STUDIES ON THE ANTIVENOM ACTIVITIES OF THE AQUEOUS EXTRACTS OF *PAULLINIA PINNATA* AND *DETARIUM MICROCARPUM* AGAINST *ECHIS CARINATUS* (CARPET VIPER) VENOM

ERIC SHALL IFUL
B.Sc. (Pharmacy); M. Sc. (Pharmacology)
PGPH/UJ/ 13767/02

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PhD Thesis, 2008

CERTIFICATION

This is to certify that the research work for this thesis and the subsequent preparation of this thesis by Eric Shall Iful (PGH/UJ/13767/02) were carried out under my supervision.

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J. C. AGUIYI Ph. D DATE

SUPERVISOR AND HEAD

---------- ----------

PROFESSOR J. KOLAWALE DATE

DEAN, FACULTY OF PHARMACEUTICAL SCIENCES

---------- ----------

PROFESSOR G. I. ADOGA DATE

INTERNAL EXAMINER

DECLARATION

I hereby declare that this work is the product of my own research efforts; undertaken under the supervision of Professor J.C. Aguiyi and has not been presented elsewhere for the award of a degree or certificate. All sources have been duly distinguished and appropriately acknowledged.

Signature and Date

ERIC SHALL IFUL

PGPH/UJ/13767/02

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DEDICATION

Mother: Mrs. K. Iful

Children: Chataimada, Valla, Miriam, Margaret, and Hyelsikya

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ABSTRACT

In rural savannah areas of Nigeria, *Echics ocellatus* (Carpet viper) is responsible for high incidence of snakebite morbidity and mortality. The snakebites are commonly treated with plant extracts in form of traditional medicine. The antivenom activity of *Paullinia pinnata* Linn and *Detarium microcarpum* Guill and Perr, popular herbs used in the treatment of *Echis ocellatus* bites are of great interest and were investigated *in vivo* and *in vitro* against the venom (2mg/kg) of *Echis carniatus.* The extracts were prepared by cold and hot extraction in water. The LD_{50} and minimum lethal dose (MLD) of the venom were first determined in mice to be 1.5mg/kg and 2mg/kg, respectively. The acute toxicity (LD_{50}) testing of the extracts indicated LD_{50} to be 600 mg/kg (i.p) and 1200 mg/kg (p.o) for *Paullinia pinnata*, while 800 mg/kg (i.p) and 1400 mg/kg (p.o) for *Detarium microcarpum*. The extract of *Detarium microcarpum* (600mg/kg) reduced mortality significantly ($p < 0.05$) in mice, whereas the extract of *Paullinia pinnata* (400mg/kg) presented a weak antivenom activity. The effective dose (ED_{50}) of the extracts was estimated as 300mg/kg (i.p) and above 400mg/kg (p.o) for *Paullinia pinnata* and 50mg/kg (i.p) and 200mg/kg (p.o) for *Detarium microcarpum* in venom pretreated mice. In animals treated with 60min pre-incubated mixtures of extracts/venom, ED_{50} of 100mg/kg and less than 50mg/kg was established for *Paullinia pinnata* and *Detarium microcarpum,* respectively. The ED_{50} of *Paullinia pinnata* in extract pretreated mice was more than 400mg/kg (i.p) and 400mg/kg (i.p), while *Detarium microcarpum* indicated 100mg/kg (i.p) and 400mg/kg (p.o) as $ED_{50.}$ The extracts of *Paullinia pinnata* and *Detarium microcarpum* inhibited all test bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa* and *Proteus mirabilis*) at various concentrations, with the extract of *Paullinia pinnata* found more active having lower MIC and MBC, and broader spectrum of activity. It also significantly ($p <0.05$) restored blood clotting time and bleeding time in rats. The same extract reversed the venom-induced increase in capillary permeability in rabbits. The extract also restored the venom-induced abnormal WBC, PCV, and platelet values to normal. The extract of *Detarium microcarpum,* however, produced significant ($p < 0.05$) inhibition of acetic acid-induced abdominal constrictions in mice, yeast-induced rectal temperature in mice, and albumin-induced inflammation in rats. The extracts of *Paullinia pinnata* produced an anti-histamine activity on guinea pig ileum, relaxation of the rabbit jejunum, and an increase in contraction of rat phrenic nerve hemi-diaphragm, while the extract of *Detarium microcarpum* caused relaxation on rabbit jejunum and increase in contraction on rat phrenic nerve hemi-diaphragm muscle. Preliminary phytochemical analysis of the extracts indicates the presence of carbohydrates, saponins, steroids and tannins in *Paullinia pinnata* root bark, while *Detarium microcarpum* leaf contains anthraquinones, flavonoids, saponins, steroids, and tannins. The trace metal analysis of the extracts also indicates the presence of Zn, Ca and Fe in *Detarium microcarpum,* while *Paullinia pinnata* contains Zn, Ca, Fe and Pb. These results therefore suggest that *Paullinia pinnata* root bark and *Detarium microcarpum* leaves contain biologically active principles, which have potentials for the treatment of carpet viper bite poisoning. Since the extracts could protect animals from death and other deleterious effects of the venom, the study has supported the use of the medicinal plants by traditional healers in the treatment of snakebite.

CHAPTER ONE

INTRODUCTION

1.1 SNAKEBITE SITUATION

Snakebite is a global medical problem especially in the rural areas of the tropics with about 40,000 deaths each year (Warrell, 1976). The annual snakebite incidence in savannah region of northern Nigeria has been estimated to be 497 per 100,000 populations, with 12.2% mortality due mainly to the carpet viper, *Echis carinatus* (Pugh and Theakston, 1980). In the recent past, studies indicate that the situation has not improved as the incidence of snakebite worldwide has been reported to be in excess of 3,000,000 per year with more than 150,000 deaths (Mustapha, 2003). In another report the incidence of snakebite is still high; with an estimated 10,000 deaths occurring every year (Mustapha, 2003) and the carpet viper still the main culprit (Mustapha, 2003; (Warrell, 1976). The incidence of snakebite is often associated with agricultural activities with the highest at the beginning of the rainy season. The other important factor responsible for the increase in snakebite cases is flood which drives out snakes from their burrows (Warrell *et al.*, 1977).

The commonest snakes of clinical importance in Nigeria comprise the cobras (*Naja* species) and the vipers (e.g. *Echis*). The carpet viper has been reported to be a very dangerous snake and its victims are mainly

farmers, hunters and herdsmen; most of who are of young productive ages. Nigerian carpet viper, *Echis carinatus ocellatus*, is commonly found in the Benue- Niger valley axis and the hilly north-eastern part of the country (Warrell and Arnett, 1976; Mustapha 2003; Warrell *et al.*, 1977).

1.2 TRADITIONAL HERBAL MEDICINES IN SNAKEBITE

For quite sometime now, the administration of antivenom has remained the mainstay in the treatment of snake venom poisoning. The supplies of antivenoms by the various governments are very unreliable; and where the products are available they are very costly. The use of antivenoms for the treatment of snake venom poisoning is further restricted by their propensity to cause hypersensitivity reactions in sensitive patients. The inability of antivenoms to resolve the local effects of the venom is also a limiting factor (Mahanta and Mukherjee, 2001). The urgent need for the discovery of new anti-snake venom from local resources such as medicinal plants has therefore been recognized.

Paullinia pinnata Linn (Family: Sapindaceae) and *Detarium microcarpum* Guill and Perr. (Family: Caesalpiniaceae) are used extensively in African traditional medicine for the treatment of various diseases (Dalziel, 1955). The most popular use of these plants is in the treatment of snakebites (Gill, 1992; Rahuta Michael, personal communication). According to these sources, the root bark of *Paullinia pinnata* is used by Okpameri people of Edo state, while the leaves of *Detarium microcarpum* are employed by traditional healers of Anaguta

people of Jos, Plateau state, Nigeria to treat snakebite patients. However, this practice lacks scientific validation and therefore this type of treatment needs thorough scientific investigation.

In the present study therefore an effort to validate the use of these plants in the treatment of bite by *Echis carinatus* was attempted. Specifically, this research work provides scientific evidence of antisnake venom properties of the root bark extract of *Paullinia pinnata* and leaf extract of *Detarium microcarpum*.

1.3 PROBLEMS OF ANTIVENOMS

The most effective and acceptable therapy for snakebite victims is the immediate administration of antivenom following envenomation (Mahanta and Mukherjee, 2001). The orthodox medical treatment of snake venom poisoning so far is limited by the use of antivenom, which is prepared from animal sera. Although, the use of anti-snake venom for the treatment of snake venom poisoning is universally accepted, therapeutic benefits are limited by the problems of hypersensitivity reactions in sensitive individuals. Furthermore, the conventional anti-snake venoms have not always been able to resolve the local effects of the venom (Russell, 1977; Warrell, 1976) such as haemorrhage, local swelling, bacterial infections, fever, pain, and bleeding. Another problem of antivenom is that of availability and cost of treatment.

Traditional remedies from plants used in the treatment of snakebite patients have a number of potential advantages. They are not expensive,

are readily available, can be grown locally, and hypersensitivity reactions to plant extracts are rare. Herbal antivenoms may alleviate the local effects of the venom, which is difficult to achieve with conventional antivenoms.

1.4 PURPOSE OF THIS STUDY

The present study was therefore conducted to determine whether or not the extracts of *Paullinia pinnata* root bark and *Detarium microcarpum* leaves could neutralize the venom and resolve local effects of *Echis carinatus* venom in animals. The results would be used to clarify the pharmacological effects of the extracts and also to ascertain the veracity of claims by traditional healers of the use of these plants in the treatment of snakebite patients.

1.5 RESEARCH QUESTIONS

Given the high usage of medicinal plants in the treatment of snakebite patients by traditional healers, the following research questions need to be answered: How effective are the extracts of *Paullinia pinnata* and *Detarium microcarpum* against carpet viper venom? Can the extracts resolve local effects of the venom, and pharmacologically neutralize the venom of *Echis carinatus* to prevent death, in animal models?

1.6 SCOPE OF THIS STUDY

In an attempt to answer the research questions, research limitations notwithstanding, this work was designed to carryout the following: (1) conduct ethnomedicinal survey on the use of these plants from oral interview, (2) collection and identification of plant materials, (3) extraction of plant materials, (4) analysis of the extracts for phytochemical compounds, (5) acute toxicological studies of the plant extracts using animal models, (6) evaluate the anti-snake (*Echis carinatus)* venom activities of the extracts of the plants with a view to ascertaining the veracity of the claims of the traditional healers, (7) undertake pharmacological screening for analgesic, antipyretic, anti-inflammatory, antibacterial, and diuretic properties of the extracts, and (8) study the mechanism of action of the plant extracts using isolated tissues.

Since the carpet viper is one of the major causes of snakebite injuries in the savannah region of Nigeria, *Echis carinatus* venom was used throughout the study.

1.7 RELEVANCE OF THE RESEARCH

The study of pharmacological properties of medicinal plants used by indigenous people is an important approach towards the discovery and development of traditional herbal medicines. The concept of testing medicinal plants is underlined by the fact that some traditional herbal medicines have proved, on investigation, to be of value in orthodox

medicine; and useful drugs have been developed from plants, some of which were originally used in traditional medicine.

In view of the limitations of conventional anti-venom in the treatment of snakebite poisoning, the need to search for better, effective, accessible and affordable remedies from indigenous plants underlines this research work.

CHAPTER TWO

LITERATURE REVIEW

2.1 BACKGROUND

Paullinia pinnata and *Detarium microcarpum* are West African savannah plants used by traditional healers in the treatment of various diseases including snakebite.

Snakebite is a global medical problem with the attendant consequences more on the people living in the rural areas of the tropics. In the recent past, some studies indicate that the situation has not improved as the incidence worldwide has been reported to be in excess of 3,000,000 per year with more than 150,000 deaths (Mustapha, 2003). In the savannah region of northern Nigeria the situation is not different either, as an estimated 10,000 deaths occurring every year have been reported with the carpet viper as the main culprit (Mustapha, 2003). The other commonest snake species of medical importance in Nigeria are the cobras (*Naja* species). The West African carpet viper, *Echis carinatus ocellatus*, is commonly found in the Benue-Niger valley and the hilly north-eastern part of the country (Warrell and Arnett, 1976; Mustapha, 2003; Warrell *et al.*, 1977). It is responsible the major snakebite fatalities in this region. The most vulnerable people are farmers, hunters and herdsmen who are of the young productive age groups. The increasing incidence of snakebite is often associated with increase in agricultural

activities. The beginning of the rainy season is also responsible for the increase in snakebite cases as flood drives out the snakes from their secure habitats.

The traditional healers use traditional medicines, most of which are herbal preparations, in the treatment of snakebite patients in their areas. Co-incidentally, high survival rate among victims of snakebites has been observed in communities where the use of traditional medicine is a common form of snakebite management (Pugh and Theakston, 1980; Rahuta Michael, personal communication). In these communities, traditional healers are popular because patients consult them in various areas of need, including snakebite. In the course of treatment of snakebite patients, the healers administer their medicaments using various treatment methods. Invariably, they employ the medicaments made from plant materials for the purpose of neutralizing the venom and also as palliatives in the treatment of snakebite poisoning. For instance, the healers apply vegetable fats to the swollen part of the limb for the swelling to subside. Some herbs are also administered to send the patient to sleep in order to relieve him of anxiety. The healers also use herbal emetics to induce vomiting so as to reassure the patient, who may believe that the venom can only be removed from the body through vomiting. The traditional healers prepare herbal medicaments by soaking powdered herbal materials in cold or hot water and are given to the patients to drink. The preparations are also made into poultices and applied to bite punctures to

promote wound healing. The medicaments are also administered by the traditional healers to relieve pain and treat fever, prevent or encourage bleeding, and to neutralize the venom to save the life of the patient.

2.2 BIOLOGY OF SNAKES

2.2.1 Description

Snake is limbless scaly reptile with very long body. Snakes are closely related to lizards, differing from them in the complete absence of limbs, movable eyelids and external ear. Like other reptiles, snakes are cold-blooded animals (Russell, 1983).

Although venomous snakes have well been described by Russell (1983), some distinctive features and common characteristics of this group of snakes are:

a. Triangular head shape. The presence of venom glands makes the heads of all poisonous snakes more triangular than oval.
b. The presence of second set of pits on the face of a snake, below the nostril, is an indication of a poisonous snake.
c. The presence of a rattle is always a guarantee that the snake is venomous.

2.2.2 Classification

Snakes are easily classified according to family and fang orientation.

1. Family

There are four major groups of venomous snakes (Russell, 1983):

i. Crotalidae (e.g. Pit viper)

ii. Elapidae (elapids) e.g. cobras and mambas

iii. Viperidae (vipers) e.g. *Echis*, and *Bitis*

iv. Hydrophiids (sea snakes)

2. Fang orientation.

Fangs are teeth and are like hypodermic needles with two openings: one near their base connecting with the venom duct and the other near the tip for the injection of venom into the prey. There are three types of venomous snakes described on the basis of fang orientation (Russell, 1983).

a) Opisthoglyphs. These are the rear-fanged snakes. The fangs are enlarged rear teeth with a "groove" through which the venom flows down while the snake swallows its prey. The members of this group are mostly harmless or mildly venomous. Example of this group is the Boomslang (*Dispholidus typus*).

b) Proteroglyphs. These are the fixed front fang snakes. They have small non-movable front fangs. Obvious examples of this group of snakes are the cobras (*Naja*), sea snakes (*Hydrophiidae*) and mambas (*Dendroaspis*).

c) Solenoglyphs. These snakes have movable front fangs. The fangs fold back into the mouth until they are needed. The poison fangs have enclosed canals within the teeth that transmit the venom out of the body,

very much like hypodermic needle. Examples of this group are puff adder (*Bitis arietans*) and carpet viper (*Echis ocellatus*).

2.2.3 Distribution

Carpet viper (*Echis carinatus*) is widely distributed in Africa, Middle East, Pakistan, India, and Sri Lanka (Warrell *et al*., 1974). In Africa, it is found in the dry savannah or desert region (Figure 1b). Snake distribution in northern Nigeria savannah region has been described elsewhere (Warrell, 1976; Pugh and Theakston, 1980). The spitting cobra is commonly found in the densely populated areas of the northern savannah region of the country, whereas carpet viper and puff adder are the major causes of snakebite fatalities in the sparsely populated areas of the region (Figure 1a). *Echis carinatus ocellatus* is one of the most medically important venomous snakes in Nigeria (Warrell *et al*., 1976; Laing and Theakston, 1993), and the commonest race found in Nigeria is *Echis carinatus ocellatus* Stemmler. The snake species are widely distributed in the savannah region of Nigeria especially in the Benue-Niger valley and the hilly areas of North eastern savannah.

2.3 *ECHIS CARINATUS* (CARPET VIPER)

Carpet viper is a small and stout dark brown snake with grayish brown markings (Plate 1). It is one of the most aggressive and feared venomous snakes in the world. It grows up to 35cm in length. Carpet viper moves around in a coiled shape when it is threatened. It is also known as saw-scaled viper because it has scales on the sides of its body

that it rubs together to produce rasping sound, very similar to that of a saw, to ward off predators.

The carpet viper (*Echis carinatus)* belongs to the family Viperidae. There are two species of the genus *Echis*: *Echis coloratus* Gunther (Bourton's carpet viper) and *Echis carinatus* (Schneider). The former occurs in the Middle East (Warrell and Arnett, 1976), whereas the later is widely distributed. *Echis carinatus ocellatus* Stemmler is the subspecies that is commonly found in Nigeria (Pugh and Theakston, 1980).

Echis carinatus is considered as the most dangerous snake in the world (Warrell and Arnett, 1976; Warrell *et al*., 1977) because of its wide distribution, abundance in farming areas, good camouflage, irritability, and its venom (Warrell *et al*., 1974), is strongly haemotoxic, affecting the vessels, blood, and heart muscle.

Bites from *Echis carinatus* is the most important cause of morbidity and mortality in man (Warrell *et al*., 1974; Mustapha, 2003), biting and killing more people than any other species of snake in the world (Warrell *et al*., 1977). The incidence of carpet viper bite in endemic areas is often associated with its prevalence (Figure 1a). It implies that its bites are correspondingly uncommon where the snake species is rare.

The endemic areas of *Echis carinatus ocellatus* bites in Nigeria are Kaltungo (Gombe State), Bambur (Taraba State), Zungeru (Niger State), Garkida-Hong-Michika area (Adamawa State), Langtang- Shendam (Plateau State), and Zaria (Kaduna State) (Warrell *et al*., 1974; Pugh *et*

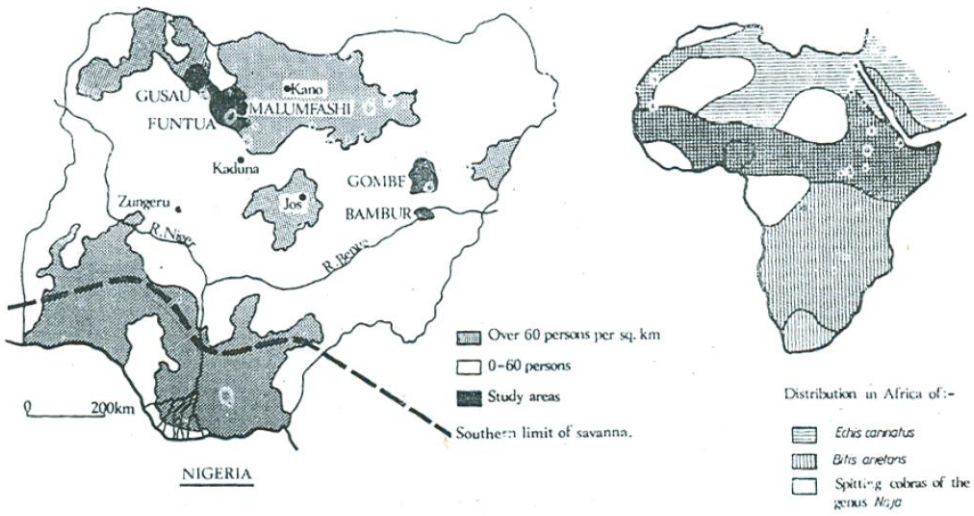


Figure 1a: Population density in Nigeria and distribution of principal snakes in Africa (from Pugh R N and Theakston RDG, 1980: Lancet 2: 1182)

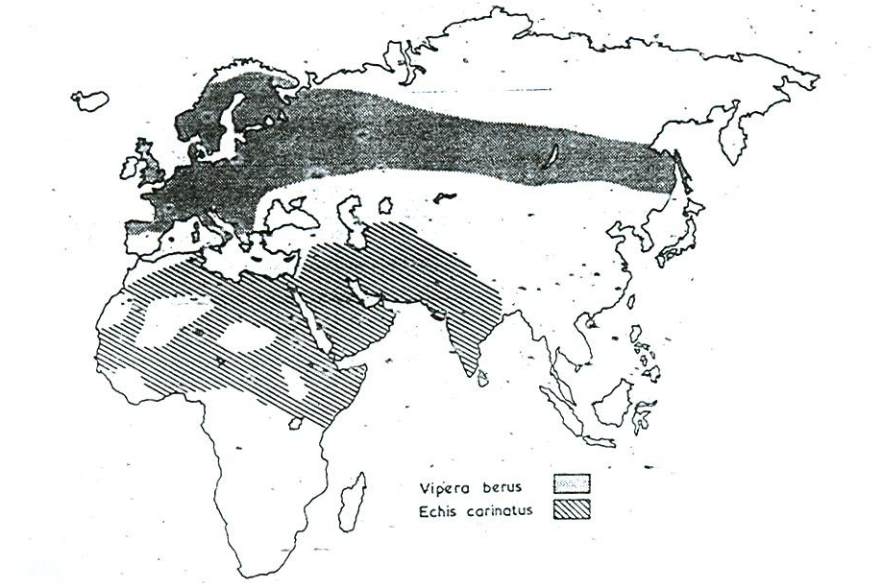


Figure 1b: World Distribution of *Echis carinatus* (From Warrell DA and Arnett C. 1976. Acta Trop. XXXIII, 4:309).

al., 1979; Reid and Theakston, 1983; Mustapha, 2003; and Warrell *et al*., 1977).

