PHARMACOGNOSTIC AND BIOLOGICAL EVALUATON OF THE ROOT BARK OF *SABA COMORENSIS* (BOJ.) PICHON (APOCYNACEAE)

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ZARIA, NIGERIA

FEBRUARY, 2009

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIRMENTS FOR THE AWARD OF MASTER OF SCIENCE IN PHARMACOGNOSY AND DRUG DEVELOPMENT

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FEBRUARY, 2009

DECLARATION

I declare that the work in the thesis entitled 'Pharmacognostic and Biological Evaluation of the root bark of *Saba comorensis* (Boj.) Pichon (Apocynaceae)' has been performed by me in the Department of Pharmacognosy and Drug development under the supervision of Dr K.Y Musa and Dr A. Agunu. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or Diploma at any University.

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CERTIFICATION

This thesis titled '**Pharmacognostic and Biological Evaluation of the Root bark of *Saba comorensis* (boj.) Pichon (Apocynaceae)'** by Attah, Francis Alfred Unuagbe meets the regulation governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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ACKNOWLEDGEMENT

I am most grateful to Jehovah God for His favour and grace to complete this work.

My sincere appreciation goes to my supervisors Dr K.Y Musa and Dr A. Agunu, all of the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU), Zaria.,who were inexplicably supportive in their comments and contributions- I appreciate the effort of my elder brother, Engr Attah Frederick and wife, my beloved mother, Mrs Funmilayo Attah. and Attah Akojima (Geologist) for their financial assistance, moral support and prayers. All other members of Attah Afhekhu's family are not left out of my appreciation list.

Additionally, I am most grateful to the following people who ,in one way or the other contributed to the success of this work;

Dr H. Ibrahim, Dr (Mrs) Nuhu Hadiza and Dr M.S. Abubakar all of the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria.

Prof. K.S. Gamaniel- Dean, Faculty of Pharmaceutical Science, University of Maiduguri

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ABSTRACT

Saba comorensis (Boj) Pichon (Apocynaceae) has been used traditionally in various parts of Tropical Africa region as an aphrodisiac, for rheumatism, arthritis, liver disease, jaundice, venereal disease, normal spleen function, as laxative, and purgative.

This study is aimed at establishing the pharmacognostic features of the root bark as well as testing its biological activity.

Macro-morphological features of *Saba comorensis* root include faint longitudinal striations with occasional cracks in the inner surface, slightly rough cork with well marked small circular ridges almost running uniformly with root hairs; the young root bark is doubled-quilled while the older one is channelled. Fracture is short and granular while the fractured surface shows a reddish-brown cork.

Microscopically, the transverse section of the root bark showed the presence of sclereids containing starch grains, pericyclic fibres, lignified sieve tubes, lignified phloem fibres (14.4-25.2μm), medullary ray usually biseriate with abundant starch grains (3.6-9.0μm) and clusters of calcium oxalate crystals (7.2-18.0μm) in the medullarly rays and parenchyma. The result of extractive values are: alcohol-soluble extractive (16% w/w ± 0.04), water–soluble extractive (22% w/w ± 0.06), total ash (13.67% w/w ± 0.03), the acid-insoluble ash (8.89% w/w ± 0.02) and moisture content (8.3% w/w ± 0.01). Preliminary phytochemical analysis revealed the presence of tannins, saponins, flavonoids, alkaloids, cardiac glycosides, anthraquinones, steroids, terpenoids, carbohydrates and proteins while the chemo-microscopy indicated the presence of cellulose, lignin, starch grains, proteins and fat and oils.

Thin-layer chromatography of the ethanolic extract showed four spots each using 10% sulphuric acid as spray reagent (heated at 100-105^{o}c) and iodine vapour, while chloroform extract gave two spots using Dragendorff spray reagent.

The root of the plant was also evaluated for the LD_{50}, anti-inflammatory, analgesic, ulcerogenic assay in animal models and its anti-oxidant properties. The LD_{50} was found to be above 770mg/kg body weight. The ethanolic extract at 50mg/kg, 100mg/kg and 150mg/kg i.p significantly decreased the number of acetic acid-induced writhing, but not in a dose dependent manner. At 100mg/kg the effect was 90.6% inhibition, which is significantly comparable to the standard drug aspirin with 91.2%. The extract elicited a dose dependent inhibition of both acute and chronic inflammation. Since results from this study strongly indicate the non-steroidal anti-inflammatory- like activity of the extract, its ulcerogenic effect was tested on fasted animals. Like most non-steroidal ant-inflammatory drugs, the extract caused gastric ulceration. The magnitude of the ulceration is dose dependent. Ulceration was higher for ethanol extract compared to the chloroform extract. The mean ulcer score of the plant extract at 500mg/kg was less than aspirin at 150 mg/kg. The scavenging activity (antioxidant properties) of the extracts using stable DPPH free radical showed that the ethanol extract has a higher antioxidant activity relative to chloroform extract. The concentration of the extract required to quench 50% of the free radical (ED_{50}) for ethanol and chloroform extracts were 0.198 and 0.284 respectively.

. The result of the pharmacognostic study could be used in setting some diagnostic indices for the proper identification and preparation of a monograph on the root bark of the plant,while the results obtained from the biological evaluation provide scientific basis for the anti-inflammatory, analgesic, and ulcerative properties of the root of *Saba comorensis.*

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CHAPTER 1

1.0 INTRODUCTION

Saba comorensis (Boj) Pichon (Family Apocynaceae) also known as *Landolphia comorensis* (Boj) K. Schum, and *Saba florida* (Bent) Bullock, is widely used in various parts of African region, for the treatment of myriads of ailments such as arthritis, lameness, rheumatism, inflammation (Kokwaro, 1976); lactation disorder, liver disease, jaundice, hepatitis, cirrhosis, adenitis, as laxative, purgative and spleen function (Bouquet, 1969). The fruit is regarded as famine food in Northern Nigeria, while the local people in Zaria take the root decoction to treat inflammation while the fruit is believed by the same people to cure Jaundice (personal communication). Mohammed (1994) also reported that a commercial citrus drink is prepared from the fruits in the Comoros Islands while the bark decoction is locally famed by the people as a potent aphrodisiac. Omino and Kokwaro (1993) reported the wide use of the root bark to treat abdominal diseases, diseases of the head, female conditions and venereal diseases.

Saba comorensis is known in English as rubber vine, *Ciwo* in Hausa and *Orombo* in Edo. It is well distributed in the Comoros, Ghana, Kenya, Malawi, Mozambique, Tanzania, Uganda (Maundu, 1999), and Nigeria (Hutchinson and Dalziel, 1963). It is a strong forest Liana up to 20cm long on other trees (FAO, 1983).

Evans (2002) reported the phytochemicals present in the sub-family Plumicroideae, to which *Saba comorensis* belongs. They include a vast range of indole alkaloids, cardioactive glycosides, and many others such as cyanogenetic glycosides, saponins, tannins, coumarins, phenolic acids and triterpenoids.

1.1 Historical perspectives of medicinal plants

Medicinal plants find application in pharmaceutical, cosmetic, agricultural and food industry. The use of the medicinal herbs for curing diseases has been documented in history of all civilizations. Before the onset of synthetic era, man was completely dependent on medicinal herbs for prevention and treatment of diseases (Kokate *et al.*, 2002). With the introduction of scientific procedures, the researchers were able to understand about toxic principles present in the green flora. The scientists isolated the active constituents of the medicinal herbs and after testing, some were found to be therapeutically active. Aconitine, atisine, lobeline, nicotine, strychnine, digoxine, atropine, morphine, are some common examples. The most recent ones are *Silybum marianum* (silymarin), *Artemisia annua* (artemesinin) and *Taxus baccata* (taxol) (Evans, 2002).

Recently, research has supported biological activities of some medicinal plants. Cancer is such a segment where researchers are expecting new molecules from herbs that can provide new tools for fighting this dreaded disease. Diabetes is another area where a lot of research is going on; while arthritis and many other inflammatory diseases are challenges that have found no satisfactory answer in modern medicine. The concept of anti-oxidants is fast catching up and latest research has shown that a number of herbal derivatives have excellent antioxidant action (Kokate *et al.*, 2002).

The plant is a biosynthetic laboratory, not only for chemical compounds, but also for a multitude of compounds like glycosides, alkaloids, etc. These constituents exert physiological and therapeutic effect. A systematic study of crude drug embraces a thorough consideration of primary and secondary metabolites derived as a result of plant metabolism. The plant material is subjected to phytochemical screening for the detection of various constituents (Kokate *et al.*, 2002).

With onset of scientific research in herbs, it is becoming clearer that medicinal herbs have a potential in today's synthetic era, as numbers of medicines are becoming resistant. It has been estimated that only 20% of the flora has been studied and 60% of synthetic medicines owe their origin to plants. Ancient knowledge, coupled with scientific principles can come to the forefront and provide us with powerful remedies to eradicate diseases (Evans, 2002).

1.2 Justification

Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the matchless availability of chemical diversity (Maria, *et al.*, 2007). A serious limitation encountered in the use and research of traditional medicine is the lack of standardization and quality control of raw material making up the drug. The ultimate objective of the pharmacognostic investigation is identification of the genuine crude drug and determining the extent of adulteration/substitution, if any. Advancement in recent years in pharmacognosy, phytochemistry and physicochemical instrumentation techniques has been of immense value in removing this major shortcoming of traditional medicine. This study is intended to provide information for the correct botanical identification of the plant as well as investigate the scientific basis for the wide ethnomedicinal use of the root of *Saba comorensis* in the tropical region. This includes studying the pharmacognostic features of the root bark as well as testing its biological activity.

1.3 Hypotheses

I. The root of *Saba comorensis* contains bioactive compounds.

II. These bioactive compounds are responsible for its biological activity.

1.4 Aims

I. To study the pharmacognostic characters of the root bark of *Saba comarensis*

II. To evaluate the biological activity of the ethanol extract of *Saba comorensis.*

1.5 Specific objectives

I. To carry out macroscopical and microscopical studies as well as determine physioco-chemical constants.

II. To conduct phytochemical tests on the various extracts of the root bark.

III. To determine the median lethal dose (LD_{50}) of the root bark (ethanol extract).

IV. To evaluate the antioxidant property of the root bark extract.

V. To carry out anti-inflammatory studies on the ethanol extract of the root bark .

1.6 The Bark

Bark is a very complex tissue that is composed of two principal zones: the inner bark and the outer bark. The outer bark, which is sometimes referred to as rhytidome and is also known as the periderm, is made up of three layers: the phellem (cork cells), phellogen (cork cambium), and the phelloderm (cork skin). The thickness of the periderm varies greatly between and within species and with the age of the bark. The inner bark, which is referred to as the phloem or bast, is complex in structure and is composed of several types of cells including sieve tubes, fiber cells, albuminose cells, companion cells, parenchyma cells, iodoblasts, and lactifers. Not all cell types occur in every bark. The bark is divided from the wood or xylem by the vascular cambium layer (Sandved *et al*., 1992). The chemical composition of bark is complex and varies between and within species, and also between the inner and outer bark. Proximate chemical analysis of bark from different species indicates that the chemical constituents of bark can be classified into four major groups: polysaccharides (cellulose, hemicellulose, and pectic materials); lignin and polyphenols; hydroxy acid complexes (suberin); and extractives (fats, oils, phytosterols, resin acids, waxes, tannins, terpenes, phlobaphenes, and flavonoids) (Roger *et al*., 2005).

1.7 Quantitative Evaluation of Crude Vegetable Drugs

The evaluation of a drug requires that it be identified both in its entire and powdered form by means of its morphological and cytomorphological characters. It is possible that faulty collection or extended storage may alter the constituents of the drug to a part where it is no longer acceptable. Therefore, drugs are subjected to evaluative procedures which will indicate their acceptability by criteria other than their morphology. (Brain and Turner, 1975).

The different determinations of quantitative analysis of crude vegetable drugs include the followings.

1.7.1 Determination of Moisture Content

The determination of moisture content is important because the presence of moisture causes the following:-

- It decreases the percentage quantity of the drug by increasing the weight.
- Causes activation of enzymes which lead to hydrolysis of the constituents.
- Causes chemical reactions and
- Harbours the growth of micro-organisms and insects.

Therefore the moisture content of all drugs should be kept reasonably low (Kokate, 1994; Evans, 2002).

1.7.2 Determination of Total Ash value

The determination of total ash is a method to measure the amount of residual substance not volatilized when the drug is ignited (AP, 1986). The total ash represents the physiological ash (i.e. ash from the extraneous matter), (AP, 1986). The physiological ash consists of the carbonates, phosphates, nitrates, sulphates, chlorides and silicates of the various metals present in the plant which it took up from the soil when growing. Example of elements taken up by the plant from the soil include sodium, potassium, calcium, magnesium, iron, copper, cobalt, manganese, carbon, phosphorous, nitrogen, sulphur, oxygen, chlorine and silicon (Kokate, 1994; Evans, 2002).

1.7.3 Determination of extractives

The determination of extractives is a method designed to measure the amount of constituents which are extractable by the solvent under specified conditions. It is applied

to those vegetable drugs for which there is no suitable method of chemical or biological assay for their active constituents (AP, 1986). The evaluation is important to guard against adulteration or substitution with drugs which have already been extracted (Kokate, 1994; Evans, 2002). The two major types of extractives commonly determined are the alcohol- soluble extractive and the water –soluble extractive.

1.8 Inflammation

Inflammation (Latin, *inflammatio*, to set on fire) is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation is not a synonym for infection. Even in cases where inflammation is caused by infection it is incorrect to use the terms as synonyms: infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen (Guyton and Hall, 2006). In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism. However, inflammation which runs unchecked can also lead to a host of diseases, such as hay fever, atherosclerosis, and rheumatoid arthritis. It is for this reason that inflammation is normally tightly regulated by the body (Guyton and Hall, 2006).

Inflammation can be classified as either *acute* or *chronic*. *Acute inflammation* is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as *chronic inflammation*, leads to a progressive shift in

the type of cells which are present at the site of inflammation and is characterised by simultaneous destruction and healing of the tissue from the inflammatory process (Guyton and Hall, 2006).

1.8.1 Causes of Inflammation

Inflammation may result from any the following;

- Burns
- Chemical irritants
- Frostbite
- Toxins
- Infection by pathogens
- Necrosis
- Physical injury
- Immune reactions due to hypersensitivity
- Ionizing radiation
- Foreign bodies, including splinters and dirt (Guyton and Hall, 2006).

1.8.2 Types of Inflammation

Table 1.1 below presents a summary of the types of inflammation.

Table 1.1: Types of inflammation

Comparison between acute and chronic inflammation:		
	Acute	**Chronic**
Causative agent	Pathogens, injured tissues	Persistent acute inflammation due to non-degradable pathogens, persistent foreign bodies, or autoimmune reactions
Major cells involved	Neutrophils	Mononuclear cells (monocytes, macrophages, lymphocytes, plasma cells), fibroblasts
Primary mediators	Vasoactive amines, eicosanoids	INF-γ and other cytokines, growth factors, reactive oxygen species, hydrolytic enzymes
Onset	Immediate	Delayed
Duration	Few days	Up to many months, or years
Outcomes	Healing, abscess formation, chronic inflammation	Tissue destruction, fibrosis

Robbins and Cotran (1985).

1.9.0 Non-steroidal anti inflammatory drugs (NSAIDS)

The history of analgesic and anti-inflammatory substances started with the use of decocted salicylate-containing plants by ancient Greek and Roman physicians. Willow

bark was already mentioned in the *Corpus Hippocraticum* (a collection of medical scripts compiled by Alexandrian scholars in approximately 300 BC) as a substance for treating fever and pain conditions. Over the past 140 years other substances have been introduced for therapy, collectively termed nonsteroidal anti-inflammatory drugs (NSAIDs), after P.S. Hench discovered the anti-inflammatory properties of glucocorticoids in 1949. NSAIDs, which possess analgesic, anti-inflammatory and antipyretic properties, are a heterogeneous group of substances without any uniform chemical properties (although most are organic acids), but nevertheless share the same therapeutic and side effects. In the past few years there have been significant advances in explaining the mechanism of action of NSAIDs (Jurgen, 2000).

1.9.1 Mechanism of Action of NSAIDs

In the 1930s, Goldblatt and von Euler showed that human seminal fluid contained a component that reduced blood pressure, the effects of which could not be classified among the tissue hormones known at the time. Von Euler termed these new, unknown substances 'prostaglandins' because he presumed that these mediators were produced in the prostate (Goldblatt, 1933; Von, 1935). After Bergström and Sjövall achieved the first chemical identification of a prostaglandin at the beginning of the 1960s, the era of prostaglandin research began (Bergstrom, 1962). It turned out that these hormones could be synthesized by many mammalian cells and that they participate in the regulation of numerous physiological functions (Jurgen, 2000). Another milestone was the discovery by Vane and coworkers that the analgesic, antipyretic and anti-inflammatory properties of acetylsalicylate were based on the inhibition of prostaglandin synthesis (Vane, 1971). Vane showed that the acidic anti-

inflammatory analgesics decreased pro-inflammatory prostaglandin concentrations by inhibiting cyclo-oxygenase. This finding made sense because the prostaglandins characterized in the 1960s were found to be substantially involved in bringing about and maintaining inflammatory processes by increasing vascular permeability and amplifying the effects of other inflammatory mediators such as kinins, serotonin and histamine. Prostaglandin E_2 (PGE_2) is also involved in the induction of fever. As we now know, prostaglandins are not themselves significant mediators of pain; instead, they increase the sensitivity of nociceptors to other stimuli in traumatized tissue. They switch normally non-excitable polymodal receptors ('silent nociceptors') into a state in which they are easily excitable (Jurgen, 2000).

1.10 Medicinal chemistry of salicylates

Salicine (salicyl alcohol glycoside) was the first compound belonging to this category that exhibited medicinal value. Salicine and Methyl salicylate may be found in the leaves, barks and fruits of many trees and plants e.g. Willow (genus Salix). These botanical services were known to Hippocrates some 2,400 years ago and were used by Galen in the second century for the treatment of certain local diseases such as ulcers, fistulas, and erusipelas. All through the middle ages salicylates were used in many medical treatments including diuresis. Willow bark was used to substitute Cinchona bark as antipyretic because the later was more expensive (Ibrahim, 1997).

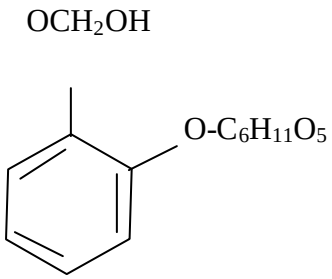
Salicine was first extracted early in the nineteenth century from Willow bark, and a few years later it was isolated in its pure state. The first preparation of salicylate was from Salicin, though it has been prepared by oxidation of salicylaldehyde and later by hydrolysis of anthranilic acid (O-aminobenzoic acid). In 1860, the commercial method of preparing salicylic acid from phenate was introduced. Salicylic acid was not

generally available until after its synthesis from phenol in 1874. Perhaps the relationship of salicylic acid to phenol led to its early use as an antiseptic. Salicylic acid (M.P 159º) occurs mainly as esters and glycosides. It is found in small amounts in cherries, strawberries and raspberries. It occurs less frequently compared to the other phenolic acids.

