staphylococcus albus, Pseudomonas aeruginosa* or *Escherichia coli* (Silva *et al*., 1997b).

2.5 The Use of Extracts and Plant Remedies in Treatment of Ulcer

A number of antiulcer drugs such as the H_{2-} receptor antagonists, proton pump inhibitor and cytoprotectants are available for ulceration, but the drugssometimes have side effects like arrhythmias, gynaecomostia, enterochromaffin, hyperplasia and hemotopoietic (Akthar *et al*.,1992) Alternative approach in recent times is the research of medicaments from medicinal plants. The use of phyto-constituents as drug therapy to treat major ailments has proved to be clinically effective and relatively less toxic than the existing drugs and also reduces the offensive factors serving as a tool in the prevention of peptic ulcer (Jainu *et al.,* 2006). In this modern era also, 75-80% of the world populations still use herbal medicine mainly indeveloping countries. For primary health care because of cost effectiveness, better cultural acceptability, better compatibility with the human body and lesser side effects. The chemical constituents

present in the herbal medicine or plants are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body (Kamboj *et al*., 2000). Natural products from plants are a rich resource used for centuries to cure various ailments. The use of natural medicine in the treatment of various diseases like peptic ulcer is an absolute requirement of our time (Sasmal *et al*., 2007).

Many studies indicate that plant products are potential agents for healing ulcers and largely preferred because of the absence of unwanted side effects and their effectiveness (Jhansi *et al.*, 2010).

Traditional forms of medicine practiced for centuries in Africa and Asia are being scientifically investigated for their potential in the treatment of ulcer related disorder (Borelli, 2000).

A larger number of medicinal plants and dietary nutrients have been shown to possess gastro-protective activity (Akthar *et al.,* 1992). Nigeria medicinal plants and their derivatives have been an invaluable source of therapeutic agents, to treat various disorders including peptic ulcer (Keay, 1989).Various plants like *Anogeissus latifolia, Alchornea castaneaefolia, Utleria salicifolia, Solanum nigrum, Ocimum sanctum, Asparagus racemosus, Scopariadulcis, Byrsonima crassa* etc. and their phyto-constituents proved active in antiulcer therapy. (Sen *et al*., 2009)

Several plant species like *Garcinia eambogia, Terminalia chebula, Napoleona vogelii, Polyalthia congifolia, Allophylus serratus, Allium sativum, Mours alba, Kielmeyera Coriacea, Cissampelos mucronata, Eruca Satina, Bauhinea racemosa, Dodonaea*

viscose, Ageratum conyzoides, Ficus arnottiana, Mouriri pusa, Piper nigrum, Solanum toruum, have all been reported to show encouraging activities (Jhansi *et al.*, 2010).

Deglycyrrhizinated Licorice (DGL) is often suggested as a treatment for stomach ulcers (Borelli, 2000). Although evidence as to whether it works is mixed, few studies have found that DGL and antacid help treat ulcers as well as some prescription drugs.

Cassia singuena leaf extract using ethanol induced Gastric ulcer model in Rat was investigated to have anti-ulcer activity (Ode andAsuzu2011)

Many scientist around the world have recently reported on Antiulcer activity of medicinal plant viz; anti-ulcerogenic activity of two extracts of *Parquetina nigrescens* and their effects on mucosal antioxidants defense system on ethanol induced ulcer in rats (Kayode *et al.,* 2009).

Garlic oil on ethanol induced gastric ulcer in rats has been reported to have anti-ulcer activity (Khoslap *et al.,* 2004).*Allophylus serratus*plant isalso reported have potential anti- ulcerogenic activity (Dharmani *et al.,* 2005).

Peptic ulcer however, can be treated at home using some natural remedies given below;

- The most effective home remedy for treating peptic ulcer is to eat bananas everyday. It is an excellent way to neutralize the hyperacidity of the gastric juices.
- Cabbage and carrot when mixed as juices have been found to be beneficial in treating peptic ulcer.
- Combining carrot juice with spinach or-beet and cucumber is effective in treating peptic ulcer.

- Lime is also beneficial in curing peptic ulcer. The citric acid and mineral salt present in it help treat the ulcer.
- Pineapple is fairly well endowed with glutamine, a compound with experimentally verified anti-ulcer effect. Pineapple also contains bromelain, a general digestive aid.
- Ginger can also be helpful when it comes to ulcers. Japanese researchers who conducted several studies on this spice found that it contains at least six anti-ulcer compounds (Oguntola, 2010)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Solutions, Chemicals and Reagents.

Freshly prepared solutions and analytical grade chemicals were used in all the experiments.As showed in table 3.1.

Table 3.1: List of the reagents used and their manufacturers

Chemical/Reagents	Manufacturers
Acetic acid,Alluminium chloride, Magnesium chloride, Methanol, Potassium hydroxide.	BDH Chemicals Ltd., Poole, England.
Chloroform, Dragendroff's, Ethanol (absolute), Ethyl acetate, Ferric chloride ,Sodium hydroxide, Formalin.	May and Baker Ltd.Dagenham England
Hydrochloric acid, Sulfuric acid, Ferric chloride	Fisons Plc., Scientific Equipment Division, Liughborough, LEII Org., England
Cimetidine	Surelife Pharmaceutical Industry Ltd. Nigeria
Distilled water	Biological Sciences Laboratory, A.B.U Zaria.
Iodine	Fisher Scientific Company, Chemical, Manufacturing Division
Safe drinking water(Faro table water)	Adama Beverages Ltd, Yola Nigeria.
Gelatin, Anisaldehyde , Chloral hydrate, Glycerol Sudan red, Vanillin.	Obtained from Pharmacognosy/drug development laboratory A.B.U. Zaria.

3.1.1 List of Apparatus and Equipments

Chromatographic chamber

Conical flasks

Crucible

Desiccator

Disecting board and kit

Filter paper (Ashless)

Filter paper (wahtman No.1)

Flat bottomed dish

Glass stoppered conical flask

Hand gloves

Heater

Measuring cylinder

Mechanical shaker

Micropipette

Microscope

Needle

Pencil

Ruler

Sieves (250 µm)

Slides and cover slips

Sprayer

Syringes 1ml (26G), 2ml, 5ml.

TLC plates (coated with silica gel)

3.2 Plant Collection and Preparations

3.2.1 Collection and Identification of Plant sample

Fresh *C. sieberiana* root was collected in Giwa town, Giwa Local Government Area of Kaduna State, in April, 2010.The plant was taxonomically authenticated by Mal Umar Shehu Gallah with Voucher specimen number 900202 deposited at the Herbarium, Department of Biological Sciences,Ahmadu Bello University, Zaria, Nigeria.

3.2.2. Preparation of Plant Materials

The roots, was removed from the plant and washed in clean water to removed sand, sliced into pieces and air dried for two weeks. It was then pulverized into coarse powder with mortar and pestle and stored in cellophane bag at room temperature until required for experiment. Sampling of plant material was done by passing the coarse powder through wire sieves used to sieve powdered medicinal plant materials with nominal aperture size expressed in µm. A 250µm sieve size powder was used to test for degree of fragmentation(sieve test) (WHO, 2011).

3.3. Pharmacognostic Studies of the Roots of *Cassia sieberiana*

3.3.1. Macroscopic Examination of root of *C. sieberiana*

Macroscopic identity of medicinal plant materials were based on shape,size,colour,surface characteristics,texture,fracture characteristics, odour and taste of fresh and powdered root. The macroscopic characters of the samples were carried out based on the method described byWallis, (1985), Evans (2008) and WHO(2011).

3.3.2.Microscopic Examinations of the root of *Cassia sieberiana*

Microscopic examinations on the fresh and powdered root were conducted to assess their anatomical features (WHO2011).

3.3.2.1 Transverse section of the root

Transverse section of the root was prepared as described by Drury and Wallington (1980). The fresh roots were fixed in FAA (formalin: acetic acid: alcohol: 1: 1: 18)upon collection from the field, following the fixation, the plant specimen was dehydrated. Gradual dehydration was performed by immersing the fixed tissues in a series of mixtures of alcohol and distilled water with increasing alcohol concentration (30%, 50%, 70%, 95%, absolute alcohol two times), for two hours each in the graded solution. Alcohol is removed from the tissue and clearing obtained by using a graded series of alcohol-chloroform solution. The plant specimen was transferred from absolute alcohol into a solution of alcohol and chloroform (3:1, 1:1, and 1:3)in turn of two hours each, and finally immersed in pure chloroform andleft over night. Once chloroform had completely replaced alcohol, paraffin was added slowly to infiltrate the tissue and remove chloroform, this was done until it gelled and was allowed to stand at room temperature for four hours. It was then placed in an embedding oven and maintained at 60^0 C until it melted. Block of paraffin was made using an L- shaped mould. After the paraffin wax was hardened, the paraffin block was trimmed by cutting away the excess paraffin; the trimmed block was placed on a piece of chock and mounted on a microtome, the microtome was set to cut sections at 12 µm thicknesses. Ribbons obtained were mounted on glass slides and held in place with Mayer egg albumen. Drops of distilled water were added to spread the ribbon on the slide. The sections were dewaxed in xylene, 2 changes of five minutes each then hydrated in absolute ethanol for 2 minutes, then 95%, 70%, 50% and 30% ethanol. The tissues were then stained and differentiated. The tissues were placed in Safranin for 30 mins, washed in water for 2 mins and then differentiated with 0.5% HCl in 70% ethanol briefly for five seconds.

Counter staining was done in fast green for few seconds and washed in tap water after which dehydration was done in 30%, 50%, 70%, 95% and absolute ethanol for 2mins in each solution. Clearing was done in xylene twice for 5 mins and to complete the preparation of the slide, cover slips were mounted over the tissue using dysterene platiciser xylene (DPX) and dried over a slide dryer at 40^0 C. Upon drying, the slides were examined under the microscope and observations made were recorded.

3.3.2.2 Histochemical detection of cell contents and cell walls

The powdered root was treated separately with appropriate chemical reagents on microscopic slide and observed under the microscope for the presence of chemical substances such as cellulose, hemicelluloses, lignin, calcium oxalate crystals, calcium carbonate, starch,mucilage, tannins, fixed oils and fats. (WHO 2011; Evans, 2008)

(a) Cell Content Materials

(i) Test for Starch

The powdered root material was stained with N/50 iodine on a glass slide. One drop of glycerol was then added and the sample observed under the microscope. Appearance of blue – black coloration will indicate presence of starch grains (WHO 2011).

(ii) Test for tannins

The powdered root was mounted in 5% ferric chloride: one drop of glycerol was then added. The sample was observed under the microscope. Appearance of blue - green colour indicate the presences of tannins (WHO 2011).

(iii) Test for fixed oils and fats

The powdered material was placed on a slide and a drop of Sudan (IV) reagent was added. Appearance of pinkish colour indicates the presence of oils (WHO 2011).

(iv) Test for calcium oxalate and calcium carbonate

The powdered root was cleared with chloral hydrate after which it was mounted in glycerol. Some bright crystals were observed, two drops of 80% sulphuric acid was then added to the sample. Disappearance of the crystals on addition 80% sulphuric acid without effervescence indicatesthe presences of calcium oxalate, while disappearance of the crystals on addition of 80% sulphuric acid with effervescences indicateethe presence of calcium carbonate (WHO 2011).

(iv) Test for hydroxyanthraquinoes

The powdered root was mounted on slide and a drop of potassium hydroxide(55g/l) was added. A red stain indicates the presence of 1,8-dihydroxyanthraquinones.

(v)Test for proteins

The powdered sample is mounted with picric acid a yellow stain on the microscopic structures indicate the presence of protein. A red stain with Million's reagent would also indicate the presence of protein.

(b) Cell Wall Material

(i) Test for cellulose

The powdered material was placed on a slide and a drop of N/50 iodine was added followed bydrop of 66% sulphuric acid. Appearance of blue color indicatesthe presence of cellulose (Evans, 2008).

(ii) Test for Hemicellulose

The powdered root was treated with N/50 iodine solution, appearance of blue colour indicate hemicelluloses (WHO 2011).

(iii) Test for lignin

The powdered root material was placed on a slide and a drop of phloroglucinol was added followed by one drop of conc. HCl. Appearance of red coloration on any tissue indicates the presence of lignin (WHO 2011).

(iv) Test for suberin or cutin

The powdered root material was treated with Sudan (iii), mounted in glycerin and warmed slightly. Appearance of red stains indicates suberin or cutins (WHO 2011).

(v) Test for mucilage

The powdered material was placed on a slide and a drop of ruthenium red was added. Appearance of pink color indicates the presence of mucilage and some gums (WHO 2011).