2.4 SNAKE VENOM PROPERTIES AND ACTIONS

Snake venom is a biological substance used by the animal for defence against enemy in an emergency. It is also used by the animal to capture and kill its prey and aids its digestion. It achieves its potent effect in part because of the venom's proteolytic activity.

The understanding of composition of snake venom would explain the diverse effects of venom and mechanism of action of antivenom. Snake neurotoxins are known to block nerve transmission at the site of neuromuscular junction but do not affect transmission within the axon (Tu, 1977). The neurotoxins of cobra venoms are post-synaptic acting. Some venom (cobra and mamba) works on the nicotinic acetylcholine receptors present on the neuromuscular junction. Other types of venom work on the pre-synaptic nerve terminal (e.g. β-bungarotoxin) and neostigmine can not reverse the action. Pre-synaptic neurotoxins inhibit the fusion of the vesicles (containing acetylcholine) with the nerve's membrane of the neuromuscular junction (Levy, 2003). This action prevents the release of the acetylcholine and results in a flaccid paralysis where the muscles are unable to contract (Garland and Bailey, 2006).

Plate 1: *Echis carinatus ocellatus* (Carpet Viper)
It was caught from Langtang plains of Plateau State, Nigeria (June 2006)

Cobra cardio-toxic venoms have an affinity for cardiac tissue and act to depolarize cardiac cell membrane, which leads to systolic arrest. The venoms of Elapid have higher concentrations of esterases such as acetyl cholinesterase, while viper venoms have higher concentrations of endopeptidases (Warrell, 1999). Phospholipases, proteases and lytic factors in snake venom cause haemolytic effects and are mainly responsible for the necrosis that follows viperid and crotalid bites.

Crotalid neurotoxins are however antagonistic to acetylcholine and act as a blocking agent at the neuromuscular junction (Russell, 1983). Proteolytic enzymes are ‘trypsin-like’ in action and help in the digestive reactions of snake venoms. Collagenase helps in degrading collagen, a major component of connective tissues, skin, and flexible vascular tissue; and the enzyme is commonly found in crotalid and viperid venoms and this explains the necrosis often seen following viper bites.

Phospholipases A and B help in degrading lipids to free fatty acids and can cause damage to the cell membrane leading to lysis and apoptosis. Phosphodiesterases break the phosphate bonds in nucleic acids, thus rendering DNA and RNA useless in the affected cells through the inhibition of protein synthesis, and have apoptosis.

Acetylcholinesterase breaks the acetate ester bond in acetylcholine, which takes place mainly in the synapse. The end result of this action is an inability to innervate smooth muscle and the inability to relax striated muscle leading to spasmodic paralysis and sometimes a concurrent drop

in blood pressure and difficulty in breathing (Levy, 2003). An anticholinesterase prevents this breakdown of acetylcholine leading to the conversion of electrical impulses from nerve to chemical energy that causes the relaxation of the innervated skeletal muscle.

Some snake venoms also contain highly competitive antagonists that prevent acetylcholine from binding at the post-synaptic nicotinic cholinoceptors (Russell, 1983). They also cause neurotoxic symptoms such as apnea and asphyxia. The non-depolarizing effect of cobra venoms and toxins is similar to that of curare. The action of cobra neurotoxins differs from that of curare because its mechanism is not one of pure competitive. While curare forms layer in front of the receptors, the snake neurotoxins are actually attached to the receptors at the post-synaptic site (Tu, 1977). The binding of the neurotoxins to cholinergic receptors is very strong and essentially irreversible. This action could explain why the post-synaptic effects are not reversible with neostigmine.

Snake venoms also contain endopolynucleotidases (DNase and RNase) which degrade DNA and RNA respectively. NAD Nucleotidase degrades nicotinamide. Cell metabolism is interrupted by inhibition of oxidative phosphorylation, which leads to insufficient supply of ATP for the cell (Levy, 2003). Consequently, cellular respiration is interrupted and death may ensue. L- Amino acid oxidase is found in all snake venoms and is oxidative.

Pro-coagulants commonly found in carpet viper venom, cause blood coagulation to occur due to its thrombin-like effect and also it can cause the activation of Factor X to Factor Xa. The anticoagulants prevent blood from clotting essentially due to the effect of the venom fibrinolysis or fibrinogenolysins or action of phospholipase on platelets or plasma phospholipids (Levy, 2003). The two chemicals may be found in the same venom. In a situation where the anticoagulant is predominant, the blood fails to clot. Conversely, when the pro-coagulant is higher the blood of the victim clots more than normal. When anticoagulant action is more frequent it will cause bleeding at the site of envenomation as well as internal bleeding and tissue oedema (Levy, 2003).

Three divalent metals (Ca, Zn, and Mg) are also available in venoms of all snake species (Tu, 1977). Copper, Fe and Mn are also present in several venoms irrespective of snake species (Levy, 2003). Monovalent cations (Na, K) are found in high concentrations in all venoms. The trace metals are assumed to be present in venoms as charge balancing ions for the proteins and inorganic salts of the venoms. However, lethality of venom is not affected by removal of trace metal, although a striking change in the degree of haemorrhagic activity has been observed (Frederich and Tu, 1971). The haemorrhagic activity disappears following the removal of divalent metals from snake venoms. Proteolytic activity is greatly reduced in EDTA treated venom while esterase activity is not affected (Friedrich and Tu, 1971). High

concentration of Mg or Zn restores considerable amount of haemorrhagic activity, proteolytic activity is restored by addition of Mg or Zn (Friedrich and Tu, 1971).

The much larger molecules of carpet viper venom are absorbed more slowly through the lymphatic system and is concentrated and bound in the kidneys and some components are eliminated in the urine. The venom is not active after oral administration and the venom components do not cross the blood-brain barrier (Tu, 1977).

2.5 PATHOGENESIS OF *ECHIS CARINATUS* (CARPET VIPER) ENVENOMATION

Snake venom is a complex mixture of about 20 different enzymes, which are proteins (Theakston and Reid, 1983). These enzymes determine the toxicity of the snake venom as to whether it is haemotoxic or neurotoxic but the venoms can not be classified as being exclusively haemotoxic or neurotoxic. Although any venomous snake should be considered dangerous, snakebite does not always result in envenomation (Warrell, 1999). However, when there is clear indication of systemic envenomation, the condition of the patient becomes an emergency, which calls for urgent medical attention. All venoms are composed of at least several toxins and are capable of producing various effects in living tissues and systems (Russell, 1983).

For the purpose of general considerations, venoms belonging to the genera viperidae, with few exceptions are mainly considered haemotoxic,

which means that they are primarily injurious to the blood and the vessels. Haemotoxic venom destroys tissue and is very painful.

The carpet viper venom, like other snake venoms, is a multi-component mixture, with more than 90% of the dry weight as protein in the form of enzymes, non-enzymatic polypeptides, toxic and non-toxic proteins (Reid and Theakston, 1983; Tu ,1977).

Echis carinatus venom acts predominantly on the haematological system particularly on the capillary endothelium, which results in instant local swelling of the area. This is as a result of an increase in vascular permeability induced by proteases, phospholipases, membrane damaging polypeptide toxins, and endogenous autacoids released by the venom. Hyaluronidase promotes the spread of the venom. Once an envenomation has taken place, the primary target is the blood system. The blood of the victim becomes incoagulable (Warrell and Arnett, 1976; Warrell *et al*., 1974) accompanied by prominent local irritation and symptoms of severe poisoning with burning pain, inflamed swelling, and formation of abscess, sudden drop in blood pressure and internal bleeding. Death ensues because the heart stops.

The venom of *Echis* species contains several toxins which are responsible for the toxic and lethal effects of the venom. The venom contains a pro-coagulant enzyme, *ecarin*, which activates prothrombin to produce thrombin, which is resistant to heparin (Tu, 1977). The activity is responsible for incoagulability of blood of the victim. The activity of the

enzyme leads to disseminated intra-vascular coagulation with the net effect being hypo-coagulable blood. This is due to the rapid consumption of coagulation factors such as fibrinogen, prothrombin, Factors V and VIII, and of platelets (Tu, 1977). The reduction in the number of platelets in the blood also leads to spontaneous bruising and prolonged bleeding after injury. The pro-coagulatory action (direct activation of prothrombin) is the principal effect of the *Echis carinatus* venom on blood coagulation in man.

The venom also contains *haemorrhagin*, which causes direct endothelial damage of blood vessel wall that leads to spontaneous bleeding (Reid and Theakston, 1983). Consequently, there is spontaneous oozing of blood into vital organs, especially the brain, causing lethality through cerebral haemorrhage (Warrell and Arnett, 1976, and Pugh *et al.*, 1979).

The combination of defibrination, thrombocytopenia and vessel wall damage can result in massive bleeding, a common cause of death after bites by carpet viper. However, administration of anti-venom temporarily stops abnormal bleeding and restores clotting process to normal (Levy, 2003).

Phospholipase A_2 is another toxic component of the *Echis carinatus* venom. The toxin causes two separate and independent actions: a non-lethal esterase activity and a toxic neurological activity where

neurotoxic phospholipase A_2s act by pre- or post- synaptic blockade of neuromuscular nerve transmission (Levy, 2003).

Echis carinatus venom also affects blood glucose level after envenomation. Tu (1977) reported that the normal blood glucose in rabbit is 0.114%. However, on administration of snake venom to rabbit, the blood sugar increased significantly (Levy, 2003). In severe poisoning, blood glucose is also derived from the conversion of muscle glycogen to glucose (Mohamed *et al*, 1965). The increase in blood sugar is due to stimulation of the central nervous system, which controls carbohydrate metabolism, and also to an increase in epinephrine secretion in the adrenal gland leading to decrease in the glands but increased concentration in the blood (Russell, 1983).

The activities of insulin are also affected by snake venom poisoning. The venom reduces insulin activity due to the inhibition of insulin release, thereby lowering glucose utilization resulting in an increase in blood glucose level (Tu, 1977).

2.6 CLINICAL FEATURES OF CARPET VIPER ENVENOMATION

Many people bitten by venomous snakes may develop no signs of poisoning because very small or no venom has been injected by the snake. However, when adequate venom is injected, the victim of *Echis carinatus* envenomation usually presents some clinical features which include local irritation and symptoms of severe blood poisoning with

burning pain, inflamed swellings, and sudden drop in blood pressure, fever, spontaneous systemic bleeding, local tissue necrosis, and formation of local blistering (Warrell *et al.*, 1977). The lethal dose of *Echis carinatus* venom in animal causes convulsions and death within a few minutes. However, such effects are rare in humans because the doses of the venom usually injected by snake are much smaller in relation to body weight. Laboratory values reveal disseminated intravascular coagulation (DIC) with thrombocytopenia as prominent feature, severely depleted fibrinogen, increased fibrin degradation products, depleted levels of clotting Factors V, VIII, II and XIII, and hyperglycaemia (Warrell *et al.*, 1977).

2.7 TREATMENT OF CARPET VIPER ENVENOMATION

Snakebite is always a terrifying experience that may cause confusion and panic in the patients (Warrell, 1976). The treatment of *Echis carinatus* bite poisoning involves the following measures:

i. Administration of first aid to the victim such as application of tourniquet above the venom in the direction of the heart to inhibit the spread of the venom. The patient should be given reassurance. The patient is given analgesic to control pain. The affected limb is immobilized so as not to aid the circulation of blood.

ii. Administration of antivenom, once there are signs of envenomation in the patient.

The basic principles of treatment of snakebite according to Warrell (1976) are:

a. to neutralize effects of the venom by injecting antivenom,
b. control of potential complications caused by antivenom, such as serum sickness, and anaphylactic shock,
c. Relieve pain with analgesic,
d. Prevent infection, using antibiotics, and
e. Treat major effects of the venom.

2.8 HISTORICAL DEVELOPMENT OF TRADITIONAL TREATMENTS FOR SNAKEBITE

Some traditional treatments and practices which were developed and used by people of the ancient times for the treatment of snakebite have been described by Russell (1983) and these included:

(1) Scarification. The treatment involved ceremonial release of the spirits, gods, or demons responsible for the bites;

(2) Excision or amputation. In this case, the bitten part of the body was removed. However, this form of cure would only be successful if excision or amputation could be carried out quickly after the bite;

(3) Cauterization. Here the ancient people resorted to burning the bite wound using chemical or heat. This measure was assumed to kill the venom;

(4) Tourniquet or ligature. This is a mechanical method of delaying the spread of the venom by applying a string above the venom. It was the most frequently used method of snakebite treatment;

(5) Heat, freezing, cold packs, and ice. The application of heat to snakebite wound, usually in the form of a poultice, hot oil or a hot implement was relatively a common procedure by middle of the 17th century. Hot stones, ashes, or poultices were widely used by the Indians. The anaesthetic property of cold was recognized by the ancient people, and was used for the relief of pain;

(6) Alcohol. The most widely used, and by far the most popular, was (and still is) alcohol. It has been a universal elixir for snakebite since the olden days;

(7) Botanical cures. Russell (1983) states that,

> one of the most popular and most widely used of all the folklore remedies for snakebites was, and still is, the application of various plants, either as a poultice to the bite, or orally as a liquid extract.

Onions, garlic, and tobacco were commonly used by the ancient people for the treatment of snakebites. Some of the folk cures are still widely employed in the treatment of snakebite in most parts of the world.

2.9 DEVELOPMENT AND USE OF HERBAL MEDICINE

The use of plants by man has a long-standing history. Plants are used for food, medicine and some other purposes (Martin, 1996). The use of plant medicine by different cultures has a long history, all over the

world. The World Health Organization (WHO) reported that about 80% of the world's population is still relying on traditional medicine (including herbal medicine) as its primary form of healthcare. This observation is particularly more relevant to people in the developing countries of the world where the majority of the populations is living in the rural areas.

The place of plants in therapeutics generally is evident by their use in all the major systems of medicine worldwide (Akerele, 1984). However, the exact time and how plants were first used in medicine is still unknown. The mystery remains with man because disease has been with him for a long time and the search for ways and means to combat it is one of his earliest and most persistent pre-occupation. The use of plants in the treatment and prevention of disease seems to have started in a form of traditional medical practice. The primitive people believed that the world was full of invisible spirits (Tella, 1979). The people, according to the same author, thought that disease was an evil work of such spirits. The people therefore resorted to the use of burnt offerings, administration of some concoctions and decoctions to ward off disease.

The search for obnoxious materials also led man to experiment with herbs. The intuitive efforts of man led to some invaluable discoveries. For instance, savages of different countries discovered the properties of the most deadly plants (e.g. curare, ouabain) as well as the virtues of some plants. Some centuries ago, the Indians of South America

discovered the value of cinchona bark for the treatment of malaria. In the same way, the natives of Brazil also discovered the value of ipecac for the treatment of amoebic dysentery.

The development of medical lore led to the emergence of individuals who demonstrated special talent for herbal preparations and employed it as a means of earning a living. Some people in the local communities have adequate knowledge of treatment with plants that abound their immediate environment. In the concept of health and disease, plants are used in the form of herbal medicine by traditional healers of different communities. The healers pass their knowledge of treatment with the plants and other remedies, usually oral, from generation to generation. The healers often use these plants in combination with other substances such as animal parts and mineral substances. The plant parts commonly used for treatment of ailments include roots, stem, leaves, seeds, flowers or bark or a combination of any of the parts (Tella, 1979). The same author noted that the use of plants in the prevention and treatment of disease might involve metaphysical processes such as prayers, invocations or incantations. The healers may also appeal to the gods in the course of their treatment procedures (Aben, 1998). The elements are mixed in traditional healing practices and are perceived by many African communities as necessary components of healing of all diseases. In this regard, the traditional healer becomes both doctor and pastor to the sick person. The traditional healer as doctor,

would try to treat physical illnesses by dispensing medications, while as priest, he tries to soothe the patient and to explain why the patient contracted the disease and not the neighbour or why the gods sent the disease to this person and not the other.

However, by 20th century there was turn of events in the health care delivery in Africa whereby governments of different countries could no longer provide healthcare in public hospitals. As a result, majority of people have resorted to private hospitals to receive modern medicine. But because of many economic reforms, prices of modern medicines went up beyond the reach of the poor. When this happened the people turned to traditional healers.

In the recent times, the use of natural products in Europe and the United States has increased enormously (Akerele, 1984). The extracts of medicinal plants are often sold and used in their purified forms for the prevention and treatment of diseases. In Africa, however, the provision of free medical care has become a mirage. This is because of high cost of health services in relation to the income of the population. Another important factor that pushes the rural settings of Africa into the use of herbal medicines is their perception of disease conditions, which is highly interwoven with socio-cultural beliefs of the people.

The origin of herbal medicine and its practices have been described by some authors (Akunyili, 2003; Getahun, 1976; Ogunyemi, 1979; Smith, 1953; and Wambebe, 1998).

1. Man used his ingenuity to select specific plant materials for the treatment of his ailments.
2. The early man used some mystical and religious rituals to allay his fear, which kept him in perpetual bondage.
3. Discovery of medicinal plants was made by accident. This occurs when a plant was used for a particular ailment and was inadventedly discovered to produce beneficial effect different from the one the plant was intended.
4. Empirical observation by man of effects of medicinal plants on domestic animals
5. That knowledge of traditional herbal medicine was given to herbalists by evil men or spirits.
6. Hunters discovered medicinal plants by observing an animal which had been shot but hurriedly eaten leave of a plant and did not die
7. Some traditional healers have claimed to make discoveries of medicinal plant when in trance as might have shown to them by the spirits of their ancestors.

In traditional settings, medicinal plants are used for therapeutic and occult purposes and are used in healing and in performing wonders. The occult forces of nature are used by healers as preventive measures against epidemic without oral medicine being taken. The occult properties of herbs are also used to prevent diseases or evil omen. Some magical implements in conjunction with plant medicines are applied to the body

such as ring, belt and other strange objects to make people invisible (Lambo, 1979). The therapeutic properties of herbs provide some healing properties or powers. However, the mystifying association with witchcraft and sorcery has greatly affected the image of the traditional herbalists and their practices (Akinkugbe, 1979).

Ampofo (1979) reported that about 80% of rural population still depends on plant medicine for treatment, and that almost all traditional healers and midwives have plants for special emergencies growing around their homes. He also noted that orthodox doctor is consulted by the patients only when a disease is rare or becomes chronic, or if complications occur.

On global perspective, the use of herbal medicine has assumed a wider dimension. In Russia, 45% of all drugs used are of plant origin (Akinkugbe, 1979).

The use of medicinal plants in Europe was first recorded in the middle ages. Their use was based on the Doctrine of Signatures or Similars, which was developed by Paracelsus (1490-1540). According to this doctrine, healing herbs or plants have physical features made by God identifying the plant with a specific disease or part of the body. Plants with heart shaped leaves were good for treating heart disease, those with liver-shaped parts were prescribed for bilious disease, and plants exuding a milky juice were believed to increase lactation in women.

In South America during the Spanish conquest (1531-1536) the settlers found medicinal plant gardens containing plants such as coca and tobacco. The use of herbal medicine among the Australian aborigines probably dates back many countries but was first observed at the end of the 18th century when the settlers found the aborigines using medicinal herbs together with ritual rites.