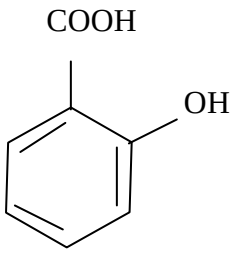
In 1876 salicylates were first used in rheumatic fever and about 20 years later acetylsalicylates-aspirin was introduced into the medical practice. Acetylsalicylic acid (ASA) was first synthesized in 1852 but more or less remained obscure until its detailed pharmacodynamic properties were studied in 1899. The name 'aspirin', was derived from 'a' of acetyl and adding to it "spirin", an old name of salicylic or spiric acid obtained from Spirea plants. Acetylsalicylic acid can be synthesized by acetylation of salicylic acid with acetic acid anhydride. The crude product may be recrystalized from benzene, mixture of acetic acid and water or various non-aqueous solvents. The methyl and ethyl esters of salicylic or acetylsalicylic acid can be prepared by treating these acids with acidified methanol or ethanol respectively. The methyl esters of these salicylates can be prepared by treating them with ethereal diazomethane. The products are prodrugs, since they yield the pharmacological active acids by hydrolysis following their absorption either from the gastrointestinal tract or through the skin (Rainsford *et al*, 1981).

ASA is used as an antipyretic, anti-inflammatory and an analgesic in a variety of conditions ranging from headache, discomfort and fever associated with the common cold, and muscular pains or aches. Aspirin is regarded as the drug of choice in the reduction of fever because of its high degree of effectiveness and wide safety margin. As aspirin inhibits platelet function, it has been employed prophylactically to minimize the incidence of myocardial infaction and transient ischemic attacks.

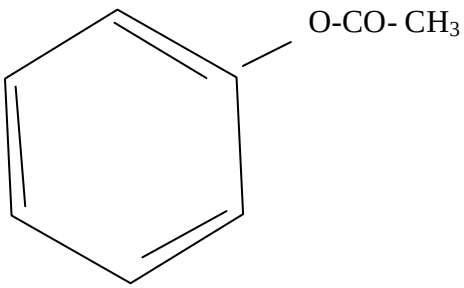
The antipyretic and anti-inflammatory actions of the salicylates are due to their inhibitory effects on prostaglandin synthesis.



Salicin
(Evans, 2002)



Salicylic Acid (Dipalma, 1971)



Acetyl Salicylic Acid(ASA)

(Diplalma, 1971)

1.11 Antioxidants

Antioxidants are molecules that slow or prevent the oxidation of other chemicals. Oxidation is a redox chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can involve the production of free radicals, which can form dangerous chain reactions. Antioxidants can terminate these chain reactions by removing radical intermediates and can inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or phenols (Matill, 1947). Although oxidation reactions are critical for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidant molecules or inhibition of these antioxidant enzymes causes oxidative stress and may damage or kill cells (Moreau and Dufraisse, 1992).

The possible mechanisms of action of antioxidants were first explored when it was recognized that a substance with anti-oxidative activity is likely to be one that is itself readily oxidized. Research into how vitamin E prevents the process of lipid peroxidation led to the identification of antioxidants as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells(Wolf, 2005)..

A paradox in metabolism is that while the vast majority of complex life requires oxygen for its existence, oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species (Davies, 2007). Consequently,

organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, proteins and lipids.(Sies, 1997; Vertuani *et al*, 2004). In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell. (Davies, 2007 Sies, 1997).

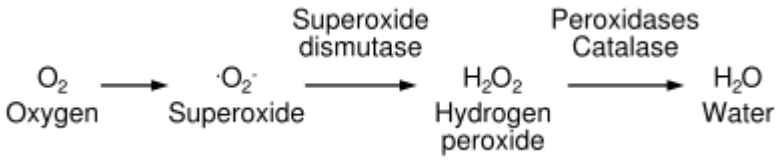
The reactive oxygen species produced in cells include hydrogen peroxide (H_2O_2), hypochlorous acid (HClO), and free radicals such as the hydroxyl radical (·OH) and the superoxide anion (O_2^-) (Valko, 2007). The hydroxyl radical is particularly unstable and will react rapidly and non-specifically with most biological molecules. This species is produced from hydrogen peroxide in metal-catalyzed redox reactions such as the Fenton reaction (Nakabeppu, *et al, 2006*). These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins (Sies, 1997). Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms, (Stohs and Bagchi,1995) while damage to proteins causes enzyme inhibition, denaturation and protein degradation (Stadtman, 1992).

The use of oxygen as part of the process for generating metabolic energy produces reactive oxygen species (Raha and Robinson, 2000). In this process, the superoxide anion is produced as a by-product of several steps in the electron transport chain (Lenaz, 2007). Reactive oxygen species are also produced during photosynthesis under conditions of high light intensity (Krieger-Linszkay, 1995). This effect is partly offset by the involvement of carotenoids in photoinhibition, which involves these antioxidants reacting with over-reduced forms of the photosynthetic reaction centres and thereby preventing superoxide production (Subo *et al*,2005).

1.12 Ascorbic acid

Ascorbic acid or "vitamin C" is a monosaccharide antioxidant found in both animals and plants. It cannot be synthesized in humans and must be obtained from the diet (Smirnoff, 2007). In cells, it is maintained in its reduced form by reaction with glutathione, which can be catalyzed by protein disulfide isomerase and glutaredoxins (Meister, 1994; Wells *et al*, 1990). Ascorbic acid is a reducing agent and can reduce and thereby neutralize reactive oxygen species such as hydrogen peroxide (Pdayatty,et al, 2003). In addition to its direct antioxidant effects, ascorbic acid is also a substrate for the antioxidant enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants (Shigeoka *et al*, 2002).

1.13 Enzyme systems



Enzymatic pathway for detoxification of reactive oxygen species.

As with the chemical antioxidants, cells are protected against oxidative stress by an interacting network of antioxidant enzymes (Davies, 2007 Sies, 1997). Here, the superoxide released by processes such as oxidative phosphorylation is first converted to hydrogen peroxide and then further reduced to give water. This detoxification pathway is the result of multiple enzymes, with superoxide dismutases catalysing the first step and then catalases and various peroxidases removing hydrogen peroxide. As with

antioxidant metabolites, the contributions of these enzymes can be hard to separate from one another, but the generation of transgenic mice lacking just one antioxidant enzyme can be informative (Ho, *et al*, 2007).

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Historical perspectives of the family Apocynaceae

The word Apocynaceae was derived from the Greek word "ALLOKUVOV (Apkunon)" meaning "dog's bane". The family is also known as Fangifani family. This

name obtained after the genus *Apocynum*, a name referring to the fruit of two separate carpels, which was thought to resemble two dog's tail (Ibrahim, 1997).

Principally, this family inhabits the intertropical zone of Old and New World especially Asia beyond the equator: Tropical Africa, extending southward to Rhodesia (now Zambia and Zimbabwe), Madagascar, Australia, Arabia, India and Indochina. They are comparatively rare in the hot and temperate regions (Hutchinson and Dalziel 1963; Irvine, 1961). They are mainly found in the tropics and .subtropics (Dutta, 1971; Hutchinson, 1973; Metcafe and Chalk, 1953).Their major habitat is terrestrial. They are usually found in thick forest, forming 20 - 30% of the forest vegetation. They are most commonly found around the mountain or hilly areas.

Apocynaceae is dicotyledonous and belongs to the Phylum Angiosperms in the Vegetative Kingdom. The Vegetative Kingdom is divided into Cryptogram, in which there are - no true flowers producing seeds, and Phenerograms in which the plants develop flowers or flower like structures in which seeds are produced. The phenerograms include all higher plants and by this is meant all present day groups which exhibit the highest development and which are usually referred to as the Spermatophytes. This group is again sub-divided into two phyla, the Gymnosperms- and the Angiosperms. Apocynaeceae is divided into two sub families Echitoidae and Plumerioideae.

The Echitoidae have corolla -lobes twisted to the right, and the stamen closely adhered to the stigma while Plumerioidae have corolla lobes twisted to the left with stamens free or slightly attached to the stigma with anther - tails absent and the seeds are usually hairless. Members of the Apocynaceae are very easy to identify. The milky latex, the twin pods or follicles and the overlapping lobes of the corolla just like a propeller. This family is related to the Asclepiadiceae in general habit, biocollateral vascular bundles,

latex tubes, general flora and fruit characters. The two however, can be easily distinguished from each other by the characters of androecium and gynoecium. In Apocynaceae stamens are distinct (not united with the stigma), corona (stamina) absent, pollen grains distinct (not in pollinia), and style one (not two) (Dutta, 1971).

2.2 Some important genera of the family Apocynaceae

The family Apocynaceae comprises 180-300 genera with 1,800 species (Hickey and King, 1981). Commonly occurring plants of the family are

Agnosma caryophyllata, (Roxb ex Shims) G. Don

Allemande cathertical

Willoughbeca edulis Roxb.

Beaumontia grandifolia Wall

Ichnocarups fructescens R.Br.

These are climbing shrubs

Nerium indicum Mill (Oleander),

Carissa congesta Will, (Bengal Currants)

Ervatamia coronaria, Stapf

Thevetia peruviana K. Schum (Pears) (Ibrahim, 1997).

2.3 The Morphological Features of the Family Apocynaceae

It is a mainly tropical and subtropical family of a very diverse habit including trees, - shrubs, climbers and a few perennial herbs with latex (Metcafe and Chalk, 1950; Irvine, 196l). The leaves are opposite or verticillate, rarely alternate, simply entire, stipules usually absent. Flowers hermaphrodite, actinomorphic. Calyx often glandular inside, lobes 5 or rarely 4, imbricate. Corolla tubular, variously shaped, lobes contorted

imbricate, very rarely valvate. Stamens 5 or rarely, inserted in the tube, filaments free or rarely united; anther often sagitate, free or connivent around the stigma, rarely adherent to the latter, two celled, opening lengthwise, connective often produced at the apex; pollen granular; disk usually present annular, copular or of separate glands. Ovary superior, one celled with two parietal placentas adnate to the septa or capels two and free or connate only at the base with ventral placentas in each carpel; style one, split at the base or entire, thickened and stigma below the apex; ovules two or more in each carpel. Fruit entire and indehiscent or of two separate carpels, baccate drupaceous or follicular. Seeds mostly with endosperm and large straight embryo, often winged or appendaged with long silky hairs (Dutta, 1970; Irvine, 1961; Ghazanfar, 1989; Hickey and King, 1981)

The leaf is usually dorsiventral or isobilateral. The outstanding anatomical characters are the universal occurrence of:

i. Laticiferous canals with variously coloured contents

ii Intraxylary phloem or a bicollateral vascular bundle (Dutta, 1971; Mclean, 1949; Metcalfe and Chalk, 1950).

2.4 Medicinal and Economic uses of the Family Apocynaceae

The members of the family Apocynaceae are mainly important as ornamentals where as some yield fruits, drugs, latex, and other articles (Dutta, 1970). The bark of *Alstonia scholaris* is astringent and forms the ‘ditta bark’ of commerce. *Aspidosperma quebracho-blanco* (Argentina) furnishes medicinal *quebracho-blanco* bark. The roots of *Evatamia coronaria* serve as antidote against scorpion sting. The bark of *Holarrhena*

antidysenterica is used in amoebic dysentery. The cortex of *Hunterria zaylamica* (Malaya) is used for treating yaws. The bark and wood of *Nerium indicum* are useful for the manufacture of rat poison. The roots of *Rauwolfia serpentina*, *Rauwolfia nitida* are used as a wonder-drug for lowering blood pressure. The seeds of *Strophanthus komb'e, S. hispidus*, and others provide strophanthin a heart stimulant and precursor of cortisone which is specific for arthritis. *Strophanthus gratis* is used as arrow poison. Some other species of strophanthus have been accepted as useful drugs in western medicine. The leaves of *Wrightia trictoria* provide wrappers for 'bidis', the seeds contain indigo-like blue dye called "goda-indrojoo" which is used in the treatment of snake bite, the seeds also yield a red oil which has medicinal applications. Some of the plants in this family are bitter and employed as a purgative, sometimes the taste is mild, scarcely bitter and simply used as laxative (Omino and Kokwaro, 1993; Ibrahim, 1997).

2.5 Phytochemical Constituents of the Family Apocynaceae

The reported constituent of the family Apocynaceae are:

- Alkaloids (e.g. Indole alkaloids, isoquinoline alkaloids and steroid alkaloids) (Alston and Turner, 1963); Omino and kokwaro, 1993).
- Cardiac glycosides or cardenolide glycosides (Alston and Turner, 1963).
- Phenolic compounds (Bentley *et al*. 1984).
- Sesquiterpènes (Ibrahim, 1997).

2.6 Saba comorensis

Current Name: *Saba comorensis*

Family: Apocynaceae

Subfamily: Plumerioideae

Synonym(s)

Landolphia comorensis (Boj) K. Schum. Var. *florida* (Benth.) K. Schum.

Saba florida (Benth.) Bullock

Common names

(English): rubber vine

(Hausa): ciwo

(Edo): orombo

Taxonomy

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Order: Gentianales

Genus: *Saba*

Species: *comorensis* (Bojer) Pichon

Variety: *Florida*

Botanical Name: *Saba comorensis var. Florida*

2.6.1 Botanic description

Saba comorensis is a strong forest liana up to 20m long on other trees. Stem lenticillate and exuding whitish sticky latex when cut. Leaves ovate or elliptic, base rounded or subcordate, apex obtuse or rounded, 7-16 × 4-8.5cm, glabrous. Flowers fragrant, borne in many short stalk terminal or auxiliary corymbs. Corolla tubular, throat yellow, petals white. Fruit subglobose 4-8cm long and 3.5-6cm wide, pitted, greenish when young,

turning orange-yellow after ripening. Fruit pulp yellow. Seeds numerous, brown-black and coated with orange-yellow pulp. The specific epithet refers to the Comoros Islands (FAO, 1983).

2.6.2 Ecology and Distribution

S. comorensis is very abundant in undisturbed forests, coastal areas and around the Great lakes region of Africa. The liana is very rare in open areas. It commonly associate with *Antriaris toxicaria*, *Milicia excelsa*, *Khaya nyasica*, *Pachystela brevipes*, *P. Msolo*, *Sterculia appendiculata*, *Sorindeia madagascariensis*, *Trema orientalis* and *Ficus spp* (FAO, 1983)**.** *S. comorensis* is native to Comoros, Ghana, Kenya, Malawi, Mozambique, Tanzania, Uganda (FAO, 1983), Nigeria.

2.6.3 Biophysical limits and fruiting time

Altitude: 0-1250m

Mean anual temperature: 20ºC

Annual rainfall: 900-2000mm

Soil type: Grows on a variety of soils (gritty sandy - clay loams – dark grey compared loamy soils) (FAO,1983)

S. comorensis does not fruit every year; moreover flowering time is not regular among populations. In Northern Nigeria, such as Niger State and Jos Plateau, flowering and fruiting time is October,while in Zaria, it is October. However, in Southern Nigeria such as Ibadan and Benin, flowering and fruiting time is December (Hutchinson and Dalziel, 1963). In Tanzania the species flower between February and November. Fruit maturing is from December- May. The seed is dispersed by birds and monkeys (FAO, 1983).

2.6.4 Propagation method

S. comorensis regenerates naturally by seed. For establishment, the seed needs fertile moist soils under partial or full shade. Artificial regeneration by cutting is also possible (Maundu, 1999).

The vine coppices on cutting the main stem (Maundu, 1999). Mature fruit never falls and must be collected once yellow in colour. For easy germination the seeds need de-pulping by hand and cleaning in water. The germination success rate is well over 90% and starts 12 days after sowing. Germination is uniform and hypogeal. Passage through animal gut greatly improves germination (FAO, 1983).

2.6.5 Medicinal uses

The fruit is edible, commonly found in market places. A refreshing sour drink can be made from the fruit. There is an export potential as the fruit does not easily rot. The flowers provide necter required in apiculture. The stems are used in hut building. The stem yields latex which is used as an inferior rubber. The bark decoction is used in treating rheumatism, etc (as ealiar listed). Its fruits are sucked for oral thrush in Kenya (Maundu,1999).

2.6.6 Other uses

Erosion control: This vine prevents river bank soil erosion. It is also a beautiful liane for shady gardens, flowers beautiful and fragrant. It is used for boundary or barrier or support as well as for making fence (FAO,1983).

CHAPTER 3

3.0 MATERIALS AND METHOD

3.1 Collection, identification and preparation of plant material

***Plant material*:** *Saba comorensis* roots were collected from their natural habitat in Samaru – Zaria, Kaduna State, Nigeria in October, 2006 and authenticated by Mr Galah

of the Biological Science Department, Ahmadu Bello University, Zaria. The plant was also identified by the same taxonomist. A herbarium sample (number 1173) was deposited at the herbarium of the university.

Extract preparation: The root bark was air dried (30-35^{o}C) and crushed to powder. The crude ethanolic extract was obtained from the powdered botanical material by maceration in cold ethanol (96%GL), for over 72 hours, before being concentrated. The ethanolic process gave a yield of 8% as measured by gravimetry. For the purpose of preliminary phytochemical investigation, some biological evaluation and chromatographic analysis, 20g each of the root bark of *Saba comorensis* were extracted successively with H_2O and $CHCl_3$. The extracts yields of the plant were 1g, and1.2g respectively. The ethanol extract was dissolved in 0.9% saline while the chloroform extract was dissolved in 2.5% Tween 80 and subsequently, normal saline.

3.2 Experimental Animals

Adult male and female Swiss mice (20-30g) and albino rats (120-180g) obtained from the animal house, Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria, were used. The animals were fed with water and food (Compressed Growers' mash) *ad libitum* and were maintained in a room with light at room temperature. Animals were acclimatized for at least 5 days before the beginning of the experiments.

3.3 List of chemicals and reagents

The reagents used were all of laboratory grade. Distilled water was used in place of water.

The following drugs/reagents were used:

- acetylsalicylic acid (ASA) (300mg/kg)
- Acetone
- Alcohol
- Ammonia solution
- Barfoed's Reagent
- Chlor-zinc-iodine
- Chloroform
- Chloroform water (0.25%)
- Copper Acetate
- Dragendorff's Reagent (Bismuth potassium iodine)
- Ethanol extract (50, 100, 150 & 500mg/kg)
- Fehling's solution I(A- Copper II oxide) and II (b- Sodium hydroxide)
- Ferric Chloride
- Formaldehyde
- Glacial acetic acid
- Hager's reagent(a saturated solution of picric acid)
- Hydrochloric acid
- N/50 Iodine
- 1.2% solution of acetic acid
- 2, 2-diphenyl-2-picrylyhdrazyl radical (DPPH)
- Lead acetate (10% w/v in carbon dioxide free water) (B.P C, 1963)
- Mayer's reagent(potassium mercuric iodide)
- Molisch's reagent (10% solution of alpha-naphthol in alcohol)
- Normal Saline
- Phloroglucinol

- Piroxicam (Pfizer).
- Ruthenium red
- Sodium Acetate
- Vehicle (water + Tween 80).

All drugs were dissolved in water, and the chloroform extract was dissolved in water with Tween 80 as an emulsifying agent.

3.4 Pharmacognostic evaluation of the root bark of *Saba comorensis*

A systematic study of *Saba comorensis* under pharmacognostic scheme was carried out which involves its description on morphological and macroscopical characters, microscopic or histological characters, determination of some physical constants, preliminary phytochemical screening and chromatography as outlined by Kokate, (2002).

3.4.1 Determination of macro-morphological features of the root bark

The general method of macroscopic examination described by Brian and Kenneth, (1948) and Kokate, (2002) was adopted in the determination of the macro-morphological features of the root bark. Such features of the root bark as size, shape, fracture, surface, colour, odour and taste were determined.