3.3.3 Quantitative Evaluation of Powdered roots of*C. sieberiana.*

Quantitative analysis was carried out on powdered drug using the modified methods described by WHO (2011). Three different determinations were carried out for each parameter and the average was taken as shown in appendix two. The detailed procedure of each of these parameters are given below;

3.3.3.1Moisture content

The moisture content of *C. sieberiana* was determined by loss on drying method. 3.0g of the root powder was accurately weighed and placed in clean, dried evaporating dish of known weight. They were placed in an oven at 105^0C for 30 minutes the weight of drug and the dish determined and returned to oven. The weight was taken subsequently after every 30 minute until the weight become constant. The weight of water lost during drying was calculated with reference to the original weight of the plant as follows (Evans, 2008).

$$\% \text{ Moisture content} = \frac{\text{Weight of moisture}}{\text{Original weight of sample}} \times 100$$

3.3.3.2 Determination of total ash

For detection of the total ash, 2 g of powdered root was placed in a suitable tarred crucible of silica previously ignited and weighed. The powdered root was spread into an even layer and weighed accurately. The materials were incinerated by gradually increasing the heat, not exceeding 45°C until free from carbon, cooled in a desicator, weighed and percentageash was calculated by taking in account the difference of empty weight of crucible andthat of crucible with total ash as follows (Evans 2008)

$$\text{Total ash} = \frac{weight\ of\ residue}{weight\ of\ sample} \times 100$$

3.3.3.3 Acid insoluble ash

The ash obtained as above was boiled for 5min with 25ml of dilute hydrochloric acid; the insoluble matter was collected on an ash less filter paper, washed with hot water and ignited toconstant weight. The percentage of acid-insoluble ash with reference to the air-dried root was calculated as follows (Evans 2008).

Total ash = $\frac{weight\ of\ residue}{weight\ of\ sample} \times 100$

3.3.3.4 Water soluble ash

The ash obtained as in 3.3.3.2 above was boiled for 5 minutes with 25 ml of water; insoluble matter collected in an ash less filter paper, washed with hot water, and ignited for 15 minutes at a temperature not exceeding45^{0}C. The weight of the insoluble matter was subtracted from the weight of the total ash; the difference in weight represents the water-soluble ash. The percentage of water-soluble ash with reference to the air-dried root was calculated as follows (Evans, 2011)

Total ash = $\frac{weight\ of\ residue}{weight\ of\ sample} \times 100$

3.3.3.5 Alcohol soluble extractive value

Five grams (5g) of coarsely powdered air-dried drug was macerated with 100 ml of methanol (70%) in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. It was then filtered rapidly; taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish at 105°C to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated as follows (Evans 2008).

Alcohol- soluble extractive = $\frac{\text{Weight of residue in 20ml extract}}{weight of powdered} \times 100$

3.3.3.6 Water soluble extractive value

Five grams (5g) of coarsely powdered air-dried drug was macerated with 100ml of chloroform water (1:400) in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent. 25ml of the filtrate was evaporated to

dryness in a tarred flat bottomed shallow dish a 105°C to constant weight and weighed. The percentage of water-soluble extractive was calculated as follows (Evans 2008).

Water- soluble extractive = $\frac{\text{Weight of residue in 20ml extract}}{weightofpowdered} \times 100$

3.3.3.7 Determination of swelling index

Two grams(2g) of fine (250µm) size powder of *C. sieberiana* root was introduced into 25ml glass stoppered measuring cylinder, 25ml of water was added and shakened thoroughly.The shaking was repeated after 10 minutes for 1 hour and then allowed to stand for 3 hours at room temperature.

The ml in volume was measured that was occupied by the plant material including any sticky mucilage. The mean value of the individual determinations was calculated (WHO 2011).

3.3.3.8 Determinations of Foaming Index

One grams (1g) of powder of *C. sieberiana* root was transferred to a 500ml conical flask containing 100ml of boiling water and was allowed to boiled for 30 minutes. It was then cooled and filtered into a 100ml flask and sufficient water was added through the filtrate to dilute to volume.

The decoction was poured into the 10 stoppered test-tube in successive portion of 1ml, 2ml, 3ml up to 10 ml. and the volume was adjusted in each tube with water to 10ml. It was then shaken for 15 seconds in lengthwise two shakes per second. It was then allowed to stand for 15 minutes and height of foam was measured (WHO 2011).

The foam index was calculated using the formula below;

$$\text{Foam index} = \frac{\mathbf{1000}}{\boldsymbol{a}}$$

When a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to height of 1cm is observed.

3.3.3.9 Determination of Tannins

Five grams(5g) of *C. sieberiana* powdered root was weighed into conical flask, 150ml of water was added and heated over a boiling water bath for 30min. cool and the mixture was then transferred to 250ml flask and diluted to volume with water. The solid material was then allowed to settle and filtered.

50 ml of the filtrate was then evaporated to dryness in an oven at 105^0C for 4 hours and weigh as (T_1) to determine the total amount of plant material that is extractable into water.

80 ml of the filtrate was taken and 6.0g of hide powder (gelatin) was added and shaken for 60 minutes. Filtered, 50ml of the clear filtrate was evaporated to dryness, Dry residue was then weigh to determine the amount of plant material not bound to hide powder (gelatin) that is extractable into water as (T_2). To determine the solubility of hide powder (gelatin) 6.0g of hide powder was added to 80ml of water and shaken for 60minutes in mechanical shaker, filtered and evaporated to dryness was done using 50ml of the clear filtrate in an oven at 105^0C and weigh to determine (T0) (WHO 2011).

Calculation of tannins quantity as a percentage was achieved using the following formular.

$$Tannins\ quantity = \frac{[T1 - (T2 - T0)] \times 500}{W}$$

Where w =- the weight of the plant material in gram.

3.3.3.10 Determination of bitterness value

One hundred milligramme of quinine hydrochloride R was dissolved in safe drinking water to produce 100ml. further dilution was made using 5ml to 500ml of safe drinking water as the stock solution of quinine hydrochloride R which contain 0.0l mg/ml. Serial dilution was then made using 9 test tube to determine the bitterness value for the initial phase show as follows (WHO 2011) .

	Tube numbers								
	1	2	3	4	5	6	7	8	9
S_T (ml)	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6	5.8
Safe drinking water(ml)	5.8	5.6	5.4	5.2	5.0	4.8	4.6	4.4	4.2
Quinine hydrochloride In 10ml 0f solution(=c)(mg)	0.042	0.044	0.046	0.048	0.05	0.052	0.054	0.056	0.058

S_T=Stock solution of quinine hydrochloride

To determine the bitterness value for the second phase, 10 test tubes were used for serial dilution for the test 5g of plant material was dissolved in 150ml of safe drinking water heated for 30min. then transfer to 250ml flask, water was then added to volume, filter then used as stock solution of the plant material for the second test as shown below.

	Tube numbers									
	1	2	3	4	5	6	7	8	910	
S_T (ml)	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0
Safe drinking water(ml)	9.0	8.0	.7.0	6.0	5.0	4.0	3.0	2-0	1.0	-

S_T=Stock solution of *C. sieberiana*

Tasting Method

Ten ml of the most diluted solution was tested by swirling near the base of the tongue for 30 sec. After 10min., the next highest concentration was tested till the last tube. After which the mouth was rinsed thoroughly until no bitter sensation remains. After 10min., the second test was carried out using the plant material solution (*C. sieberiana*). The threshold bitter concentration was then determined in both test. The threshold bitter concentration is the lowest concentration at which a material continues to provoke a bitter sensation after 30 seconds.

Calculation of bitterness value in units per g was achieved using the following formula:

$$Bitterness\ value = \frac{2000 \times c}{a \times b}$$

a = the concentration of the stock solution (S_T)mg/ml

b = the volume of S_T (in ml) in the tube with the threshold bitter concentration

c = the quantity of quinine hydrochloride R(in mg) in the tube with the threshold bitter concentration.

3.4 Chemical Studies of Extract on the *Cassia sieberiana*Root

3.4.1 Extraction and fractionation of powdered roots

The extract was prepared according to the method described by (Modusolumuo *et al.,* 1999). The coarse powdered root (2.5kg) was extracted exhaustively with absolute ethanol (maceration) three times (w/v 1:3) at room temperature for a week. The ethanol crude extract was combined and concentrated on a Büchi rotary evaporator at 45°C giving the crude ethanol extract (80g). It was stored in sterile container for further used. Sixty grams (60 g) of the ethanolicextract was suspended in distilled water (500ml).

The aqueous portion was partitioned with ethyl acetate (6x 200 ml) and *n*-butanol (5 x 200 ml). Percentage yield for the extract and fractions is calculated using the formular :$\% \ yield \ = \frac{Mass \ of \ dry \ extract}{250 \ 0g} \times 100$

Fractionation of plant sample was adapted from Cho *et al*. (2003).The fractionation flow chart isshown below;

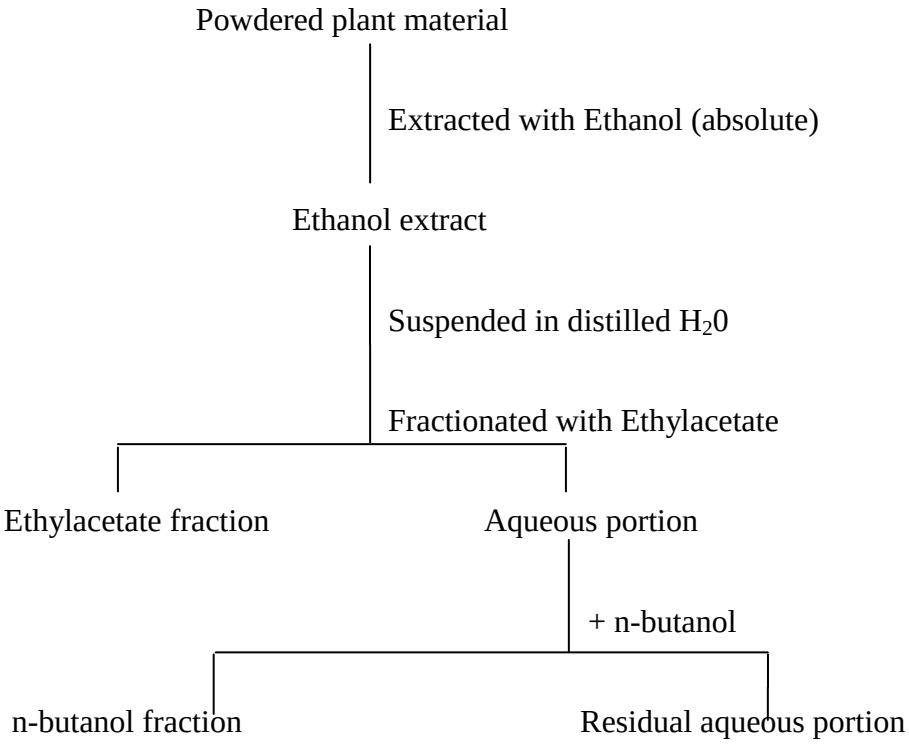


Fig 3.1 Fractionation of *C. sieberiana* powdered root

3.4.2 Preliminary Phytochemical Screening of Extract of *C. sieberiana*

Phytochemical screening of the crude ethanol and fractionated extracts were performed for thepresence of secondary metabolites usingstandard phytochemical methods as described by Wall,(1952),Sofowara (1993)and Evans (2008).

3.4.2.1 Test for Alkaloids

The powdered roots (2 g) were boiled in a water bath with 20 ml of 5% sulphuric acid in 50% ethanol. The mixture was cooled and filtered. A portion was reserved. Another portion of the filtrate was put in 100 ml of separating funnel and the solution was made alkaline by adding two drops of concentrated ammonia solution. Equal volume of chloroform was added and shaken gently to allow the layer to separate. The lower chloroform layer was run off into a second separating funnel. The ammoniacal layer was reserved. The chloroform layer was extracted with two quantities each of 5 ml of dilute sulphuric acid. The various extracts were then used for the following test:

Mayer's test: To the filtrate in test tube I, 1 ml of mayer's reagent was added drop by drop. Formation of a greenish coloured or cream precipitate indicates the presence of alkaloids (Evans, 2008).

Dragendoff's test: To the filtrate in test tube II, 1 ml of dragendoff's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2008).

Wagner's test: To the filtrate in tube III, 1 ml of wagner's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2008).

3.4.2.2Test for tannins

Two grams of the powdered roots was extracted with 10 ml of 50% alcohol, it was then filtered and the filtrate was divided into three portions for the following tests. Ferric chloride test: Three drops of diluted solution of $FeCl_3$ was added to the test tube I, production of a blue or greenish-black colour that changes to olive green as more ferric chloride is added indicates the presence of tannins (Evans, 2008).

Bromine water test: Three drops of bromine water was added to the second portion of the filtrate. A buff coloured precipitate indicates condensed tannins while hydrolysable tannins gave none (Evans, 2008). Lead sub-acetate test: Three drops of lead sub acetate solution was added to the third portion. Occurrence of a coloured precipitate indicates the presence of tannins (Evans, 2008).

3.4.2.3 Identification of anthraquinones

Borntrager's test (for free anthracene derivatives): The powdered roots (0.5 g) was taken in a test tube and 5 ml of chloroform was added and shaken for 5 min. The mixture was filtered and the filtrate shaken with equal volume of 10% ammonia solution. A pink, red or violet colour in the aqueous layer after shaken indicates the presence of free anthraquinone (Evans, 2008).

Modified Borntrager's test (for combined anthracene derivatives): One gram of the powdered roots was boiled with 5 ml of 10% hydrochloric acid for 3 min. The hot solution was filtered in a test tube, cooled and extracted gently with 5 ml of benzene. The upper benzene layer was pipetted off and shaken gently in a test tube with half of its volume of 10% ammonium hydroxide solution. A rose pink to cherry red colour in the ammonia layer indicates the presence of anthraquinone (Evans, 2008).

3.4.2.4 Test for saponins

Frothing test: The powdered roots (0.5 g) was placed in a test tube and 10 ml of distilled water was added and shaken vigorously for 30 s. It was then allowed to stand for 30 min and observed. Formation of honey comb froth indicates the presence of saponins (Safowora, 1993).