2.10 NATURAL PROPERTIES OF MEDICINAL PLANTS

The therapeutic properties are owed to the presence of some chemical compounds produced naturally by plants. These chemical compounds are required by the plants for their growth and survival. Such compounds include macronutrients, e.g. hydrogen, carbon and oxygen. Others are potassium, phosphorous and nitrogen from soil, and also molecules such as amino acids, sugars and carbohydrates. They also contain trace elements and are only needed in small quantities for plant growth. These include heavy metals, e.g. mercury (Hg); copper (Cu), manganese (Mn), zinc (Zn), iron and cobalt.

Plants also produce primary metabolites. These compounds are essential for biochemical pathways, which control growth, photosynthesis, respiration, or flowering.

Plants again produce secondary metabolites which aid them to adapt to environmental conditions, competing with other plants, and warding off attacks by predatory insects and animals or attracting ones that play role in pollination, fruit dispersal or protection. Essential oils,

for instance, reduce water loss in plant growing in arid zone, as well as repel insects and deter grazing animals. Also alkaloids, the bitter tasting compounds, are often poisonous to discourage predators. They also serve as storehouse of nitrogen for plants growing in poor soils which can not source nitrogen elsewhere. In humans, the compounds can be used to ward off, ameliorate, or cure some deadlier diseases often by acting as specific toxins against the causal organisms, aberrant cells, or abnormal physiological processes.

The secondary plant products, which are phytochemical compounds, are responsible for the characteristic odours, and pungencies and colours of plants, and others give a particular plant its culinary, medicinal or poisonous virtues and to aid the survival of the producers (Evans, 2002).

2.11 THE ROLE OF MEDICINAL PLANTS IN TREATMENT OF SNAKEBITE POISONING

The use of plant medicines against snake and snakebites worldwide dates back to the prehistoric days and was probably borne out of sheer need for prevention and cure. The treatment of snakebite by traditional healers usually involves the soaking of powdered herbal preparations in cold or hot water or mixed with saliva and given to patient to swallow. The primary aim of use of medicinal plant is to neutralize the effect of the venom to prevent death. They are also used to treat snake envenomation because they alleviate some of the signs/symptoms of poisoning

(Houghton and Osibogun, 1993). For instance, the terrifying experience by patient may be accompanied by fear and panic, which could be managed by the use of plants with tranquilizing properties. They are also used in the treatment of accompanying conditions such as inflammation, pyrexia, local swelling, and for stoppage of bleeding by the application of plants with haemostatic effect. Similarly, plant that can enhance or stimulate body immune system may give some beneficial effect in the treatment of snake envenomation, which often compromises immune system of the patient.

In African setting, however, the treatment of disease conditions including snake envenomation is often associated with magic or spiritual connotations as it is the general belief that the disease condition is caused by some unseen forces (Tella, 1979; Tella, 1986).

2.12 ETHNOMEDICINAL USES OF *PAULLINIA PINNATA* AND *DETARIUM MICROCARPUM* EXTRACTS IN CARPET VIPER (*ECHIS CARINATUS*) ENVENOMATION

The Okpameri people of Edo state of Nigeria use the leaf juice of *Paullinia pinnata* for the treatment of black tongue and sore throat (Gill, 1992). Dry powdered root bark of *Paullinia pinnata* is soaked in cold or warm water and the solution is given orally, and powder applied in form of poultice to wound. Ritual ceremonies such as incantations or religious ceremonies are not required during collection of the plant, preparation and administration of the medicament. Other plants or ingredients are not required for the preparation of recipes. Water is the only solvent required

for the preparation of the recipe /medicine. The plant is commonly found in the riverside of the Naraguta hills of Jos. The information on the ethnomedicinal uses of the plant was obtained from Gill, 1992. The taxonomists and the textbook did not state that the plant parts should be collected at any specific time of the day or season.

The taxonomist also informed the researcher that *Detarium microcarpum* leaf powder is used by Anaguta people of Jos for the treatment of *Echis carinatus ocellatus* envenomation. According to the traditional healers, the dry powder is soaked in cold or warm water and the infusion administered to patient orally. The dry powder is also applied in form of poultice to the wound or an incision. The taxonomist confirmed that, no ritual incantations were required in the process of collection and administration of the plant materials. It was also stated that no other ingredients are required for the preparation of the medicine.

2.13 ETHNOBOTANICAL DESCRIPTION OF THE PLANTS

The following medicinal plants, commonly used by traditional healers, were selected for the study and their ethnobotanical descriptions are as summarized.

2.13.1 ***Paullinia pinnata*** Linn. Family: Sapindaceae

The plant is known with various local names: Hausa- *hannu biyar* or *yatsa biyar*. Yoruba- *kakashe nla*, ogb- *okuje* (*ogbe*= wound).

It is a tropical African plant, regarded as terrible poison. Its chief medicinal use in West Africa is as an agent to stop bleeding (Dalziel,

1955). Leaves and roots ground and mixed with local spices for dysentery; the powder is also mixed with Guinea grains and used to arrest threatened abortion.

In Ghana, roots are crushed with oil and pepper, for local application to bleeding wound. Roots, leaves and seeds together pounded with ginger or Guinea grains are used to heal open sores. Infusion of leaves is used for fever. Parts used are leaves, seed and roots. Roots are used as aphrodisiac, diuretic, and for the treatment of leprosy, jaundice, oral wounds and snakebite (Gill, 1992).

Although much work has been carried out using other parts of the plant (Dayom *et al*., 2004), there is no record of any scientific study on the use of the root as an anti-snake remedy.

The phytochemical compounds present in the plant are alkaloids, saponins, tannins (Gill, 1992).

2.13.2 ***Detarium microcarpum*** Guill and Perr. Family: Caesalpiniaceae

The common local names of the tree are: Hausa- *taura*; Nupe- *gungorochi*; Fulfulde- *konkehi*; Yoruba- *ogbogbo*; and Ibo- *ofo*.

The bark and leaves are used in dressing wounds, ulcers and fresh cuts (Dalziel, 1955), and for their diuretic and astringent properties (Kouyate and va Demme, 2006). The tree is commonly found in open savannah woodland, growing as high as 15-20 feet. The bark is bluish and exudes a slightly fragrant gum or gum resin.

The fruit is rounded or oval, flattened, about 1½ inches in diameter, containing one seed, with edible sweet greenish mesocarp which is penetrated by a fibrous network attached to the hard bony shell of the seed (Dalziel, 1955). The kernel of the seed is deep purple brown, and is more or less oily and edible.

The plant parts are employed by the people for traditional treatment and domestic uses. The seeds are burnt to drive away mosquitoes. In Nigeria, the young seed is given to persons wounded by poisoned arrows, and is said to induce vomiting. The fruit is also used in some parts of West Africa as a remedy for 'chest disease'. The cold infusion of bark, roots and wood is given as a restorative for weakness and anaemia. In some parts of West Africa, the young shoots are boiled and given with food as a fever medicine. The bark is also used in parturition in cases of retained placenta.

The infusion of leaves is given to snakebite victims for the treatment of *Echis ocellatus* envenomation (Rahuta Michael, personal communication). According to the information, the powdered leaf is also applied to the wound of the bite.

Similarly, some work has been carried out on the plant. Kouyate and van Damme (2006) reported some antibacterial action of *Detarium microcarpum* bark extract against some bacteria including *Pseudomonas aeruginosa* and *Staphylococcus aureus*. However, there is no known scientific study on the use of the leaves as an anti-snake remedy.

2.14 RESEARCH LIMITATIONS

In the process of carrying out this work, a number of problems were encountered. For instance there was little information on various research works carried by other researchers on the plants. One of the factors that militated against this work was the secrecy surrounding information on the use of the traditional medicaments of the plants. For example, because most of the time traditional medicines are used as complex mixtures, other ingredients were not disclosed. The vagueness of therapeutic claims made by the traditional healers was also misleading.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Animals

Mice, rats, guinea pigs, rabbits, and cats were purchased from the University of Jos Animal house, National Veterinary Research Institute Vom, and Ahmadu Bello University (ABU) Animal House, and *Echis carinatus ocellatus* (Carpet viper) was caught from the Langtang plains of Plateau state by Mr. Victor D. Simon and identified in the Zoology Department of University of Jos, and clinical isolates of *Proteus mirabilis, Pseudomonas aeruginosa, and Staphylococcus aureus* were obtained from the Microbiology Laboratory of University of Jos.

3.1.2 Drugs

The following drugs were used in the experiments: acetylcholine, histamine, adrenaline, noradrenaline, and *d*-tubocurarine were obtained from Pharmacology laboratories of University of Jos and ABU Zaria. *Echis carinatus* venom (Sigma) was purchased from Zayo Nigeria Ltd Jos; propranolol, prazosin, gentamycin, indomethacin, thiopental sodium (BP), promethazine, cyproheptadine, and castor oil were purchased at Miconny Pharmacy Ltd, Jos and Lamed Pharmacy Ltd, Jos.

3.1.3 Chemicals and reagents

Formalin was obtained from Human Anatomy department of University of Jos. Department Pharmaceutical Chemistry University of Jos supplied methylated spirit, and distilled water. Nutrient agar (Biotec) was supplied by the Pharmaceutical Microbiology Department University of Jos. Crystal violet, gum acacia, acetic acid, ammonia solution, hydrochloric acid, chloroform, sulphuric acid were obtained from Department of Pharmaceutical Technology University of Jos. Albumin was obtained from Pharmacology Laboratory ABU Zaria. Water for injection (Sam Pharmaceutical ltd), and normal saline (Dana) were purchased from Lamed Pharmacy Ltd Jos. Benedict's solution, picric acid solution, carbon tetrachloride, potassium hydroxide solution, Fehling's solution, methanol, sodium hydroxide, dinitrobenzoic acid, glacial acetic acid, lead sub acetate, Wagner's reagent, Mayer's reagent, Dragendorff's reagent, and ferric chloride were of analytical grade and used as received from Pharmacognosy Laboratory, University of Jos.

3.2. METHODOLOGY

3.2.1 The Medicinal Plant Selection

Plants for phyto-therapeutic investigation have been described by some authors (Sofowora, 1984; and Souza Brito, 1996). The decision criteria for the selection of antisnake venom candidates for evaluation were based on evidence of safety and efficacy from traditional use. This

is because the more widespread the use of a given preparation, the likely it is to be effective and the less likely it is to be toxic. The determination of criteria for the selection of medicinal plants is as important as the investigation into the plants. Since the traditional healers claimed success in the treatment of *Echis carinatus* envenomation, it is expected that some chemical constituent(s) with anti-venom activities will be found in the plant extracts. Although many suggestions have been made, the selection of *Paullinia pinnata* and *Detarium microcarpum* for this study was based on ethnopharmacological information.

3.2.2 Collection and Identification of the Plant Materials

The plant materials were collected from the Naraguta hills of Jos with the aid of traditional taxonomist and herb seller, Rahuta Michael. The information on the plants as supplied by the taxonomist was recorded as described by Sofowora (1984). Fresh roots of *Paullinia pinnata* Linn (Family: Sapindaceae) and leaves of *Detarium microcarpum* Guill and Perr, (family: Caesalpiniaceae) were collected.

The plants, *Paullinia pinnata* and *Detarium microcarpum*, were collected using their local names. In *Hausa*, *Paullinia pinnata* is known as *hannu biyar or yatsa biyar*. The collection of plant samples was carried out carefully so as to avoid over-harvesting the species. The plant, *Paullinia pinnata,* is commonly distributed around the riverside areas, while *Detarium microcarpum* is found in the savannah areas.

The roots of *Paullinia pinnata* along with the leaves (Plate 2) and fresh leaves of *Detarium microcarpum* (Plate 3) were collected and identified by a horticulturist, A. I. Kareem, of the Federal College of Forestry, Jos and authenticated by S.W.H. Hussaini of the Department of Botany, University of Jos. The voucher specimens (№ A6/RT/03, and A11/L/03, respectively) were deposited in the herbarium of the Faculty of Pharmaceutical Sciences, University of Jos.

3.2.3 Preparation of Materials

The fresh root bark of *Paullinia pinnata* Linn (Family: Sapindaceae) was washed and shade dried at room temperature and pounded to a coarse powder using pestle and mortar. The leaves of *Detarium microcarpum* Guill and Perr (family: Caesalpiniaceae) were also shade dried and pounded to powder. The powdered materials were sieved, labeled and stored at 4°C.

3.2.4 Extraction Process

For each extraction process, 20g of powdered materials were transferred to extraction flask to which was added 25ml of distilled water. This was left for 24 hours and filtered. Filtration was done twice; first with a coarse sieve, then with Whatman filter paper (No. 1). The filtrate was collected and freeze-dried, producing lyophilized powder. The percentage yield was calculated and sample was stored at 4˚C.

For the hot water extraction, the same process used for the cold-water extraction was employed except that the powdered samples were boiled for 10 minutes before filtration and freeze-drying.

3.2.5 Phytochemical Studies

Preliminary analyses were carried out on the aqueous (cold and hot) extracts of *Paullinia pinnata* root bark and *Detarium microcarpum* leaves for their phytochemical constituents using standard methods (Sofowora, 1982; Evans, 2002).

Test for alkaloids

0.5g of each extract was stirred with 5ml of 1% aqueous hydrochloric acid on steam bath and filtered; 1 ml of each of the filtrates was treated with a few drops of Mayer's reagent, Dragendorf's reagent, and picric acid solution. Precipitation with any of the reagents was taken as preliminary evidence for the presence of alkaloid in the extracts.

Test for saponins

About 0.5g of each extract was shaken with water in a test tube. Frothing, which persists on warming was taken as preliminary evidence for the presence of saponins.

Test for tannins

About 0.5g of each plant extract was stirred with 1ml of distilled water, filtered, and ferric chloride reagent added to the filtrate. A blue-black, green, or blue green precipitate was taken as evidence for the presence of tannins.

Test for anthraquinones

i. Borntrager's test was used for the detection of anthraquinones in the extracts.

0.5g of each extract was shaken with 5ml carbon tetrachloride; filtered and equal volumes of 10% dilute ammonia solution added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammonical (lower layer) phase indicates the presence of free anthraquinones.

ii. Combined Anthraquinones.

0.5g of each plant extract was boiled with 5ml aqueous sulphuric acid and filtered while hot. The filtrate was shaken with 5ml benzene, the benzene layer separated and ½ it's own volume of 10% ammonia solution was added. A pink, red, or violet colouration in the ammonia phase (lower layer) indicates the presence of anthraquinone derivatives in the extract.

Test for glycosides

i. Legal test for reducing sugar

To about 100mg of each extract was added 2.5ml of dilute sulphuric acid and boiled in a water bath for 15 minutes. It was cooled and neutralized with 20% potassium hydroxide solution. 5ml of mixture of Fehling's solution A and B was added and boiled for 5 minutes. The presence of a brick red precipitate indicates the hydrolysis of a reducing sugar, an indication of glycoside.

Plate 2: The root and leaves of *Paullinia pinnata* Linn.
Linn.
(Family: Sapindaceae).

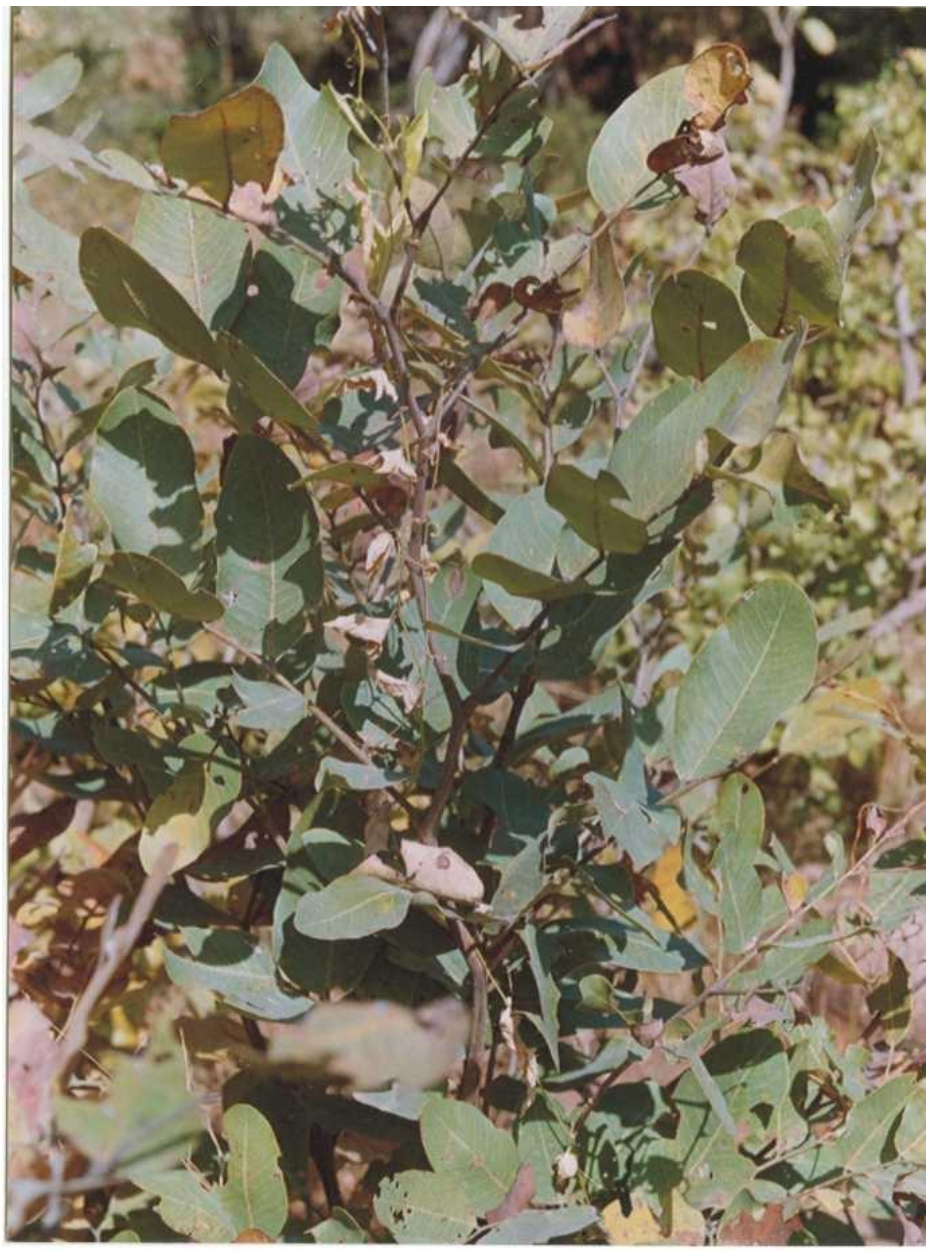


Plate 3: Detarium microcarpum Guill and Perr.
(Family: Caesalpiniaceae).

ii. Salkowski test for steroidal ring

0.5g of each extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer (chloroform layer). A reddish-brown colour at the interface indicates the presence of a steroidal ring (i.e. aglycone portion of the cardiac glycoside).

Test for carbohydrates

100mg of each extract was dissolved in 3ml of distilled water and mixed with a few drops of Molisch reagent (10% solution of α-naphthol in alcohol). 1ml of concentrated sulphuric acid was then carefully added down the side of the inclined tube so that the acid forms a layer beneath the aqueous solution without mixing it. A reddish or violet ring at the junction of the liquids was observed, which indicates the presence of carbohydrates.

Test for flavonoids

Lead-sub acetate test was used. 100mg of each extract was dissolved in 5ml water and filtered. To the filtrate, 2-3 drops of lead-sub acetate solution were added. A yellow coloured precipitate indicates the presence of flavonoids. The results of the phytochemical screening are as presented in Table 3.

3.2.6 Test for Trace Metals

The extracts were analyzed for trace metals using Atomic Absorption Spectrophotometer (Model: Zeeman 180/60/80 Hitachi Japan). Solutions of the extracts were introduced in a flame in which the metallic elements were reduced to atomic vapour by thermal energy. The vapour absorbed thermal energy from the flame and became excited. The atomic vapour in excited state fell back to the ground state within a short period of time, and emitted energy at the wavelength specific to the metallic elements. The intensity of the energy emitted was taken as proportional to the number of atoms in excited state or the concentration of the metallic elements.

3.2.7 Antibacterial Activity

The test bacteria used to screen for the antibacterial activity of the extracts were *Proteus mirabilis*, *Ps. aeruginosa* and *Staphylococcus aureus*. The organisms were chosen to represent wound causing pathogens.