3.4.2 Determination of the micro-morphological features of the root bark

Microscopy

Microscopic examination was also done using fresh bark of *Saba Comnensis* that has been previously soaked in water (for 24hours) until sufficiently soft; transverse

sections were made and mounted in the following reagent as described by Brian and Kenneth (1948) and Evans, (2002).

a. Glycerin
b. Chloral hydrates solution and warmed gently in order to clear the sections.
c. Iodine water (50% Iodine solution) to stain starch.
d. Phloroglucinol and hydrochloric acid to stain lignified tissues.
e. Corallin Soda and allowed to stand to stain the callus of the sieve tubes pink.

The result obtained from test 'b' and'd' were used to draw the plan of tissues as seen under the low power of the Microscope.

Small pieces of the bark were also macerated in 5% potassium hydroxide solution in water until sufficiently soft and subsequently washed with water, teased out under alcohol, mounted in glycerin and sketches of isolated elements were taken.

The powered bark was also examined by mounting a sample in reagents used for the transverse sections. Permanent slides were prepared from the freshly collected root and root barks of *Saba comorensis*.

Permanent preparation of microscopic sections

The following were the techniques employed for the study of the various sections of the root bark of *Saba comorensis* as outlined by Ibrahim, (1997) and Dutta, (2005).

Fixation: The root of *Saba comorensis* were collected and cut into pieces not more than 3mm long fixed in Formalin-Acetic acid –Alcohol (FAA) making sure damage is minimized when cutting them. This was left overnight.

FAA has the following composition:

70% alcohol - 90ml

40% formaldehyde - 5ml

Glacial acetic acid - 5ml

Dehydration: The materials were transferred into 70% alcohol to dehydrate for 2 hours, to absolute alcohol for 2 hours, to 25% Chloroform/alcohol for 2 hours, to 50% Chloroform/alcohol for 2 hours, to 70% Chloroform/alcohol for 2 hours and to pure Chloroform – two changes for 2 hours.

Infiltration: Infiltration was done by adding chips of 58-60ºC paraffin wax and kept adding until the wax started to gel in the chloroform, left overnight to allow infiltration to take place properly and chloroform evaporated by pouring the material with gelled paraffin wax into a shallow dish and left on top of embedding oven at 60ºC for one hour and transferred to embedding oven to allow evaporation to take place better for half an hour, making sure there was no more smell of chloroform in the wax, transferred material into pure molten wax in embedding oven at 60ºC for half an hour and to another pure molten wax for half an hour.

Embedding (Preparation of Paraffin block): 'L' angle mould, an embedding mould was used. The inner surface of the embedding mould was smeared with dilute glycerin. Molten wax, slightly heated above 60ºC, was poured into the mould to about half its volume, and transferred into the wax with the aid of warm forceps and oriented them into their cutting angle. A skin film was allowed to form on top of wax after orientation by blowing air on it. Warm spatula was used to transfer them into mould and oriented with warm fine forceps and Submerged in cold water when the thin film formed on top of wax. Water was left running to allow the block to harden. The mould was removed from the block and placed in a cool dry and dust free atmosphere ready for sectioning.

Sectioning: A trimmed cut piece of block with specimen in equal sides was obtained so as to get a straight ribbon when sectioning, i.e. top or bottom edges straight and parallel.

Care was taken to ensure that the sides are at right angles and seen that the object was carefully placed in the centre of the block to be sectioned. This was mounted on a microtome specimen holder by using warm blunt scarpel to melt the wax on the surface of object holder, submerged in water to stick to the block firmly before sectioning. Sections were cut with a sharp microtome knife to enable sections to ribbon properly.

Mounting of Ribbon on Slide: *A* clean slide was smeared with a drop of egg albumen. A portion of ribbon was cut and mounted on the albumenized slide with some few drops of distilled water on the slide. A section was stretched on top of a spirit lamp flame making sure not to melt the section. The water was drained carefully after stretching and the section pressed to stick on the slide with the aid of several thicknesses of filter papers using the side of the hand as pressure, and were left to dry in a dust free atmosphere for three days before staining.

Staining Method: The slides were dewaxed in xylene (two changes – 5minutes each). Section was treated with absolute alcohol for 2 minutes, 95% alcohol for 2 minutes, 70% alcohol for 2 minutes, in Safranin for 20minutes and stained in 50% alcohol for 1 minute. This was washed in 70% alcohol for 1 minute; washed in 95% alcohol for 1 minute, washed in absolute alcohol for 1 minute, counter stained in fast green prepared in 95% alcohol for 1 minute, differentiated in 95% alcohol for ½ minute, transferred to absolute alcohol for ½ minute and cleared in Xylene I and II**.** This was then mounted in Di-n-butylphthalate in Xylene (D.P.X) using a clean cover slip, dried on a warm hot plate, labeled and kept in slide cabinet for observation under the phase contrast Microscope.

Chemo-microscopy of the powered root

Chemomicroscopical analysis was carried out on the powdered root bark of *Saba comorensis* according to the methods described by Brain and Turner (1975) and Evans (2002).

i. Chloral hydrate solution was used to clear the drug sample in order to observe the calcium oxalate crystals present.

ii. The powdered root bark was mounted in dilute glycerol to observe the orange – brown starch grains present.

iii. Phloroglucinol and concentrated hydrochloric acid was also used to mount the powdered *Saba comorensis* root bark and the red colour and its intensity were observed, this indicated the extent of lignifications.

iv. N/50 Iodine solution was used to mount the powder and Blue – black colouration was noted indicating the presence of starch.

v. The powder was again mounted in Sudan IV followed by warming and a distinct red colouration was expected to indicate the presence of fat and oils.

vi. Concentrated hydrochloric acid was added and observed under the microscope for the presence of Calcium carbonate.

vii. Picric acid and millons reagent were also used to test for the presence of proteins. A yellow colouration indicates the of proteins using picric acid while it is red for millon's reagent.

viii. Ammonia Solution of copper II oxide was added to detect the presence of cellulose.

3.4.3 Determination of some Physical Constants of the Root Bark of *Saba comorensis*

i. *Determination of the Moisture Content*

The method of "Loss on drying" as described by Brain and Turner (1975) was

adopted in the moisture content determination of the root bark powder. Excess moisture in a sample suggests the presence of unwanted water which may be due to incorrect preparation or wrong storage. Excess moisture can result in the breakdown of important constituents by enzymatic activity and may encourage the growth of yeasts and fungi during storage (brain and turner, 1975).

Procedure: 3g of accurately weighed powdered root bark of *Saba comorensis* was put in a glass dish. This was heated for 1 hour at 105^{o}C, cooled in a desiccator, and weighed. The procedure was repeated until there was no further loss in weight. The moisture content determination was repeated for at least three times and the average moisture content was calculated.

ii. ***Determination of the ash values***

The method of Kokate (1993) was adopted in the determination of the ash values of the powered drug.

a. ***Total Ash Determination***: 3g of the powdered drug was accurately weighed into a tarred silica crucible. This was then incinerated gradually increasing the heat until free from carbon and cooled. It was then transferred and kept in a desssicator. The ash was weighed and the percentage total ash calculated with reference to the air dried sample. The whole procedure was repeated for for at least three times and the average total ash value calculated.

b. ***Determination of acid insoluble ash****:* The total ash obtained was boiled for 5 minutes with 25ml of dilute HCl. This was then filtered and the insoluble matter collected on ashless filter paper, washed with hot water, and ignited in tarred crucible. It was subsequently cooled, kept in a dessicator and weighed. The percentage acid insoluble ash with reference to the air dried drug was calculated. The entire procedure was repeated for at least three times and the average taken (Kokate, 1993).

iii. ***Determination of extractive values***

The method described by Kokate (1993) was used for the determination of the extractive values.

a. ***Alcohol – soluble extractive determination:*** 5g of accurately weighed coarsely powdered air-dried root bark of *Saba comorensis* was macerated in 100ml of Alcohol (90%) in a stoppered flask for 24hours, shaking frequently during the first six hours. This was filtered rapidly through filter paper taking precaution against excessive loss of alcohol. 25ml of the alcoholic extract was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105^{o} and weighed. The percentage weight by weight of the 90% Alcohol soluble extractive with reference to the air-dried drug was calculated. The entire procedure was again repeated and the average of at least four determinations taken as the Alcohol soluble extractive.

b. ***Water-soluble extractive determination****:* The procedure used in the determination of the alcohol-soluble extractive was again followed but this time, Chloroform water (0.25% Chloroform in distilled water) was used instead of alcohol.

3.4.4 Chemical evaluation of the root bark extract

a. ***Preliminary phytochemical screening on the root bark extracts of Saba comorensis***

Preliminary phytochemical tests were performed on the extracts (Chloroform, Ethanolic, and aqueous extracts) to identify the presence or absence of organic metabolites such as tannins, saponins, flavonoids, cyanogenic glycoside, starch, reducing sugars, proteins, alkaloids, resins, sterols and terpenes and oils. The tests were

carried out according to the methods of Brain and Turner (1975), Sofowara (1993) and Evans (2002). The following procedures were used to test for organic metabolites:

i. Test for carbohydrates

Molisch's test (General test): 0.5g of the extracts were dissolved in 3ml of water and heated, a few drops of Molisch's reagent was added and a small amount of concentrated H_2SO_4 from the side of the test tube to form a lower layer. The colour (red or purple) of the interfacial ring was noted and recorded.

Barfoed's test (General test for monosaccharides: 1ml of extract and 1ml of Barfoed's reagent were put in a test tube and heated in a beaker of boiling water. Red precipitate of cuprous oxide is formed within 2 minutes if a monosaccharide is present.

Test for reducing sugars: Reducing sugar was also tested for by dissolving 0.1g of the drug in 5ml of water; 5ml of an equal mixture of Fehling's solution A and B was added and boiled.

Standard test for combined reducing sugars: 1ml of extract was hydrolysed with 5ml of dilute HCl. The solution was then neutralized with NaOH solution and Fehlings test (described above) was carried out on it. Production of rose colour is indicative of the presence of combined reducing sugars.

Selivanoff's test for Ketoses: To a small portion of extract was added a crystal of resorcinol and an equal volume of HCl containing a little Phloroglucinol. The solution was then heated. The production of a rose colour is indicative of Ketoses.

Standard test for Pentoses: To a small amount of extract an equal volume of HCl containing a little amount of phloroglucinol was added, the solution was heated on a small flame. A red colour indicates the presence of pentoses.

ii. Test for saponins

Frothing test: About 2g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed vigorously and observed for the formation of a stable persistent froth.

Heamolysis test: 0.2g of the powdered root bark was extracted with 10ml of warm water, filtered and the filtrate retained. 2ml of 1.8% Sodium chloride was added to two test tubes. To one of the test tube was added 2ml of the extract. Blood was obtained by pricking the thumb at the base of the nail and the blood was drawn into a small pipette. Five drops of the blood was added to each of the test tubes. The tubes were stoppered and inverted gently to mix the contents. Heamolysis in the test tube containing extract and not in the control indicates presence of saponins (Brain and Turner, 1975).

iii. Test for flavonoids

Shinoda's test: 0.5g of each of the powdered sample was extracted in ethanol by boiling in a water bath for 5min, filtered and cooled. To the filtrate was added four pieces of magnesium filings followed by few drops of concentrated hydrochloric acid. A pink or red colour indicates the presence of flavonoids.

Sodium hydroxide Test: 5ml of 10% NaOH was added to an equal volume of the ethanolic extract. A yellow colouration indicates the presence of flavonoids (Evans, 2002).

Ammonia test: 5ml of dilute ammonia solution was added to a portion of each plant extract followed by the addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicates the presence of flavonoids. The yellow colouration disappeared on standing (Brain and Turner, 1975).

iv. Test for tannins and polyphenolic compounds

Lead sub-acetate test: To a small portion of a solution of the extracts was added lead subacetate solution and observed for a coloured precipitate.

Ferric chloride test: To a small portion of a solution of the extracts was added 3-5 drops of ferric chloride solution. This was then observed for a greenish-black to a brownish–blue precipitate.

Bromine Water Test: To another portion of the solution of extract was added a few drops of bromine water. A buff coloured precipitate indicates the presence of condensed or pseudo-tannins.

v. Test for anthraquinones (Borntrager's test): 2.5g of each plant extract was shaken with 5ml of benzene, filtered and 35ml of 10% ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink, red, or violet colour in the ammoniacal (lower) layer indicated the presence of free anthraquinones. Combined anthraquinones were tested for by boiling 2.5g of each extract with 2.5ml of benzene, the benzene layer separated and half its own volume of 10% NH_3 solution added. A pink, red or violet coloration in the ammonia lower phase indicated the presence of derivatives of anthraquinone (Evans 2002).

vi. Test for cardiac glycosides and terpenes

a. Keller–Killiani's Test: 0.5g of extract was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayed with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicates the presence of a deoxy sugar characteristic of cardenolides. A violet ring appeared below the brown ring while in the acetic acid layer a greenish ring formed just above the brown ring and gradually spread throughout this layer (Evans, 2002).

b. Kedde test: 1ml of an 8% solution of the extract in methanol was mixed with 1ml of a 2% solution of 3,5-dinitrobenzoic acid in methanol and 1ml of a 5.7% aqueous NaOH. An immediate violet colour indicated the presence of cardenolides in the extract, the colour fading gradually through reddish – brown to brownish – yellow with the

precipitation of a whitish crystalline solid. This test indicates the presence of a lactone ring in the cardenolides.

C. Salkowski's Test: 0.5g of the extract was dissolved in 2ml chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish – brown colour at the interface indicated the presence of a steroidal ring (i.e.an aglycone portion of the cardiac glycoside).

d. Liebermann – Burchard's Test: To a solution of 0.5g of the extract was added 3 drops of strong lead subacetate solution, mixed thoroughly and filtered. The filtrate then extracted with 10ml of chloroform. To the chloroform portion was added equal volume of acetic acid anhydride. 1ml of concentrated H_2SO_4 was added down the side of the test tube to form a lower layer. Colour change ranging from immediate to over a period of one hour was observed. A red – violet colour at the interface of the two liquids and a green colour in the chloroform layer indicate the presence of terpenes. .

vii. Test for cyanogenic glycoside

1g of the powdered root bark of *Saba comorensis* was placed in a clean test tube and a few drops of water added. About 5ml of chloroform was then added. A freshly prepared sodium picrate paper was suspended with the aid of a cork ensuring that the paper strip does not touch the test tube wall. The closed test tube was placed in a water bath for 15minutes. Colour changes were observed.

viii. Test for alkaloids: About 0.5g of each extract was stirred with 5ml of 1% aqueous hydrochloric acid on a water bath; 1ml of the filtrate was treated with a few drops of Mayer's reagent, and a second 1ml portion was treated similarly with Dragendorff's reagent as well as Wagner's reagent. Turbidity or precipitation with either of these reagents was taken as evidence for the presence of alkaloids in the extract evaluated.

3.5 Determination of some Biological activity of the root bark extract

3.5.1 Acute toxicity determination (LD_{50})

The acute toxicity (LD_{50}) of the ethanolic extract (EE) was determined in mice by the method of Lorke (1983) using the intraperitoneal route. The acute toxicity was tested in two steps:

a. Initial investigations were carried out to determine the range of doses producing the toxic effects.

b. Based on these results, further specific doses were administered to calculate the LD_{50}. The initial investigation involved the use of nine animals divided into 3 animals per group. Each of this group received widely differing doses in order to determine the approximate range of acute toxicity. Initial doses given were 10mg/kg, 100mg/kg and 1000mg/kg body weights. Following from the results of the initial doses administered, the new doses chosen were 600, 1000, 1,600 and 2,900 mg/kg body weights. The median lethal dose was calculated from the administered doses. Animals were also observed for signs of toxicity.

LD_{50} = √Lowest Lethal Dose x Highest non-lethal dose.

3.5.2 *In-vitro* antioxidant assay (DPPH Scavenging Activity).

The method described by Poteract [1997] modified by Juntachote and Berghofer [2005] was used. Effect of the ethanol and chloroform extracts on the content of 2, 2-diphenyl-2-picrylyhydrazyl radical (DPPH) was estimated. The aliquots of the different concentrations (10-500Ug/ml) of the extract were added to 3.0ml of 3.9mg of DPPH in 50ml of methanol. Absorbance at 517nm was determined after 30mins, and the ED_{50} was then derived. ED_{50} value denotes the concentration of sample required to scavenge

50% of the DPPH free radical Juntachote and Berghofer, 2005); the formula for percentage inhibition is as given below

$$\% \text{ inhibition} = \frac{\text{0ptical Density of DPPH} - \text{Optical Density of sample}}{\text{Optical Density of DPPH}} \times \frac{100}{1}$$

3.5.3 Evaluation of analgesic activity (Acetic acid induced writhing)

The response to an intraperitoneal injection of acetic acid solution, manifesting as a contraction of the abdominal muscles and stretching of the hind limbs, was studied according to procedures described by Koster *et al* (1959) and Owoleye *et al* (2001). Swiss albino mice (5per group) were administered the following:

Group 1: Normal Saline

Group 2: 50mg/kg, ip extract.

Group 3: 100mg/kg ip extract

Group 4: 150mg/kg ip extract.

Group 5: 150mg/kg ip acetylsalicylic acid (ASA).

After thirty minutes, 10mg/kg of 0.6% v/v acetic acid solution was administered intraperitoneally to all groups. The number of writhing movements produced in each group for the succeeding 5 – 15 minutes after the administration of acetic acid was counted (Aliyu *et al., 2005*) and the results were expressed as percentages relative to the control group. A significant reduction in the number of writing movements in the groups tested with Ethanol extract compared with control was considered to be a positive analgesic response.

$$\% \text{ inhibition of Writhing} = \frac{\text{mean number (Control) x mean number (test)}}{\text{Mean number of Control}} \times \frac{100}{1}$$

3.5.5 Acute & chronic anti-inflammatory analysis of the root bark extract of *Saba comorensis*

Acute and chronic anti – inflammatory studies was carried out in Formalin – induced paw oedema in Wistar rats as described by Ashoke *et al* (2006). Overnight – fasted, Wister rat were randomly assigned to five groups of five animals each. Such animals received Normal Saline, Piroxicam, and graded doses of the Ethanol extract of the root bark. Inflammation was produced in these animals by injection of 0.1ml of 1% w/v formalin into the subplanter region of left hind paw. The volume was measured using Venier Calliper, at 0, 1, 2, and 4 hours after formalin injection; this represents the acute phase. The difference between the 0 and 4 hours reading were taken as the volume of oedema and the percentage of oedema inhibition was calculated for each group. In the chronic phase, the Drug treatment started 30 minutes before the injection of formaldehyde and continued every 24 hours (just after measurement of paw volume) until day 4. The paw volume was measured on day zero and continued every day until the fifth day. The volume of oedema and percentage of oedema inhibition were calculated for each group. The difference between day 0 and the fifth day was taken as the volume of oedema.

3.5.5 Ulcerogenic assay

The method described by Dimo *et al* (2006) was employed for this assay.

Method: Food was withheld from the animals for eighteen hours before the start of the experiment. The fasted animals (n = 4) received the ethanol extract (EE) & the Chloroform extract (CE). 150mg/kg and 500mg/kg were the administered doses each for the EE and the CE. Control animals received equivalent volume of vehicle (Normal Saline) and 150mg/kg of acetylsalicylic acid (Aspirin). Three hours later animals were

sacrificed and the stomachs removed and cut open at regions of less curvature. Normal saline was used to wash the open stomach and viewed with a magnifying lens (x 10). The arbitrary scales described below were used to score the presence or absence of lesions in the mucosal surface.