Two grams (2g) of the powdered root was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drop of olive oil and shaken vigorously, then observed for the formation of emulsion.(Wall *et al*.,1952).

3.4.2.5 Test forflavonoids

Two gram of the powdered roots sample was completely detaned with acetone. The residue was extracted with ethanol. The mixture was then filtered and used for the following tests:

Shinoda's test: Few magnesium chips were added to 3 ml of the ethanol solution and 2 drops of concetrated hydrochloric acid was added. A pink or red colour indicates the presence of flavonoids (Evans, 2008).

Sodium hydroxide test: To test tube II, 2 ml of 10% NaOH solution was added, yellow solution indicates the presence of flavonoids which on adding dilute hydrochloric acid becomes colourless (Evans, 2008).

$FeCl_3$ test: To test tube III, 3 drops of $FeCl_3$ solution was added, production of greenish-black colour indicates the presence of phenolic nucleus (Sofowora, 1993).

3.4.2.6 Test forsterols and triterpenes

Three grams of the powdered root was placed in a test tube and 10 ml of 50% alcohol was added, the tube was then placed on a water bath and heated for 3 min. It was then allowed to cool to room temperature and filtered. The filtrate was then evaporated in an evaporating dish to dryness and 5 ml of petroleum ether was added to the dish and stirred for 5 min, the petroleum ether portion was then decanted and discarded. 10 ml of chloroform was then added and stirred for about 5 min, it was then transferred into test tube and 0.5 mg of anhydrous sodium sulphate was added and shaken gently and filtered, the filtrate was then divided into two test tubes and used for the following tests

Lieberman-Burchard's reaction: To test tube I, equal volume of acetic anhydride was added and gently mixed. Then 1 ml of concentrated H_2S0_4 was added down the side of the tube. The appearance of a brownish-red ring at the contact zone of the two liquids and a greenish colour in the separation layer indicates the presence of sterols and triterpenes(Evans, 2008).

Salkowski's test: To test tube II, 2 to 3 drops of concentrated sulphuric acid was added to form a lower layer. Reddish-brown colour at the inter phase indicates the presence of steroidal ring (Evans, 2008).

3.4.2.7Test for cardiac glycosides(Keller- Killani test)

Five ml of each extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicates the presences of deoxysugar characteristic of cardenolides. A violet colour may appear below the brown ring, while in the acetic acid layer, a greenish colour may form gradually throughout the layer.(Evans, 2008)

3.4.3Thin layer chromatographic studies of extracts of *C. sieberiana*

Small quantity of the dried fractionated extract was reconstituted in methanol to get a light mixture of the fraction in liquid form.TLC plate(silica gel $G_{600.}$ 0.25mm thickness) was cut into strips, sufficient quantity of a previously mixed and homogeneous mobile phase such asEthylacetate(100%), Chloroform- Methanol(8:2), Chloroform-Ethylacetate-Methanol-Water(15:8:4:1) were used. The plates were sprayed with 10% H_2SO_4 and Iodine as standard spraying reagent for TLC. Number of spots and $R_f$values were determined. (WHO 2008)

Chromatography of various class of compounds previously screened were then confirmed according to the standard screening method (Evans, 2008) by using thin layer chromatography (TLC)using related mobile system,chloroform- Ethylacetate – methanol- water(15:8:4:1) spraying of the TLC plates using various chemical reactants such as Dragendroff's test for alkaloids, ferric chloride test for tannins,anisaldehyde/H_2SO_4 test for terpenoids,aluminium chloride test or 5%NaOH for flavonoids,methanolic KOH for anthraquinoes and vanillin spray was also used (Evans 2008).

3.5 Evaluation of Anti-Ulcer activity of Extracts of *Cassia sieberiana*

3.5.1. Experimental Animals

A total of 45 male Wister rats weighing 100 – 170g bred in the laboratory animal unit of the Faculty of Pharmaceutical Science A.B.U Zaria, were used for the experiment. The animal were housed under similar condition of temperature and relative humidity, light, dark cycle. The rats were kept in stainless steel wire mesh cages which separated them from their faeces to prevent coprophagy.They were fed on standard diet, grower

mesh (ECWA feeds, Jos) and water *ad. Libitum.*Ethical rules guiding the use of animals for experimentation were strictly adhered to (DHHS 1985).

3.5.2Acute Toxicity Study of Extracts of *Cassia sieberiana*

The median lethal dose (LD_{50}) of the plant extract was determined by the method of Lorke, (1983)using 9 rats in the first phase rats were divided into 3 groups of 3 rats each andwere given a graded doses of 10, 100 and 1000 mg/kg body weight orally. They were observed for 24hr for signs of toxicity. In the second phase 4 rats were divided into 4 groups of 1 rat each and were also treated with the crude extract at doses of 1000, 1600, 2900 and 5000 mg/kg bodyweight *(i. p).*The oral median lethal dose (LD_{50}) was calculated as the geometric mean of the minimum toxic dose and maximum tolerated dose using the second phase. This procedure was repeated for ethylacetate, n-butanol and aqueous fractions.

3.5.3Experimental design for ulcer studies

Ethanol-induced ulcers were evaluated in rats as described by Morimoto *et al.*, (1991). Sixty adult rats were weighed, marked and randomly assorted into 9 groups (1-9) with each group containing 5 rats each. Therats were fasted for48hours.Animals in groups 1, 2 and 3 were given distilled water (1ml), ethanol (1 ml) and standard drug as positive control(cimetidine 100mg/kg) respectively, while the remaining groups were given200, 400 and 800 mg/kg doses of ethyl acetate fraction (groups 4-6) and n-butanol fraction (7-9).After 30mins of administration 1 ml of absolute ethanol was administered orally to all the rats. The animals were sacrificed by cervical dislocation and dissected after one hour. Their stomach were carefully removed, each stomach was cut open through the greater curvature with a scissor and rinsed, stretched lightly and spread on a filter paper for proper viewing and assessment of ulcers. The stomachs were examined for ulcer macroscopically. The extent of the mucosal damage were measured by using a

calibrated meter rule (in millimeters) and the ulcer indices measurement was done from left to right of each tissue and Raju (2009)Scoring of ulcer was adopted as showed below

0 = Normal coloured stomach

0.5 = Red colouration

1 = Spot ulcer

1.5 = Haemorrhagic streaks

2 = Ulcers ≥ 3 but ≤ 5

3 = Ulcers >5

The average mucosal damage was determined and the ulcer index (U.I) was calculated. The effectiveness of the extract and drugs was calculated using the following formula (Ode and Asuzu, 2011)

Preventive index % = $\frac{U.Iof\ negative\ control - U.I\ of\ treated}{U.I\ of\ negative\ control} \times 100$

Tissues were then kept in air tight containers and preserved with formalin for reference and further study.

3.6.Statistical Analysis

One way Analysis of variance (ANOVA) was carried out to test for significant differences between the means of samples and control. A difference was considered statistically significant when $p < 0.05$.Using SPSS Version 20, followed by multiple comparisons using Duncan Multiple Range Test (DMRT) to separate the means.

CHAPTER FOUR

4.0 RESULTS

4.1 Pharmacognostic Studies of Roots of *Cassia sieberiana*

4.1.1 Macroscopic studies

Roots arecylindricalin shape, branched, thickness varying with age, odour characteristic, and very bitter taste.

Table 4.1Macroscopic Characters of *Cassia sieberiana* Roots

Characters		**Fresh**	**Powdered**
Shape		Cylindrical	
Size		5-20mm in diameter	
Surface characteristics		Smooth	Coarse
Texture		Hard	Rough
	Odour	Characteristics	Choking
Organoleptic characters	Taste	Very bitter	Very bitter
	Colour	Brown	Cream

4.I.2Microscopic studies

Microscopic studies of the root showed the following tissue systems:

4.1.2.1 Transverse section (T.S)

The T.S of the root is circular in outline (plate III, IV and V).Its outer layer is composed of a lignified cork with several layer of thick walled,flat,polygonal cells with reddish brown content, which are impregnated with suberin. This is followed by thin walled epidermal cells. The phellogen part is bilayered, immediately below the cork and cells tangentially elongated. The cortex portion of the root is composed of horizontally elongated parenchyma cells, containing starch grains, which are abundant, simple, mostly spherical,reniform – oval with central hilum.The phloem is a continuous ring consisting of sieve tubes, fibres, aseptate with pointed end, companion cells and phloem parenchyma. Medullary rays are well developed and are biseriate. The xylem occupies the entire central portion with prominent vessels.The longitudinal section revealed similar cellular contents (Plate VI).

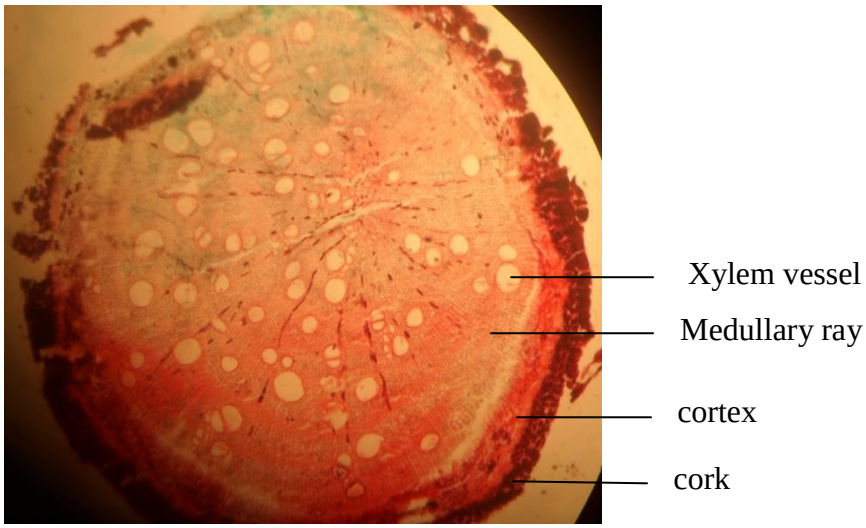


Plate III:T.S of *Cassia sieberiana* root showing the whole section (Mag. X40)

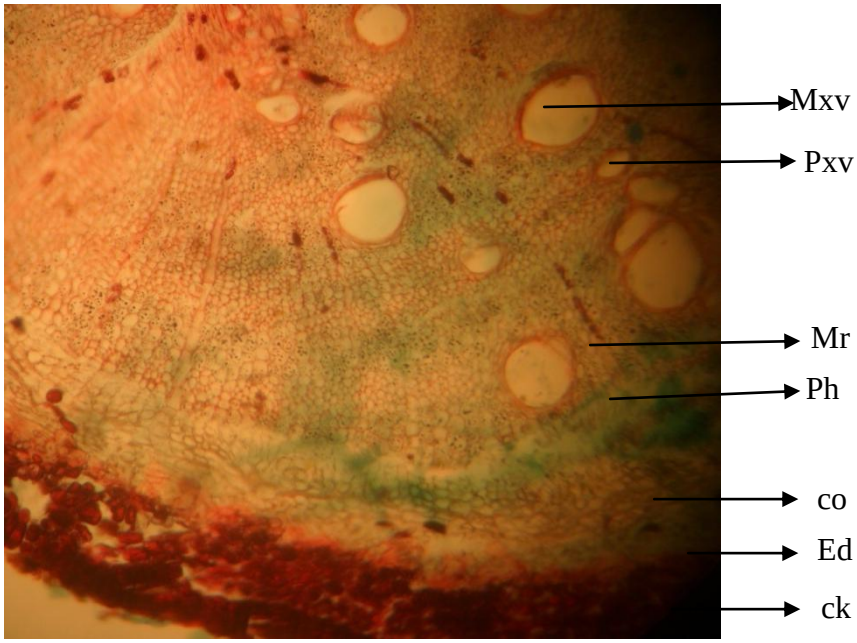


Plate IV: T. S of *Cassia sieberiana* rootshowing the central portion.*Where, Ck-Cork,Ed- Epidermis, Co- Cortex, Ph- Phloem, Mr-Medullary ray, Pxv- Protoxylem vessel, Mxv- Metaxylem vessel,* (Mag. X100)

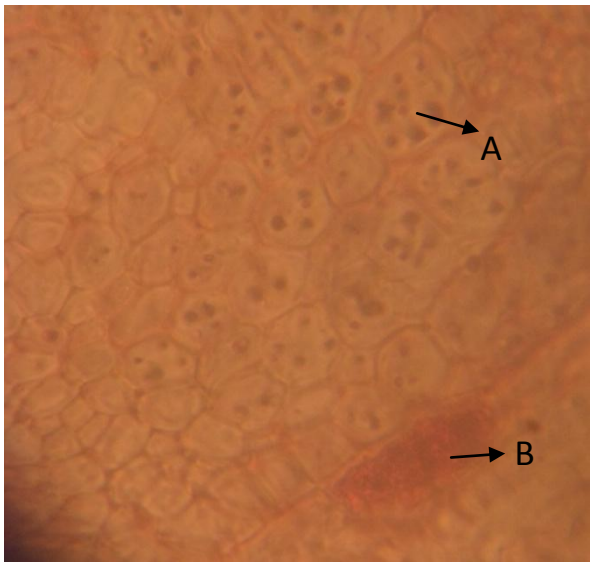


Plate V: T S of *Cassia sieberiana* root, showing A-Starch grains, B-ligninified cell in Parenchyma cells. (Mag. X400)

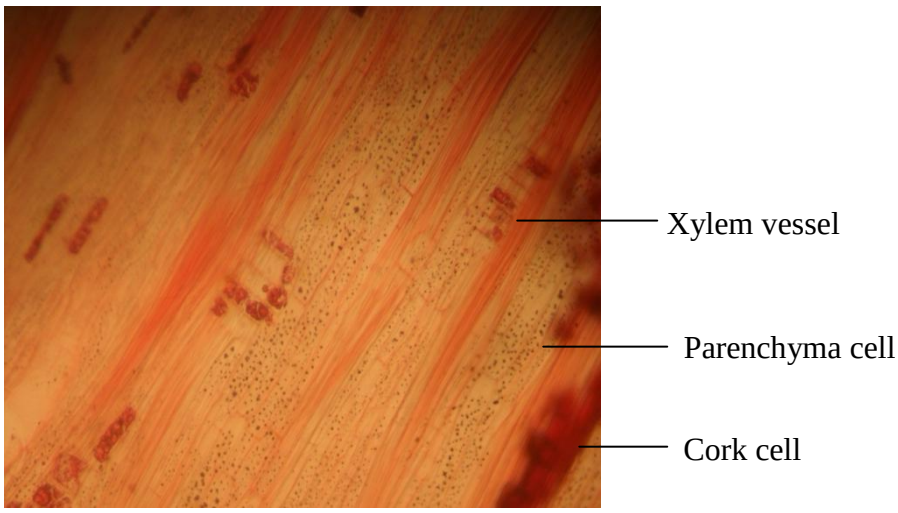


Plate VI; Longitudinal Section of *C. sieberiana* root showing the central portion. (Mag. X100)

4.1.2.2 Histochemical studies on powdered roots

Chemomicroscopic studies of the root revealed the presence of starch, calcium oxalate, tannins cellulose, hemicellulose,lignin, suberin and mucilage.As given in table 4.2 below

Table 4.2 Histochemical detection of cell and cell wall material of powdered root.