Preliminary sensitivity testing to determine the comparative potency of each extract against the test microorganisms was carried out using the following methods:

Agar Diffusion method: Plate method

The extracts were reconstituted with sterile distilled water to 10 and 15% w/v. The antibacterial activity was evaluated using the Kirby-

Bauer method (Aguiyi *et al*., 1996). The experiment was carried out by preparing nutrient agar plates and seeded with viable cells of the test micro-organisms, *Proteus mirabilis, Ps. aeruginosa* and *Staphylococcus aureus*. Gentamycin was used as a standard drug. On each seeded plate was bored four cups with sterile № 4-cork borer, then each of the three cups was filled with 0.1ml of each extract of graded concentrations (125, 250, 500mg/ml) and the fourth with gentamycin (600µg/ml) solution. The plates were left for two hours at room temperature (37^oC) for diffusion, after which they were incubated at 37^oC for 24 hours. The zones of inhibition were recorded to the nearest whole number (mm). A zone of 10mm excluding diameter of the cup was taken as indication of active inhibition.

Agar dilution method: Tube method

(i) Determination of Minimum Inhibitory Concentration (MIC)

The MICs of the extracts as well as that of the standard drug (gentamycin) were determined by the Tube Dilution method. The extracts and drug were serially diluted two-fold in a row of tubes. Drop of 24-hour broth cultures of the test organisms diluted to yield 10^6 Colony-Forming Units (CFU) per ml was then added to each test tube. The tubes were incubated at 37^oC for 24 hours after which the tubes were examined for growth as determined by turbidity in comparison with the controls. The lowest concentration of extract/drug showing no visible growth was recorded as MIC (bacteriostatic concentration).

(ii) Minimum Bactericidal Concentration (MBC)

Tubes that exhibited no visible growth during the MIC experiments were selected for the determination of the Minimum Bactericidal Concentration (MBC). Drop from each of the tubes was sub-cultured over the surface of extract or drug-free glucose mineral salts agar (pH 7.4) and incubated for further 24 hours at 37^{o}C. The lowest concentration at which no growth was observed was recorded as the MBC (Tables 7 and 8). There were two controls for each experiment, a nutrient broth control and the other (test organisms incubated) without extract or standard drug.

3.2.8 Acute Toxicity Study of Aqueous Extracts of *Paullinia pinnata* Root Bark and *Detarium microcarpum* Leaf in Mice

The toxicity of extracts of the root of *Paullinia pinnata* and *Detarium microcarpum* leaves was investigated in mice for the determination of the median lethal dose (LD_{50}) using the method Abubakar *et al*., (2000). Four-week-old mice (15 – 18g body wt) from the University of Jos Animal House were used. The mice were housed, with equal day/night cycle at room temperature of 28^{o}C and 60% relative humidity. All animals had free access to water and fed with standard feeds (Grand Cereal™).

The lyophilized extracts were dissolved in distilled water and given either by oral or intraperitoneal route. The animals were divided into ten (10) groups (6 mice per group) and given the extract by gastric gavage at doses of 100, 200, 400, 600, 800, 1000, 1200, 1400, 1600 and 2000

mg/kg, respectively. For the intraperitoneal route, the mice were allotted into 8 groups (6 mice per group) and administered the extract at doses of 100, 200, 400, 600, 800, 1000, 1200, 1400 mg/kg as single dose, respectively. The mice were observed for 24 hours following administration of the extracts and all signs of toxicity or deaths in each group were recorded. The LD_{50} of the extract was estimated from the graph of percentage (%) death converted to Probit against Log-dose of the extract (Akah and Odita, 2001).

3.2.9 Lethality (LD_{50} and MLD) Assay of *Echis carinatus* Venom

The study was carried out to determine the median lethal dose (LD_{50}) and minimum lethal dose (MLD) of the venom.

The venom was reconstituted with normal saline and concentration of 1mg/ml was obtained. The methods of Theakston and Reid (1983) and Abubakar *et al* (2000) were adopted for the experiment. The median Lethal dose (LD_{50}) of *Echis carinatus* venom was assayed by injecting different concentrations of venom in 0.2 ml normal saline into the peritoneum of albino mice (Theakston and Reid, 1983).

Adult female mice (15-18 g) were allotted into six groups (6 mice per group) and injected intraperitoneally (i.p) with the following doses 0.5, 1, 1.5, 2.0, and 2.5 mg/kg of the reconstituted venom. The control group (6 mice) received normal saline (i.p.). The mice were returned to the cages and were allowed food and water *ad libitum*. The number of death was recorded over a period of 24 hours after the administration of

the venom. The median lethal dose (LD_{50}) and minimum lethal dose (MLD) of the venom were estimated using the probit analyses (Akah and Odita, 2001).

3.2.10 Evaluation of Activity of Aqueous Extracts of *Paullinia pinanata* and *Detarium microcarpum* on *Echis carinatus* Venom in Mice

In order to evaluate the effect of the extracts on the venom, adult mice were used for the experiment. The experiment was carried out using the different methods of Mahanta and Mukherjee (2001), Aguiyi *et al* (1998), Theakston and Reid (1983), and Abubakar *et al* (2000).

The lyophilized root bark powder of *Paullinia pinnata* and leaf powder of *Detarium microcarpum* were reconstituted with normal saline and referred to as the extract. Eight mice were used per group for each dose (Mahanta and Mukherjee, 2001). The mice were returned to the cages and were allowed food and water *ad libitum*. The experiment was carried out using three different experimental models.

Venom pretreated mice group

The experiments in this group of animals were carried out using intraperitoneal and oral routes to administer the extracts to the mice.

i. Intraperitoneal administration

The animals were divided into five groups (8 mice per group) and pretreated with the minimum lethal dose (2mg/kg) of the venom (i.p) and challenged after 5 minutes of venom administration with the extract (i.p) at doses of 50-400mg/kg. The treatment groups (2, 3, 4, and 5) received

the extract (i.p.) and the control group (1) received the minimum lethal dose (2 mg/kg) of the venom.

ii. Oral administration

For this route, the above experiment was repeated except that the extracts were administered to the animals using intragastric method.

Mice treated with incubated mixture of venom/extracts

The mice were also allotted into five groups (8 per group) and were administered (i.p.) the pre-incubated mixture of the venom (2 mg/kg) and the extract at the doses of 50, 100, 200, and 400 mg/kg body weight. The control group (1) received the venom, while groups 2, 3, 4, and 5 received pre-incubated mixture of the venom and extract. The venom was first pre-incubated with various doses of the extract (50-400 m/kg) at 37^{o}c for 60 minutes to obtain venom/extract.

Extract pretreated animal group

The animals were pretreated with different doses of extracts (50-400mg/kg) using intraperitoneal and oral routes respectively, and were challenged with the venom (2mg/kg), 120 minutes after the administration of the extracts.

The animals in the experiments, involving the two extracts, were observed for toxicity signs and mortality up to 24 hours. The effective doses (ED_{50}) of the extracts were estimated using probit analyses.

3.2.11 Evaluation of Analgesic Activity of Aqueous Extracts of *Paullinia pinnata* Root Bark and *Detarium microcarpum* Leaf: Writhing Test in Mice

The methods of Bisignano *et al* (1994) and Okpo *et al* (2001) were used. Albino mice of either sex (18-22g) were kept in an animal house (25± 2^{o}c) with a 12 hour light/dark cycle. The mice were starved for 18 hours but given water *ad libitum*. The aqueous extract of *Paullinia pinnata* root bark and *Detarium microcarpum* leaves were administered in different doses (100-400 mg/kg, orally) to five groups of mice (4 per group). Animals in group 1 received normal saline (p.o) and served as negative control. The animals were given 0.3% acetic acid (i.p), one hour after the administration of the extract (100 - 400mg/kg, p.o) or normal saline. The abdominal muscle constrictions together with stretching of hind limbs, resulting from intraperitoneal injection of acetic acid (0.3%), were observed. The number of writhing movements within 15 minutes after the administration of acetic acid was recorded. For the purpose of comparison, indomethacin (2.5 mg/kg, s c) as a reference drug, was administered to animals in group 5 and served as positive control.

3.2.12 Evaluation of Antipyretic Activity of the Extracts of *Paullinia pinnata* and *Detarium microcarpum* in Mice

The method of Bisignano *et al* (1994) was used to evaluate the antipyretic activity of the extracts. A total of sixteen (16) mice were used in four groups (4 mice per group). The mice were fasted and after 18

hours their rectal temperatures were recorded using digital thermometer (CE0197) with rectal probe. Pyrexia was induced by SC injection of 20% aqueous suspension of Brewer's yeast (20ml/kg). The extracts of *Paullinia pinnata* and *Detarium microcarpum* were administered to the animals in doses of 100 and 400mg/kg, and 100 and 600mg/kg p.o, respectively. The temperatures of the animals were recorded before and after 1, 2 and 3 hours after administration of the extracts. The first group was administered (p.o.) same volume of gum acacia (GA) suspension that served as negative control. Animals in groups 2 and 3 received the extracts of *Paullinia pinnata* (100 and 400 mg/kg, orally) and *Detarium microcarpum* (100 and 600 mg/kg, p.o) respectively. The fourth group was administered indomethacin (2.5 mg/kg p.o) and served as positive control.

3.2.13 Evaluation of Anti-Inflammatory Activity of the Extracts in Rats

The anti-inflammatory effect was evaluated by measuring the albumin-induced oedema in rat hind paw according to the method of Okpo et al (2001), and Bisignano (1994). Paw oedema was induced in albino rats (180-200 g) by injection of albumin.

The animals were divided into four groups (6 rats / group) for each extract. The first group served as the negative control and received normal saline (0.1 ml/100 g rat) p. o., the second and third groups received aqueous extract of *Paullinia pinnata* (100 mg/kg, 400 mg/kg)

p.o, and aqueous extract of *Detarium microcarpum* (100 and 600 mg/kg), p.o., respectively; the fourth group served as positive control and received subcutaneous indomethacin (2.5 mg/kg). These were all administered **before** the injection of albumin.

The albumin (0.1 ml of a 1% w/v suspension in normal saline) was injected subcutaneous 60 minutes later into the sub-plantar region of the right hind paw of the animals. The linear paw circumference was measured before and 1, 2, 3, 4, and 5 hours **after** administration of albumin using the cotton thread method (Bamgbose and Noamesi, 1981). The value of inflammation (i.e. increase in foot circumference) was considered against that of negative control limb (Warrell *et al*., 1977).

3.2.14 Determination of the Activity of Extracts of *Paullinia pinnata* and *Detarium microcarpum* on Influence of *Echis carinatus* Venom on Blood Coagulation System (Clotting Time, and Bleeding Time) in Rats

This work evaluated the effect of crude extracts of the two plants on effect of venom on whole blood system, *in vitro* and *in vivo*. The *in vivo* experiment was used to determine the bleeding time, while the *in vitro* exercise was for the determination of the clotting time.

Bleeding Time Tests

For the *in vivo* determination of bleeding time, procedures similar to the methods of Igboechi and Anuforo (1986), Essien *et al* (1985), and Mohamed *et al* (1969) were used. Four groups of rats (140-200 g), of four rats per group, were used. The rats of the first group were

intraperitoneally injected with normal saline (2 ml/kg) and served as control. Animals in the second and third groups received pre-incubated mixtures of extracts of *Paullinia pinnata* and venom and *Detarium microcarpum* and venom, respectively. In the fourth group, the animals were injected with a sub lethal dose of the venom (1mg/kg body weight). Four hours after the treatment of the animals, the tail of each rat was gently shaved and pierced with lancet. A piece of white filter paper was used to blot the blood gently from the punctured surface of the body. The time of puncture and the first appearance of the blood were noted. The readings were taken every 15 seconds. The end result occurs when the paper was no longer stained by blood or a blood clot. The bleeding time was recorded for control and treatment groups by accepting the control bleeding time as 100%. The results were expressed as the mean of observations from four animals.

Clotting Time Test

The experiment was used to determine the effect of the venom on blood clotting time and the influence of the extracts on this reaction. For the *in vitro* experiment, a modification of the method of Igboechi and Anuforo (1986) was used. Clotting time is the time required for a firm clot to be formed in fresh blood placed on glass slides. In this experiment, rats were allotted into four groups (4 rats per group). Animals in group 1 were injected with the normal saline, group 2 received venom, and groups 3 and 4 were given incubated venom-extract mixtures, respectively.

The blood sample was collected from the rats and a drop was placed on a clean plain slide and every 15 seconds, a tip of office pin was passed through the blood until a thread like structure was observed between the drop of the blood and tip of the pin. The thread-like structure was an indication of a fibrin clot. The time was recorded for each animal group.

3.2.15 Evaluation of Activity of Aqueous Extracts of *Paullinia pinnata* and *Detarium microcarpum* on the Influence of *Echis carinatus* Venom on Blood cells in Rats

The ability of the extracts of *Paullinia pinnata* and *Detarium microcarpum* to restore normal haematological characteristics in rats injected with the *Echis carinatus* venom (1 mg/kg) was evaluated. The animals (180-220 g) were divided into four groups (4 rats per group). Animals in the first, 2^{nd}, 3^{rd}, and 4^{th} group were injected (i.p) normal saline (2 ml/kg), water, *Paullinia pinnata* and *Detarium microcarpum* extracts respectively; and 30 minutes later animals in groups 2, 3 and 4 were injected the venom (1 mg/kg) i.p. Blood samples were collected from the animals by cardiac puncture four hours later, into heparinized containers.

For the purpose of examining pathophysiological state of the animal, blood morphology was analysed as described by some workers (Warrell *et al*, 1977; Hardy, 1967). The following parameters were measured.

Haemoglobin concentration

The haemoglobin content was determined by colorimetric method. In the experiment, 0.02 ml of blood was diluted in 4ml of 0.04 % ammonia solution and was allowed to stand for 5 minutes for the formation of oxyhaemoglobin. The optical density of the solution was read in a colorimeter at a wavelength of 520 nm. The results in King Armstrong (Å) were converted to the SI units of g/100ml haemoglobin concentration.

White Blood Cell (WBC) counts

For the total white blood cell count, 0.01 ml of blood was diluted in to 0.19ml of Tuerks solution (20 ml glacial acetic acid) to give a diluting factor of 1:20. The solution was filled into a Neubauer chamber and placed on a microscope stage and the cells were allowed to settle for 3 minutes. The cells were counted in the four outer 1mm squares and recorded.

Red Blood Cell (RBC) counts

The total red blood cell (RBC) count was determined using the same technique of WBC count, where 0.005ml of blood was diluted in 0.995ml of Hayem's solution to give the diluting factor of 1:200. However, the cells were counted under the microscope within 80 of the 400 small squares of the central 1mm squares (5groups of 16 small squares evenly spaced). The counts were recorded.

Platelet counts

For the platelet count, 0.01 ml of blood was diluted into 0.19 ml of Boa's fluid to give a diluting factor of 1:20. The solution was allowed to sediment for a few minutes.

3.2.16 Evaluation of Activity of the Aqueous Extracts of *Paullinia pinnata* and *Detarium microcarpum* on Capillary Permeability after *Echis Carinatus* venom in Rabbits

The method of Osman and Gumaa (1974) was used to evaluate the effect of extracts of *Paullinia pinnata* and *Detarium microcarpum* on capillary permeability after the administration of *Echis carinatus* venom. The animals (1.5-2.5 kg) were divided into four groups (2 rabbits per group). The abdominal skin of the rabbits was shaved and 24 hours later, the animals were injected intradermaly on both sides of the midline of the shaved area with incubated mixtures of the extract (400 and 600 mg/kg) and venom (0.1 mg/kg), venom (0.1 mg/kg) and promethazine (5 mg/kg), and venom only, and normal saline, respectively. The pretreated animals were injected intravenously with crystal violet solution 45 minutes later. The leakage of the dye was estimated, to determine the extent of increase in capillary permeability, 30 minutes later using physical examination of the skin.

3.2.17 Diuretic Action of Aqueous Extracts of *Paullinia pinnata* and *Detarium microcarpum* in Rats

The procedures of Navarro *et al* (1994) and Aguiyi and Obi (1998) were adapted for the study of the effect of the extracts of *Paullinia*

pinnata and *Detarium microcarpum* on urine excretion. Albino rats (200-220 g) were randomly assigned to four groups of animals (4 rats per group).

The animals were starved for 12 hours prior to the experiment but were adequately hydrated by giving normal saline (50 ml/kg body weight), orally. This was followed by the administration of the extracts of Paullinia *pinnata* and *Detarium microcarpum* to animals in groups 2 and 3 in the doses of 400 and 600 mg/kg, i.p., respectively. The control group (1) received only normal saline i.p and the same volume of vehicle (H_20), which was used to dissolve the extract. Animals in group 4 received furosemide as positive control. The rats were then placed individually in metabolism cages and their urine was collected over 12 hours, using a graduated measuring cylinder and tested for Na and K ion content. The urinary excretion was calculated in relation to the body weight of the experimental animals (ml/kg). The concentration of the electrolytes (Na^+ and K^+) were estimated from the urine samples of each of the four groups 12 hours after administration of the extracts. The pH meter was used to measure the pH of the urine samples.

3.2.18 Determination of Activity of *Paullinia pinnata* and *Detarium microcarpum* on Blood Glucose after *Echis carinatus* Venom in Rabbits

The effect of the extracts of *Paullinia pinnata* and *Detarium microcarpum* on blood glucose was determined in rabbits with average

weight of 1.0 ± 0.12 kg. The methods of Osman and Gumaa (1974) and Mohamed et al (1963) were adopted and used.

The rabbits were divided into four groups (2 per group) and fasted overnight. The animals in groups 1, 2, 3, and 4 were then injected subcutaneously with normal saline, venom (1.0 mg/kg), venom/extract mixtures of *Paullinia pinnata a*nd *Detarium microcarpum* The blood samples were drawn from a marginal ear vein before and two hours after treatment. The samples were analysed.

3.2.19 Evaluation of Activity of Extracts of *Paullinia pinnata a*nd *Detarium microcarpum* on the Local Effects of *Echis carinatus* venom in Rabbits

An *in vivo* study of the influence of the extracts on local effects produced by *Echis carinatus* venom poisoning was carried out using rabbits (1.5-2kg). The method of Osman and Gumaa (1974) was adopted and used. The hind legs of the rabbits were shaved gently 24 hours before the experiment. A sub lethal but deleterious dose (1mg/kg) of the venom in 1ml normal saline was then injected into the right leg while the left leg received a similar dose of the venom with effective doses of the extracts (400mg/kg, 600mg/kg *Paullinia pinnata* and *Detarium microcarpum*, respectively). In order to verify the effect of the extracts on histamine reactions in rabbits, the experiment was repeated by injecting the venom/standard antihistamine (promethazine) preparation into one leg and venom/saline into the other leg. The sites of injection were constantly observed for local reaction up to 24hours.

3.2.20 Evaluation of Effect of Aqueous Extracts of *Paullinia pinnata* and *Detarium microcarpum* on Blood Pressure of Anaesthetised Cats

The blood pressure activities of the aqueous extracts of *Paullinia pinnata* and *Detarium microcarpum* were examined in an experiment using anesthetized cats.

The methods of Achola and Munenge (1999) and Ismail *et al* (1972) were adopted. Cats of either sex weighing between 1 and 2kg were initially anaesthetized intraperitoneally with sodium thiopentone (25 mg/kg). The femoral vein was cannulated through which the extracts and drugs were administered and washed into the circulation with heparinized (1000 IU/kg) normal saline (0.9%); and artificial respiration was maintained with a pump. One arm of the cannulated carotid artery was connected to a pressure transducer and a pressure gauge for recording changes in blood pressure. The following readings were taken during the experiments: 1) systolic blood pressure (SBP), before and after administration of extracts, and 2) diastolic blood pressure (DBP), before and after administration of extracts. The Mean Arterial Pressure (MAP) was estimated from the systolic blood pressure (SBP) and diastolic blood pressure (DBP) as described by Bowman and Rand (1982).

Further studies were carried out using standard drugs to elucidate the possible mechanism by which the extracts elicited the observed hypotensive effects. The standard drugs employed were cyproheptadine, adrenaline, promethazine, and indomethacin. Injections were made after

taking record of the control. The MAP was calculated thus: MAP=DBP+ (SBP-DBP)/3.

3.2.21 Evaluation of Effect of *Paullinia pinnata* and *Detarium microcarpum* on Isolated Rat Phrenic Nerve Hemi-Diaphragm

The adult albino rats (180-200g) were sacrificed and the chest wall was opened to remove the phrenic nerve-diaphragm. The tissue preparation was attached to a Perspex holder carrying a pair of electrodes. It was then placed in a double walled 50ml organ bath containing Tyrode's solution (containing double strength glucose) and the bath was aerated with 95% 0_2 and 5% CO_2 at 37^o C.