Description	Scale (score)
No Lesion	0
Hyperemia	0.5
One or two Lesions	1
Severe Lesions	2
Very Severe Lesions	3
Mucosa full of lesions	4

(Bani *et al*, 2000).

CHAPTER 4

4.0 RESULTS

4.1 Macro-morphological features of the root bark of *S. comorensis*

The identified macro morphological features of *Saba comorensis* root were slightly rough cork with well marked small circular ridges almost running uniformly with root hairs (plates II and III); faint longitudinal striations with occasional cracks in the inner surface (plate IV),the young root back is doubled-quilled while the older one is channeled (plates IV and V). Fracture is short and granular while the fractured surface

shows a reddish-brown cork. Organoleptic features of the root include a dark brown outer surface and a reddish brown inner surface, faint aromatic odour, and a mildly astrigent taste. Plates I-V present the result of the Morphological features of the root bark.



Plate I: *Saba comorensis* showing fruit and flowers



Plate II: Fresh root of *Saba comorensis*



Plate III: Outer surface of the root bark of *Saba Comorensis*



Plate IV: Inner surface of the root bark



Plate V: Young root bark of *S. comorensi*

4.2 Micro-morphological features of the root bark of *S. comorensis*

Microscopically, the transverse section showed the presence of cork cells (Figures 4a and 5a, plate IX) sclereids containing starch grains, pericyclic fibres, lignified sieve tubes, lignified phloem fibres, (14.4-25.2µm), pitted vessels (Figures 5a-6a, plates XII and XIV), medullary ray usually biseriate with abundant starch grains (3.6-9.0µm), thin prisms and clusters of calcium oxalate crystals (7.2-18.0µm) in the modularly ray parenchyma, stone cells and secretion cells (Figures 1a-3a, plates VII-XI). Xylem elements were absent in the bark which conforms to the theory which says that bark is that part of the stem or root outside the Cambium (Evans, 2002).

Characteristics of the powdered root indicated slender fibres, stone cells, starch grains, parenchyma, and crystals of calcium oxalate (Figure 5a).

Figure 1a : Transverse section of the root bark of *S comorensis* showing the plan of tissues

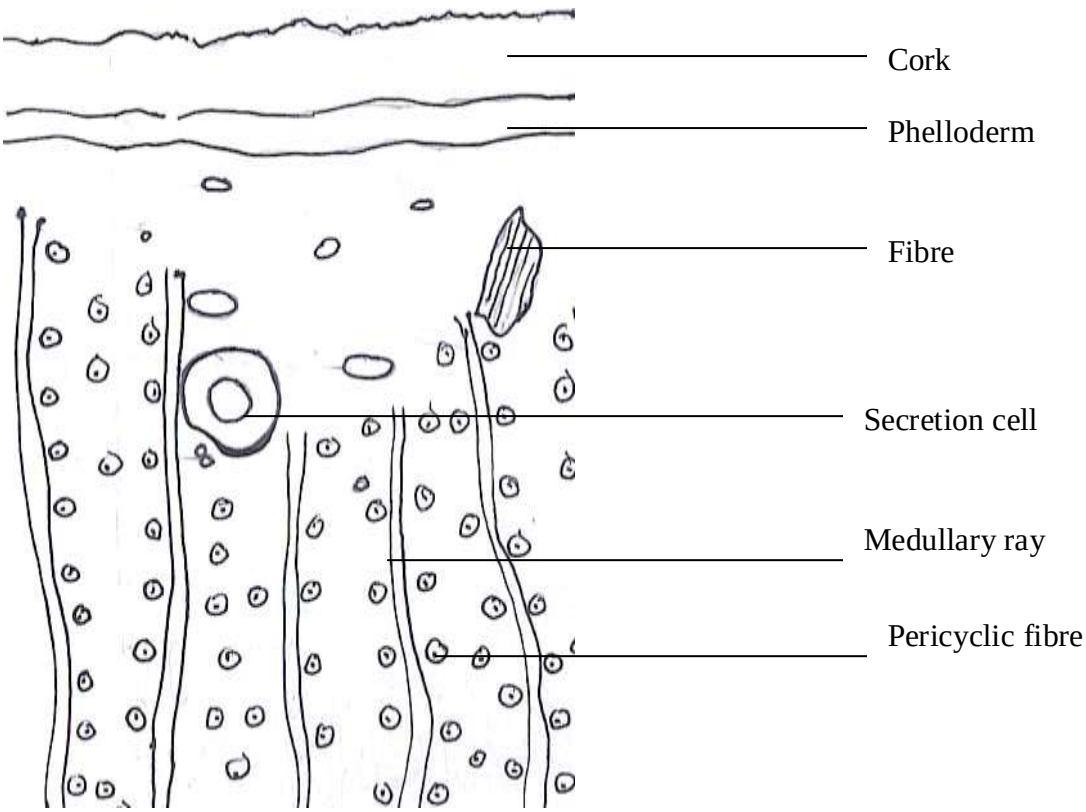


Figure 2a: Tranverse Section of the bark of *S. comorensis* (x 400)

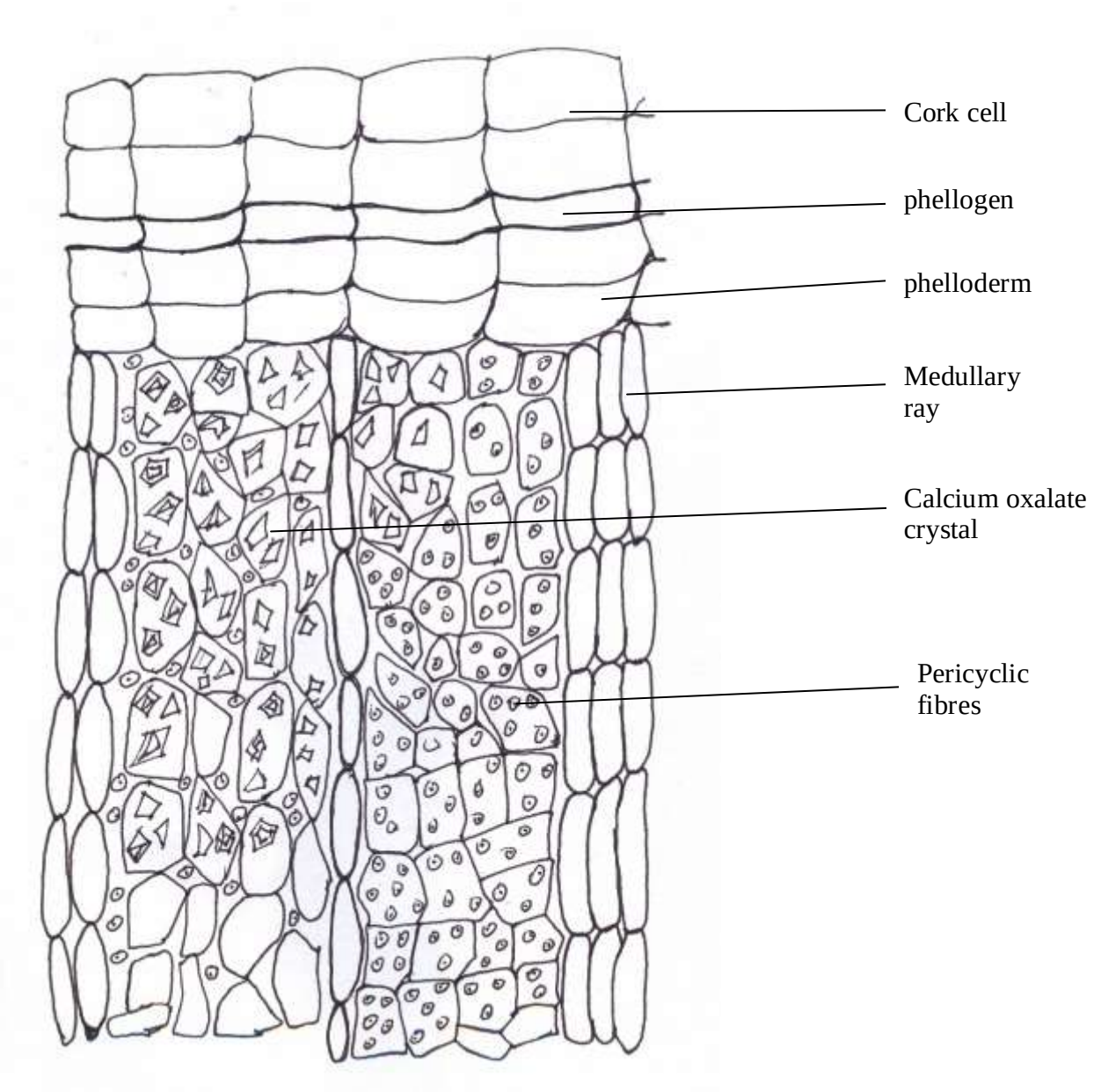
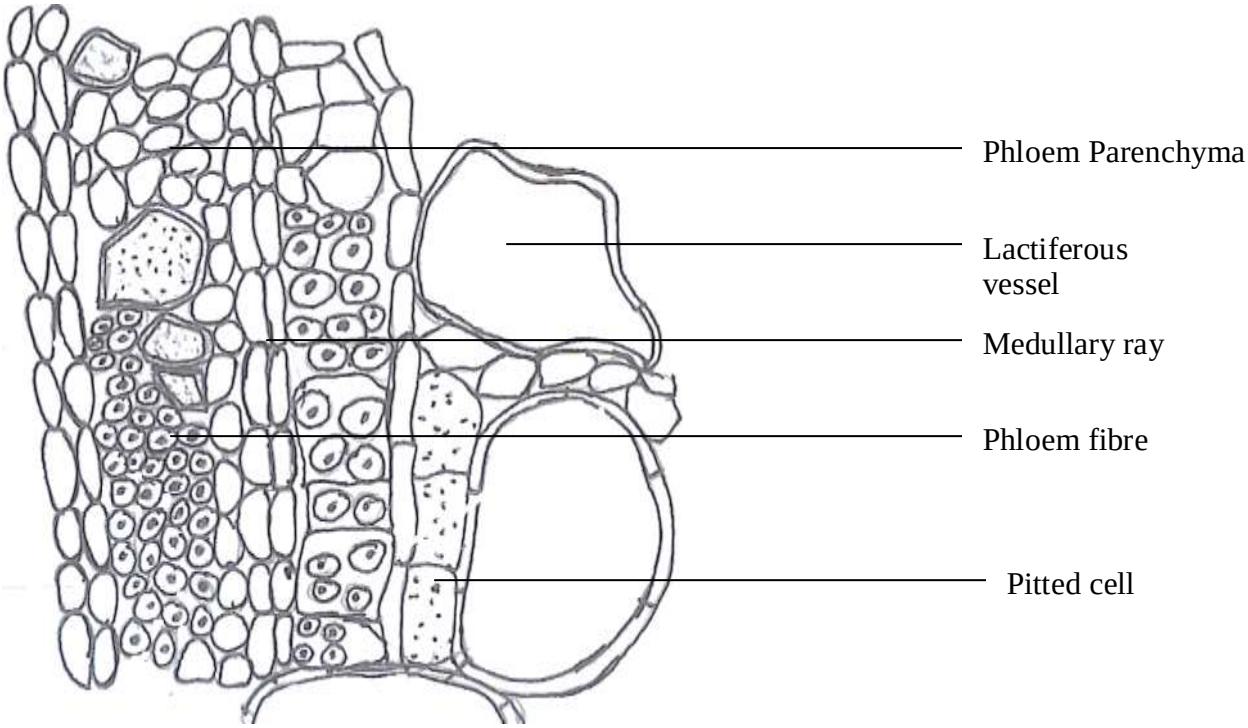
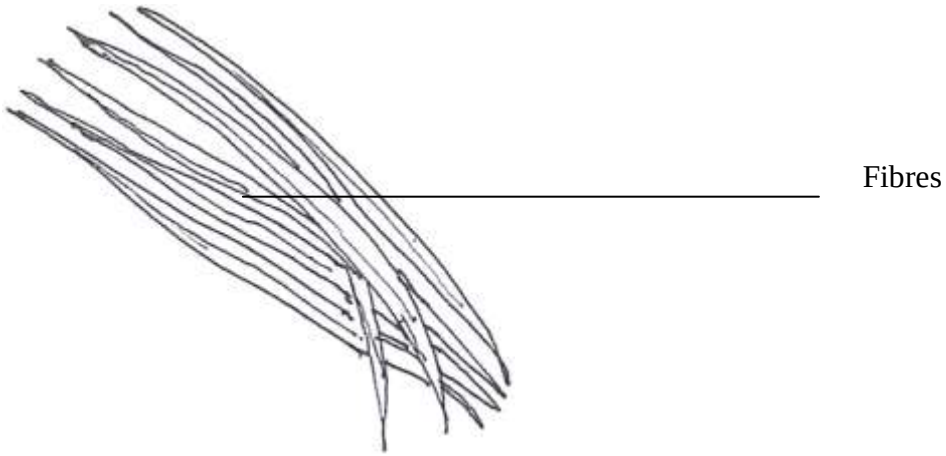


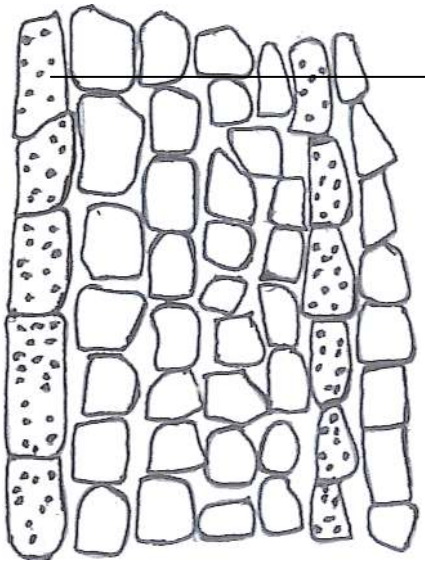
Figure 3a: Transverse Section of the inner bark of *S. comorensis (x 400)*

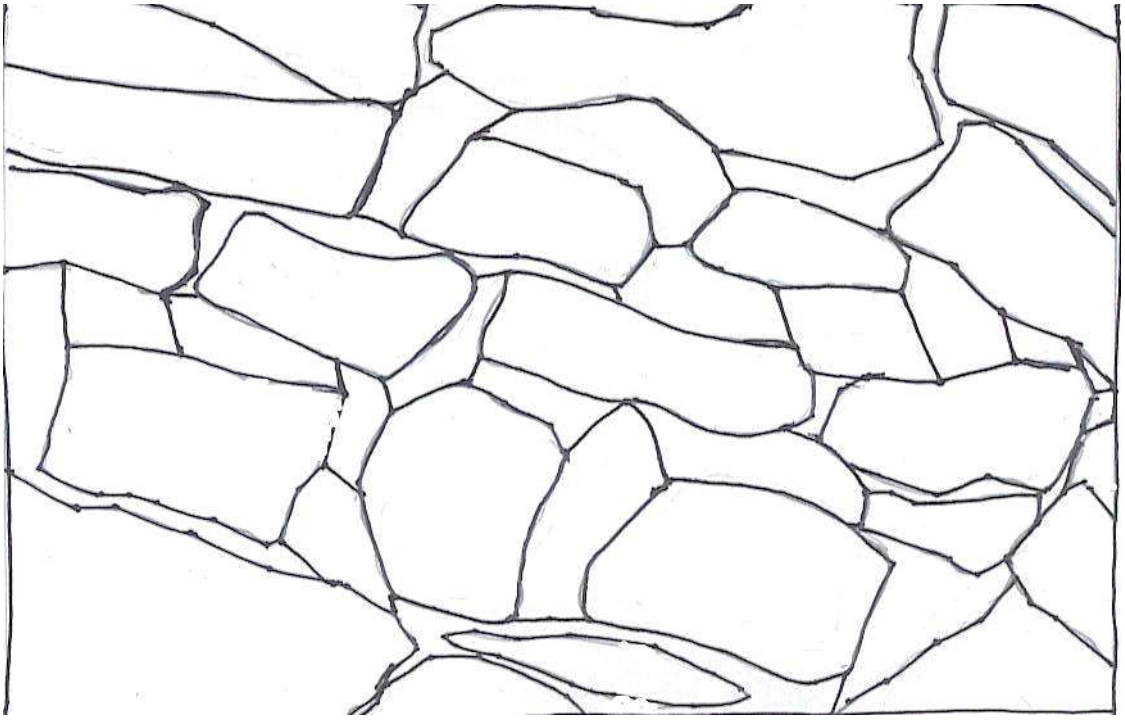




Fibres from the powder

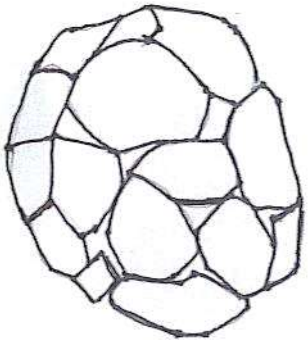
Figure 4a : Longitudinal section of the root bark of *S. comorensis* (x 400)



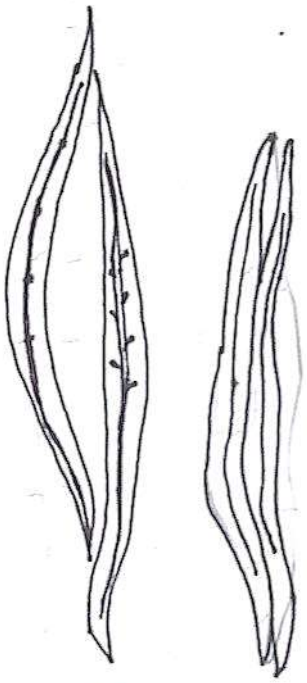


Cork cells in section (x 600)

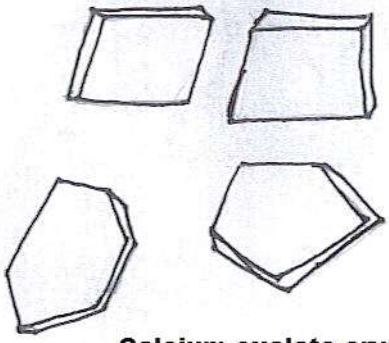
Figure 5a: features from powder



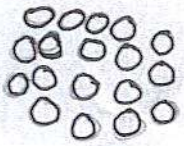
suface view of
the cork cells



Fibres

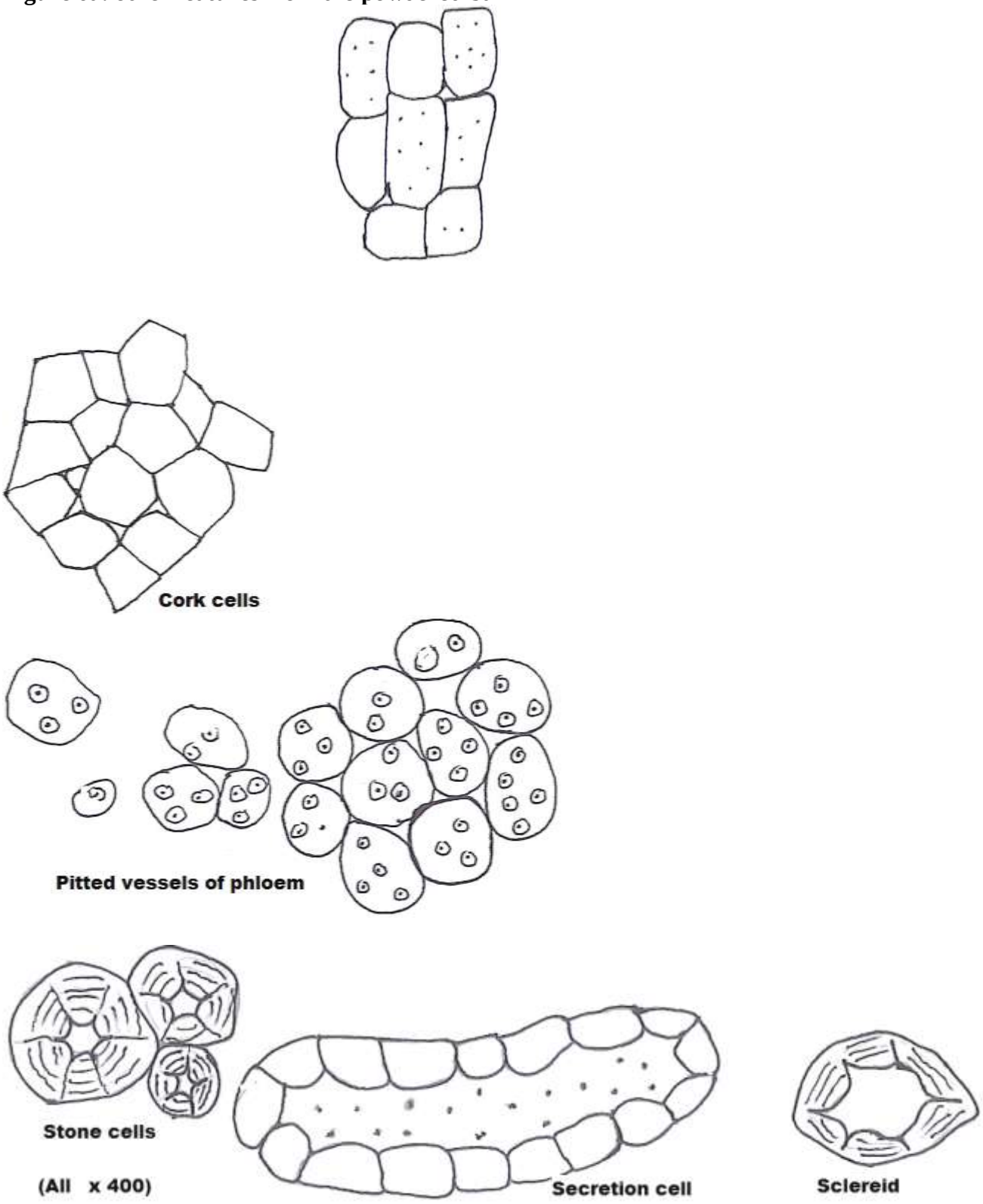


Calcium oxalate crystals



Starch grains

Figure 6a: other features from the powdered bark



4.3 Chemomicroscopical evaluation of the root bark of *S. comorensis*

The application of chemicals to small quantities of the drug in powdered form or to histological sections of the drug gave the following result;

Lignin: A drop of phloroglucinol and concentrated hydrochloric acid gave a red stain to show the presence of lignin.