Text	Observations	Inference	Histological zones
Starch	Blue-black color	Present	Epidermis, Parenchyma,
Calcium oxalate	No effervescence	Present	Parenchyma
Tannin	Greenish black color	Present	Endodermis, Cork cells
Fixed oil & fat	No observation	Absent	
Protein	Yellow stain	Present	Epidermis, Cork cells
Cellulose	Bluish color	Present	Cell wall
Hemicellulose	Blue color	Present	Cell wall
Lignin	Cherry red	Present	Cell wall
Suberin	Orange red	Present	Cork cells
Mucilage	Pink color	Present	Simple tissues
Aleurine grains	Brown	Present	Parenchyma

4.1.2.3Quantitatve powdered microscopy of the roots

The length of fibre is 74.48 µm. The starch grains are simple and oval with diameter 10.64µm, calcium oxalate crystals are prisms with base diagonal of 19.29µm as showed in table 4.3

Table 4.3 Quantitatve powdered microscopy of the root

Cellular element	**Measurement**
Fibres	74.48 µm
Calcium oxalates	19.29µm
Starch grains	10.64µm

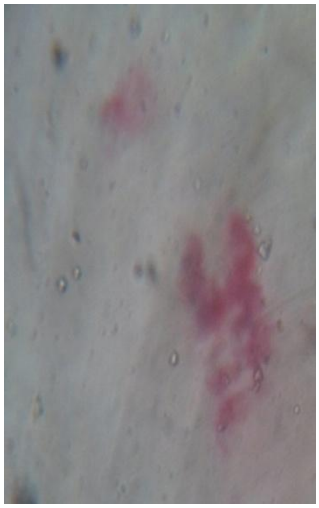


Plate VII: Lignified cells



Plate VIII:Calcium oxalate crystals in groups

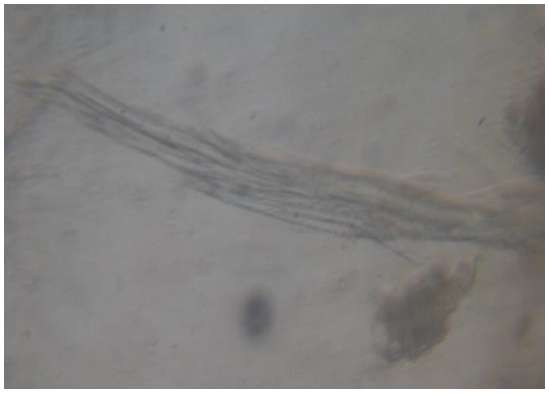


Plate VIX: Pericyclic fibres



Plate X:Calcium oxalate crystals

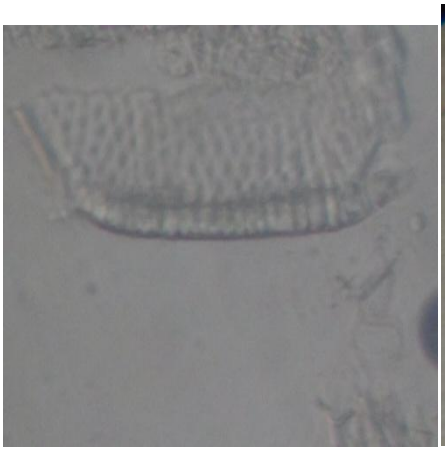


Plate XI: Boarded pitted Xylem vessels



Plate XII: Cork cells

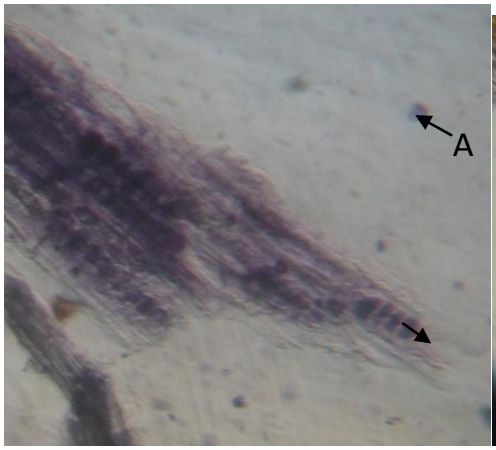


Plate XIII: A- Starch grains

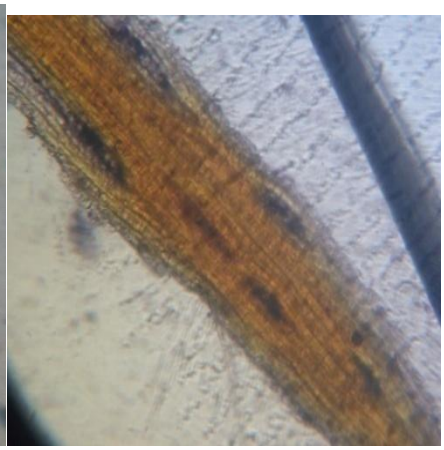


Plate XIV: Wood element containing cell content

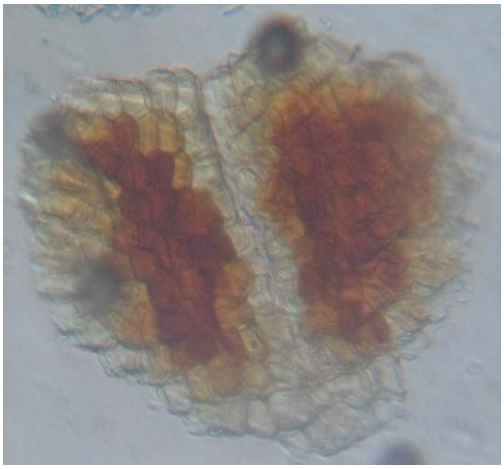


Plate XV: Cork cells with cortical parenchyma

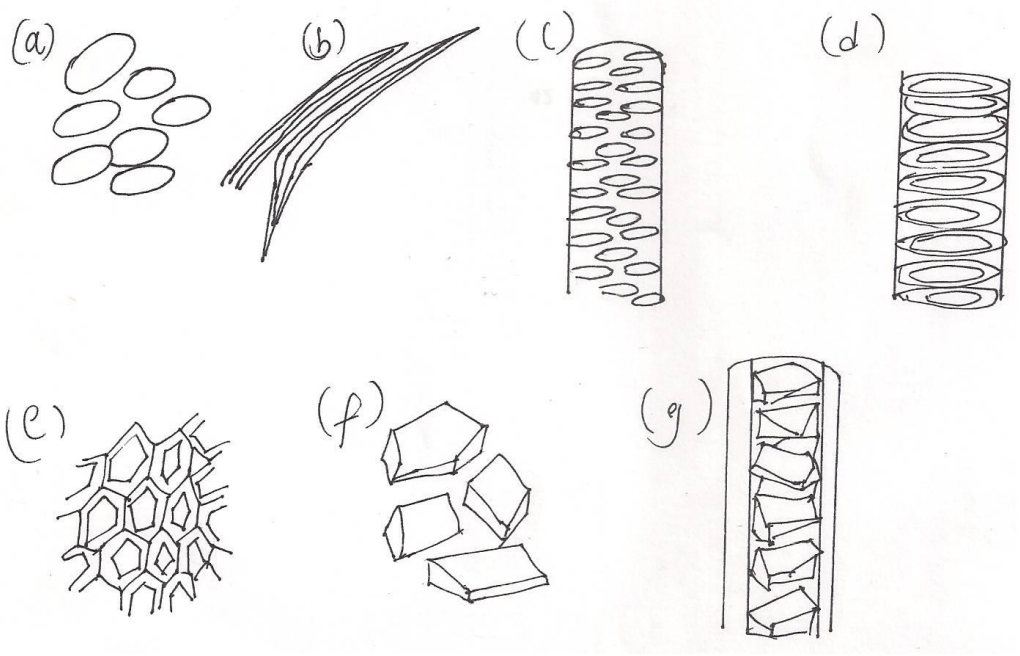


Fig 4 .1 Some features of the powdered roots of *C. sieberiana;* (a) Starch grains,(b) Pericyclic fibres,(c) Reticulatexylem vessel,(d)Annular xylem vessel, (e)Cork cells, (f) Calcium oxalate crystals,(g)Medullary ray containing crystals.(Mag. X100)

4.1.3 Quantitative Evaluation of Powdered Roots

A moisture content of 6.2 ± 0.3% was observed for the powdered root. The total ash was 5.8 ± 0.43% while acid insoluble ash and water soluble ash were found to be 1.0 ± 0.24 and 3.5 ± 0.24% respectively. Alcohol soluble extractivewas found to be 12 ± 0.47% and water soluble extractive6± 0.47%., foaming index(<100), swelling index (3.5ml) tannins content (39%),the bitterness value(8400unit/g). Detailed calculations of quantitative studies are showed in appendix II.

Table: 4.4. Quantitative Parameters of *C sieberiana*

Parameter	**Results***
Moisture content	6.2±0.30(%w/w)
Total ash	5.8 ± 0.43 (%w/w)
Acid insoluble ash	1.0 ± 0.24 (%w/w)
Water soluble ash	3.5 ±0.24 (%w/w)
Alcohol soluble extractive	12 ± 0.47 (%w/w)
Water soluble extractive	6 ± 0.47 (%w/w)
Foaming index	Less than 100
Swelling index	3.5 ml
Tannins content	39(%w/w)
Bitterness value	8400 units/g

*Mean of three determinations

4.2Chemical Studies of Extracts *Cassia sieberiana* Roots

4.2.1 Extraction of Plant Material

C. sieberiana crude extract is dark brown in colour with a pleasant smell and a pasty consistency. The total solid recovered from ethanol extraction was 80g having percentage yield of 3.2%. The partitioning of the fractions weighed 14.01 g, 9.98 g and 7.80 g for Ethylacetate,n-butanol, andAqueousrespectively.The percentage yields for the fractions are 0.6%, 0.4%, and 0.3% for Ethyl acetate, n- butanol and Aqueous respectively as indicated in Table 4.5.Ethylacetate fraction was found to be higher than the other fraction.

Table 4.5 Percentage yield of the Extract and Fractions

Extract/fractions	Colour	Mass(g)	Percentage Yield (%)
Ethanol Extract	Dark brown	80.00	3.2
Ethyl acetate	Lightbrown	14.01	0.6
n-Butanol	Deep brown	9.98	0.4
Aqueous	Pale brown	7.80	0.3

4.2.2. Phytochemical Evaluation

Phytochemical constituents detected in the extract include anthraquinones, saponins, steroids, triterpenes, flavonoid, tannins andcardiac glycoside. Ethylacetate fraction revealed the presence of anthraquinones, flavonoid, tannins,glycoside and steroid/terpenoids. Then- butanolfractions showed the presences of tannins, and saponins, while the aqueous fractions show the presence of flavonoids, tannins and saponins(Table 4.6).These secondary plant metabolites are known to possess various pharmacological effects and may be responsible for various action of *C. sieberiana*.

Table 4.6 Phytochemical Screening of Ethanol Extract, Ethylacetate, n-Butanol and Aqueous fraction of *C. sieberiana* root.