The rat phrenic nerve-diaphragm muscle preparation was stimulated either indirectly through the phrenic nerve (NS) or directly (MS) at 24mm/minute at a frequency of 0.4 Hz and voltage of 8. The drug/extract exposure time was 30 seconds. The tissue was allowed to equilibrate for 30 minutes. The extracts were added to the organ bath at concentrations ranging from 4×10^{-4}g/ml – 6.4×10^{-3}g/ml at 30 second tissue drug contact time cycle. The preload of 800mg was attached in all cases. The neuromuscular activities were recorded on a micro-dynamometer (model 7050 Ugo Basile, Milan Italy) recorder. The tissue was washed three times with Tyrode's solution and the experiment was repeated four times for each concentration of extract. A competitive blocker of Ach receptor, *d*-tubocurarine solution (1.6×10^{-5}g/ml), was used as a control. The time course of the experiment was also examined

having subjected the muscle preparation to more drug-contact time cycle of 120-200 seconds. An anticholinesterase, neostigmine solution, was used in an attempt to reverse the neuromuscular blocking activity of *d*-tubocurarine in the presence of the extracts.

3.2.22 Evaluation of Effect of Aqueous Extracts of *Paullinia pinnata* and *Detarium microcarpum* on Isolated Rabbit Jejunum

The rabbits (1-2kg) were killed by a sharp blow on the head, their abdomens opened and sections of the jejunum (2-3cm) were removed. The tissue was suspended in a 50 ml organ bath containing Tyrode's solution. The muscle preparation was aerated with air and maintained at 37^{o}C. The preparation was allowed to equilibrate for 30 minutes before it was challenged with the extracts.

3.2.23 Evaluation of Effect of Aqueous Extracts of *Puallinia pinnata* and *Detarium microcarpum* on Guinea Pig Ileum

Guinea pigs (300-400 g) were starved for 24 hours, killed, exsanguinated and the abdomen opened. Pieces of ileum were removed and a suitable length (2-3 cm) was suspended in an organ bath (50 ml) containing Tyrode's solution. The bath was aerated and maintained at 37^{o}C. The tissue was allowed to equilibrate for about 60minutes. The tissues were challenged with the plant extracts. The effects of acetylcholine (Ach) (1x10^{-5}g/ml), and histamine (1x 10^{-5}g/ml) were investigated.

3.3 STATISTICAL ANALYSIS

Student’s t-test was used for the comparison between the mean study groups with $p < 0.01$ or $p < 0.05$ being considered significant.

CHAPTER FOUR

RESULTS

4.1 RESULTS

The summaries of the pharmacological effects of the extracts of *Paullinia pinnata* and *Detarium microcarpum* are presented in this section.

4.1.1 Preparation of Crude Aqueous Extracts

The results in table 1 showed that the extracts of *Paullinia pinnata* plant material produced higher yield than those of *Detarium microcarpum* in both cold and hot water.

Table 1: Crude aqueous extracts of *Paullinia pinnata* and *Detarium microcarpum* showing percentage yield after cold and hot water extraction methods

Type of extraction	Sample	Wt of material used (g)	Wt of extract (g)	% Yield
CW	*Paullinia pinnata* root	200	63.2	32
CW	*Detarium microcarpum* leaf	200	36.4	18
HW	*Paullinia pinnata* root	200	68.1	34
HW	*Detarium microcarpum* leaf	200	38.2	19

Key: CW= Cold water; HW= Hot water

4.1.2 Phytochemical Constituents of *Paullinia pinnata and Detarium microcarpum* extracts

The results of the preliminary phytochemical analyses (Table 2) showed the presence of carbohydrates, saponins, steroids, and tannins in both cold and hot water extracts of *Paullinia pinnata*; and anthraquinones, flavonoids, saponins, steroids and tannins in cold and hot water extracts of *Detarium microcarpum*. The aqueous root bark extract of *Paullina pinnata* contained that carbohydrates and steroids sparingly, while saponins and tannins were found in abundance. However, traces of anthraquinones, saponins, and steroids were found in extract of *Detarium microcarpum*, and flavonoids and tannins in abundance. The persistent frothing of the solution of *Paullinia pinnata,* which was in higher proportion than that observed with the solution of *Detarium microcarpum,* corroborated the presence of saponins in the crude extracts. The constituents of the two medicinal plants were found to be present in equal proportions in both cold and hot water extracts, respectively.

Table 2: Phytochemical analyses of aqueous extracts of *Paullinia pinnata* and *Detarium microcarpum* showing the constituents

Chemical compounds	***Paullinia pinnata***		***Detarium microcarpum***	
	Cold water extract	**Hot water extract**	**Cold water extract**	**Hot water extract**
Alkaloids	-	-	-	-
Anthraquinones	-	-	+	+
Carbohydrates	+	+	-	-
Cardiac glycosides	-	-	-	-
Flavonoids	-	-	++	++
Saponins	++	++	+	+
Steroids	+	+	+	+
Tannins	++	++	++	++

Legend: + = compound present; ++ = greater presence; - = the compound not present

4.1.3 Trace Metal contents of Extracts of *Paullinia pinnata a*nd *Detarium microcarpum*

The results in table 3 show the presence of iron, calcium, and zinc ions in the two extracts. In addition lead was found in the extract of *Paullinia pinnata*. The two extracts were devoid of Magnesium ions.

Table 3: The metal element contents (ppm) of the extracts of *Paullinia pinnata* root bark and *Detarium microcarpum* leaves.

Extract	Fe	Mg	Ca	Zn	Pb
Dm	0.0903	-	0.2834	0.0070	-
Pp	0.0277	-	0.0146	0.0202	0.1331

Legend: - = element not present; Dm = *Detarium microcarpum*; Pp = *Paullinia pinnata*.

4.1.4 Antibacterial Activity of Extracts of *Paullinia pinnata* and *Detarium microcarpum* against wound causing bacteria

The results in tables 4, 5, 6 and 7, and plates 4 and 5 represent summary of the antibacterial activities of the extracts of *Paullinia pinnata* and *Detarium microcarpum*. The comparable potency test results of the extracts showed that the extracts of *Paullinia pinnata* and *Detarium microcarpum* have some antibacterial activity against the test microorganisms. The aqueous extracts of *Paullinia pinnata* exhibited activity against all test organisms. Similarly, extracts of *Detarium microcarpum* showed significant activity against all organisms but in higher concentrations.

However, the MIC and MBC of extracts of *Detarium microcarpum* were higher compared to those of *Paullinia pinnata* extract. The hot water extract of *Paullinia pinnata* exhibited effect against *Staphylococcus aureus* but at higher concentration. Conversely, the cold-water extract of *Detarium microcarpum* did not inhibit growth of *Staphylococcus aureus* even at a fairly high concentration (500mg/ml).

The minimum concentrations of the cold water extract of *Paullinia pinnata* needed to inhibit visually discernable growth of the bacterial suspension of *Staphylococcus aureus* and *Proteus mirabilis* were very significantly ($p < 0.05$) lower, while for *Ps. aeruginosa* they were significantly ($p > 0.05$) higher. The bactericidal effect was most

pronounced for *Prot. mirabilis*, and least for *Staphylococcus aureus* (Tables 6 and 7).

Table 4: Antibacterial spectrum of crude aqueous root extract of *Paullinia pinnata and* gentamycin against test organisms of *Proteus mirabilis, Pseudomonas aeruginosa*, and *Staphylococcus aureus* showing zones of inhibition (mm)

Test organisms	Cold water extract (mg/ml)			Gent. µg/ml	Hot water extract (mg/ml)			Gent. µg/ml
	125	**250**	**500**	**600**	**125**	**250**	**500**	**600**
Proteus mirabilis	12±0.2	13±0.4	16±0.2	46±0.1	12±0.1	13±0.2	15±0.3	47±0.1
Pseudomonas aeruginosa	13±0.1	16±0.1	18±0.4	34±0.3	11±0.4	13±0.2	21±02	36±0.3
Staphylococcus aureus	14±0.1	16±0.2	19±0.3	42±0.3	0	0	14±0.3	45±0.3

SEM ± of four experiments

Table 5: Antibacterial spectrum of crude aqueous leaf extract of *Detarium microcarpum* against *Proteus mirabilis, Pseudomonas aeruginosa* and *Staphylococcus aureus*

Test organisms	Cold water extract (mg/ml)			Gent. µg/ml	Hot water extract (mg/ml)			Gent µg/ml
	125	**250**	**500**	**600**	**125**	**250**	**500**	**600**
Proteus mirabilis	0	12±0.2	14±0.1	44±0.4	0	0	15±0.4	50±0.4
Pseudomonas aeruginosa	0	13±0.3	21±0.2	35±0.3	0	13±0.2	18±0.3	36±0.2
Staphylococcus aureus	0	0	0	45±0.5	0	15±0.2	23±0.2	45±0.5

SEM± of 4 experiments

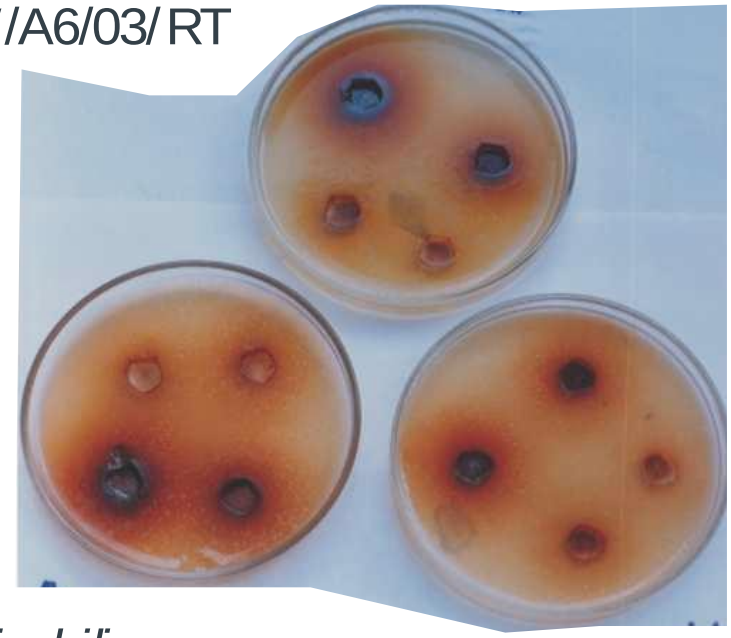
Pseudomonas aeruginosa CW/A6/03/RT

Plate 4a

Proteus mirabilis CW/A6/03/RT

Pseudomonas aeruginosa CW/A6/03/RT

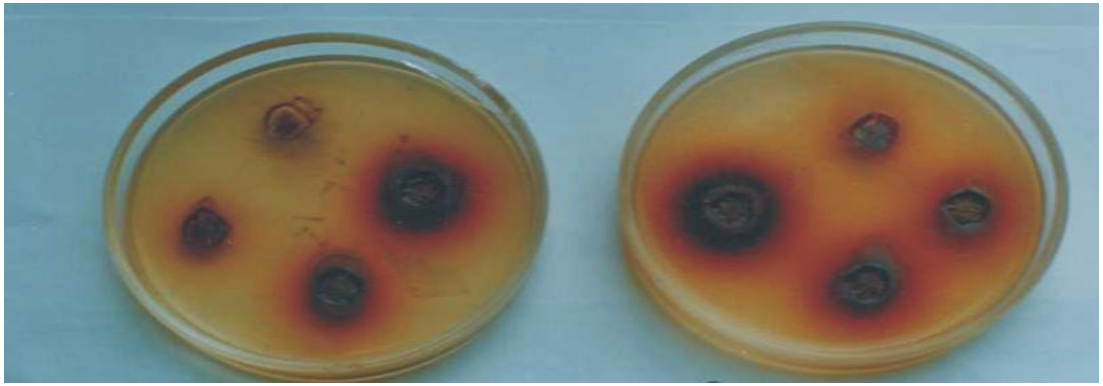
Pseudomonas aeruginosa CW/A6/03/RT



Proteus mirabilis CW/A6/03/RT

Proteus mirabilis CW/A6/03/RT

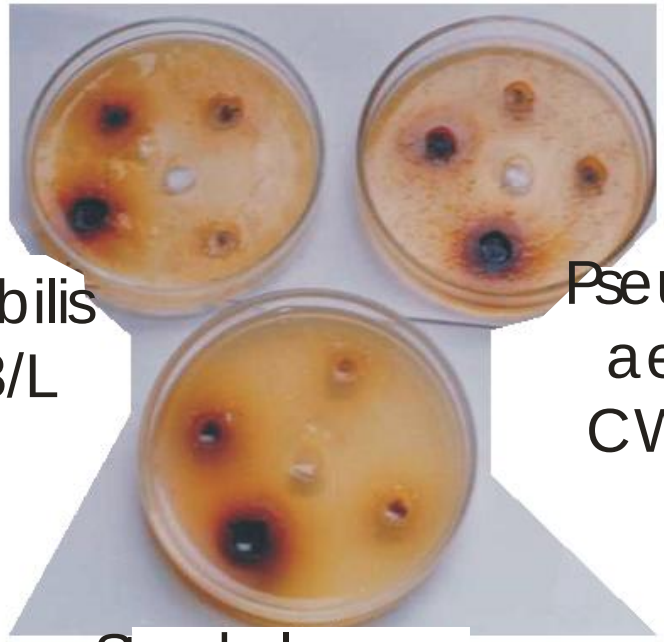
Plate 4b



Staphylococcus aureus CW/A6/03/RT

Plate 4c

Plate 4: Effect of Paullinia pinnata root bark extract and gentamycin on test organisms: Proteus mirabilis, Pseudomonas aeruginosa, and Staphylococcus aureus

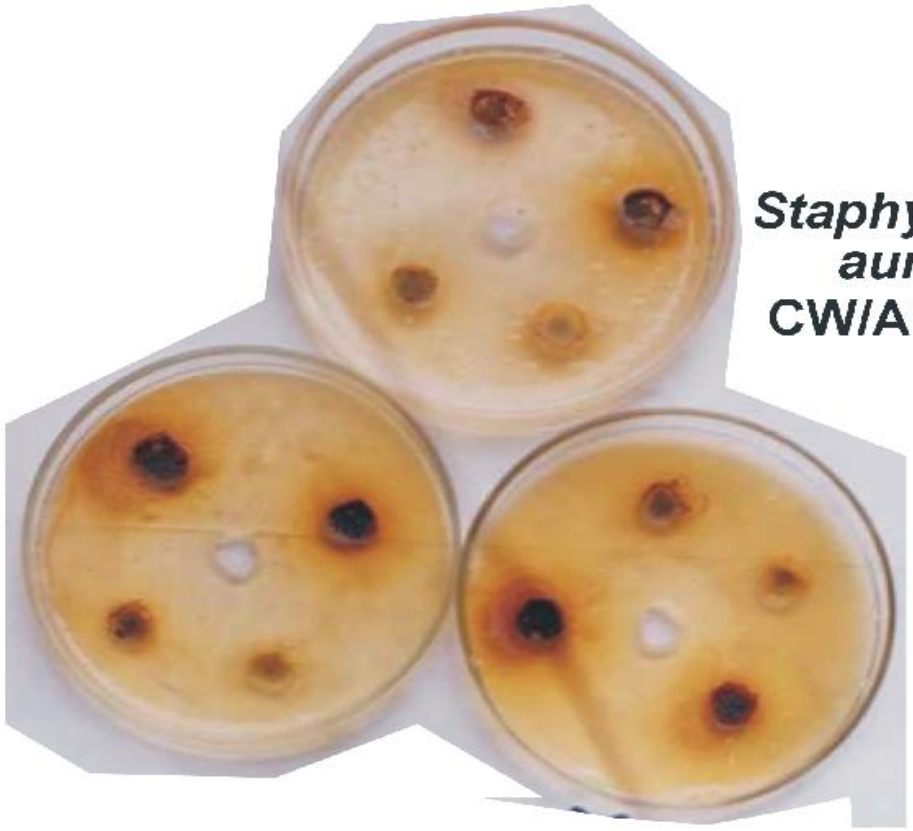


Proteus mirabilis
CW/A11/03/L

Pseudomonas
aeruginosa
CW/A11/03/L

Staphylococcus
aureus
CW/A11/03/L

Plate 5a



Staphylococcus aureus CW/A11/03/L

Proteus mirabilis CW/A11/03/L

Pseudomonas aeruginosa CW/A11/03/L

Plate 5b

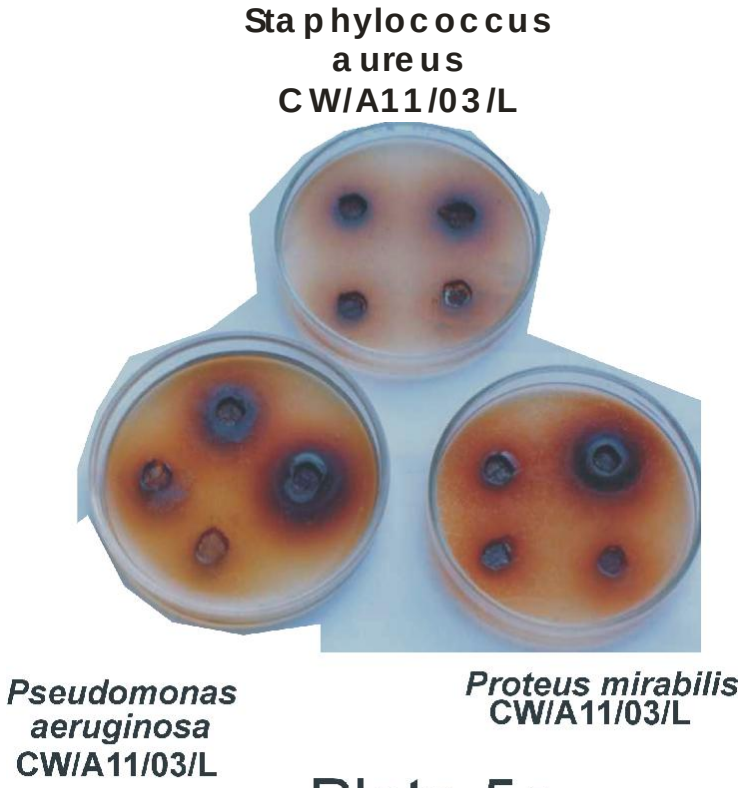


Plate 5c

Plate 5: Effect of ***Detarium microcarpum*** **leaf extract** *and gentamycin on test organisms:* ***Proteus mirabilis, Pseudomonas aeruginosa, and Staphylococcus aureus***

Table 6: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of cold-water extracts of *Paullinia pinnata* and *Detarium microcarpum* against *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*

Organism	***Paullinia pinnata*** (mg/ml)		***Detarium microcarpum*** (mg/ml)		**Gentamicin**(µg/ml)	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Proteus mirabilis	4±0.1	20±0.2	50±0.3	100±0.4	20±0.2	40±0.2
Pseudomonas aeruginosa	20±0.2	100±0.4	50±0.2	200±0.4	35±0.3	55±0.3
Staphylococcus aureus	0.8±0.01	20±0.3	50±0.3	200±0.5	5±0.3	10±0.1

Table 7: MIC and MBC of hot water extracts of *Paullinia pinnata* and *Detarium microcarpum* against *Proteus mirabilis, Pseudomonas aeruginosa* and *Staphylococcus aureus*

Organism	***Paullinia pinnata*** (mg/ml)		***Detarium microcarpum*** (mg/ml)		**Gentamicin**(µg/ml)	
	MC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Proteus mirabilis	25±0.1	50±0.3	50±0.3	100±0.5	20±0.3	40±0.3
Pseudomonas aeruginosa	50±0.2	60±0.2	50±0.6	200±0.4	30±0.2	60±0.5
Staphylococcus aureus	25±0.3	75±0.4	25±0.4	200±0.3	5±0.1	10±0.5

4.1.5 Acute Toxicity Studies of the Extracts of *Paullinia pinnata* and *Detarium microcarpum* in Mice

In acute toxicity studies of the extracts, mice of certain body weight category (15-18g) were deliberately chosen because this group of animals is more sensitive to snake venom than the males (Russell, 1983). The mice injected intraperitoneally with *Paullinia pinnata* root bark aqueous extract and *Detarium microcarpum* leaf extract displayed physical signs and symptoms of toxicity. Toxic effects evoked in the mice were persistent licking of the limbs, restlessness and uncoordinated movements, and coarse hair. Animals treated with *Detarium microcarpum* extract exhibited sign of peritoneal irritation at the sites of injection. Death started to manifest 3 h after intraperitoneal injection of the extracts of *Paullinia pinnata* and *Detarium microcarpum.* This effect was first noticed 6 h after oral administration of extracts of *Paullinia pinnata* and *Detarium microcarpum*. On the whole, except for those transient adverse effects, no other major toxic effects preceding death were observed.