Cellulose: Cellulose swells and dissolved in Cuoxam when treated with corallin soda and few drops of sodium carbonate solution.

Starch: N/50 iodine solution stained starch and hemicellulose blue.

Tannins: Some cells had brown or yellowish brown contents which indicate the presence of tannins. This was confirmed by the appearance of greenish black and green colour when mounted in Ferric chloride solution.

Calcium oxalate: The bright structures (prism shaped) observed disappeared on the addition of 80% H_2SO_4. This confirmed the presence of calcium oxalate crystals.

Oils: Some secretory cells stained red with Sudan IV indicating the presence of oils or lipids.

Proteins: Red stains with millions' reagent and yellow stains with picric acid were observed which indicated the presence of proteins.

Table 4.1: Result of Chemo-microscopy

constituent	Test	result
Calcium oxalate	Chloral hydrate solution.	++
Starch grains	Dilute glycerol	++
Lignin	Phloroglucinol & dilute HCl.	+
Cellulose	Ammonia solution of Copper II oxide	+
Fats and Oils	Sudan IV Solution	+
Calcium Carbonate	Conc. HCl.	+
Proteins	Picric acid	+

+ Means present

- means absent

++ means abundant

4.4 Preliminary phytochemical screening of the extracts of *S. comorensis*

The present study carried out on the plant sample revealed the presence of chemical constituents.

i. Test for carbohydrates

Molisch's test (General test): Molisch's reagent gave a positive test for carbohydrates (a reddish ring at the interface). After shaking and being allowed to stand for 2 minutes and then a further dilution, a final dull-violet precipitate was observed which is indicative of the presence of carbohydrates.

Barfoed's test (General test for monosaccharides)*:* No red precipitate of cuprous oxide was formed within 2 minutes indicating the absence of monosaccharides.

*Test for Reducing sugars:*A red precipitate was formed indicating presence of free reducing sugar.

Standard test for combined reducing sugars: The root bark extract gave positive reactions (rose colour) to show the presence of combined reducing sugars in the extracts.

Selivanoff's test for Ketoses: A rose colour was produced on heating the solution. The production of a rose colour is indicative of Ketoses.

Standard test for Pentoses: A red colour was observed when the solution was heated on a small flame to indicate the presence of pentoses.

ii. Test for saponins

Frothing test: A stable persistent froth was formed when shaken vigorously indicating the presence of saponins.

Heamolysis test:.There was heamolysis in the test tube containing extract and no observable change in the control indicating the presence of saponins.

iii. Test for flavonoids

Shinoda's test: A pink colour was observed indicating the presence of flavonoids.

Sodium hydroxide Test: A yellow colouration indicated the presence of flavonoids.

Ammonia test: A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

iv. Test for tannins and polyphenolic compounds

Lead sub-acetate test: A brown precipitate was observed in the ethanol extract of the root bark indicating presence of tannins.

Ferric chloride test: A greenish-black to a brownish–blue precipitate was observed showing the presence of tannins.

Bromine Water Test: A buff coloured precipitate which indicates the presence of condensed or pseudotannins was observed when few drops of bromine water was added to the extract.

v. Test for anthraquinones (Borntrager's test): A pink colouration in the ammonia lower phase indicated the presence of derivatives of anthraquinone.

vi. Test for cardiac glycosides

a. Keller–Killiani's Test: A brown ring was obtained at the interface indicating the presence of a deoxy sugar characteristic of cardenolides. A violet ring appeared below the brown ring while in the acetic acid layer a greenish ring formed just above the brown ring and gradually spread throughout this layer.

b. kedde's test: An immediate violet colour indicated the presence of cardenolides in the extract, the colour fading gradually through reddish – brown to brownish – yellow with the precipitation of a whitish crystalline solid. This test indicates the presence of a lactone ring in the cardenolides.

C. Salkowski's Test: A reddish – brown colour at the interface indicated the presence of a steroidal ring (i.e.an aglycone portion of the cardiac glycoside).

d. Liebermann – Burchard's Test: A colour change ranging from immediate to over a period of one hour was observed. A red – violet colour at the interface of the two liquids and a green colour in the chloroform layer indicated the presence of terpenes.

vii. Test for cyanogenic glycoside: No colour changes were observed indicating the absence of cyanogenic glycosides.

viii. Test for alkaloids:

(a) Wagner's test: Brownish precipitate was observed in the extracts of the root bark of the plant indicating presence of alkaloids.

(b) Mayer's test: A whitish precipitate was observed in extracts of the different plant tested. This indicates presence of alkaloids.

(c) Dragendorff's test: Orange precipitate was observed on addition of the reagent to the extract of all the plant parts. This indicates presence of alkaloids.

Table 4.2: Summary of preliminary phytochemical test on the root bark extract of *Saba comorensis*

S/no	Chemical Component	Test	Solvent Extracts		
			EtOH	**$CHCl_3$**	**H_20**
1	Carbohydrates	Molisch's reagent	+	+	+
	- Reducing sugars	Fehling's solution	+	-	+
	- Combined reducing sugars	Hydrolysis + Fehling's solution	+	-	+
	-Ketoses	Selivanoff's test	+	-	+
	-Pentoses	HCl + phloroglucinol	+	-	+
2	Saponins	Frothing test	+	-	+
		Haemolysis test	+	-	+
3	Flavonoids	Shinoda's test (red colouration)	+	-	+
		Sodium hydroxide test	+	-	+
4	Tannins and other polyphenolic compounds	Lead subacetate test	+	-	+
		Ferric chloride test	+	-	+
		Bromine water test	+	-	+
5	Anthraquinones	Borntrager's test	+	+	+
6	Cardiac glycosides and Terpenes	Kedde's test	+	+	+
		Keller- Killiani's test	+	+	+
		Salkowski's test	+	+	-
		Lieberman –burchard's test	-	+	-
8	Cyanogenic clycosides	(Sodium Picrate paper) Guignard test	-	-	-
9	Alkaloids	Dragendorff's reagent	-	+	-
		Mayers reagent	-	+	-
		Wagner's reagent	-	+	-

+ Means positive while - means negative

4.5 Chromatographic analysis of the extracts of *S. comorensis*

Considering the solvent system used for the chromatographic analysis, the water – methanol - Ethylacetate (75:5:5) and water - chloroform - n-hexane - methanol (5:2:1:5:1) were the most effective in separating the mixtures of the ethanol extracts. Four spots or separations were respectively obtained using Iodine vapour and sulphuric acid (heated to 100 ^{o}c), while two spots were observed with the first solvent system using ferric chloride spray reagent, while Chloroform - n-butanol – ethylacetate – water (4:3:2:1) was most effective in separating the chloroform extract mixtures. Two spots were observed using Dragendorff spray reagent. The colour of the spots ranges from light brown, dirty green, brownish-black to dark brown. Table 4.3 and plates xvi-xix presents a summary of the Chromatographic result. The result also indicates the presence of some of the compounds earlier identified in the preliminary screening.

Table 4.3: **Chromatographic analysis of Solvent extracts of the root bark of *Saba comorensis***

Extract	**Solvent system**	**Spray reagent**	**Number of spots**	**R_f (cm)**	**Colour of spot**
Ethanol	H_2O: Meoh : Etac (75:5:5)	Iodine vapour	4	0.504, 0.510, 0.520, 0.514	Light-brown, dirty green, greenish-brown and brownish-black
		Ferric chloride	2	0.200 0.419	dirty green dirty green
	EtoH:H_2O:$CHCl_3$: n-Hex (5:2:1.5:1)	10% Sulphuric acid (100-105^{o}c)	4	0.507 0.513 0.520 0.538	Dirty green light green light green greenish black
Chloroform	$CHCl_3$:n-But:EtAC: H_2O (4:3:2:1)	Dragendorff's reagent	2	0.200, 0.940,	Orange

4.6 Physical constants (Quantitative Evaluation)

The evaluation of some physical constants showed that the root bark of *Saba comorensis* has a total ash value of 13.67% and acid insoluble ash of 8.89%; 16% as the ethanol soluble extractive, 22% as the water-soluble extractive, and moisture content of 8.30%.Table 4.4 presents a summary of the result.

Table 4.4: Results of some Physical constants of the powdered root bark of *Saba comorensis*.

Evaluative parameters	**Value (%w/w)***
Moisture content	8.30 ± 0.01
Total ash value	13.67 ± 0.03
Acid-insoluble ash value	8.89 ± 0.02
Alcohol-soluble extractive value	16.00 ± 0.04
Water-soluble extractive value	22.0 ± 0.06

*Mean value of at least four counts.

4.7 Acute toxicity test on the root bark extract

A summary of the acute toxicity test result is presented in table 8. The extract produced overt toxic effects at a dose greater than 770mg/ body weight in mice. The mice also showed some signs of toxicity at a dose of 600mg/kg which were later overcome. Those that did not die within twenty four – hour period died within three days after extract administration. Some of the signs include restlessness, stretching movements, aggressiveness and unusual consecutive stomach and abdominal movements.

Table 4.5: Summary of result of acute toxicity test of the ethanol extract of *S. comorensis*

first part of test		**2nd phase of investigation**	
Doses(mg/kg)	**mortality**	**Doses(mg/kg)**	**mortality**
10	0/3	600	0/2
100	0/3	1,000	1/1
1000	1/3	1,600	1/1
		2,900	1/1

4.8 Antioxidant assay

Results of the scavenging activity of the extracts using stable DPPH free radical showed that the ethanol extract has a higher antioxidant activity relative to chloroform extract. This is shown in tables 4.6 and 4.7. The concentration of the extract required to quench 50% of the free radical (ED_{50}) for ethanol and chloroform extracts were 0.198 and 0.284 respectively as shown on Table 4.8. The value of the ED_{50} of ethanolic extract is smaller than that of chloroform extract. Figures 1and 2 show the graphical demonstration of the free radical scavenging activity of the ethanol and the chloroform Extracts. Figure 3 is a comparative graphical illustration of the free radical scavenging activities of ethanolic and chloroform extracts.

Table 4.6: Free Radical scavenging activity of Ethanolic and Chloroform Extracts of the root bark S*aba comorensis*

Concentration (ug/ml)	Ethanol extract		Chloroform extract	
	Optical density	% Inhibition	Optical density	% Inhibition
10	0.283 ± 0.009	12.9	0.305 ± 0.003	6.2
50	0.183 ± 0.014	14.2	0.294 ± 0.002	9.5
250	0.133 ± 0.013	59.1	0.188 ± 0.004	42.2
500	0.004 ± 0.002	98.8	0.068 ± 0.01	79.1
Ascorbic acid(200)	0.004 ± 0.003	98.8	0.004 ± 0.003	98.8
Blank(Control)	0.325 ± 0.017	-	0.325 ± 0.017	-

± represents the S.E.M

Table 4.7: ED_{50} of crude ethanol extracts and chloroform portion of the root bark of *Saba comorensis* against DPPH.

Extracts	ED_{50} (mg extracts/ ml)
Crude ethanol	0.198
Chloroform portion	0.284

ED_{50} is the concentration of the various extracts required to quench 50% DPPH under chosen experimental conditions.

4.9 Analgesic activity

The effect of the Ethanol extract of S*aba comorensis* on acetic acid-induced writhing in mice is demonstrated in Table 4.9. The Extract (50, 100 and 150mg/kg, i.p) showed a significant ($P < 0.05$) reduction in the number of writhes induced by acetic acid, but not in a dose-dependent manner. The inhibition was highest at 100mg/kg and lowest at 150mg/kg. The effect of the extract at 100mg/kg was comparable to that of the Standard drug –Aspirin (150mg/kg). Figure 4 shows the effect of Ethanol extract on acetic acid induced abdominal writhing test in mice. From the graph it can be observed that the mean number of writhing movements was lowest at 100mg/kg and highest at 150mg/kg and Control respectively.

Table 4.8: Effect of the ethanolic extract on acetic – acid induced writhing test in mice.

Treatment (mg/kg)	No of writhings	Mean no.of writhing	% intribution
Normal Saline	170	34.0± 3.4	-
Extract 50	63	12.6± 6.7	62.9
" 100	16	3.2 ± 3.6	90.6
" 150	91	18.2 ± 3.5	46.5
Aspirin 150	15	3.0± 1.0	91.2

N=5 *t-test against control at P<0.05

4.10 Anti-inflammatory Studies

The activity of the ethanol extract of S*aba comorensis* on Formalin-induced in Rats is shown in Tables 4.9 and 4.10 for the acute and chronic phases. The extracts dose-dependently elicited Significant ((P ‹ 0.05) inhibitory effect on acute oedema formation at 2h and 4h after formalin injection. However, at 150mg/kg, it produced a significant anti-inflammatory effect comparable to the standard drug-Piroxicam. In the Chronic Phase, the inhibition was also in a dose-dependent manner. Comparing the phases of inflammation, the extracts produced more effect during the acute phase. Figures 5 and 6 demonstrate the time-dependent response pattern of the Effect of Ethanolic extracts of the root bark of *Saba comorensis* on Formalin-induced paw oedema in hind paw of rats for the Acute phase and Chronic phases respectively. While Figures 7 and 8 graphically illustrate the effect of the administration of varying doses of Ethanol extracts of *Saba comorensis* on paw thickness of rats during the acute and chronic phases of inflammation. The standard Drug produced the highest effect having the lowest paw thickness at both phases of inflammation. This is closely followed by the 150mg/kg extract. From the graphs, the effects produced are dose dependent.

Table 4.9: Acute anti inflammatry activity of ethanolic Extracts of *Saba comorences* in formalin – induced inflammation.

Groups	Initial paw thickness (cm)	Paw thickness after 4 hrs	Increase in paw thickness (cm)	% Inhibition
i. 50mg/kg	0.42 ± 0.02	0.60 ± 0.09	0.18± 0.05	50.0
ii. 100mg/kg	0.42± 0.02	0.544 ± 0.03	0.12 ± 0.025	66.7
iii. 150mg/kg	0.44 ± 0.28	0.52 ± 0.02	0.08 ±0.15	77.8
Piroxicam 150mg/kg	0.43 ± 0.03	0.48 ± 0.15	0.05 ± 0.09	86.1
Control	0.42 ± 0.02	0.78 ± 0.04	0.36 ± 0.03	-

n= 5 t-test of extract versus control at $P<0.05$

Table 4.10: chronic antiinflammatory activity of ethanolic extract of s*aba comorensis* in formalin – induced inflammation.

Groups	Initial paw thickness (cm)	Paw thickness after 5 days	Increase in paw thickness (cm)	Inhibition (%)
I. 50mg/kg	0.42 ±0.02	0.64 ± 0.06	0.22 ± 0.04	38.9
II. 100mg/kg	0.42 ±0.02	0.64 ± 0.06	0.22 ± 0.04	38.9
III. 150mg/kg	0.44 ± 0.28	0.60 ± 0.05	0.16 ± 0.16	55.6
Piroxicam 150mg/kg	0.43 ±0.03	0.56 ± 0.04	0.13 ± 0.03	63.9
Control	0.42 ±0.02	0.78 ± 0.04	0.36 ± 0.03	-

n=6 t-test of extracts versus control at $P<0.05$.

4.11 Ulcerogenic Studies

The ethanol and chloroform extracts both elicited varying degrees of gastric irritation in rats. The effect is dose- dependent. The ethanol extract produced a higher effect compared to the chloroform extract. At 500mg/kg, the Ethanol extract produced severe lesions while the chloroform extract produced "one or two" lesions. However, the standard drug (acetylsalicylic acid) produced very severe lesions at a dose of 150mg/kg when compared to the extracts. The result is summarized in table 4.11.

Table 4.11: Effect of Extracts on Gastric Irritation In Rats

Extract	Dose (mg/kg)	Ulcer incidence	Ulcer score
EE	500	4/4	2.75 ± 0.7
	150	2/4	0.5 ± 0.7
CE	500	4/4	1 ± 0.7
	150	2/4	1 ± 0.7
ASA	150	4/4	3.00 ± 0.9
Control	-	0/4	0.00 ± 0.00

N = 4. Lesions and the mucosal surface were scored according to arbitrary scale: 0=no lesion; 0.5 = hyperemia; 1= 0ne or two lesions; 2 = severe lesion; 3 = very server lesions; 4 Mucosal full of lesions.
EE = Ethanolic Extract

CE = Chloroform Extract

ASA = Acetylsalicylic acid

CHAPTER 5

5.0 DISCUSSION

5.1 Macro-morphological features of the root bark of *S. comorensis*

The general appearance of crude drugs often indicates whether it is likely to comply with prescribed standards (Kokate *et al*., 2002). The faint longitudinal striations, slightly rough cork, marked circular ridges, double-quilled young bark,channelled old bark, dark-brown outer surface and reddis-brown inner surface, faint aromatic odour as well as characteristic taste (slightly astrigent) are all important diagnostic characters. Ibrahim, (1997) reported some morphological and organoleptic characters of the root bark of *Carrissa edulis* to include the following; curved to channeled shape, light brown inner surface and dark brown outer surface with striations,short fracture, smooth fractured surface, pungent odour and salty taste. For Cinnamon bark, Kokate (1994) reported a pale brown colour , inner surface darker than the outer surface, aromatic odour, sweet taste, short fracture and splintery, while that of Cinchona bark is reported to possess flat pieces or quills, grayish green outer surface, presence of cracks, wrinkles, reddish brown inner surface, short fracture, odourless and intensely bitter taste (Kokate,1994).