Constituent	**Crude extract**	**EAF**	**NBF**	**AQ**
Anthraquinones	Present	Present	Absent	Absent
Tannins	Present	Present	Present	Present
Flavonoids	Present	Present	Absent	Present
Saponins	Present	Absent	Present	Present
Terpernoids/Steroid	Present	Present	Present	Absent
Cardiac Glycoside	Present	Present	Absent	Absent
Alkaloid	Absent	Absent	Absent	Absent

NB: Ethylacetate,(EAF),Butanol, (NBF), Aqueous,(AQ)

4.2.3 Chromatographic Assay/TLC

TLC profiles using CHL: EAA : MEOH : W solvent system in ratio (15 : 8 : 4 : 1) revealed eight , four and three spots in ethyl acetate, n- butanol and aqueous fractions portionof the TLC plate respectively. At chloroform: methanol (8: 2) revealed five spots at ethyl acetate fraction portion and there was no separation at n – butanol and aqueous fractions portion. Ethyl acetate (100%) revealed two spots at ethyl acetate and n – butanol fractions portion. Ethyl acetate: methanol (8: 2) solvent system, revealed eight spots infraction portion (Table 4.7). Spraying the TLC plates with specific spraying reagents confirmed one spot and yellow colour for flavonoids (Plate), tannins has two spots and they are blue black in colour at ethyl acetate and n – butanol fractions portion (Plate) . Terpenoids, cardiac glycoside, anthraquinone has one spot each at ethyl acetate fraction portion. (Table 4.8).

Table 4.7TLC profiles of various fractions of *C. sieberiana*sprayed with 10% H_2S0_4

	Number of Spots: (R_F Values, color)		
Solvent system	**Ethylacetate**	**n- Butanol**	**Aqueous**
CHL: EAA: MEOH:W (15 : 8 : 4 : 1)	Eight	Four	Three
	0.02(brown)	0.12(brown)	0.89(brown)
	0.12(brown)	0.18(brown)	0.93(brown)
	0.24(pink)	0.85(brown)	0.95(brown)
	0.30(red)	0.95(brown)	
	0.43(brown)		
	0.60(purple)		
	0.85(brown)		
	0.95(brown)		
CHL : MEOH (8 : 2)	Five		
	0.19(brown)	No separation	No separation
	0.33(brown)		
	0.43(brown)		
	0.57(brown)		
	0.71(brown)		
Ethylacetate (100%)	Two	Two	
	0.75(pink)	0.75(pink)	No separation
	0.81(brown)	0.81(brown)	
CHL: EAA (8: 2)	Eight		
	0.07 (brown)	No separation	No separation
	0.13(brown)		
	0.20 (brown)		
	0.27(brown)		
	0.36(brown)		
	0.40 (yellow)		
	0.47(brown)		
	0.53(brown)		

EAA-Ethylacetate, CHL-Chloroform, MEOH-Methanol,W-water.

Table 4.8; Chromatography separations of phytochemicals of various fractions by TLC using CHL: EAA: MEOH:W(15:8:4:1)

Phytochemicals	Spot	Colour	R_f	Fraction portion
Flavonoid	1	Yellow	0.23	EAA
Tannins	2	Blue black	0.13, 0.04	EAA , B
Terpenoids	1	Brown	0.4	EAA
Cardiac Glycoside	1	Purple	0.5	EAA
Anthraquinones	1	Red	0.3	EAA

EAA-Ethylacetate, B-Butanol

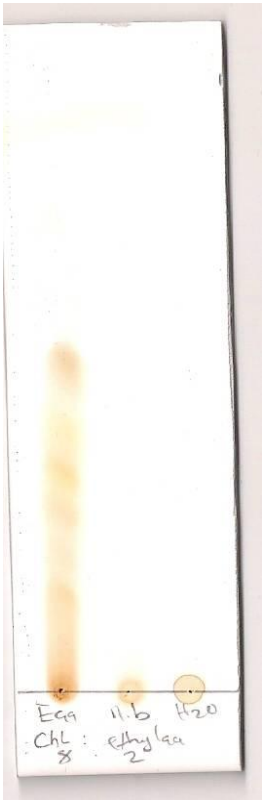


Plate XVI: TLC profile of Ethylacetate, Butanol and Aqueousfraction using Chloroform- Ethylacetate (8:2)as the developing solvent system sprayed with 10% H_2SO_4 in methanol and heated at 105^0C for 2 minutes.

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Plate XVII: TLC profile of Ethylacetate, Butanol and Aqueous fraction using Chloroform - Methanol (8:2)as the developing solvent system sprayed with 10% H_2SO_4 in methanol and heated at 105^0C for 2 minutes.

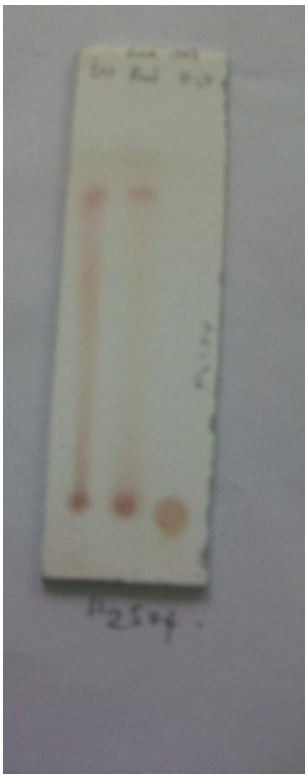


Plate XVIII: TLC profile of Ethylacetate, Butanol and Aqueous fraction using Ethylacetate (100%)as the developing solvent system sprayed with 10% H_2SO_4 in methanol and heated at 105^0C for 2 minutes.



Plate XIX: TLC profile of Ethylacetate, Butanol and Aqueousfraction using chloroform- ethyl acetate-methanol-water (15:8:4:1)as the developing solvent system sprayed with 10% H_2SO_4in methanol and heated at 105^0C.

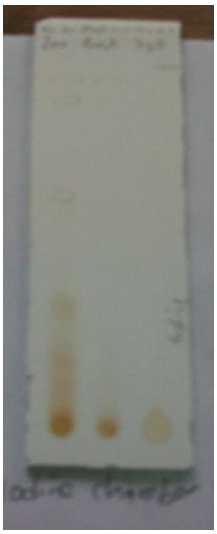


Plate XX: TLC profile of Ethylacetate, Butanol and Aqueous fractions using chloroform- ethyl acetate- methanol- water (15:8:4:1) as the developing solvent system placed in iodine chamber.

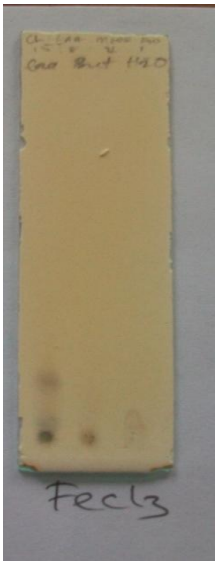


Plate XXI: TLC profile of Ethylacetate, Butanol and Aqueous fractions using chloroform- ethyl acetate- methanol- water (15:8:4:1) as the developing solvent system sprayed with $FeCl_3$for Tannins.

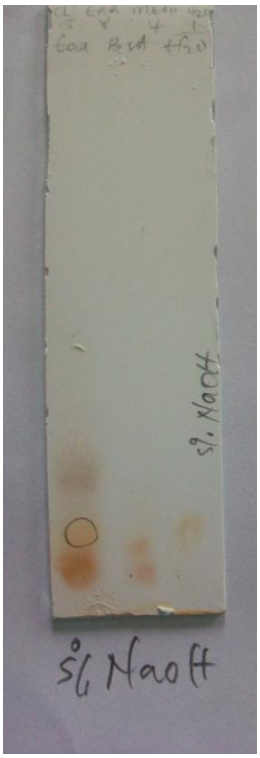


Plate XXII: TLC profile of Ethylacetate, Butanol and Aqueous fractions using chloroform- ethyl acetate- methanol- water (15:8:4:1) as the developing solvent system sprayed with 5% NaOH for flavonoids.



Plate XXIII: TLC profile of Ethylacetate, Butanol and Aqueous fractions using chloroform- ethyl acetate- methanol- water (15:8:4:1) as the developing solvent system sprayed spray with vanillin.



Plate XXIV: TLC profile of Ethylacetate, Butanol and Aqueous fractions using chloroform- ethyl acetate- methanol- water (15:8:4:1) as the developing solvent system sprayed with methanolic KOH for Anthraquinones.

4.3. Evaluation ofAnti-Ulcer Activity of Fractions of *C. sieberiana*

4.3.1 Acute Toxicity Studies

The fractions(ethylacetate,nbutanol and aqueous) werecharacterized by a very low degree of toxicity. Their acutetoxicity LD_{50} in Wister rats was found to be above 5000 mgkg-1as presented in table 4.9 and 4.10. The weights of all the Wister rats used for the toxicity study are showed in appendix three.

Table; 4.9The percentage mortality of different doses of ethanol extract, ethylacetate, n-butanol and aqueous fraction of *C. sieberiana* root administered in rats during the first phase.

	Ethanolextract		**Ethylacetate**		**n- Butanol**		**Aqueous**	
T (Mg/kg)	**M**	**%M**	**M**	**% M**	**M**	**% M**	**M**	**% M**
10	0/3	0	0/ 3	0	0/ 3	0	0/ 3	0
100	0/3	0	0/ 3	0	0/ 3	0	0/ 3	0
1000	0/3	0	0/ 3	0	0/ 3	0	0/ 3	0

T -Treatment, M - Mortality.

Table; 4.10 the percentage of different mortality doses of ethanol extract, ethyl acetate n-butanol and aqueous fraction of *C. sieberiana* root administered in rat during the second phase

	Ethanol extract		**Ethyl acetate**		**n- Butanol**		**Aqueous**	
T (Mg/kg)	**M**	**%M**	**M**	**% M**	**M**	**% M**	**M**	**% M**
10 00	0/1	0	0/ 1	0	0/ 1	0	0/ 1	0
1600	0/1	0	0/1	0	0/1	0	0/1	0
2900	0/1	0	0/ 1	0	0/ 1	0	0/ 1	0
5000	0/1	0	0/ 1	0	0/ 1	0	0/ 1	0

T -Treatment, M - Mortality.

4.3.2 Anti- Ulcer Studies

C. sieberiana fraction produced significant dose- dependent antiulcer activity in all the dose levels.Ethylacetate portion produced a significant reduction in the mean ulcer index of 15.2 ± 0.68, 4.6± 0.21, 0.0±0.0 at 200, 400, 800mg/kg respectively when compared with 53. 8 ± 2.40mg/kg in Ethanol treated rats. The n- butanol fraction shows significant reduction in the mean ulcer index of 27.4 ± 2.4, 10.27 ± 0.46, 1.4 ± 0.06 at 200, 400 and 800 mg/kg respectively. The preventive index% of various groups are Ethanol(0) Cimetidine(88),Ethanolextract at 200, 400, and 800 mg/kg (61, 79 and 96)respectively.Ethylacetate fraction at 200, 400, and 800mg/kg (72, 91 and100)respectively.n- Butanol 200,400,and 800mg/kg(49,81,97)respectively as showed in figure 4.2 and 4.3 below. The weights and ulcers indices of each of the Wister rats used for anti-ulcer activity are indicated in appendix four.

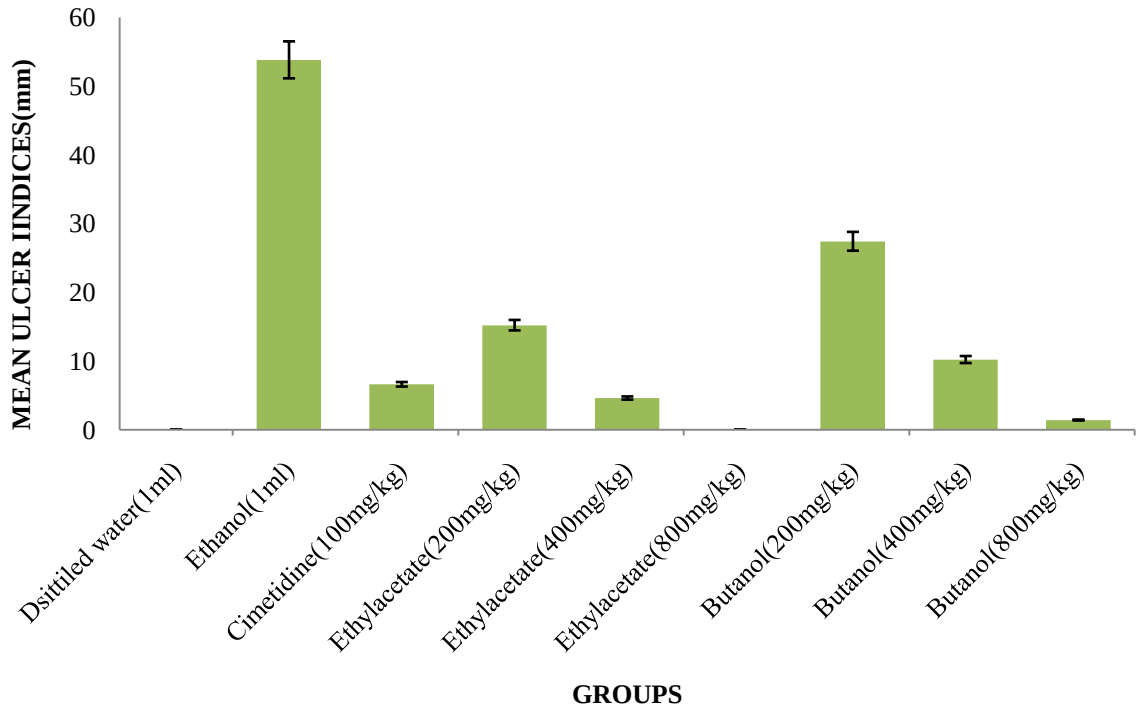


Figure 4.2: Mean ulcer indices for ethanol, cimetidine, ethyl acetate and n-butanol fraction on ethanol- induced Wister rats