The median lethal dose (LD_{50}) of the extracts was estimated from the tables (Tables 8a-d). The percentage response (death) was converted to *probit*, and *Probit* five (5) was considered 50% response (mortality).

The lethal dose 50% (LD_{50}) of *Paullinia pinnata* root bark extract after intraperitoneal injection in mice was calculated to be 600 ± 2.56mg/kg, while that of *Detarium microcarpum* leaf extract was 800 ± 5.7mg/kg. Similarly, the lethal dose 50% (LD_{50}) of *Paullinia pinnata* root bark extract after oral administration was estimated to be 1200 ± 2.3mg/kg and of *Detarium microcarpum* leaf extract was found to be 1400 ± 12.5mg/kg.

Table 8a: Determination of LD_{50} of aqueous extract of *Paullinia pinnata* root bark after intraperitoneal injection in mice

Group	Dose (mg/kg)	No. death	% death	Probit
1	100	0	0	-
2	200	1/6	16.67	4.0339
3	400	0/6	0	-
4	600	3/6	50	5.0000
5	800	4/6	66.67	5.4316
6	1000	4/6	66.67	5.4316
7.	1200	6.6	100	8.7190
8	1400	6/6	100	8.7190

Table 8b: Determination of the LD_{50} of aqueous leaf extract of *Detarium microcarpum* after intraperitoneal injection in mice

Group	Dose (mg/kg)	No. of death	% death	Probit
1	100	0	0	-
2	200	0/6	0	-
3	400	0/6	0	-
4	600	2/6	33.33	4.5684
5	800	3/6	50	5.0000
6	1000	5/6	83.33	5.9661
7	1200	6/6	100	8.7190
8	1400	6/6	100	8.7190

Table 8c: Effect of aqueous root bark extract of *Paullinia pinnata* to determine the LD_{50} after oral administration

Group	Dose (mg/kg)	No. of death	% death	Probit
1	100	0/6	0	-
2	200	0/6	0	-
3	400	0/6	0	-
4	600	1/6	16.67	4.0339
5	800	1/6	16.67	4.0339
6	1000	2/6	33.33	4.5684
7	1200	3/6	50	5.0000
8	1400	3/6	50	5.0000
9	1600	4/6	66.67	5.4316
10	2000	6/6	100	8.1214

Table 8d: Effect of aqueous extract of *Detarium microcarpum* to determine the LD_{50} after oral administration

Group	Dose (mg/kg)	No. of death	% death	Probit
1	100	0/6	0	-
2	200	0/6	0	-
3	400	2/6	33.33	4.5684
4	600	0/6	0	-
5	800	0/6	0	-
6	1000	1/6	16.67	4.0339
7	1200	2/6	33.33	4.5684
8	1400	3/6	50	5.0000
9	1600	3/6	50	5.0000
10	2000	5/6	83.33	5.9661

4.1.6 Lethality Assay of *Echis carinatus* Venom in Mice

The table (9) presents the results of lethality tests on *Echis carinatus* venom for determination of median lethal dose (LD_{50}) and the minimum lethal dose (MLD) after intraperitoneal injection in mice. The median lethal dose (LD_{50}) of the venom was estimated from the data, and *Probit* five (5) was considered 50% response (mortality). The LD_{50} and MLD were calculated to be 1.5mg/kg and 2mg/kg, respectively.

Table 9: Effect of *Echis carinatus* venom on mice to determine the median lethal dose (LD_{50}) and minimum lethal dose (MLD) of the venom

Group	venom Dose (mg/kg)	No. of deaths/№ mice used	% death	Probit
1	-	0/6	0	-
2	0.5	0/6	0	-
3	1.0	1/6	16.67	4.0339
4	1.5	3/6	50	5.0000
5	2.0	6/6	100	8.1214
7	2.5	6/6	100	8.1214

4.1.7 Inhibitory Effect of the Extracts of *Paullinia pinnata* and *Detarium microcarpum* on *Echis carinatus* venom in Mice

The effectiveness of the extracts of *Paullinia pinnata* and *Detarium microcarpum* against *Echis carinatus* venom was determined after intraperitoneal and oral administration in venom pretreated animals (Tables 10a-j). The median effective dose (ED_{50}) of the extracts was estimated from the data. The percentage cure was converted to *probit,* and *probit* five (5) was considered 50% response (survival).

The effective dose 50% (ED_{50}) of *Paullinia pinnata* extract after intraperitoneal injection was estimated to be 300mg/kg body weight mouse, while the therapeutic index (TI) was calculated to be 2. Similar result (ED_{50}) after oral administration was estimated to be more than 400mg/kg body weight, and the therapeutic index (TI) of the extract was found to be less than 3. *Detarium microcarpum* extract had its effective dose 50% (ED_{50}) as 50mg/kg body weight after intraperitoneal injection, and therapeutic index (IT) was 16. However, the effective dose 50% (ED_{50}) of the same extract after oral administration was estimated to be 200mg/kg body weight with therapeutic index of 7.

In the animal groups pretreated with pre-incubated mixture of venom and the extracts, the effective dose 50% (ED_{50}) of *Paullinia pinnata* extract administered intraperitoneally was estimated to be 100mg/kg body weight, and therapeutic index (TI) was 6. The effective

dose 50% (ED_{50}) of *Detarium microcarpum* extract was found to be less than 50mg/kg body weight, with therapeutic index (TI) more than 16.

The effective dose 50% (ED_{50}) of *Paullinia pinnata* extract after intraperitoneal injection in extract pretreated mice was estimated to be more than 400mg/kg body weight, with therapeutic index (TI) less than 1.5. The effective dose 50% (ED_{50}) of *Detarium microcarpum* extract was estimated to be 100mg/kg body weight and therapeutic index (TI) 8. The effective dose 50% (ED_{50}) of *Paullinia pinnata* extract after oral administration in extract pretreated mice was also estimated to be more than 400mg/kg body weight, with therapeutic index (TI) less than 1.5. However, the effective dose 50% (ED_{50}) of *Detarium microcarpum* extract was estimated to be 400mg/kg body weight and therapeutic index (TI) of 3.5.

The method of Okpako *et al* (2002) was used to calculate the Therapeutic Index (TI) of the extracts.

Table 10a: Effect of *Echis carinatus* venom (2mg/kg) in mice challenged (i.p) with root bark aqueous extract of *Paullinia pinnata*

Group	Dose (mg/kg) extract	Mortality (24h) (no. of death/ no. of mice used)	% Survival (24h)	Probit of % survival
1	0	8/8	0	-
2	50	8/8	0	-
3	100	7/8	13	3.8736
4	200	5/8	38	4.6945
5	400	2/8	75	5.6745

Table 10b: Effect of *Echis carinatus* venom (2mg/kg) in mice challenged (p. o) with root bark aqueous extract of *Paullinia pinnata*

Group	Dose (mg/kg) extract	Mortality (24h) (no. of death / no. of mice used)	% Survival (24h)	Probit of % survival
1	0	8/8	0	-
2	50	8/8	0	-
3	100	8/8	0	-
4	200	6/8	25	4.3255
5	400	5/8	37.5	4.6814

Table 10c: Effect of *Echis carinatus* venom (2mg/kg) in mice challenged (i.p) with leaf extract of *Detarium microcarpum*

Group	Dose (mg/kg) extract	Mortality (24h) (no. of death/ no. of mice used)	% Survival (24h)	Probit of % survival
1	0	8/8	0	-
2	50	4/8	50	5.0000
3	100	4/8	50	5.0000
4	200	2/8	75	5.6745
5	400	1/8	87.5	6.1503

Table 10d: Effect of *Echis carinatus* venom (2mg/kg) in mice challenged (p.o) with leaf extract of *Detarium microcarpum*

Group	Dose (mg/kg) extract	Mortality (24h) (no. of death/ no. of mice used)	% Survival (24h)	Probit of % survival
1	0	8/8	0	-
2	50	8/8	0	-
3	100	6/8	25	4.3255
4	200	4/8	50.0	5.0000
5	400	4/8	50.0	5.0000
6	600	3/8	62.5	5.3186
7	800	3/8	62.5	5.3186

Table 10e: Effect of administration (i.p.) of pre- incubated mixture of *Echis carinatus* venom (2mg/kg) and root bark aqueous extract of *Paullinia pinnata* in mice.

Group	Dose (mg/kg) extract	Mortality (24h) (no. of death/no. of mice used)	% Survival (24h)	Probit of % survival
1	0	8/8	0	-
2	50	6/8	25	4.3255
3	100	4/8	50	5.0000
4	200	2/8	75	5.6745
5	400	2/8	75	5.6745

Table 10f: Effect of administration (i.p.) of pre-incubated mixture of *Echis carinatus* venom and leaf extract of *Detarium microcarpum* in mice

Group	Dose (mg/kg) extract	Mortality (24h) (no. of death/ no. of mice used)	% Survival (24h)	Probit of % survival
1	0	8/8	0	-
2	50	2/8	75	5.6745
3	100	2/8	75	5.6745
4	200	1/8	87.5	6.1503
5	400	1/8	87.5	6.1503

Table 10g: Effect of root bark aqueous extract of *Paullinia pinnata* administration (i.p.) on effect of *Echis carinatus* venom in extract pretreated mice.

Group	Dose (mg/kg) extract	Mortality (24h) (no. of death/ no. of mice used)	% Survival (24h)	Probit of % survival
1	0	8/8	0	-
2	50	8/8	0	-
3	100	7/8	13	3.8736
4	200	6/8	25	4.3255
5	400	6/8	25	4.3255

Table 10h: Effect of root bark aqueous extract of *Paullinia pinnata* administration (p.o) on effect of *Echis carinatus* venom in extract pretreated mice.

Group	Dose (mg/kg) extract	Mortality (24h) (no. of death/ no. of mice used)	% Survival (24h)	Probit of % survival
1	0	8/8	0	-
2	50	8/8	0	-
3	100	7/8	12.5	3.8497
4	200	6/8	25	4.3255
5	400	6/8	25	4.3255

Table 10i: Effect of leaf extract of *Detarium microcarpum* administration (i.p.) on effect of *Echis carinatus* venom in extract pretreated mice

Group	Dose (mg/kg) extract	Mortality (24h) (no. of death/ no. of mice used)	% Survival (24h)	Probit of % survival
1	0	8/8	0	-
2	50	8/8	0	-
3	100	4/8	50	5.0000
4	200	2/8	75	5.6745
5	400	2/8	75	5.6745

Table 10j: Effect of leaf extract of *Detarium microcarpum* administration (p.o) on effect of *Echis carinatus* venom in extract pretreated mice

Group	Dose (mg/kg) extract	Mortality (24h) (no. of death/ no. of mice used)	% Survival (24h)	Probit of % survival
1	0	8/8	0	-
2	50	7/8	12.5	3.8497
3	100	7/8	12.5	3.8497
4	200	5/8	37.5	4.6814
5	400	4/8	50	5.0000

4.1.8 Analgesic Activity of the Extracts of *Paullinia pinnata* and *Detarium microcarpum* in Mice

The results (Tables 11a-b) indicate that *Paullinia pinnata* extract treated animal groups did not inhibit writhing movements. This is because the results of the treatment groups were not significantly different from what was obtained in the negative control group. The treatment of mice with *Detarium microcarpum* aqueous extract (100-400 mg/kg) however, produced marked and dose dependent inhibition of the writhing movements in the animals. This is because the percentage inhibition by extract of *Detarium microcarpum* (400 mg/kg) was comparable to that produced by the standard drug, indomethacin (2.5mg/kg), 51.9 and 65.4% respectively.

Table 11a: Analgesic effect of aqueous extract of *Paullinia pinnata*, and indomethacin against acetic acid-induced abdominal constrictions in mice: writhing test in mice

Animal group	Dose (mg/kg)	No. of writhes per 15 min	% Inhibition of Writhing movement
Normal saline	-	50±0.82	0
Paullinia pinnata	100	50±0.82	0
	200	45±2.86	10
	400	48±1.41	4
Indomethacin	2.5	20±0.82	60*

Values are mean ± SEM of 4 experiments.

*$p < 0.05$ compared to the negative control

Table 11b: Analgesic effect of aqueous extract of *Detarium microcarpum* administration against acetic acid-induced abdominal constrictions in mice: Writhing test in mice.

Animal group	Dose (mg/kg)	No. of writhes	% Inhibition of writhing movement
Normal saline	-	52±0.82	0
Detarium microcarpum	100	45±2.86	13.5
	200	40±0.41	23.1
	400	25±0.91	51.9*
Indomethacin	2.5	18±0.71	65.4

SEM of 4 experiments

*$p < 0.05$ compared to the controls

4.1.9 Antipyretic Activity of the Extracts of *Paullinia pinnata* and *Detarium microcarpum* in Mice

The results in tables 12 a-b represent the effects of the extracts of *Paullinia pinnata* and *Detarium microcarpum* against Brewer's yeast-induced hyperpyrexia in mice. There was no significant ($p > 0.05$) decrease in yeast-induced temperature in animals treated with extract of *Paullinia pinnata*. The rectal temperature of the animals, instead increased from 1 h of administration of yeast, which was comparable to the result in the positive control group.

The pharmacological activity of the extract of *Detarium microcarpum* showed significant ($p < 0.05$) reduction in the rectal temperature of the animals, that manifested one h after the administration of the extract and this effect was noticed with both lower and higher concentrations of the extract.

Table 12a: Effect of aqueous extract of *Paullinia pinnata* on Brewer's yeast- induced hyperpyrexia in mice.

Animal group	Dose (mg/kg)	Temp. before yeast.	Temp. after the extract, indom., and GA		
			1 h	2 h	3 h
GA	-	37.00±0.01	37.90±0.03	37.70±0.03	37.65±0.03
Extract	100	37.10±0.04	37.60±0.01*	38.10±0.02*	37.80±0.02*
	400	37.00±0.06	37.40±0.07*	37.70±0.04*	37.80±0.03*
Indom.	2.5	36.00±0.02	36.30±0.05	36.20±0.03	36.20±0.02

Legend: GA= Gum acacia; Indom. = Indomethacin

The values are mean ± SE of 4 experiments.

*p > 0.05 compared the controls

Table 12b: Effect of aqueous extract of *Detarium microcarpum* on Brewer's yeast-induced hyperpyrexia in mice.

Animal group	Dose (mg/kg)	Temp. before yeast	Temp. after the extract, indom., and GA		
			1 h	2 h	3 h
GA	-	37.00±0.01	38.40±0.04	38.30±0.04	38.40±0.04
Extract	100	36.70±0.04	35.60±0.02*	35.40±0.02*	35.30±0.03*
	600	37.10±0.03	36.80±0.05*	36.40±0.03*	36.20±0.02*
Indom.	2.5	37.00±0.02	36.40±0.01	36.40±0.01	36.30±0.05

Legend: GA= Gum acacia; Indom. = Indomethacin

*$p < 0.05$ compared to the controls

SEM (n = 4).

4.1.10 Anti-Inflammatory Activity of the Extracts of *Paullinia pinnata* and *Detarium microcarpum* in Rats

The results in tables 13a-b summarize the effect of extracts of *Paullinia pinnata* and *Detarium microcarpum* on albumin-induced oedema in rat paw. The extract of *Paullinia pinnata*, did not inhibit the formation of oedema, the result of which was not much different from that of the negative control group. The extract of *Detarium microcarpum* however, produced a dose dependent inhibition of the oedema, which was comparable to the inhibition in indomethacin control group.

The albumin produced a swelling of the rat paw reaching peak in 3 h and gradually subsided over 2 h by *Detarium microcarpum*. The extract of *Detarium microcarpum* exhibited the maximum inhibition of the inflammation at 600mg/kg compared to the lower dose of 100mg/kg. Indomethacin (2.5mg/kg) greatly inhibited the swelling which was not significantly different from that of the extract of *Detarium microcarpum* (600mg/kg) within the same period of time.

Table 13a: Effect of the aqueous extract of root bark of *Paullinia pinnata* on albumin-induced inflammation in rats

Rat group	Dose (mg/kg)	Foot circum-ference (cm) before albumin	Foot circumference (cm)				
			1h	2h	3h	4h	5h
Normal saline	-	1.5±0.01	1.6±0.03	1.6±0.02	1.7±0.05	1.6±0.03	1.6±0.03
Paullinia pinnata	100	1.5±0.03	2.6±0.02	2.7±0.03	2.8±0.02	2.7±0.03	2.7±0.04♦
	400	1.5±0.02	2.5±0.04	2.5±0.02	2.8±0.03	2.7±0.03	2.7±0.02♦
Indome-thacin	2.5	1.6±0.04	2.0±0.04	1.7±0.03	1.6±0.02	1.6±0.02	1.6±0.03

SEM (n= 4)

♦$p > 0.05$ compared to the controls

Table 13b: Effect of the aqueous extract of root bark of *Detarium microcarpum* in albumin-induced inflammation in rats.

Rat group	Dose (mg/kg)	Foot circum-ference (cm) before albumin	Foot circumference (cm)				
			1h	2h	3h	4h	5h
Normal saline	-	1.6±0.03	1.8±0.05	1.8±0.02	1.7±0.03	1.8±0.04	1.7±0.02
Detarium microcar-pum	100	2.0±0.04	2.8±0.03	2.9±0.03	3.0±0.02	2.5±0.02	2.3±0.03
	600	1.9±0.02	2.2±0.02	2.2±0.01	2.1±0.05	2.0±0.03	1.9±0.02♦
Indometh-acin	2.5	1.8±0.05	2.0±0.03	1.9±0.01	1.8±0.03	1.8±0.03	1.8±0.02

SEM (n= 4)

♦$p < 0.05$ compared to controls

4.1.11 Effect of Extracts of *Paullinia pinnata* and *Detarium microcarpum* on Blood Coagulation Time in Rats injected with *Echis carinatus* Venom

The table (14) presents summary of the results of clotting time and bleeding time analyses of blood drawn from rats treated with normal saline, venom, venom/ *Paullinia pinnata* mixture, and venom/ *Detarium microcarpum* mixture, respectively. The animal group treated with normal saline was considered as negative control. The incubation of the venom with the extract of *Paullinia pinnata* before injecting into rats neutralized the effect of venom on blood clotting time and bleeding time. The average blood clotting time and bleeding time in animals treated with normal saline was 75±2.5 and 80±2.0 sec, respectively. However, the blood clotting time and clottind time were markedly increased in animal groups treated with venom (100±2.0 sec), and venom/*Detarium microcarpum* mixture (120±2.5 sec.), respectively.

Table 14: Effect of extracts of *Paullinia pinnata* and *Detarium microcarpum* on blood coagulation time in rats injected with *Echis carinatus* venom

Rat group	Clotting Time (sec)	Bleeding Time (sec)
Normal saline	75±2.5	80±2.0
Venom	100±2.0	120±2.2
Venom+ *Paullinia pinnata*	85±3.5▪	100±2.3♦
Venom+ *Detarium microcarpum*	120±2.5	130±2.1

SEM (n=4)

♦$p < 0.01$; ▪$p < 0.05$

4.1.12 Effect of Extracts of *Paullinia pinnata* and *Detarium microcarpum* on Blood Cell Values in Rats injected with *Echis carinatus* venom

The blood analysis of the blood cell parameters showed that the PCV, RBC, and Platelet counts were markedly reduced in venom treated animals. However, WBC in the blood of this group of animals was significantly increased. With the exception of PCV in *Detarium microcarpum* extract treated rats, all the blood cell values were restored to normal in the presence of the extracts (*Paullinia pinnata* and *Detarium microcarpum*). Haemoglobin was not significantly ($p > 0.05$) affected in all animal treatment groups. The blood sample analyses showed that the PCV is slightly lower in venom treated animals, while in the presence of the extract of *Paullinia pinnata* this was not significantly affected. However, this value was significantly increased in animals treated with *Detarium microcarpum*. The WBC count was restored in animals treated with the venom/*Detarium microcarpum* mixture and venom/*Paullinia pinnata* mixture. In the case of haemoglobin (Hb) level, there was no appreciable difference in the results from all animal groups. The results were all within the normal range, as compared with the negative control of normal saline. The red blood cells as well as the platelet counts were restored in the presence of the two extracts.