5.2 Micro-morphological features of the root bark

Microscopic evaluation allows detailed examination of a drug and it can be used to identify the organized drug by their known histological characters (Kokate *et al*., 2002). Microscope, by virtue of its property to magnify, permits the minute structure under study to be enlarged and can be used to confirm the structural details of drugs

from plants origin (Kokate *et al*., 2002). For effective results, double staining and permanent preparation of microscopic sections were used to distinguish cellular structure. Safranin stains lignified elements deep red, haematoxylin stains cellulose elements purplish while light green stains cellulose elements bright green (Dutta, 2005).

Chemomicroscopy involves the approach of chemical methods to small quantities of drugs in powder form or to histological sections of the drug. Result from chemo-microscopy together with that of preliminary phytochemical screening further establish the chemical data of the root bark.

Quantitative microscopy and linear measurements is another important histological aspect. The measured diameters of lignified phloem fibres (14.4- 25.2 µm), starch grains (3.6-9.0 µm) and calcium oxalate crystals (7.2-18.0 µm) may be useful for detecting adulterants. For instance, Kokate *et al* (2002) reported the diameter of starch grains in the bark of *Cinnamonium cassia* to be 10 microns.

5.3 Physical constants of the root bark (Quantitative Evaluation)

The moisture content of the drug was determined to be 8.30%. This will not encourage bacteria, fungi or yeast growth as the general requirement for moisture content in most crude drugs is not more than 14% (AP, 1986). It also means the root bark was properly dried. Some reported moisture contents are *Carissa edulis* stem and root bark 10.4% and 8.0% respectively (Ibrahim, 1997), Acacia is 15% and Digitalis is 6.0% (B.P, 1980). The total ash and acid insoluble ash values obtained (13.67% and 8.89%) were reasonably high to indicate the physiological state of the root. The inorganic (ash) content of barks can be as high as 13% and, in general, the inner bark contains more inorganics as compared to the outer bark (Young 1971, Choong *et al*. 1976, Hattula and Johanson 1978, Harper and Einspahr 1980). For example, the outer

bark of willow contains 11.5% ash, the inner bark 13.1%, and the sapwood 0.9%; sweetgum outer bark is 10.4%, inner bark 12.8%, and sapwood 0.5% ash; red oak outer bark is 8.9%, inner bark 11.1%, and sapwood 0.9% ash. The major inorganic elements in bark are Na, K, Ca, Mg, Mn, Zn, and P (Choong *et al.* 1976). Some other reported total ash of some drugs are *Carissa edulis* stem and root barks are 13.5% and 15.0% respectively (Ibrahim, 1997), Catechu 9%,Peeled liquorice 6%, unpeeled liquorice 10% (B.P, 1980). Both water and alcohol soluble extractives values were also reasonably high to indicate the solubility of the active constituents in these extractive solvents. Results obtained for the Physico-chemical constants are therefore diagnostic of the drug.

5.4 Preliminary phytochemical screening and Chromatography

The presence of alkaloids, tannins, saponins, flavonoids, cardiac glycosides, and steroids as obtained from the preliminary phytochemical screening and some later confirmed in the chromatographic analysis was supported by the literature (Evans, 2002), which states the presence of a vast range of indole alkaloids, steroidal alkaloids, cardioactive glycosides and many others such as cyanogenetic glycosides, saponins, tannins, phenolic acids and triterpenoids in the subfamily Plumicroideae to which *Saba comorensis* belongs. The absence of cyanogenetic glycosides may also inform the wide use of the root as this gives information about its relative low toxicity. It also suggests that cyanogenic glycosides may not be the only toxic compound present in the root of *S. comorensis*. The result obtained from the chromatography agrees with the result from the preliminary phytochemical screening for phytochemicals.

Free sugars are extracted from bark (Roger *et al*, 2005). Hot water extraction yields about 5% free sugar fraction, which is mainly composed of glucose and fructose; this amount varies depending on the growing season. For example, the free sugar

content is low in early spring and increases during the growing season, reaching a maximum in the fall (Laks 1991). Other minor free sugars found in bark include galactose, xylose, mannose, and sucrose. Hydrolysis of the hot water extract of bark yields more free sugars, the most abundant one being arabinose. These sugars are tied up as glycosides or in the hemicelluloses. Other sugars released during hydrolysis are glucose, fructose, galactose, xylose, mannose, and rhamnose (Roger *et al,* 2005).

Hydrolyzable and condensed tannins are also major extractives from bark (Roger *et al.*, 2005). The hydrolyzable tannins are esters of carboxylic acids and sugars that are easily hydrolyzed to give benzoic acid derivatives and sugars. The condensed tannins are a group of polymers based on a hydroxylated C-15 flavonoid monomer unit. Low degree of polymerization tannins are soluble in polar solvents, whereas the high degree of polymerization tannins are soluble in dilute alkali solutions (Hemingway *et al.* ,1983). It is difficult to isolate pure fractions of tannins and the structure can be altered by the extraction procedure. Tannins are antiseptic in nature. They have astringent properties and can hasten healing of wounds in inflamed membrane (Tigar 1998). Tannic acid has been found to have antibacterial, antiseptic, anti-ulcer and antiviral properties (Tigar 1998). Commercially, tannins give ink (stains) when mixed with iron salt. It is also used in tanning of hides and skins, into leather (Wallis, 1985).

Terpenes are a condensation of two or more five-carbon isoprene (2-methy-1, 3-butadiene) units in a linear or cyclic structure. They can also contain various functional groups. The most common of the monoterpenes are α- and β-pinenes found in firs and pines. Birch bark has been reported to contain up to 25% terpenes, by total dry weight (Seshadri and Vedantham, 1971).

Flavonoids are a group of compounds based on a 15-carbon hydroxylated tricyclic unit (Laks, 1991). They are often found as glycosides. Many tree barks are rich

in mono- and polyflavonoids (Hergert, 1960, 1962). Their function seems to be as an antioxidant, pigment, and growth regulator (Laks, 1991). Flavonoids are most commonly known for their anti-oxidant activity. Flavonoids have been referred to as "nature's biological response modifiers" because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses, and carcinogens. They show anti-allergic, anti-inflammatory (Yamamoto and Gaynor, 2006), anti-microbial and anti-cancer activity. In addition, flavonoids act as powerful antioxidants, protecting against oxidative and free radical damage.

Glycosides are used for the treatment of various illnesses. Cardiac glycosides are used as cardiac stimulant. Cardiac glycosides exhibit their action on heart. They increase the force of systolic contraction and decrease the heart rate. Anthraquinone glycosides are used as laxative (Kokwaro, 1976).

The usefulness of the active constituent of plants as the main source of drugs has made serious in–road into the medical profession. Saponins possess specific physical, chemical and biological activities that make them useful as drugs. Some of these biological properties include anti-microbial (Yamamoto and Gaynor, 2006), anti-inflammatory, anti-feedant (Dharam, 1987) and heamolytic effects (Abe *et al*., 1981).

5.5 Acute toxicity studies of the root bark extract

The relatively high LD_{50} range (above 770mg/kg i.p) obtained for S*aba comorensis* root extract in this study probably suggest that the plant extract is relatively safe in mice (Lorke, 1983).

5.6 Antioxidant Assay

Oxidation reactions can involve the production of free radicals, which can form dangerous chain reaction. Antioxidants can terminate these chain reactions by removing radical intermediates and can inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents, such as thiols and phenols. Low levels of antioxidant molecules or inhibition of antioxidant enzymes (catalase, superoxide dismutase and various peroxides) cause oxidative stress and may damage or kill cells. Antioxidants have been identified as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells (Wolf, 2005). The 2, 2 – diphenyl-2-picrylhydrazyl radical has been used widely to evaluate the free radical scavenging capacity of antioxidants (Harbone, 1973). The determination of scavenging activity using DPPH is a very fast method to evaluate the antioxidant activity of the extracts. The decrease in absorbance of DPPH at 517nm resulting from a colour change from purple to yellow is a measure of the antioxidant activity. This resulted from the acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Matthaus, 2002).The decoulorization is stoichoimetric with the number of electrons taken up (Owoyele, 2001). ED_{50} is the concentration of ethanol extracts as well as the chloroform extract from root bark of *Saba comorensis* required to quench 50% of DPPH under the chosen experimental conditions.

As shown in table 2, the ED_{50} of ethanol extracts showed better scavenging capacity than the chloroform extracts. The ED_{50} of ethanol and chloroform extracts required to quench DPPH were 0.198 and 0.284mg extracts/ml respectively. The data obtained reveal that both extracts are free radical inhibitors, thus primary antioxidants that react with free radicals. The significant in-vitro antioxidant activity of *Saba comorensis* was in a concentration dependent manner. This may be due to the presence of chemical constituents such as flavonoids and Saponins. It has been suggested that prostaglandin $E_{2,}$ mediate an increase in Nitric Oxide (NO) - production. Superoxide is also known to participate in the formation of chemotactic factors and recruitment of Poly Morpho Nuclear cells (PMNs.). Hence, this study indicated that S*aba comorensis*, which could scavenge free radicals, might inhibit the recruitment of PMNs and thereby reduce inflammation. Moreover, recent studies suggest that inflammatory tissue damage is due to liberation of reactive oxygen species from phagocytes invading the inflammation sites (Conner and Grishama 1996). In addition nitric oxide is also implicated in inflammation, cancer and other pathological condition. Interaction between superoxide and nitric oxide regulates the vascular tone or inflammation (Conner and Grishama, 1996).

5.7 Analgesic, anti-inflammatory and Ulcerogenic Studies

The result obtained in this study showed that the Ethanolic extract of the root bark of S*aba comorensis* possesses anti-inflammatory and Analgesic activities. These effects were observed in acetic acid-induced writhing and Formalin-induced oedema tests in animals. The acetic acid-induced writhing tests is also called the abnormal constriction test (Aliyu *et al., 2005).*This method is a sensitive procedure to establish peripherally acting analgesics, and such a response involve local peritoneal receptors

(Bentley *et al*., 1983;Ashok *et al.*, 2006). Vogel and Vogel (1997) have shown that non-steroidal anti-inflammatory agents can be determined by the writhing test. The method is also able to detect antinociceptive compounds at doses that may be inactive with other models, such as the tail flick method (Aliyu *et al., 2005)*.

Formalin induces Paw oedema which closely resembles human Arthritis (Greenwald, 1991). Arthritis induced by formalin is a model used for the evaluation of an agent with probable anti-proliferative activity (Banergee *et al.*, 2000). As the extract significantly inhibited this model of inflammation, it can be thought to possess anti-proliferative and anti-arthritic activities similar to Piroxicam, a cyclooxygenase inhibitor. The ability of the extracts to reduce inflammation in acute and chronic phases may be due to the inhibition of the release of pro-inflammatory mediators of acute inflammation such as histamine and prostaglandin. As results from this study strongly indicate the non-steroidal, anti-inflammatory-like activity of the extract, its ulcerogenic effect was tested on fasted animals. Non-steroidal, anti-inflammatory drugs are thought to impair the mucosal defense of the stomach (Jain *et al.*, 2002). They act by inhibition of cyclooxygenase and, therefore, inhibit the production of gastric prostaglandins. This leads to a reduction in production of gastric mucus and an increase in mucosal permeability (Jain *et al.*, 2002). The mild ulceration of the gastric mucosa caused by the extract could be due to inhibition of cyclo-oxygenase typical of anti-inflammatory prostaglandin inhibitors such as the non-steroidal anti-inflammatory drugs NSAIDS (Range and Dale, 1988). Thus, the extract may exert anti-inflammatory effects by inhibiting the synthesis of prostaglandin. It is a well established fact that NSAIDS exert their analgesic and anti-inflammatory activity by the inhibition of cyclooxygenase activity (Vane, 1971). Therefore, it is clear that the association of analgesic and anti-inflammatory effect observed with the extract has been shown in Non-Steroidal anti-

inflammatory drugs (NSAIDS). These pharmacological effects may be due to their content of flavonoids, tannins, alkaloids, and Saponins. Many compounds from these classes of compounds have been found to exhibit anti-inflammatory effects (Dharam, 1987; Tigar, 1998; Martini *et al.*, 2004; Toker *et a*l., 2004). However, the chemical constituents and mechanism(s) responsible for the pharmacological activities remain to be investigated.

CHAPTER 6

6.1 Conclusion and recommendation

The relevance of medicinal plants cannot be over emphasized. *Saba cmorensis* has varied medicinal uses. The details of organoleptic, macro- and microscopic characters, various evaluative parameters, results of preliminary photochemical, and Chromatographic analysis established in the present study will facilitate in identifying the genuine drug from any substitute or spurious samples and will also be useful in preparation of monographs on this plant.

The rich chemical constituent of the root bark of *Saba comorensis* may be the reason why it has been helpful to locally achieve the treatment of certain diseases in Nigeria and African region as a whole. The absence of cyanogenic glycosides in the root of *Saba comorensis* which the literature reported present in the sub-family (*Plumiroideae*),could be helpful in the chemotaxonomy of the plant.

Saba comorensis studied here can be seen as a potential source of useful drugs. The results presented here suggest the possible usefulness of *Saba comorensis* in the treatment of inflammation-related diseases like arthritis. The chemical constituents and mechanism(s) responsible for the pharmacological activities remain to be investigated. A further study is also encouraged on the root of the plant in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds.

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APPENDIX A

Acute Toxicity evaluation

First step:

Doses is mg/kg Body weight

10	100	1000
0/3*	0/3	1/3 (a death occurred after few hours)

Where '*' = number of animals which died/number used.

Doses Chosen for the Second Test

600	1000	1,600	2,900
0\2	1/1 (after 5hrs)	1/1 (after 3 hrs)	1/1 (after many hours)

LD_{50} = √Lowest Lethal Dose x Highest non-lethal dose

Lowest lethal dose = 1000mg/kg body weight

Highest non-lethal dose = 600mg/kg body weight.

LD_{50} = √1000 X 600

= 1774.5967 mg/kg body weight.

= 770mg/kg body weight.

APPENDIX B

Acute and Chronic Anti-inflammatory activities.

Group A – 150 mg/kg.

DAYS	O		1		2	3	4	5
HOURS	0	1	2	4	24	48	72	96
Paw measurement(cm) ± S.E.M	0.44 ±0.28	0.84 ±0.12	0.54 ±0.04	0.52 ±0.02	0.58 ±0.10	0.70 ±0.08	0.62 ±0.08	0.60 ±0.05

Group B - 100mg/kg.

DAYS	0		1		2	3	4	5
HOURS	0	1	2	4	24	48	72	96
Paw measurement(cm) ± SEM	0.42 0.02	0.82 0.07	0.64 0.06	0.54 0.03	0.74 0.08	0.62 0.08	0.70 0.04	0.64 0.06

Group C – 50mg/kg

DAYS	0		1		2	3	4	5
Hours	0	1	2	4	24	48	72	96
paw measurement (cm)	0.42	0.70	0.58	0.60	0.70	0.68	0.74	0.64
± SEM	0.02	0.07	0.05	0.09	0.07	0.01	0.13	0.06

Group D - Piroxicam

DAYS	0		1		2	3	4	5
Hours	0	1	2	4	24	48	72	96
paw measurement (cm)	0.43	0.73	0.60	0.48	0.58	0.73	0.60	0 .56
± SEM	0.03	0.06	0.09	0.15	0.03	0.08	0.12	0.04

Group E - control

DAYS	0		1		2	3	4	5
Hours	0	1	2	4	24	48	72	96
paw measurement (cm)	0.42	0.63	0.78	0.78	0.70	0.92	0.82	0.78
± SEM	0.02	0.07	0.10	0.04	0.12	0.10	0.14	0.04

APPENDIX C

Result of Moisture content

a. Weight of Evaporating dish (W_1) = 210g

Initial weight of dish + Drug (W2) = 213g

Initial weight of Drug (W_3) = 3g

Final weight of evaporating dish + drug (W_4) = 212.75G

Loss in weight = $W_2 - W_4$ = 213 - 212.75g

= 0.25g

b. W_1 = 151g

W_2 = 154g

W_3 = 3g

W_4 = 153.72g

Loss in weight = Weight = W2 - W_4 => 154.00 - 153.72

= 0.28g

c. W_1 = 51.00g

W_2 = 54.00g

W_3 = 3.00g

W_4 = 53.77g

Loss in weight = Weight = W2 - W_4 = 54.00 -53.77

= 0.23g

d. W_1 = 51.00g

W_2 = 54.00g

W_3 = 3.00g

W_4 = 53.75g

Loss in weight = Weight = W2 - W_4 = 54.00 – 53.75

= 0.25g

Average loss in Weight = $\frac{0.25 + 0.28 + 0.23 + 0.25}{4}$ = 0.25g ± 0.01

Moisture content (%) = $\frac{0.25 \times 100}{3}$ = 8.3 ± 0.01

Result of Ash value

a. Weight of Crucible (W_1) = 37

Initial weight of Crucible + Drug (W_2) = 40g

Initial weight of powdered drug (W_3) = 3g

Final weight of crucible + Drug (W_4) = 39.45g

Loss in weight = $W_2 - W_4$

=40.00 – 39.45g

=0.45g

b. W_1 = 37.0g

W_2 = 40.0g

W_3 = 3g

W_4 = 39.50g

Loss in weight = W2 - W_4

= 0.5g

c. W_1 = 370g

W_2 = 40.00g

W_3 = 3.00g

W_4 = 39.6g

Loss in weight = W2 - W_4

= 0.40g

d. W_1 = 37.0g

W_2 = 39.72g

W_3 = 3.00

W_4 = 39.4g

Loss in weight = W2 - W_4

= 0.32g

Average total ash = $\frac{0.45 + 0.5 + 0.40 + 0.32 + 0.38}{5}$ = 0.41 ±0.03

% Total ash = $\frac{0.41 \times 100}{3}$ = 13.67 ± 0.03

Result of acid insoluble ash

a. Weight of Crucible (W_1) = 39.75g

Weight of powered drug W_2 = 3g

Weight of crucible + residue W_3 = 40g

Weight of residue = W3– W_1 => 40-39.75 = 0.25g

b. W_1 = 40.00g,

W_2 = 3.00g

W_3 = 39.80g

Weight of residue = (40 – 39.80) g

= 0.20g

c. W_1 = 39.46g

W_2 = 3.00g

W_3 = 39.75g

Weight of residue = 39.75g -39.45g

= 0.29g

d. W_1 = 39.60g

W_2 = 3.00g

W_3 = 39.87g

Weight of residue = 39.87g – 39.60g

= 0.27g

Average acid-insoluble ash value = $\frac{(0.25+ 0.27 + 0.26 + 0.29)}{4}$ = 0.26 ± 0.015