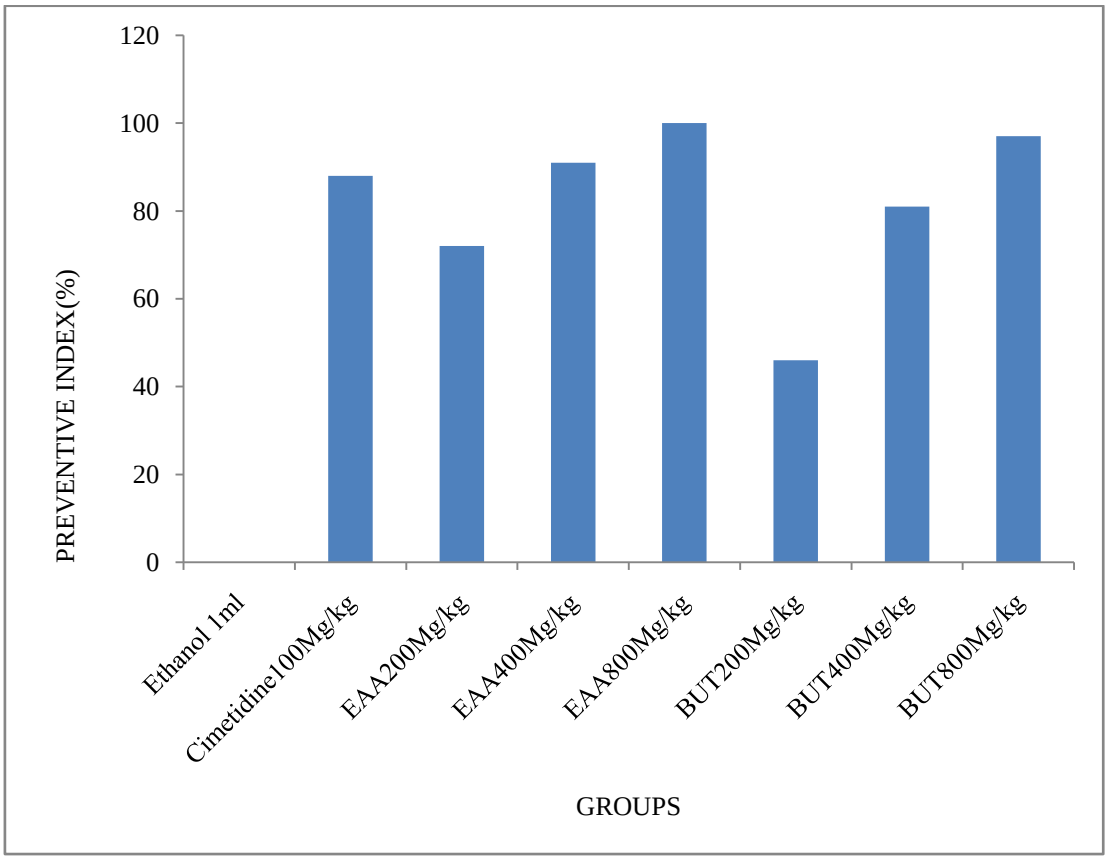


Figure 4.3: Preventive indices percentage for ethanol, cimetidine, ethyl acetate and n- butanol fraction on ethanol- induced Wister rats.

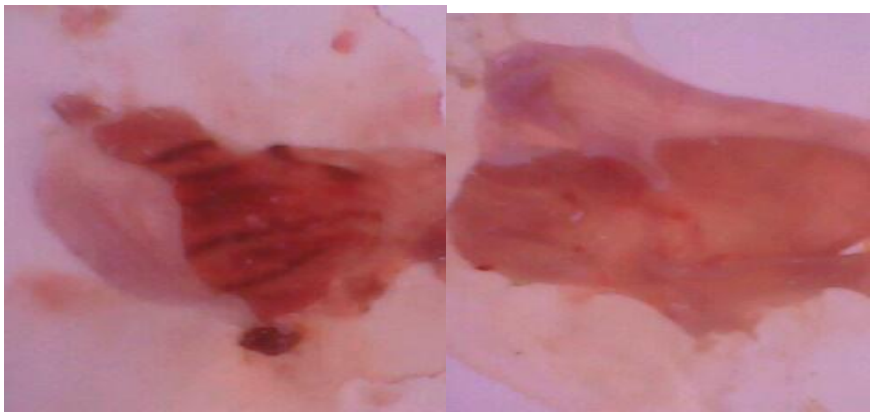


Plate XXV. Photograph of rat stomach showing severe ulcer lesions by administration of ethanol (1ml)

Plate XXVI. Photograph of rat stomach showing highly protected by administration of 100 mg/kg of cimetidine of *C. sieberiana* from ethanol- induced ulceration.



Plate XXVII. Photograph of rat stomach showing completely protected by administration of 800 mg/kg of ethyl acetatefraction of *C. sieberiana* from ethanol-induced ulceration.



Plate XXVIII. Photograph of rat stomach showing fairly protected byadministration of 200mg/kg of ethyl acetatefraction of *C. sieberiana* from ethanol-induced ulceration.



Plate XXIX. Photograph of rat stomach showing highly protected by administration of 800 mg/kg of n- butanolfraction of *C. sieberiana* from ethanol-induced ulceration.



Plate XXX. Photograph of rat stomach showing normal stomach by administration distilled water on rats.

CHAPTER FIVE

5.0 DISCUSSION

The primary steps for establishing the quality control profile of any plant drug are the macroscopic and microscopic evaluations and According to WHO (2011), botanical standards should be proposed as a protocol for the diagnosis of herbal drugs. Macroscopic identity of medicinal plant materials is based on shape, size, colour, surface characteristics texture, fracture, characteristics and appearance of the cut surface.Theseare notable features used as diagnostic characters to distinguish the plant from other species. However, since these characteristics are judged subjectively and substitutes or adulterants may closely resemble the genuine material, these findings are usually substantiated by microscopy. According to WHO publication 2011, microscopic characters are necessary to establish the botanical identity of commercial samples of medicinal plants and play an important role in checking adulteration and substitution.

The powdered roots histochemistry gives a preliminary idea about the type of compounds and their accumulation in the plant tissues. The resultsof this study is supported by the report of Sam *et al*., (2011) .Histochemical studies of *C. sieberiana* is of great interest for quality control in basic research and drug production, especially for imported items and for raw material sold by traditional herbalists.

Physicochemical evaluation of powdered root of*C. sieberiana* is useful towards establishing pharmacognostic standards on identification, purityand quality of the plant which is gaining relevance in plant drug research (WHO 2011)

The ash value indicates the presence of inorganic ions. During ash preparation organic matter gets oxidized and certainamount of volatile elements are lost. The residue

remaining after incineration of plant material is ash content or ash value, which simply represent inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a form of adulteration (WHO 2011).

. The ash value was determined by 3 different methods which measured total ash, acid insoluble ash and water soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition. It includes both physiological (which is derived from plant tissue itself), and from non- physiological ash (residue of the extraneous matter, like sand etc adhering to the plant substances) (WHO, 2011). The total ash determined was 5.8 ± 0.43 (% w/w) for*C. sieberiana* root in contrast withAjayi *et al*., (2015)determination having 2.1 ± 0.07 total ash this variation is due to the geographical locations attributed to possible varying mineral contents in the soil.Acid – insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter and was found to be 1.0±0.24 (%w/w). This low value shows that the amount of silica present especially as sand and siliceous earth are in very minute quantity. In contrast to the study of Sam*et al.*, (2011) having 4.8 acid- insoluble ash,this difference may be explained by the fact that variation may occur in same plant found in different environment. Water soluble determined for *C. sieberiana* was 3.5±0.24 (%w/w) as is the difference in weight between the total ash and the residue after treatment of the total ash with water, which was higher than acid insoluble ash.

Loss on drying test is a method to measure the loss in mass of the sample, when dried under the specific conditions. This method is applied to determine the amount of water all or a part of water for crystallization or volatile matter in the sample. The loss on drying was6.2 ± 0.3 (% w/w), the less the value of moisture content,the lesser it prevent bacterial, fungal or yeast growth. An excess of water in medicinal plant materials will

encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis (WHO 2011).

Extractive values help us in determine the amount of active constituents and is done on plant materials for which as yet no suitable chemical or biological assay exists. Water soluble and alcohol (70% methanol) soluble extractive values were found to be 6.0 ± 0.47 (% w/w) and 12.0 ±0.47 (% w/w) respectively. The higher value of alcohol soluble extractives shows that alcohol is a better solvent for extracting the root of *C. sieberiana* plant. However this result was in contrast to the findings of Sam *et al*., (2011) who showed that ethanol(70%) extractive14.28% w/w was lower than chloroform -water extractive 15.99% w/w,this may be due to different strength of alcohol used and also differences in geographical locations.

Many medicinal plant materials are of specific therapeutic or pharmaceutical utility because of their swelling properties, especially gums and those containing an appreciable amount of mucilage, pectin or hemicelluloses (WHO 2011). The swelling index is the volume in ml taken up by the swelling of 1g of plant material under specific condition, the swelling index of *C. sieberiana*powdered root was found to be 3.5 ± 0.00 ml, thisshowed that the root has swelling ability which is due to an appreciable amount of mucilage and hemicelluloses present. The foaming ability of an aqueous decoction of plant materials and their extractsis measured in terms of a foaming index. The foamingindex of *C. sieberiana* determined was less than 100. This implied that the persistent foam that was observed when an aqueous decoction of the root was shaken shows that the plant contains little saponins.Tannins (or tanning substances) are substances capable of turning animal hides into leather by binding proteins to form water insoluble substances that are resistant to proteolytic enzymes.

This process when applied to living tissue is known as an “astringent” action and is the reason for the therapeutic application of tannins.Chemically, tannins are complex substances, they usually occur as mixtures of polyphenols that are difficult to separate and crystallize. They are easily oxidized and polymerized in solution. If this happens they lose much of their astringent effect and are therefore of little therapeutic value. The tannins content was 39% w/w which shows that *C. sieberiana* root has astringency (WHO 2011).

Plant materials with medicinal propertythat have a strong bitter taste (‘bitters”) are employed therapeutically, mostly as appetizing agents. Their bitterness stimulates secretion in the gastrointestinal tract especially of gastric juice (WHO 2011). Bitter substances can be determined chemically. The bitter properties of plant are determined by comparing the threshold bitter concentration of extract of the materials with that of a dilute solution of quinine hydrochloride (WHO 2011).

The bitterness value is expressed in unit equivalent to the bitterness of a solution containing 1g of quinine hydrochloride R. in 2000ml. The bitterness value of *C. sieberiana* root was found to be 8400unit/g. This shows that the plant roots possess a strong bitter taste and it stimulates the secretion of gastrointestinal tract gastric juices (WHO 2011).

Therapeutic efficacies of medicinal plants are attributed to the quality and quantity of active principles they contain (Evans, 2008).

Preliminary phytochemical screening of *C. sieberiana*roots extracts revealed the presence of anthraquinones, tannins, cardiac glycoside, sterol, saponin, flavonoids and triterpenes.(Table 4.5).Ethylacetate fraction revealed the presence of athraquinones,

flavonoid, tannins,sterol,glycoside and terpenoids. n- butanol showed the presences of tannins, and saponins,aqueous fraction showed the presence of tannins, flavonoids and saponins. The ethylacetate extract contains the highest phyto constituents. The result of this study is supported by the report of Modusolomuo *et al.*, (1999), who also reported that the roots and pods(fruit) pulps shows the presence of four active principles; tannins, saponins and alkaloids, which are of varying concentrations. However, in contrast to his study, this study showed that the roots did not show the presence of alkaloids. Also in contrast to the study of Sam *et al.*, (2011), this study showed that the roots have flavonoids in ethylacetate fraction. This difference may be explained by thefact that variation may sometimes occur in bioactive compound of different parts of the same plant and even in same plant parts found in different environment. (Elujoba,1999) reported that variation may occur in bioactive compound of the same plant due to different environment where they are found. These secondary plant metabolites are known to possess various pharmacological effects and may be responsible for various action of *C. sieberiana.* Also the phytochemical components in this plant root provide an empirical basis for its traditional medicinal uses. Phytochemicals have been reported to have medicinal uses (Tella and Ojo, 2005). Presence of Tannins and phenols act as an astringent of great therapeutic value (WHO, 2011). Some medicinal plants have been reported to have antimicrobial activity because of presence of phenol (Silva *et al.* 1997a; Modusolumuo *et al.,* 1999). Gums and mucilage are characteristic of certain natural families like Leguminosae and also used as demulcents and bulk laxative (Abo *et al.*,1999). Glycosides because of the different chemical nature of aglycones have different pharmacological activity (Kinjo *et al.,* 1994). Flavonoids are important groups of polyphenolic compounds. A number of

flavonoids are reported to possess anti – inflammatory activity and anti ulcerogenic activity (Sannomiya *et al.*,2005).

Presence of biological compounds like terpenoids, alkaloids, glycoside, flavonoid and saponins are known to contribute to anti- microbial, anti-ulcer activity of the plants containing them (Brain and Turner 1975).

TLC studies performed with ethylacetate, butanol and aqueous extracts of *C. sieberiana* root revealed clues for identifying and distinguishing the plant from other species. The spots produced were sprayed with different reagent to confirm the phyto constituents. Flavonoids, tannins saponin, tritepenes present encouraging finding which implies the definite presence of certain constituents in the sample.