Table 15: Effect of extracts of *Paullinia pinnata* and *Detarium microcarpum* on blood cell values in rats injected with *Echis carinatus* venom

Rat group	WBC(/mm^3)	PCV (%)	Hb(g/100ml)	RBC(/mm^3)	Platelets(/mm^3)
N/s	5,250±200	40±1.0	15.91±0.2	8,510,000±1200	161,000±400
V	18,050±205	38±2.0	15.33±0.5	4,800,000±1300	88,000±450
V +A6	6,150±110*	42±5.0*	16.57±0.6	8,360,000±1255*	141,000±350*
V+A11	6,500±100*	59±2.0	16.16±0.4	7,310,000±1300*	137,000±380*

Legend: N/s = normal saline; V = venom; A6= *Paullinia pinnata;* A11 = *Detarium microcarpum.*

*$p < 0.05$ compared to controls

4.1.13 Effect of the Extracts on Capillary Permeability after *Echis carinatus* venom

The results in tables 16a-b present summaries of effects of *Paullinia pinnata* and *Detarium microcarpum* extracts on capillary permeability in venom treated rabbits. The dye permeated the entire body surface of the animals treated with the venom. In animals treated with pre-incubated mixtures of venom/ *Paullinia pinnata* and venom/promethazine the dye was found to be localized to the areas of injection as in animals of the negative control group. In animals treated with pre-incubated mixtures of venom/ *Detarium microcarpum*, however, the dye permeated very large surface area of the body.

Table 16a: Effect of the aqueous extract of *Paullinia pinnata (400mg/kg)* on capillary permeability in venom treated rabbits

Rabbit group	Area of diffusion (cm^2)
Normal saline	6
Venom	Entire body (>10) ▫
Venom+ *Paullinia pinnata*	7▪
Venom+ promethazine	8

▪P<0.05; p >0.05

Table 16b: Effect of the aqueous extract of *Detarium microcarpum* (600mg/kg) on capillary permeability after *Echis carinatus* venom

Rabbit group	**Area of diffusion (cm^2)**
Normal saline	6
Venom	Entire body (>250) ▫
Venom+ *Detarium microcarpum*	240▫
Venom+ Promethazine	7.5

▫p >0.05

4.1.14 Diuretic Effect of Extracts *Paullinia pinnata* and *Detarium microcarpum* in Rats

The aqueous extract of *Detarium microcarpum* (600mg/kg) produced a significant increase in urinary excretion of water comparable to the volume of water excreted in animals treated with the standard drug, furosemide. The extract produced a steady increase in the urinary excretion of water 4 h after the administration, reaching peak over the period of 12 h. Similarly, the extract caused a significant increase in sodium and potassium excretion with respect to the negative control. The extract of *Paullinia pinnata* however, did not affect water and the electrolyte excretion in the animals. There was also no modification of the pH of the pooled urine samples in all the rat groups (pH for normal saline group, 9.35±2; *Paullinia pinnata*, 9.24±1; *Detarium microcarpum*, 9.43±2; and Furosemide, 9.43±3). The maximum urinary water output in the treatment groups of *Detarium microcarpum* and *Paullinia pinnata* were 86.2±2.5 and 34.1±2.0 ml, respectively.

Table 17: The diuretic effect of aqueous extracts of *Paullinia pinnata* (400 mg/kg) and *Detarium microcarpum* (600 mg/kg) in rats

Group	Volume of urine (ml)	pH of urine	Conc.$Na^{+(mmole/L)}$	Conc. $K^{+(mmole/L)}$
Normal saline	3.9±0.5	9.35±0.1	48.41±1.2	50.13±0.6
Paullinia pinnata	2.9±0.6	9.24±0.3	36.86±1.5	47.36±1.2
Detarium microcarpum	5.6±1.5•	9.43±0.1	120.34±1.7•	81.43±1.5•
Furosemide	8.6±0.3	9.43±0.1	114.01±1.4	132.58±0.6

SEM (n = 4)

•$p < 0.01$ compared to the controls

4.1.15 Effect of *Paullinia pinnata* and *Detarium microcarpum* Extracts on Blood Glucose in Rabbits treated with *Echis carinatus* venom

The *Echis carinatus* venom produced hyperglycemic effect on the rabbits after 6 h as compared with the control. The venom increased blood glucose level in animals treated with *Paullinia pinnata* extract comparable to that produced by venom alone (Table 18). The glucose level in venom treated rabbits and treated with *Detarium microcarpum* extract however, remained within normal range as compared to the one produced in normal saline treated rabbits.

Table 18: Effect of the aqueous extracts of *Paullinia pinnata* (400 mg/kg) and *Detarium microcarpum* (600 mg/kg) on blood glucose in rabbits treated with *Echis carinatus* venom (1.5 mg/kg).

Animal group	**Conc. of glucose (mg/100 ml)**
Normal saline	1.5±0.6
Venom + *P. pinnata*	2.88±0.2
Venom	2.75±0.5
Venom + *D. microcarpum*	1.38* ±0.2

*$p < 0.01$; SEM (n = 4)

4.1.16 Effect of extracts of *Paullinia pinnata* and *Detarium microcarpum* on the Local Effects produced by *Echis carinatus* venom in rabbits

There were no local reactions (such as oedema, haemorrhage, and necrotic ulceration) in the legs injected with the pre-incubated mixtures of the venom/extract of *Paullinia pinnata*, and venom/promethazine, respectively. The legs injected with the pre-incubated mixture of the venom/extract of *Detarium microcarpum* however, presented oedema, haemorrhage, and necrotic ulceration of the skin at sites of injection. There was also oedema in the two right legs injected with the venom. Haemorrhage and necrotized tissues were also observed at the injection sites in the affected legs injected with venom.

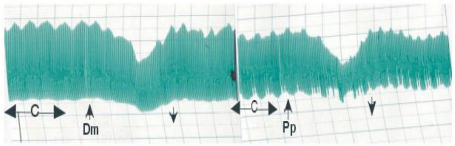
4.1.17 Effects of the aqueous extracts of *Paullinia pinnata* and *Detarium microcarpum* on Anaesthetized Cat Blood Pressure

The hypotensive responses obtained after administration of the extracts are presented in figure 2 and tables 19a-c. The slow intravenous administration of aqueous extracts of *Paullinia pinnata* (30µg/kg) and *Detarium microcarpum* (15µg/kg) in sodium thiopental anaesthetized cats depressed the blood pressure (Figure 2a). The extract of *Detarium microcarpum* (15µg/kg cat) produced a marked fall in blood pressure, which started ten to 20 sec after administration, lasted 5 sec. and returned to normal within 20 min (Figure 2a). Similarly, *Paullinia pinnata* (30µg/kg cat) produced gradual and marked fall in the blood pressure which lasted for 10 sec and returned to normal within 2 min (Figure 2a).

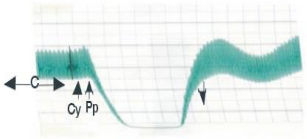
Further studies were carried out to elucidate the possible mechanisms by which the extracts elicited the observed hypotensive effect. Injection of the extracts of *Paullinia pinnata* (30µg/kg) and *Detarium microcarpum* (15µg/kg) into the cats, ten min after the intravenous injection of cyproheptadine Hcl (2mg/kg), produced a marked and prolonged fall in blood pressure which started 5-10 sec, reaching maximal reduction within 2 min and returned to normal within 30 to 40 min (Figure 2b).

The hypotensive effect of *Detarium microcarpum* was not affected by cyproheptadine as it was neither blocked nor potentiated by the

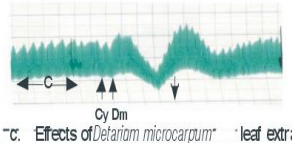
drug(Figure 2c). However, the hypotensive effect of *Paullinia pinnata* was potentiated by cyproheptadine (1mg/kg) (Figures 2b and 2c). The pretreatment of the animals with indomethacin (2.5mg/kg, i.p) 10 min before injecting the aqueous extracts of *Paullinia pinnata* and *Detarium microcarpum*, markedly blocked the hypotensive effect of *Detarium microcarpum* but not the effect of *Paullinia pinnata* (Figures 2f and 2g). However, injection of promethazine (2.5mg/kg) 10 min before the administration of the extracts did not affect the hypotensive activity of the two extracts (Figures 2d and 2e). The pretreatment of the cats with adrenaline (1µg/kg) ten minutes before injection of the extracts, markedly blocked the hypotensive effect of *Paullinia pinnata* but no effect on the response of the animal to *Detarium microcarpum* (Figures 2h and 2i). The pretreatment of the cats with small dose of *Paullinia pinnata* blocked the hypotensive action of the venom (5µg/kg). But higher dose of the venom (10 µg/kg) after the extract, reduced the BP remarkably which could not be reversed by the extract of *Paullinia pinnata* and adrenaline, and the animal died (Figures 2j and 2k).



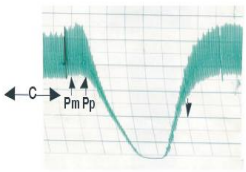
a. Effect of *Paullinia pinnata* (Pp) (30µg/kg) and Detarium *microcarpum* (Dm) (15µg/kg) on blood pressure of anaesthetized cat.



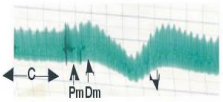
b. Effects of *Paullinia pinnata* (Pp) root extract (30-g/kg) in the presence of cyproheptadine (Cy) (2mg/kg) on blood pressure of an anaesthetized cat.



c. Effects of *Detarium microcarpum* leaf extract (15µg/kg) in the presence of cyproheptadine (Cy) (2mg/kg) on blood pressure of an anaesthetized cat.



d. Effect of *Paullinia pinnata* (Pp) root extract (30µg/kg) in the presence of promethazine (Pm) (2.5mg/kg) on blood pressure of anaesthetized cat.

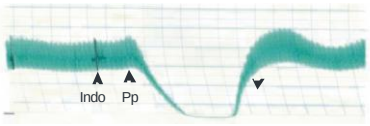


e. Effects of *Detarium microcarpum* (Dm) leaf extract (15µg/kg) in the presence of promethazine (Pm) (2.5mg/kg) on blood pressure of an anaesthetized cat.

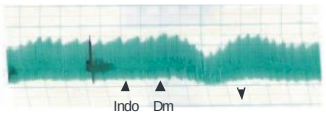
▼ = *Washout*

C = *control*

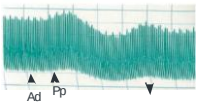
Fig. **2:** Effect of *Paullinia pinnata* (Pp) (30µg/kg) and *Detarium microcarpum* (Dm) (15µg/kg) on blood pressure of anaesthetized cat.



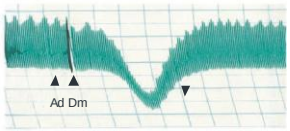
f. Effects of *Paullinia pinnata* (Pp) root extract (30µ g/kg) in the presence of indomethacin (Indo) (2.5mg/kg) on blood pressure of an anaesthetized cat.



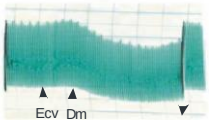
g. Effects of *Detarium microcarpum* (Dm) leaf extract (15µ g/kg) in the presence of indomethacin (Indo) (2.5mg/kg) on blood pressure of an anaesthetized cat.



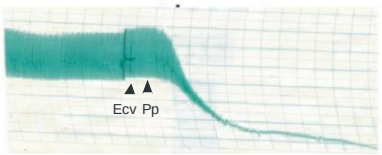
h. Effect of *Paullinia pinnata*(Pp) root extract (30µg/kg) in the presence of adrenaline (Ad) (µ g/kg) on blood pressure of anaesthetized cat.



i. Effect of *Detarium microcaroum*(Dm) leaf extract (15 µg/kg) in the presence of adrenaline (Ad) (µ g/kg) on blood pressure of anaesthetized cat.



j. Effect of *Detarium microcarpum* leaf extract (15µ g/kg) on blood pressure of an anaesthetized cat pretreated with *Echis carinatus* venom (10µg/kg).



k. Effect of *Paullinia pinnata* (Pp) root extract (30µ g/kg) on blood pressure of an anaesthetized cat pretreated with *Echis carinatus* venom (10µg/kg).

Fig. 2 contd: Effect of *Paullinia pinnata* (Pp) (30µ g/kg) and *Detarium microcarpum* (Dm) (15µg/kg on blood pressure of anaesthetized cat.

Table 19a: Effect of extracts of *Paullinia pinnata* (30µ/kg) and *Detarium microcarpum* (15µg/kg) on blood pressure of anaesthetized cats

Treatment group	Systolic pressure (mmHg)		Diastolic pressure (mmHg)		MAP
	Basal	Extract treatment	basal	Extract treatment	
Pp	190±1.6	120±4.5	140±2.0	80±1.6	93±2.0
Dm	190±1.0	150±3.5	140±1.5	90±1.8	110±2.5

Legend: Pp = *Paullinia pinnata*; Dm = *Detarium microcarpum;*
MAP = mean arterial blood pressure

The results are mean ± SE of 4 experiments.

Table 19b: Effect of standard antagonists on the blood pressure effect of the extract of *Paullinia pinnata* (30µg/kg)

Treatment group	Systolic pressure(mmHg)	% fall in SBP	Diastolic pressure(mmHg)	% fall in DBP	MAP
Control	190	0	150	0	-
^{pp}	120±2.5	36.8	80±1.5	46.7	93
Cypro^{pp}	20±1.8	89.5	10±2.1	93.3	13
Indo^{pp}	25±2.2	86.8	15±1.7	90	18
Prom^{pp}	20±2.6	89.5	10±2.0	93.3	13
Adren^{pp}	180±1.5	5.3	130±1.2	13.3	147
ECV^{pp}	185±1.5	2.6	85±1.8	43.3	118

Legend: pp = *Paullinia pinnata*; Cypr = cyproheptadine; Indo = indomethacin; Prom = promethazine; Adre = adrenaline; *ECV* = *Echis carinatus* venom

The values are mean ± of 4 experiments.

Table 19c: Effect of standard antagonists on the blood pressure effect of the extract of *Detarium microcarpum* (15µg/kg)

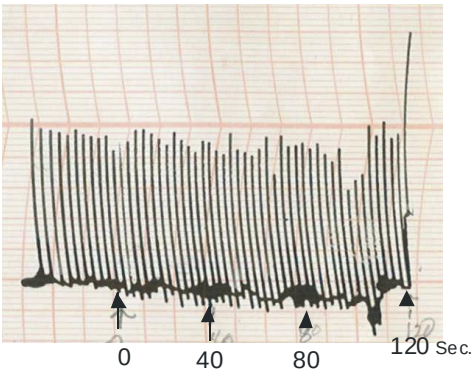
Treatment group	Systolic pressure(mmHg)	% fall in SBP	Diastolic pressure(mmHg)	% fall in DBP	MAP
Control	185	0	145	0	-
^{dm}	150±1.4	18.9	90±2.8	37.9	110
Cypr^{dm}	100±2.2	46	50±1.2	65.5	67
Indo^{dm}	120±2.2	35	80±1.3	44.8	93
Prom^{dm}	40±1.1	78.4	20±1.5	86.2	27
Adre^{dm}	90±2.1	51.4	40±1.6	72.4	57
ECV^{dm}	145±2.5	21.6	80±2.1	44.8	122

Legend: dm = *Detarium microcarpum;* Cypr =cyproheptadine; Indo = indomethacin; Prom = promethazine; Adre = adrenaline; *ECV = Echis carinatus* venom

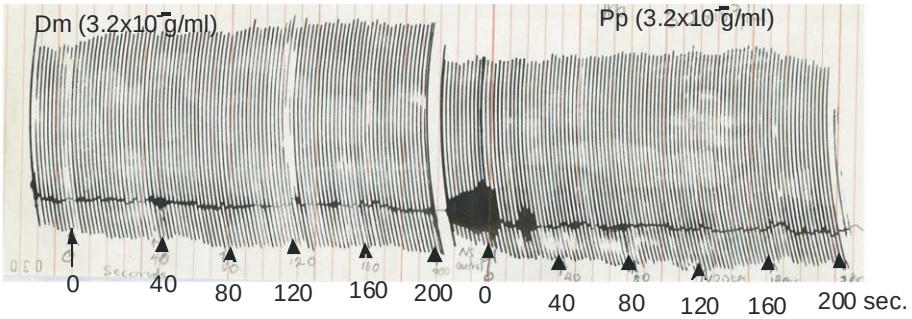
The effects recorded in the present study are typical responses and each obtained from at least four experiments.

4.1.18 Effect of Extracts of *Paullinia pinnata* and *Detarium microcarpum* on Rat Phrenic Nerve Hemi-Diaphragm Muscle Preparation

Single maximal nerve stimulation of the diaphragm muscle increased the muscle contraction (Figure 3i). The extracts of *Detarium microcarpum* (3.2 $x10^{-3}$g/ml) and *Paullinia pinnata* (3.2$x10^{-3}$g/ml) increased contraction on the nerve stimulated diaphragm muscle and the increase was dose dependent (Figure 3ii). The increase was 55% and 45% greater than that produced by the control (3i), respectively. The *d*-tubocurarine solution (1.6$x10^{-5}$g/ml) did not reverse the increase in contraction of the phrenic nerve diaphragm muscle by the leaf extract of *Detarium microcarpum* (3iii). However, it reversed the increase in contraction by the extract of *Paullinia pinnata*. The effect of the extract was not significantly influenced by neostigmine (Figure 3 iv).

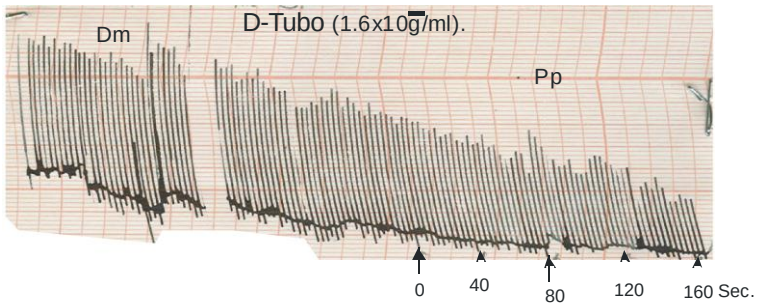


i. Control

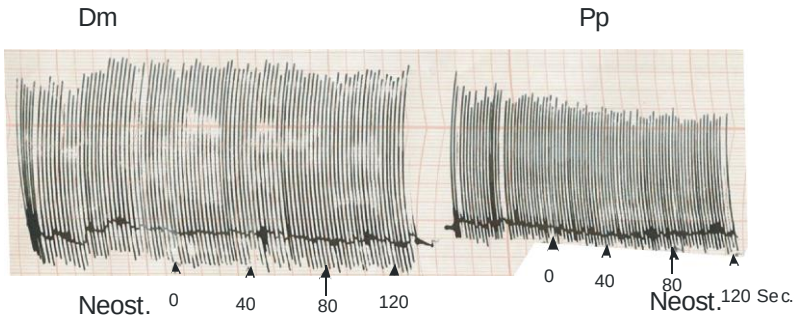


ii. Effect fo aqueous extracts of (Dm) *Detarium microcarpum and Paullinia pinnata* (Pp) on isolated rat phrenic-hemidiaphragm

Fig. 3 Effect of aqueous extracts of (Pp) *Paullinia pinnata* *Detarium microcarpum* And (Dm) on isolated rat phrenic-hemidiaphragm



iii. Effect fo aqueous extracts of (Dm) *Detarium microcarpum* *Paullinia pinnata* and (Pp) on isolated rat phrenic-hemidiaphragm in the presence of d-tubocurare (1.6x10g/ml).



iv. Effect fo aqueous extracts of (Dm) *Detarium microcarpum* *Paullinia pinnata* and (Pp) on isolated rat phrenic-hemidiaphragm in the presence of neostigmine

Fig. 3 contd.: Effect fo aqueous extracts of (Dm) *Detarium microcarpum* *Paullinia pinnata* and (Pp) on isolated rat phrenic-hemidiaphragm

Table 20a: Effect of aqueous extract of *Paullinia pinnata* on rat phrenic nerve hemi-diaphragm preparation

Voltage = 8V, Frequency = 0.4Hz, Stock Conc. = 100mg/ml.

S/№	FBC (g/ml)	Contraction height (cm)	Response (% increase in twitch height in 15min.)
1	Control	3.8	0
2	$4x10^{-4}$	3.9±2.4	2.63
3	$8x10^{-4}$	4.2±1.5	10.52*
4	$1.6x10^{-3}$	4.5±2.2	18.42*
5	$3.2x10^{-3}$	5.4±2.3	42.11*

The values are mean ± SE of four experiments.

*$p < 0.05$ compared to the control.