% acid-insoluble ash = $\frac{0.26 \times 100}{3}$ = 8.89% ± 0.015

Result of alcohol-soluble extractive values

a. Weight of empty dish (W_1) = 47.0g

Weight of dish + alcoholic extract (W_2) = 47.15g

Weight of air-dried drug (W_3) = 5g

Weight of alcohol – soluble extract = $W_2 - W_1$

= 0.15g

0.15g in 20ml

$\frac{0.15 \times 100}{20}$ = 0.75g in 100ml

b. . W_1 = 47.00g

W_2 = 47.18g

W_3 = 5.00g

$W_2 - W_1$ = 47.18g – 47.00g = 0.18g

0.18g in 20ml

$\frac{0.18g \times 100}{20}$ in 100ml = 0.90g

c. . W_1 = 47.00g

W_2 = 47.16g

W_3 = 5.00g

$W_2 - W_1 = 47.16g - 47.00g = 0.16g$

$W_2 = 47.15g$

0.16g in 20ml

$\frac{0.16 \times 100}{20}$ in 100ml $= 0.80g$

d. $W_1 = 47.00g$

$W_3 = 5.00g$

$W_2 - W_1 = 47.15g - 47.00g = 0.15g$

0.15g in 20ml

$\frac{0.15 \times 100}{20}$ in 100ml $= 0.75g$

Average alcohol-soluble extractive $= \frac{0.75 + 0.90 + 0.80 + 0.75}{4} = 0.18 \pm 0.04$

% alcohol-soluble extractive $= \frac{0.8 \times 100}{5} = 16\% \pm 0.04$

Result of water-soluble extractive values

a. Weight of empty dish, W_1 = 178.78g

Weight of dish +water-soluble extractive, W_2 = 178.99g

Weight of water-soluble extractive, W3 = 0.21g

Weight of air-dried Drug, W4 = 5.00g

$\frac{0.21 \times 100}{20}$ in 100 ml

= 1.05g

b. W_1 = 47.00g

W_2 = 47.22g

W_3 = 0.22g

W_4 = 5.00g

0.22g in 20ml

$\frac{0.22 \times 100}{20}$ in 100ml

= 1.10g

c. W_1 = 47.00g

W_2 = 47.25g

W_3 = 0.25g

W_4 = 5.00g

0.25g in 20ml

$\frac{0.25 \times 100}{20}$ in 100ml

= 1.25g

d. W_1 = 47.00g

W_2 = 47.20g

W_3 = 0.20g

W_4 = 5.00g

0.20g in 20ml

$\frac{0.20 \times 100}{20}$ in 100ml

= 1.00g

Average water-soluble extractive = $\frac{1.05 + 1.10 + 1.25 + 1.00}{4}$

= 1.1 ± 0.06

% water-soluble extractive = $\frac{1.1 \times 100}{5}$ = 22% ±0.06

APPENDIX E

Result of Microscopy

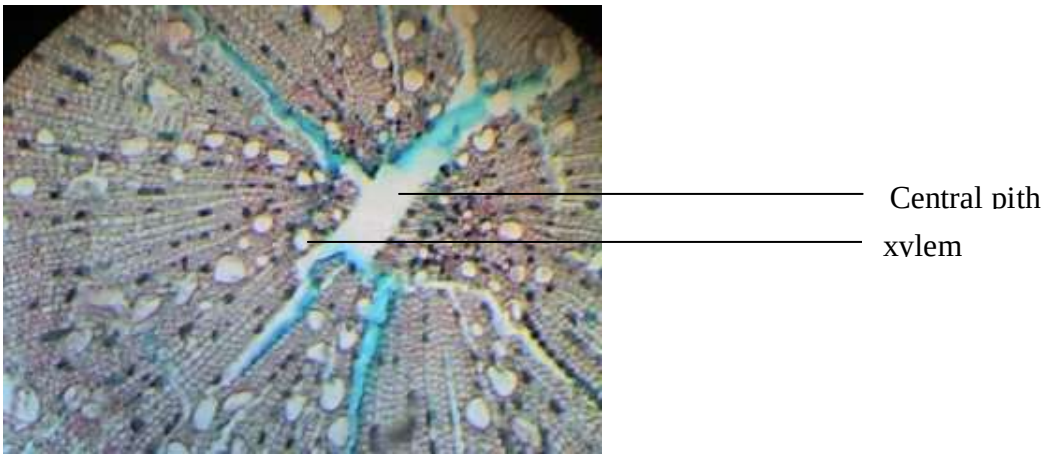


Plate VI: Transverse Section of the innermost part of the old root of Saba comorensis

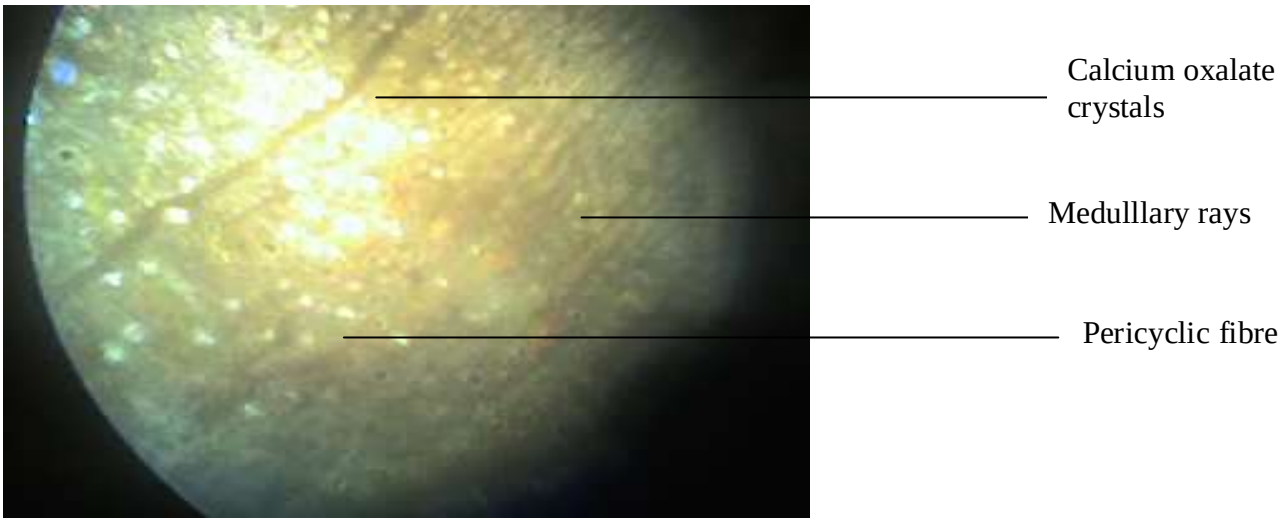


Plate VII: Transverse Section of the root bark of *S. comorensis* showing abundant calcium oxalate crystals (x 150)

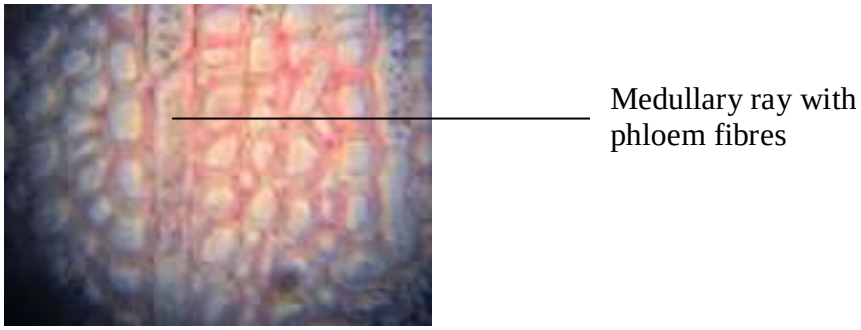


Plate VIII: Longitudinal Section of the root bark of *S. comorensis* (x 250)

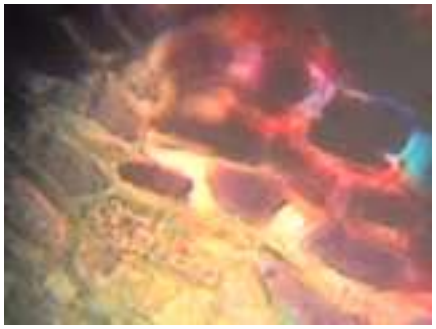


Plate IX: Cork cells from old root of *S. comorensis* (x 250)

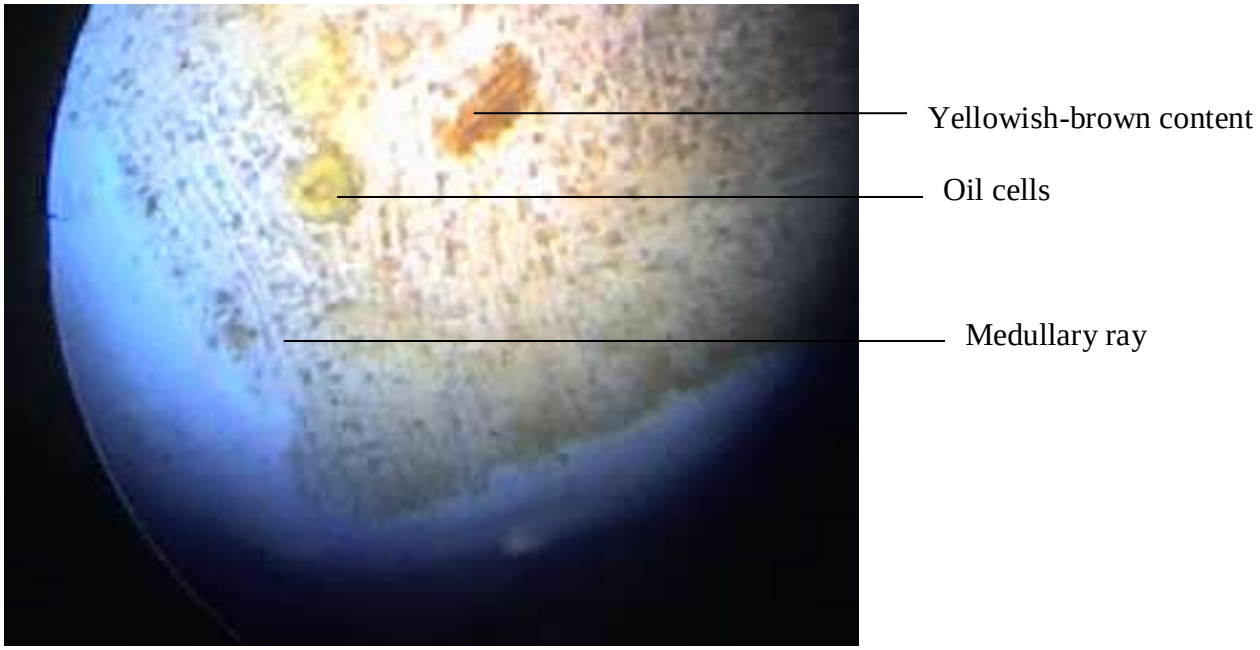


Plate X: Transverse Section showing the plan of tissues of the root bark of *S. comorensis* (x 250)



Plate XI: Secretion cells from powder of the root bark of *S. comorensis* (X 250)

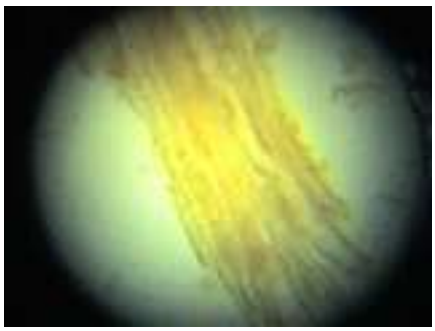


Plate XII: Fibres with calcium oxalates crystals (X 250)



Plate XIII: Fibres from the same powder (X 250)

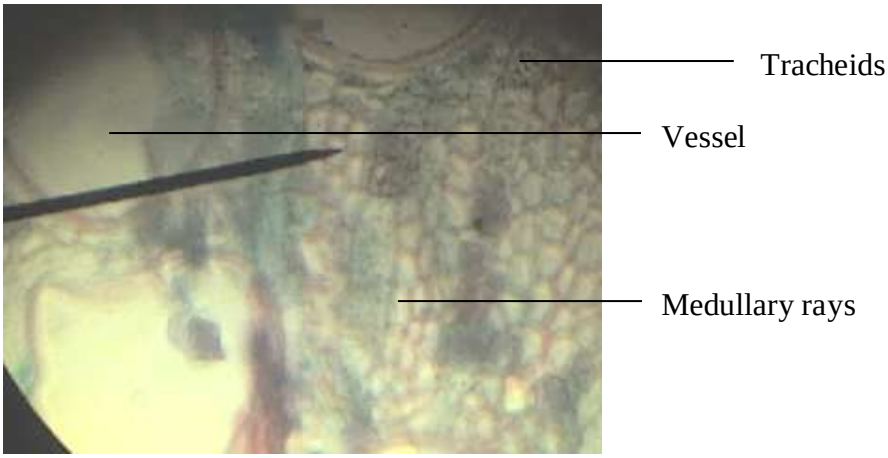


Plate XIV: Phloem elements from the bark of *S. comorensis* (X 250)



Plate XV: (a) Fragment of phloem from powdered root

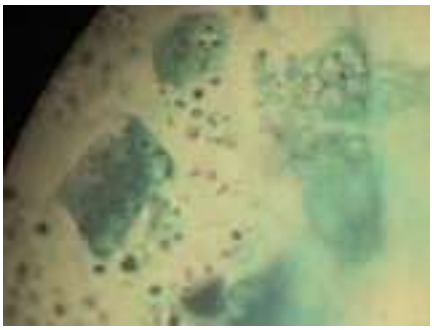


Plate XV: (b) Isolated pitted and sieve tubes of Phloem (x 250)

APPENDIX

Result of chromatographic analysis of the root of *S. comorensis*

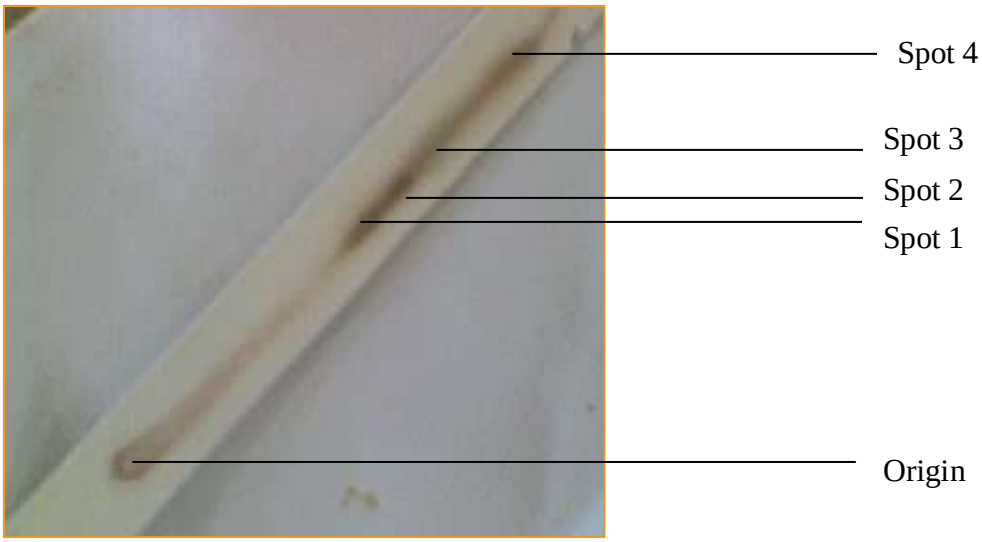
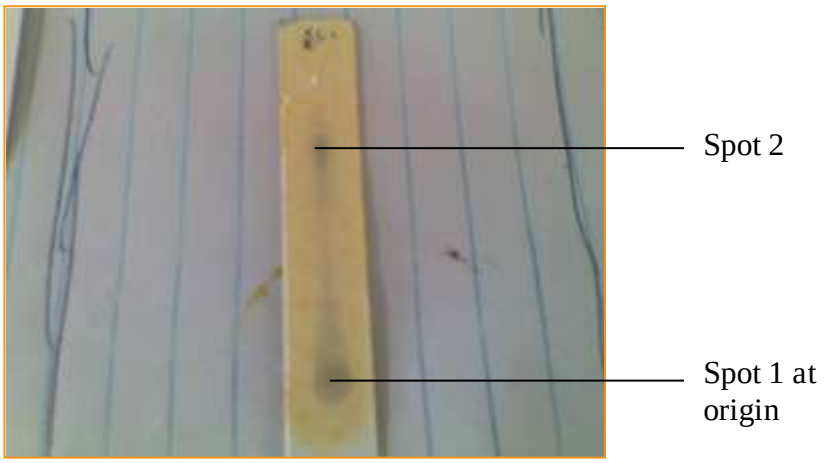


Plate XVI: Ethanol extract of *S comorensis* using iodine vapour



PlateXVII: Ethanol extract of the root bark of *S comorensis* using ferric Chloride

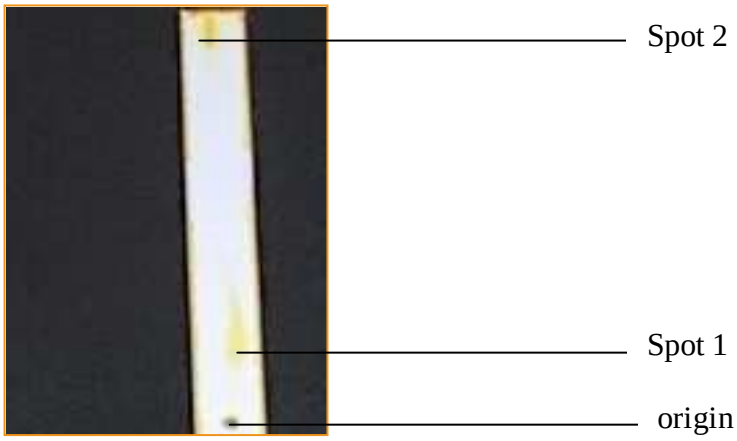


Plate XVIII: Chloroform extract of the root bark using Dragendorff ’s spray reagent



Plate XIX: Ethanol extracts using 10% sulphuric acid

APPENDIX F

Graphical illustration of the biological activity of the root bark extract of *S. comorenses.*

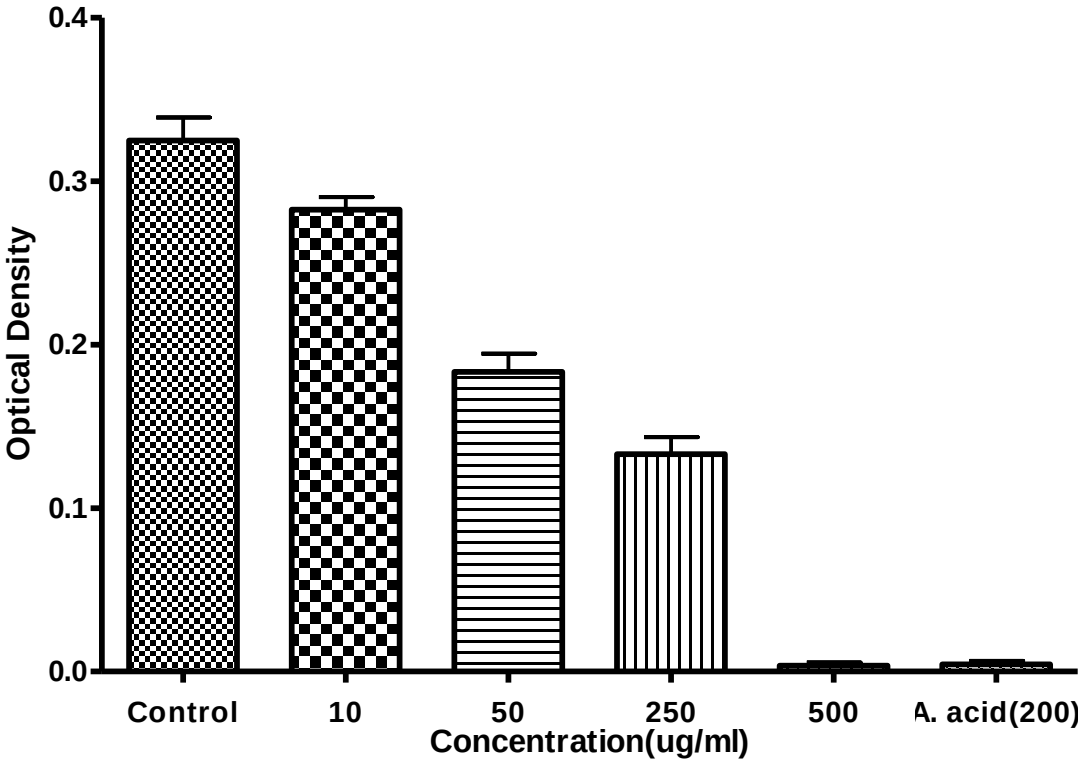


Figure 1: Antioxidant activity of Ethanolic extract of the root bark of *Saba comorensis*