The median acute toxicity testing (LD_{50}) was above 5000mg/kg and the extracts did not cause any significant changes in the behavioural patterm. According to toxicity scale of Lorke, (1983), any compound with an LD_{50} above 5000mg/kg should be considered of no practical toxicity and is said to be non- toxic. This could be attributed to the fact that orally administered drugs and compounds do undergo some biochemical processes that potentially decrease the amount reaching systemic circulation for pharmacological effects (Brander *et al*.,1991). These results is in support by the work of Weremfo, *et al*., (2007) who evaluated the ethanolic root extract of *C. sieberiana*for their behavioural and pharmaco-toxicological effect. Ajayi *et al.,* (2015) also reported the LD_{50} of*C.sieberiana*to be above 5000 mg/kg.

Ethanol is widely used to induced ulcers (Kayode *et al.,* 2009). This are done by suppressing the protective action of the mucus secreted by mucus membrane the increased synthesiss of mucus can be explained as the probable cytoprotective

mechanism in this case. It disrupts the gastric mucosal barrier and cause profound micro – vascular changes with strong vasoconstriction accompanied by arteriolar dilation responsible for engorgement of mucosal capillaries (Cho and Ogle, 1992). The pathogenesis of mucosal damage in the stomach include the generation of reactive oxygen system that seem to play a vital role in the formation of lipid peroxide accompanied by impairement of anti – oxidative enzyme activity of cells (Konturek *et al.*,2000).

C. sieberiana extract produced significant anti-ulcer activity in all the dose levels as showed in figure 4.2 and 4.3. Ethylacetate fraction shows complete gastro protective effect with preventive index of 100% at 800 mg/kg over 88% with cimetidine. Ethylacetate fraction at 400 mg/kg and butanol fractions at 800 mg/kg also shows better gastro – protective effect over cimetidine, preventive index percentage increases with increase in dose of the extracts, it produced a dose dependent effect .These may be due to the facts that the extracts produced its effect by forming a cytoprotective barrier, which may be useful against peptic ulcers. Fractions of *C. sieberiana* may be said to suppress the ulcerogenic tendencies of ethanol probably by stimulating the production of the mucus coverage on the intestine. It could also be through anti –oxidants potentials of the constituents, although this was not evaluated of Therefore *C. sieberiana* fractionated root possesses strong gastrocytoprotective properties against ethanol-induced gastric ulcers. These observations confirmed to the findings of Nartey *et al.*,(2012) but with lower percentage inhibition of the extract given as 27.50%, 50.00% and 85.38% respectively for animals pretreated with 500 mg/kg, 750 mg/kg and 1000 mg/kg body weight of *C. sieberiana* root bark.The roots of *C. sieberiana* were found to contain flavonoids and tannins which were extracted into aqueous solution (Guata-Yoro *et al.*,2009). Some phytochemical compounds such as flavonoid

groups may prevent or suppress ulcerogenic process. It was shown that *C.* singueana leaf has flavonoid compound which exhibit a gastro protective effect against ethanol-induced stomach ulcers. (Ode and Asuzu,2011).

Previous studies has showed that plant drugs containing saponins , terpenoids, flavonoid and phenolic compound are known to be in anti- ulcer activity (Sen *et al*., 2009).

Tannins may prevent ulcer development due to their protein precipitation and also constricting effect (Aguwa *et al*., 1988). Their astringing action can help precipitating micro proteins on the ulcer site forming an impervious layer over the lining that hinders induced gastric ulcer in rats as evidenced by the guts secretions and protects the underlying mucosa from reduction in the ulcer score.(Aguwa *et al*., 1988).

The protective effect of root extract of gastric ulcers many be due to the strengthening of gastric mucosa (Goel *et al,* 1991) or by other mechanisms like increased gastric and duodenal alkaline secretion (Kim *et al*. 2002) by increased luminal prostaglandin levels (Konturek *et al,* 2000).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6. ISUMMARY

This study was designed to determine the Pharmacognostical, Phytochemical and Pharmacological evaluation of *C. sieberiana* root.

In pharmacognostic studies the macroscopy, microscopy, histochemical and physico-chemical constants were carried out. The pharmacognostical details evolved from the present study would help to fix up the standards for ***C. sieberiana*** in relation to its identification, authentication and differentiation from other related species and adulterants.

The macroscopic study had provided a characteristic identity of the fresh and powdered root which was brown and cream in colour respectively, very bitter taste and characteristic odour for powdered root these are the notable feature of the root.

The transvers section of the root is circular in outline and has thin continuous epidermal layer, a narrow cortex with homogenous layers of parenchyma cells and prominent xylem vessel were observed.

Quantitative microscopical examination of the powder root showed oval starch grains, prism calcium oxalate crystals and pericyclic fibres these are diagnostic features of the root.

Histochemistry of this study showed the presence of lignin, cellulose, hemicellose, starch, mucilages, tannin and suberin.

Various physico-chemical parameters such as ash values, extractive values, loss on drying, tannin content, swelling index, bitterness value and foaming index were found to substantiate its standard values to check for adulteration and purity control of the plant.

The qualitative preliminary phytochemical analysis was performed to detect the nature of the phyto-constituent and their presence in ethanol extract and various fractions. Ethanol extracts showed the presence of anthraquinones, tannins, flavonoids, saponins, terpenoids/steroids and cardiac glycosides. Ethyl acetate fractions was found to contain flavanoids, anthraquinones, tannins, terpenoids/steroids and cardiac glycosides, n-butanol fraction showed the presence of tannins, saponins and terpenoids/steroids while the aqueous fractions tannins, flavonoids and saponins.

Qualitative chromatographic analysis (TLC) was performed for the identification of different components in the fractions. It is also a diagnostic method to find out the adulterants and to check the purity.

The pharmacological activity of this study dealt with the screening of oral acute toxicity study and anti – ulcer activities of ethyl acetate and n-butanol fractions of the root of *C.sieberiana*

The ethanolic extract and fractions were found to be very safe up to 5000 mg/kg body weight by median acute toxicity study.

The ethyl acetate and n- butanol fraction showed promising anti- ulcer activities in a dose dependent manner.

6.2 CONCLUSIONS

In conclusion the results of this investigation on *C.sieberiana* could, therefore, serve as a basis for proper identification, collection and investigation of the plant.The quantitative parametersof this study which are being reported for the first time in Zaria could also be useful in the preparation of a monograph in the proposed Nigeria Pharmacopoeia.

The anti-ulcer results determined using ethanol induced gastric ulcer model in rats of this study lend credence to traditional use of the plant root in ulcer treatment.

The present study may be useful to supplement the information with regard to its standardization and in carrying out further research and its use in traditional system of medicine.

6.3 RECOMMENDATIONS

Further studies on *C. sieberiana* extract are recommended to evaluate its anti-oxidant potentials.

Furtherstudiesisrecommended to isolate the active principle and determine its mechanism of action.

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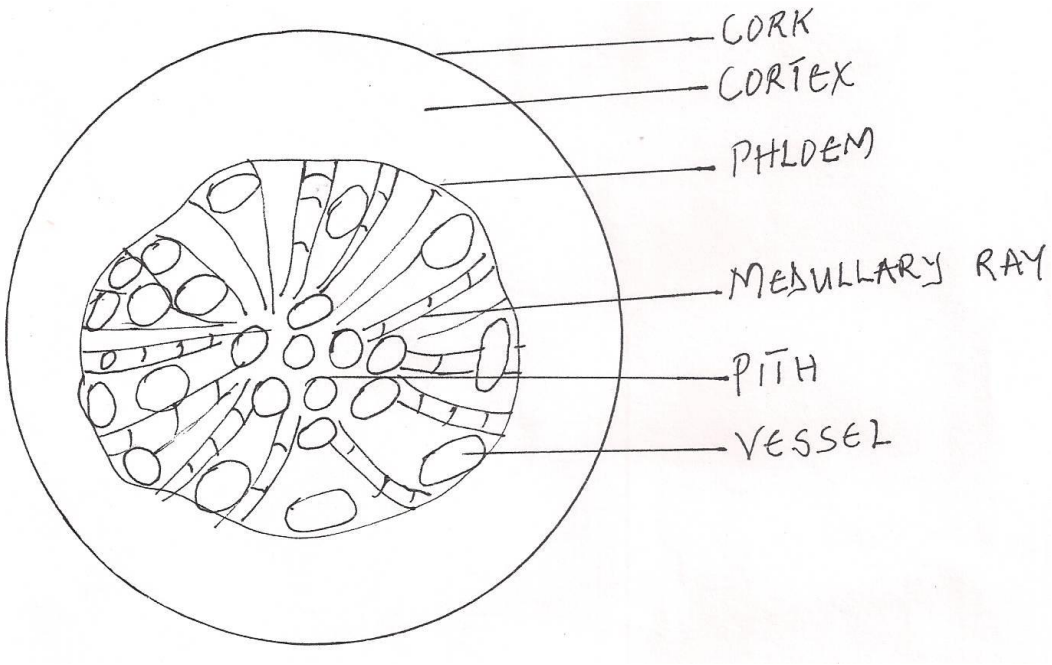
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APPENDICES



APPENDIX 1;Transverse section of *Cassia sieberiana* root.(MG X100)

APPENDIX 2; Quantitative studies of *Cassia sieberiana* **powdered Root**

- AshValue determination as ;$\frac{w2}{w}$×100

  w=weight of powdered root(2g)

  w2=weight of residual ash

  percentage ash value for the 1st determination

  w0= constant weight of crucible =25.88g

  w1=weight of crucible and ash after heating =25.98g

  w2 = (w1-w0) =25.98-25.88 =0.1g

  Ash value =$\frac{0.1}{2}$×100 = 5%

percentage ash value for the 2nd determination

w0= constant weight of crucible =51.22g

w1=weight of crucible and ash after heating =51.35g

w2 = (w1-w0) =51.35-51.22 =0.13g

$$\text{Ash value} = \frac{0.13}{2} \times 100 = 6.5\%$$

percentage ash value for the 3rd determination

w0= constant weight of crucible =51.22g

w1=weight of crucible and ash after heating =51.34g

w2 = (w1-w0) =51.34-51.22 =0.12g

$$\text{Ash value} = \frac{0.12}{2} \times 100 = 6\%$$

Average Ash value determination$= \frac{5+6.5+6}{3} = 5.8 \pm 3.4\%(w/w)$

- Determination of Acid-insoluble Ash:

percentage forAcid-insoluble ash the 1st determination

w0= constant weight of crucible =25.87g

w1=weight of crucible and acid insoluble ash after heating =25.88g

w2 = (w1-w0) =25.88-25.87 =0.01g

$$\text{Acid insoluble Ash value} = \frac{o.o1}{2} \times 100 = 0.5\%(w/w)$$

percentageAcid-insoluble ash for the 2nd determination

w0= constant weight of crucible =51.22g

w1=weight of crucible and acid insoluble ash after heating =51.24g

w2 = (w1-w0) =51.24-51.22 =0.02g

$$\text{Acid insoluble Ash value} = \frac{o.o2}{2} \times 100 = 1.0\%(w/w)$$

percentage Acid-insoluble ash for the 3rd determination.

w0= constant weight of crucible =51.22g

w1=weight of crucible and acid insoluble ash after heating =51.25g

w2 = (w1-w0) =51.25-51.22 =0.03g

Acid insoluble Ash value =$\frac{0.03}{2}$×100 = 1.5%(w/w)

Average Acid-insoluble Ash determination=$\frac{0.5+1.0+1.5}{3}$ =1±2.12%(w/w)

- Determination of Water-soluble Ash:

  percentage forwater-soluble ash for the 1st determination

  w0= constant weight of crucible =25.88g

  w1=weight of crucible and water-soluble ash after heating =25.96g

  w2 = (w1-w0) =25.96-25.88 =0.08g

  Water-soluble ash value =$\frac{0.08}{2}$×100 = 4.0%(w/w)

  percentageWater-soluble ash for the 2nd determination

  w0= constant weight of crucible =51.22g

  w1=weight of crucible and Water-soluble ash after heating =51.29g

  w2 = (w1-w0) =51.29-51.22 =0.07g

  Water-soluble ash value =$\frac{0.07}{2}$×100 = 3.5%(w/w)

percentage Water-soluble ash for the 3rd determination.

w0= constant weight of crucible =25.88g

w1=weight of crucible and Water-soluble ash after heating =25.94g

w2 = (w1-w0) =25.94-25.88 =0.06g

Water-soluble ash value =$\frac{0.06}{2}$×100 = 3.0%(w/w)

Average Water-soluble ash determination=$\frac{4.0+3.5+3.0}{3}$ =3.5±2.02%(w/w)

- Determination of Moisture content;

$$\frac{weightloss}{powderedweight} \times 100$$

Weight loss = initial weight of dish and powdered (w1) – final weight of dish and powdered.(w2)

Powdered weight = 3g

Percentage moisture content for 1st determination.

W1 =54.29 g

W2 =54.12g

Weight loss = 54.29-54.12 = 0.17g

Percentage moisture content$\frac{0.17}{3}$ ×100 =5.67 %(w/w)

Percentage moisture content for 2nd determination.

W1 =54.22 g

W2 =54.02g

Weight loss = 54.22-54.02 = 0.2g

Percentage moisture content$\frac{0.2}{3}$ ×100 =6.7 %(w/w)

Percentage moisture content for 3rd determination.