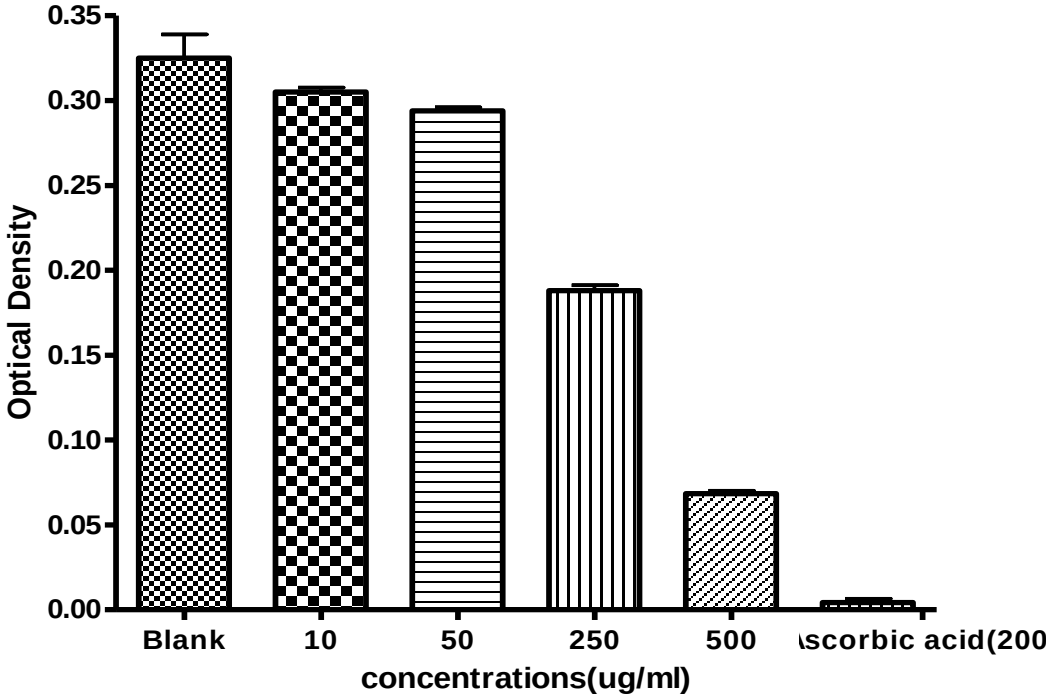


Figure 2: Antioxidant activity of Chloroform extract of the root bark of S*aba comorensis*

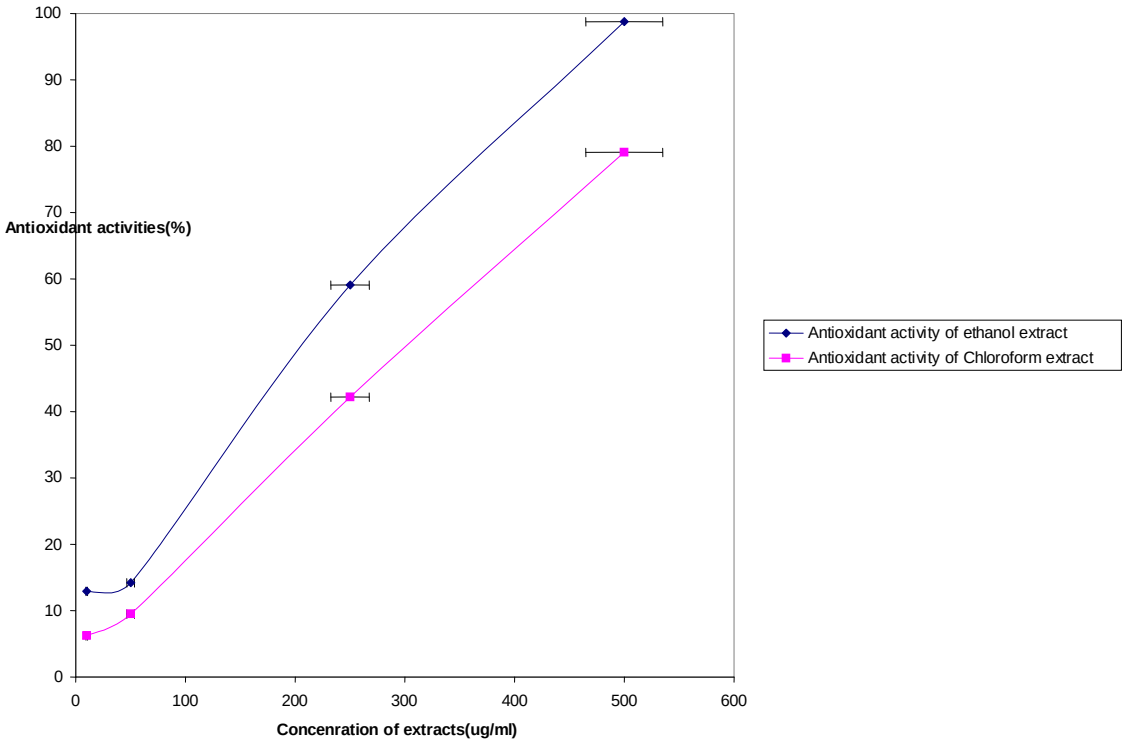


Figure 3: Comparative Antioxidant activities of Ethanol and Chloroform extracts.

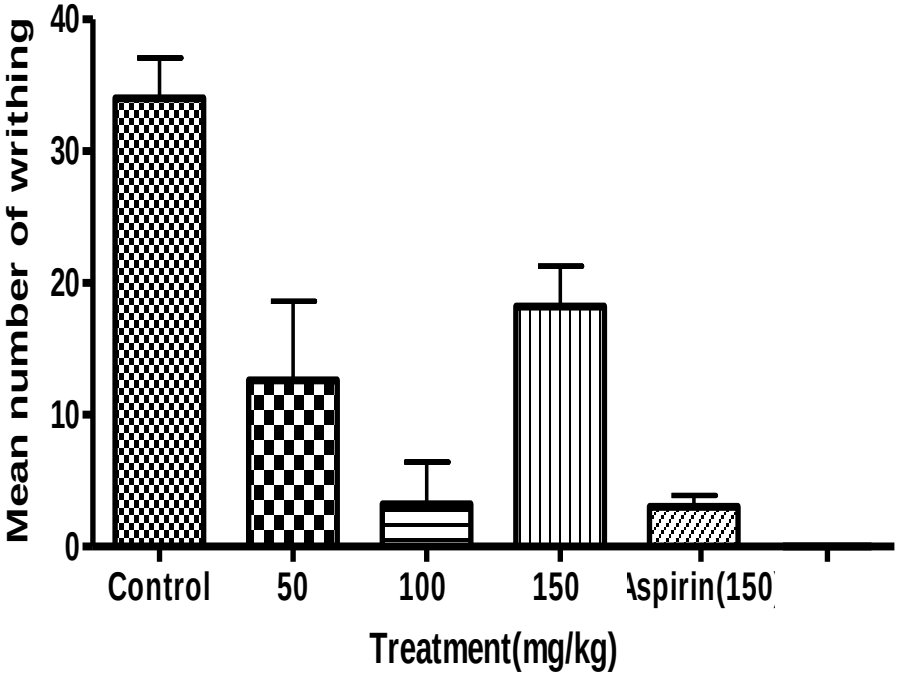


Figure 4: Effect of Ethanol extract on acetic acid-induced Writhing test in mice

APPENDIX F

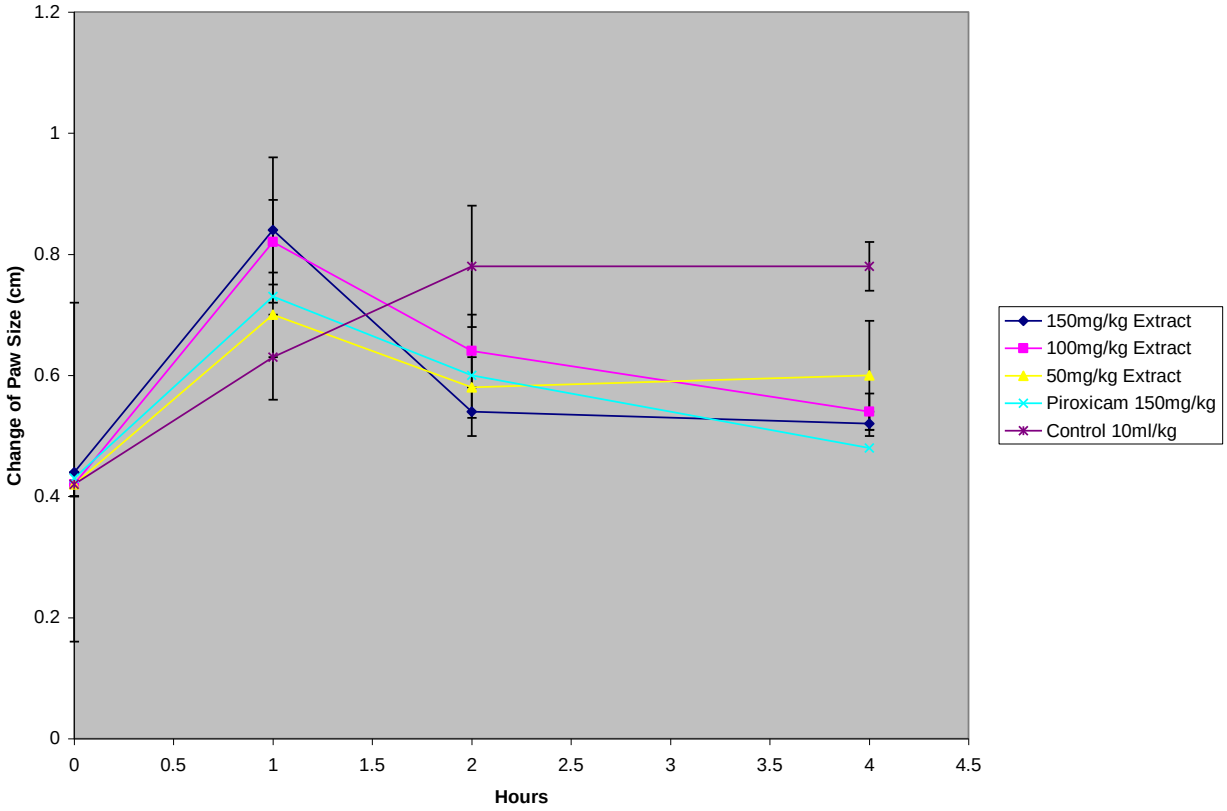


Figure 5: Effect of Ethanolic extracts of the root bark of *Saba comorensis* on Formalin-induced paw oedema in hind paw of rats (Acute phase). Each point represents the mean ± SEM of change of hind paw for five rat

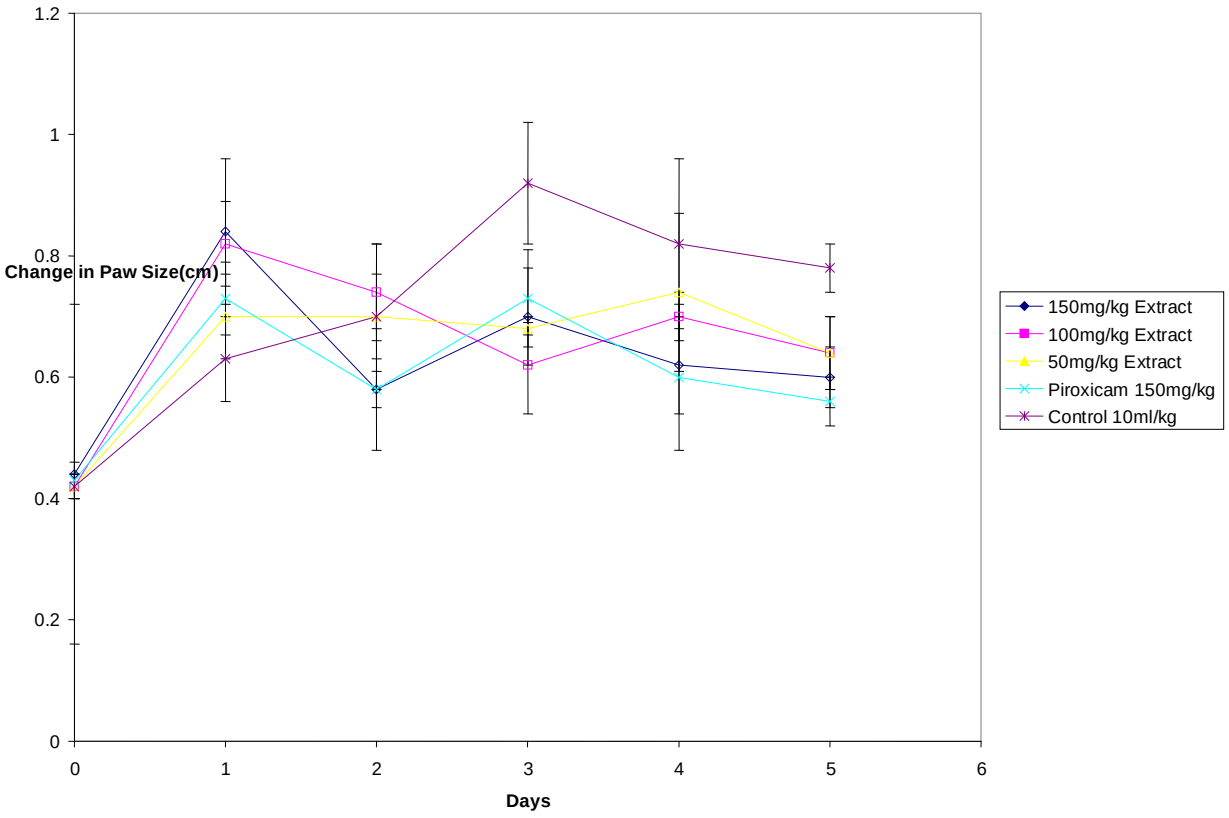


Figure 6: Effect of Ethanolic extracts of the root bark of *Saba comorensis* on Formalin-induced paw oedema in hind paw of rats (Chronic phase). Each point represents the mean ± SEM of change of hind paw for five rats

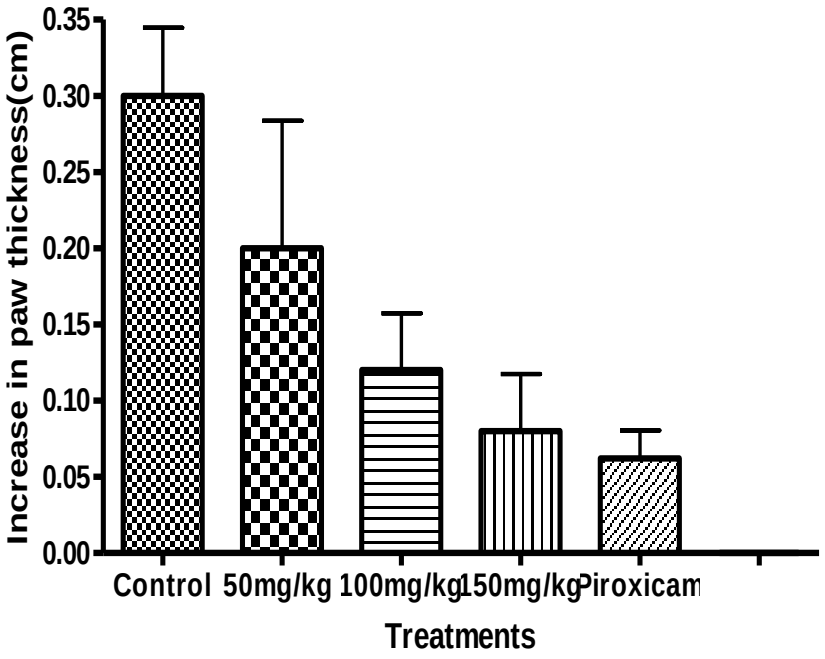


Figure 7: Acute Anti-inflammatory activity of Ethanolic Extracts of the root bark of *Saba comorensis* in Formalin-induced inflammation in Rats.

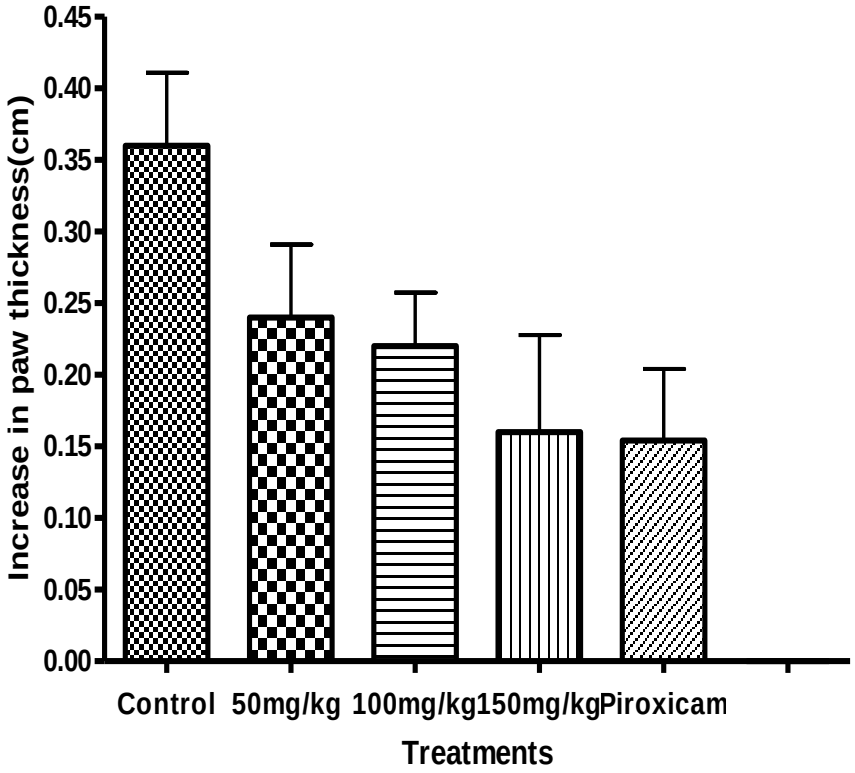


Figure 8:Chronic Anti-inflammatory activity of the Ethanol Extract of the root bark of S*aba comorensis*