W1 =54.22 g

W2 =54.02g

Weight loss = 54.22-54.02 = 0.2g

Percentage moisture content$\frac{0.2}{3}$ ×100 =6.7 %(w/w)

Percentage moisture content for 4th determination.

W1 =54.29 g

W2 =54.12g

Weight loss = 54.29-54.12 = 0.17g

Percentage moisture content$\frac{0.17}{3}$ ×100 =5.67 %(w/w)

Average moisture content =$\frac{5.67+6.7+6.7+5.67}{4}$= 6.2±4.4%(w/w)

- Alcohol soluble Extractive Determination; as

$$\frac{\text{Weight of residue in 20ml extract}}{weight of powdered} \times 100$$

Alcohol soluble Extractive for 1st determination

Weight of powdered =5g

W1 of clean crucible =144.71g

W2 of crucible+extract(dried) =144.83g

Weight of residue in 20ml=144.83-144.71 =0.12g

Alcohol soluble extractive = $\frac{0.12}{5}$ ×100 =2.4%(w/w) .In 100ml =2.4×5 =12%(w/w)

Alcohol soluble Extractive for 2nd determination

Weight of powdered =5g

W1 of clean crucible =144.71g

W2 of crucible+extract(dried) =144.83g

Weight of residue in 20ml=144.84-144.71 =0.13g

Alcohol soluble extractive = $\frac{0.13}{5}$ ×100 =2.6%(w/w) .In 100ml =2.6×5 =13%(w/w)

Alcohol soluble Extractive for 3rd determination

Weight of powdered =5g

W1 of clean crucible =144.71g

W2 of crucible+extract(dried) =144.82g

Weight of residue in 20ml=144.82-144.71 =0.11g

Alcohol soluble extractive = $\frac{0.11}{5}$ ×100 =2.2%(w/w) .In 100ml =2.2×5 =11%(w/w)

AverageAlcohol soluble extractive =$\frac{12+13+11}{3}$= 12±6.9%(w/w)

- Water soluble Extractive Determination; as

$\frac{\text{Weight of residue in 20ml extract}}{weight of powdered}$ ×100

Water soluble Extractive for 1st determination

Weight of powdered =5g

W1 of clean crucible =144.71g

W2 of crucible+extract(dried) =144.77g

Weight of residue in 20ml=144.77-144.71 =0.06g

Water soluble extractive = $\frac{0.06}{5}$ ×100 =1.2%(w/w) .In 100ml =1.2×5 =6%(w/w)

Water soluble Extractive for 2nd determination

Weight of powdered =5g

W1 of clean crucible =144.71g

W2 of crucible+extract(dried) =144.76g

Weight of residue in 20ml=144.76-144.71 =0.05g

Water soluble extractive = $\frac{0.05}{5}$ ×100 =1.0%(w/w) .In 100ml =1.0×5 =5%(w/w)

Water soluble Extractive for 3rd determination

Weight of powdered =5g

W1 of clean crucible =144.71g

W2 of crucible+extract(dried) =144.78g

Weight of residue in 20ml=144.78-144.71 =0.07g

Water soluble extractive = $\frac{0.07}{5}$ ×100 =1.4%(w/w) .In 100ml =1.4×5 =7%(w/w)

AverageWater soluble extractive =$\frac{6+5+7}{3}$= 6±3.5%(w/w)

- Determination of swelling index

Test 1;

Weight of powdered =2g

h1= Initial height of plant material in ml =7.5

h2 =final height of plant material in ml =11.0

swelling index =11-7.5 =3.5ml

Test 2;

h1= Initial height of plant material in ml =7.5

h2 =final height of plant material in ml =11.0

swelling index =11-7.5 =3.5ml

test3;

using 1g of plant material .

h1= Initial height of plant material in ml =4

h2 =final height of plant material in ml =7.5

swelling index =7.5-4 =3.5ml

Average value for swelling index =$\frac{3.5+3.5+3.5}{3}$ =$\frac{10.5}{3}$=3.5± 2.02□□

- Determination of foreign matter.

  By Visual inspection and suitable sieve

  Sand = 1g ;dust =15g

  Foreign matter =1+15 =16g

  % Foreign matter = $\frac{16}{100}$ =0.16g

- Determination of Tannins content;

  Using the following formular ;$\frac{□1-(□2-□0)\times 500}{□}$

  W =weight of plant material in grams =5g

  T1 =weight of container(w1) =134.92g

  Final weight after dryness(w2) =135.1g

  T1 =(w2-w1) =135.1-134.92 =0.18g

  T2 =weight of container(w1) =25.51g

  Final weight after dryness(w2) =27.86g

  T2 =(w2-w1) =27.86-25.51 =2.35g

  T0 =weight of container(w1) =25.68g

  Final weight after dryness(w2) =28.42g

  T0 =(w2-w1) =28.42-25.68=2.56g

  Total Tannins content ;$\frac{(0.18-(2.35-2.56)\times 500}{5}$

  $$\frac{(0.18-(-0.21)\times 500}{5}$$

  $\frac{0.39\times 500}{5}$ =0.39×100 =39%w/w

- Determination of Bitterness value;

Using the formular ; $\frac{2000\times\square}{\square\times\square}$

a = 0.01mg/ml

b=1ml

c =0.042mg= $\frac{2000\times0.042}{0.01\times1}$ =$\frac{84}{0.01}$ =8.400unit/g

Appendix 3;Evaluation of Anti Ulcer Studies

- Acute toxicity testing/LD_{50}

  1st part of investigation

  Group 1;Aqueous extract.

BW	Doses(mg/kg)	Dose/bw(ml)	mortality
198		0.396	
207	1000	0.414	0/3
184		0.368	
204		0.204	
204	100	0.204	0/3
157		0.157	
219		0.219	
210	10	0.210	0/3
169		0.169	

Group 2; Ethylacetate extract.

BW	Doses(mg/kg)	Dose/bw(ml)	mortality
218		0.436	
212	1000	0.424	0/3
193		0.386	
189		0.189	
235	100	0.235	0/3
216		0.216	
195		0.195	
197	10	0.197	0/3
213		0.213	

Group 3;Butanol extract.

BW	Doses(mg/kg)	Dose/bw(ml)	mortality
161		0.322	
175	1000	0.350	0/3
146		0.292	
217		0.217	
206	100	0.206	0/3
166		0.166	
278		0.278	
202	10	0.202	0/3
191		0.191	

2nd Phase for Acute toxicity testing;

Ethanol extract

BW	Doses(mg/kg)	dose/bw(ml)	mortality
147	5000	0.755	0/1
167	2900		0/1
191	1600		0/1
190	1000		0/1

Ethylacetate extract

BW	Doses(mg/kg)	dose/bw(ml)	mortality
147	5000	0.755	0/1
167	2900		0/1
191	1600		0/1
160	1000		0/1

Butanol extract

BW	Doses(mg/kg)	dose/bw(ml)	mortality
140	5000	0.700/1	
189	2900	0.548	0/1
151	1600	0.240/1	
151	1000		0/1

Ethylacetate extract

BW	Doses(mg/kg)	dose/bw(ml)	mortality
148	5000	0.740/1	
187	2900	0.540/1	
126	1600	0.202	0/1
125	1000		0/1

- **Anti ulcer studies**

Group 1(Distilled water 1ml)

Bw	Dose	ulcer Assesment	total
101		-	
124		-	
130		-	
111		-	
147		-	

Mean ulcer index =0.0

Group 2(Ethanol 1ml negative control)

Bw	ulcer Assesment	total
147	0,3,0.3,0.2,0.3,0.1	1.2
119	2.0,2.0,2.0,1.5.2.0,2.0,2.0,2.0,1.4	14.9
113	1.0,0.2,1.0	2.2
141	2.0,1.0,1.0,0.8,0.5,0.3,0.1	5.7
101	0.7,0.8,0.5 0.6,0.3	2.9
		26.9

Mean ulcer index =$\frac{£x}{n}$ = $\frac{26.9}{5}$ =5.38cm ± 2.4

Group 3(Cimetidine 100mg/kg positive control Ethanol)

Bw	ulcer Assesment	total
132	0.0	-
100	0.2,0.3,0.1	0.6
102	0.1 0.1	0.2
144	0.1,1.0	1.1
113	0.5 0.6,0.3	1.4
		3.3

Mean ulcer index $=\frac{£x}{n} = \frac{3.3}{5}$ =0.66cm ± 0.30

Group 4 (Ethylacetate fraction of *Cassia sieberiana* extract 200mg/kg)

Bw	ulcer Assesment	total
122	1.5,0,3,0.7,0.8,1.0,0.03	4.6
122	1.0,1.0,0.2,0.5	2.7
105	-	-
100	0.1,0.1,0.1	0.3
121	-	-
		7.6

Mean ulcer index $=\frac{£x}{n} = \frac{7.6}{5}$ =1.52cm ± 0.68

Group 5 (Ethylacetate fraction of *Cassia sieberiana* extract 400mg/kg)

Bw	ulcer Assesment	total
122	0.1	0.1
114	-	-
120	-	-
116	0.1,0.2	0.3

148	1.0,0.6,0.2,0.1	1.9
		2.3

Mean ulcer index $=\frac{£x}{n} = \frac{2.3}{5} = 0.46\text{cm} \pm 0.21$

Group 6 (Ethylacetate fraction of *Cassia sieberiana* extract 800mg/kg)

Bw	ulcer Assesment	total
135	-	-
114	-	-
162	-	-
114	-	
	-	
111	-	-
0.0		

Mean ulcer index $=\frac{£x}{n} = \frac{0.0}{5} = 0.0\text{cm} \pm 0.0$

Group 7 (Butanol fraction of *Cassia sieberiana* extract 200mg/kg)

Bw	ulcer Assesment	total
120	1.0,2.0,0.6,0.1	3.7
124	0.6,0.1	0.7
111	-	-
105	1.0,2.0,0.6,0.5, 0.8	4.9
149	2.0,1.0,0.6,0.8	4.4
		13.7

Mean ulcer index $=\frac{£x}{n} = \frac{13.7}{5} = 2.74\text{cm} \pm 1.22$

Group 8 (Butanol fraction of *Cassia sieberiana* extract 400mg/kg)

Bw	ulcer Assesment	total
105	0.1,0.1,0.1	3.7
102	-	-
130	2.0 0.1 0.4	2.5
140	1.0,0.5 0.1	1.6
150	0.1 0.1 0.5	0.7
		5.1

Mean ulcer index $=\frac{Ex}{n} = \frac{5.1}{5}$ =1.02cm ± 0.46

Group 9(Butanol fraction of *Cassia sieberiana* extract 800mg/kg)

Bw	ulcer Assesment	total
130	0.1,0.1	0.2
141	0.1	0.1
112	0.1 0.1	0.2
160	0.1	0.1
100	0.1	0.1
		0.7

Mean ulcer index $=\frac{£x}{n} = \frac{0.7}{5}$ =0.14cm ± 0.06

Preventive index(%) calculated as;P.i(%) = $\frac{U.Iof\ negative\ control - U.I\ of\ treated}{U.I\ of\ negative\ control}$

U.I of control = 53.8

Group 1(distilled water) P.I $=\frac{53.8-0}{53.8}\times 100 = 100$

Group 2(1ml ethanol) P.I $=\frac{53.8-53.8}{53.8}\times 100 = 0$

Group 3(100mg/kg cimetidine) P.I $=\frac{53.8-6.6}{53.8}\times 100 = 88$

Group 4(EAA of CSE 200mg/kg) P.I $=\frac{53.8-15.2}{53.8}\times 100 = 72$

Group 5(EAA of CSE 400mg/kg) P.I $=\frac{53.8-4.6}{53.8}\times 100 = 91$

Group 6(EAA of CSE 800mg/kg) P.I $=\frac{53.8-0}{53.8}\times 100 = 100$

Group7(butanol of CSE 200mg/kg) P.I $=\frac{53.8-27.4}{53.8}\times 100 = 49$

Group 8(butanol of CSE 400mg/kg) P.I $=\frac{53.8-10.2}{53.8}\times 100 = 81$

Group 9(butanol of CSE 800mg/kg) P.I $=\frac{53.8-1.4}{53.8}\times 100 = 97$

Table 4.11 Effect of *Cassia sieberiana* ethylacetate & n-butanol extract on Ethano linduced gastric ulcer.

Treatment	**Dose**	**Mean ulcer index± SEM**	**Preventive index (%)**
Distilled water	1ml	0.0 ± 0.0^{a}	--
Ethanol	1ml	53.8 ± 2.40^{e}	0
Cimetidine	100 mg/kg	6.6 ± 0.30^{b}	88
Ethylacetate	200 mg/kg	15.2 ± 0.68^{c}	72
	400 mg/kg	4.6 ± 0.21^{b}	91
	800 mg/kg	0.0 ± 0.0^{a}	100
Butanol	200 mg/kg	27.4 ± 2.40^{d}	46
	400 mg/kg	10.2 ± 0.46^{c}	81
	800 mg/kg	1.4 ± 0.06^{a}	97

N.B: MUI-Mean ulcer index.SEM-Standard error mean.PI-Preventive index, Mean with same alphabets along the column are not differences at $p < 0.05$, N = 5 per group

ANOVA TABLE

Source of variations	Df	SS	MS	f	p-value	FCRIT
Between groups	8	123.58	15.44	3.19	0.00767	2.209
Within groups	36	174.14	4.84			
Total	44	297.73				