Table 20b: Effect of aqueous extract of *Detarium microcarpum* on rat phrenic nerve hemi-diaphragm preparations

Stock conc. = 100mg/ml

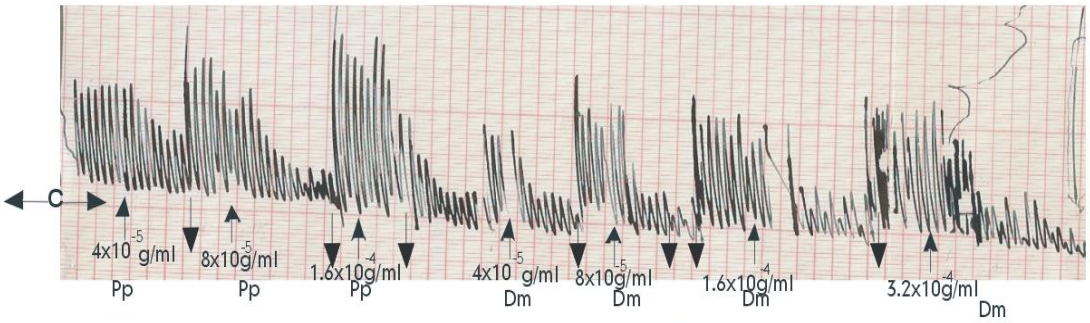
S/№	FBC (g/ml)	Contraction height (cm)	Response (% increase in twitch height in 15min.)
1	control	2.5	0
2	4×10^{-4}	4.0±1.8	60♦
3	8×10^{-4}	4.5±2.1	80♦
4	1.6×10^{-3}	5.0±1.5	100♦
5	3.2×10^{-3}	5.6±1.2	124♦

SEM (n= 4)

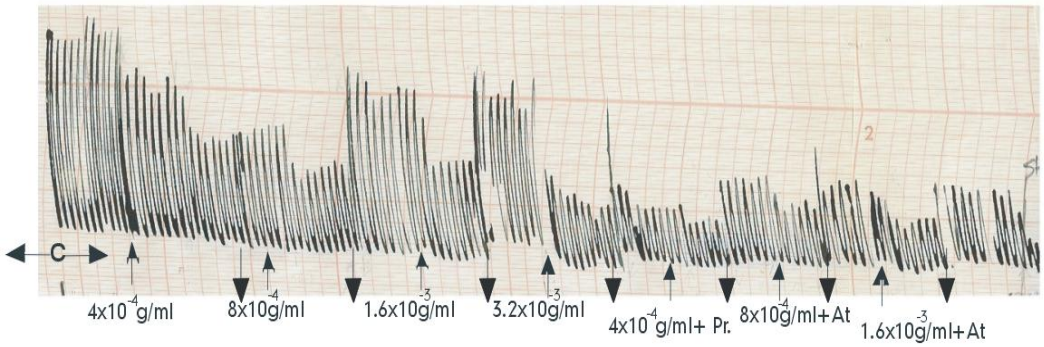
♦$p < 0.05$ compared to controls.

4.1.19 Effect of Extracts of *Paullinia pinnata* and *Detarium microcarpum* on Rabbit Jejunum

The extracts of *Paullinia pinnata* and *Detarium microcarpum*, depressed the tone and amplitude of the spontaneous contractions of the isolated rabbit jejunum in a manner similar to that of adrenaline. The spontaneous contractile activity of the tissue was depressed markedly (60%) by *Paullinia pinnata* and was then blocked by atenolol. The agonist effect of *Detarium microcarpum* on the muscle preparation was however blocked by prazosin.



a. Effects of *Paullinia pinnata* root bark extract and *Detarium microcarpum* leaf extract on isolated rabbit jejunum.

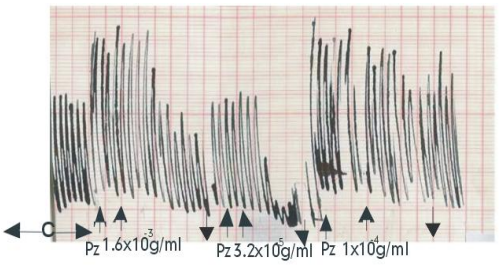


b. Effects of extracts of *Paullinia pinnata* in the presence of atenolol ($1x10^{-6}$ g/ml) on isolated jejunum.

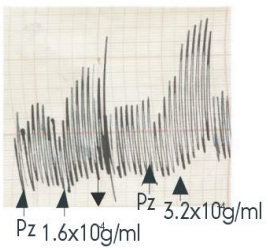
▼ = Washout

C = control

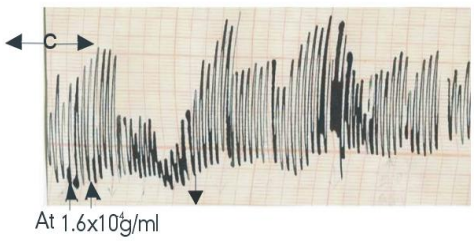
Fig. 4: The effects of Paullinia pinnata rock bark extract and Detarium microcarpum leaf extract on isolated rabbit jejunum



c. Effect of *Paullinia pinnata* (P p) root extract (30µg/kg) in the presence of prazosin (Pz) (1x10⁻⁶g/ml) on rabbit jejunum.



d. Effect of *Detarium microcarpum* (Dm) leaf extract (15µg/kg) in the presence of prazosin (Pz) (1x10⁻⁶g/ml) on rabbit jejunum.



e. Effect of *Detarium microcarpum* extract on isolated rabbit jejunum in the presence of atenolol (1x10⁻⁶g/ml).

↓ = Washout

C = control

Fig. 4 contd. The effects of Paullinia pinnata root bark extract and Detarium microcarpum leaf extract on isolated rabbit jejunum

Table 21a: Effect of the extract of *Paullinia pinnata (*$1.6x10^{-8}$gm/ml*)* on isolated rabbit jejunum

Substance	Conc.(g/ml)	Amplitude(mm)	% decrease	Remarks
Control	-	50	0	Normal response
^{p}	$1.6x10^{-3}$	20±2.5	60•	Agonist effect
Prazosin^{p}	$1x10^{-6}$	20±2.0	60	No blockade
Atenolol^{p}	$1x10^{-6}$	15±1.2	70	Partial blockade

Legend: p = *Paullinia pinnata (*$1.6x10^{-8}$gm/ml*)* was added.

SEM (n = 4).

•$p < 0.05$

Table 21b: Effect of the extract of *Detarium microcarpum (*$8x10^{-4}$gm/ml) on isolated rabbit jejunum

Substance	Conc.(g/ml)	Amplitude(mm)	% decrease	Remarks
Control	-	60	0	Normal response
[d]	$8x10^{-4}$	25±2.2	58.33*	Agonist
Prazosin[d]	$1x10^{-6}$	40±2.5	33.33	Blockade
Atenolol[d]	$1x10^{-6}$	25±2.1	58.33	No blockade

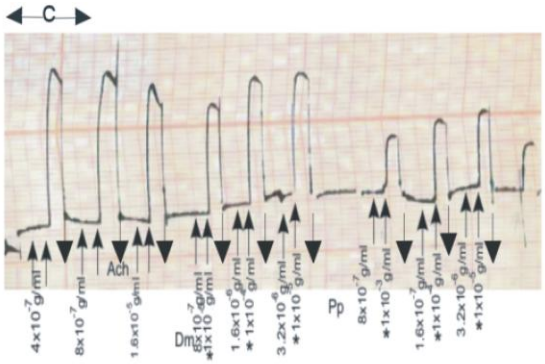
Legend: [d] = *Detarium microcarpum (*$8x10^{-4}$gm/ml*)* was added.

SEM (n = 4)

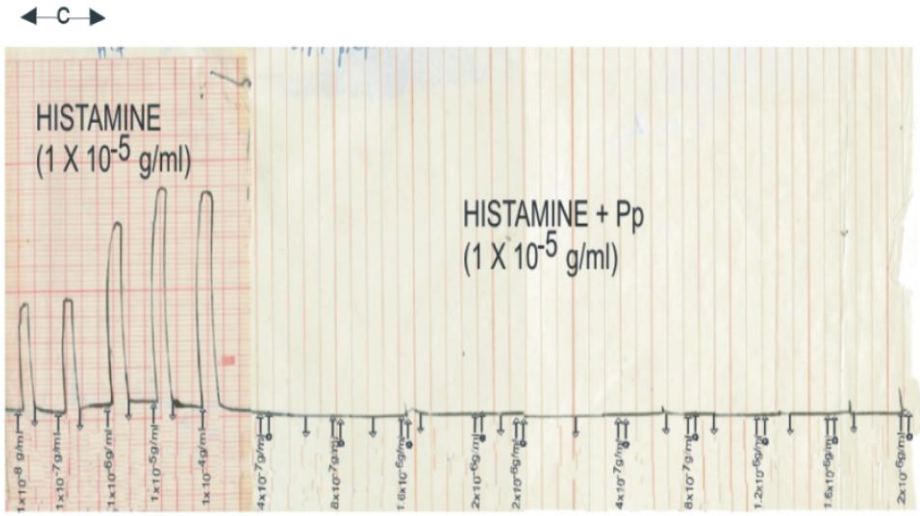
*$p<0.05$

4.1.20 Effect of Extracts of *Paullinia pinnata* and *Detarium microcarpum* on Guinea pig Ileum

The extract of *Paullinia pinnata* inhibited responses of the tissue to two agonists, acetylcholine and histamine. The extract of *Detarium microcarpum* inhibited the responses of the animal tissue to acetylcholine. However, there was no effect against histamine-induced muscle contraction.



a. Effect of *Paullinia pinnata* (Pp) and *Detarium microcarpum* (Dm) leaf extract on isolated guinea pig ileum in th presence of Ach. * = Ach added.



b. Effect of aqueous extract of *Paullinia pinnata* (Pp) on isolated Guinea-pig ileum. N=4.
The effect of pretreatment with *Paullinia pinnata* (Pp) on histamine activity

Legend: = Extract (Pp) added.

▼ = Washout

C = control

Fig. 5: The effect of extract of Paullinia pinnata (Pp) and Detarium microcarpum (Dm) on isolated guinea pig ileum

Table 22a: The effect of *Paullinia pinnata (*$1.6x10^{-8}$ g/ml) on the agonist activity of Ach and histamine on guinea pig ileum

Substance	Conc.(g/ml)	Amplitude(mm)	% response	Remarks
Control (Ach)	$5x10^{-8}$	24±1.0	100	Normal contractions
Ach*	$5x10^{-8}$	12±1.5	50	Anti-cholinergic
Control (Hist)	$5x10^{-8}$	18±1.2	100	Normal contractions
Hist*	$5x10^{-8}$	0	0	Anti-histaminic effect

Legend: * = *Paullinia pinnata (*$1.6x10^{-8}$ g/ml) was added.

Table 22b: Effect of *Detarium microcarpum (*$1.6x10^{-8}$ g/ml) on the agonist activity of Ach and histamine on guinea pig ileum

Substance	Conc.(g/ml)	Amplitude(mm)	% response	Remarks
Control (Ach)	$5x10^{-8}$	25±1.2	100	Normal contractions
Ach#	$5x10^{-8}$	15±1.5	40	Anti-cholinergic
Control (Hist)	$5x10^{-8}$	24±1.6	100	contractions
Hist#	$5x10^{-8}$	24±2.0	100	Not anti-histaminic

Legend: Ach = acetylcholine; Hist. = Histamine;

= *Detarium microcarpum (*$1.6x10^{-8}$ g/ml) was added

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION AND CONCLUSION

Paullinia pinnata Lnn (Family: Sapindaceae) *and Detarium microcarpum* Guill and Perr (Family: Caesalpiniaceae) are found throughout the savannah region of Nigeria. The root bark of *Paullinia pinnata* and leaves of *Detarium microcarpum* are used by traditional healers in Nigeria for the treatment of snakebite patients. This study indicates that aqueous extracts of root of *Paullinia pinnata* and the leaves of *Detarium microcarpum* are effective in neutralizing the lethality and local effects of *Echis carinatus* venom in animals.

The pathological properties of *Echis carinatus* venom are mainly associated with haematological disturbances leading to haemorrhage and incoagulability of blood. Some local pathological effects such as pain, tissue necrosis, oedema, and fever always accompany envenomation from this species of snake. Many of the conventional antivenoms are ineffective in antagonizing the local effects of the venom. The neutralization of the activity of *Echis carinatus* venom by aqueous extracts of *Paullinia pinnata* root and *Detarium microcarpum* leaves therefore may have provided an insight into the possible usefulness of the plants in the treatment of snakebite in humans. Furthermore, an aqueous extract of *Detarium microcarpum* leaves is more effective in neutralizing

the lethality of *Echis carinatus* venom in view of its tremendous effect on the effect of the venom in mice.

The extraction yields of these plants indicate that *Paullinia pinnata root* and *Detarium microcarpum* leaves are readily soluble in water. The product yields however, indicate that the root bark of *Paullinia pinnata* is more soluble in water than the leaves of *Detarium microcarpum*. Notwithstanding, the plant materials are soluble in the same proportion in both cold and hot water, respectively. The solubility of the crude extracts of the medicinal plants suggests that the active pricinples in the herbal extracts are readily available in their traditional preparations, which may be in part, the basis for the success being recorded by the healers in the treatment of snakebite patients.

The analysis for ionic content of the two plants also indicates that they contain calcium and zinc. These ions may have influenced the effect of snake venom activity *in vitro* and *in vivo* as trace metal ions have been implicated in the potential toxic effects of snake venoms (Friederich and Tu, 1971) such as haemorrhage. Consequently, plant extract with high trace metal content may affect pathological level of metal ions in the venom as more of the toxic metals will be added to that of the venom. The extracts being devoid of magnesium (Mg) ions, they are not likely to potentiate the local or neurotoxic effects of the venom *in vivo*. However, the presence of zinc in the extracts, albeit in small quantities, may influence the haemorrhagic activity of the venom. It therefore suggests

that plant extracts with high zinc content may negate the anti-haemorrhagic effect of *Paullinia pinnat*a extract in the management of *Echis carinatus* envenomation if the trace metal is not removed from the extract before administration. The development of anti-venom from this plant may therefore require careful elimination of zinc ions from the materials.

The results of the preliminary phytochemical screening indicate the presence of carbohydrates, saponins, steroids, and tannins in both the cold and hot water extracts of *Paullinia pinnata* root bark*;* and anthraquinones, flavonoids, saponins, steroids, and tannins in extracts of *Detarium microcarpum* leaves. This conforms to the reports of Dalziel (1955) and Gill (1992) both of which linked the presence of these compounds in the plants. The presence of tannins in the two plant extracts only confirms their large distribution in plants (Anton, 1988).

This study also shows that the aqueous extracts of *Paullinia pinnata* root bark and *Detarium microcarpum* leaf possess strong anti-bacterial activity against *Staphylococcus aureus, Proteus mirabilis,* and *Pseudomonas aeruginosa*. The comparable antibacterial potency tests against these microorganisms (Tables 4 and 5) demonstrate the activity of the extracts against wound infection causing bacteria. The microorganisms are said to be the major bacteria that play important role in wound infections (Gruickshank *et al*., 1973). The crude aqueous extracts of the two medicinal plants exhibited good antibacterial

activities. This finding corroborates the use of *Paullinia pinnata* root and *Detarium microcarpum* leaf decoctions by traditional healers in the treatment of some secondary infections such as wounds and ulcers (Dalziel, 1955; Gill, 1992).

The Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) tests also support the sensitivity test results. The concentration with which the extracts can inhibit the test organisms is an important factor as the lower the MIC and MBC of a product, the higher the potency. The cold water extract of *Paullinia pinnata* seems to be more potent antibacterial agent, as the concentrations needed to inhibit visually discernable growth of bacterial suspension of *Staphylococcus aureus* and *Proteus mirabilis* are very low. However, the *Detarium microcarpum* extract appears to be a weak antibacterial agent as the minimum concentrations needed to inhibit growth of all the organisms are significantly high. The potent antibacterial activity of *Paullinia pinnata* over *Detarium microcarpum* against the microorganisms may be attributed to the difference in their phytochemical constituents. This is because saponins and tannins, both of which are well known for their antibacterial properties (Egwari, 1999), are the major constituents of *Paullinia pinnata*. These findings therefore, suggest that the traditional therapeutic indications of *Paullinia pinnata* appear to have fairly good degree of correlation with their specific antibacterial activity. The antibacterial compounds in the plant extracts

therefore may possess some therapeutic potential which can be used effectively without any side effects that are often associated with synthetic antibacterial agents. The study therefore justifies the use of the plants by traditional healers as cure for wounds.

The median lethal doses (LD_{50}) of the aqueous extract of *Paullinia pinnata* (1200mg/kg p.o. and 600mg/kg i.p) are relatively lower than those of *Detarium microcarpum* (1400mg/kg p.o.; 800mg/kg i.p). In order to substantiate this assumption, the therapeutic index (TI), which is the ratio of the dose of the extract that is required to kill 50% of the population to the dose that would cure 50% of the population, of each treatment was estimated. The values of the therapeutic index for *Detarium microcarpum* are bigger than those of *Paullinia pinnata*, irrespective of route of administration. These findings therefore suggest that the extract of *Detarium microcarpum* is safer than that of *Paullinia pinnata*; and that the extracts of the two plants are better tolerated after oral administration. It is further suggested that any anti-snake venom developed from *Paullinia pinnata* must be used with great care because of its smaller therapeutic index values.

The venom of *Echis carinatus* is responsible for haemorrhage, tissue necrosis, and blood coagulation system disturbances and death (Warrell *et al*., 1977). The results of the assay tests on *Echis carinatus* venom for determination of the median lethal dose (LD_{50}) and the minimum lethal dose (MLD) show that the venom was least tolerated by

the animals. These findings suggest that the venom is highly toxic; the results of which are in accord with the reports of Russell (1977).

The study of inhibitory effect of the extracts on *Echis carinatus* venom was designed to investigate the anti-snake venom activity of the two medicinal plants. The effectiveness of the extracts against *Echis carinatus* venom was described in terms of comparison of inhibition of deaths in extract-treated animal groups with that in venom-treated controls. An inhibition of 30% or more was considered significant ($p < 0.05$).

In the experimental modes, the extracts were able to neutralize the MLD (2mg/kg) (i.e. 1.3 LD_{50}) of the venom, and this was expressed by the ability of the mice to survive the lethality of the venom.

The results of pharmacological evaluation of the extracts show that *Detarium microcarpum* (600mg/kg, i.p) seems to be more potent in the reduction of mortality in mice pretreated with the MLD of the *Echis carinatus* venom (2mg/kg). The percentage survival in venom pretreated mice and injected with *Paullinia pinnata* (400mg/kg) appears to be poor (ED_{50} = 300mg/kg) compared to the effect of *Detarium microcarpum* (ED_{50} = 50mg/kg). Similarly, the potency of the extracts after oral administration are not very much different, except that the amounts of the extracts required for cure are higher (*Paullinia pinnata,* ED_{50} >400mg/kg; *Detarium microcarpum* ED_{50} = 100mg/kg). The effect of the extract in animals treated with pre-incubated venom/extract mixture seems to

demonstrate some chemical interaction between the toxic components of *Echis carinatus* venom and certain compounds of the extracts; leading to the neutralization of the lethal components of the venom. This interactive effect between the venom and the extracts appears to be better with *Detarium microcarpum* (ED_{50} < 50mg/kg; *Paullinia pinnata,* ED_{50} = 100mg/kg). This suggests that certain chemical compounds of the extracts may be responsible for the neutralization of the lethal components of the venom. Tannins, being potent precipitants of proteins, may be the possible mechanism of action of the extracts of the two plants against the venom.

Although the animals pretreated with the extracts (i.p) before administration of *Echis carinatus* venom enjoyed protection, the extract of *Detarium microcarpum* (ED_{50} = 100mg/kg) appears to be more effective in providing protection than that of *Paullinia pinnata* (ED_{50} > 400mg/kg). In the animals with the extracts (p.o) before injection of the venom, higher doses of the extracts may be required (*Paullinia pinnata*, ED_{50} >400mg/kg and *Detarium microcarpum,* ED_{50} = 400mg/kg) to produce same level of protection. On the whole, the effective doses 50% (ED_{50}) of the extract of *Detarium microcarpum* are lower than those of *Paullinia pinnata*. The implication is that the extracts are more effective after incubation of venom and extracts before administration. This suggests that some chemical compounds of the extracts react to deactivate the lethal components of the venom *in vitro*, thereby rendering the venom

less toxic. At the pharmacodynamic sphere the extracts give better protection to the animals after parenteral administration of the extracts irrespective of the treatment model. This suggests that better results are achieved during treatment of snakebite patients following parenteral administration of the extracts.

The results of the pharmacological effects of the extracts also suggest that their inhibitory effect on snake venom-induced pathological symptoms could be, in part, due to the active constituents of the phytochemical compounds (Welton *et al*, 1988). The effectiveness of *Detarium microcarpum* over *Paullinia pinnata* may be attributed to the chemical composition of the two extracts. *Detarium microcarpum* extract appears to derive its potency from this theory because it contains tannins, potent protein precipitants, and flavonoids which are known to inhibit phospholipase A_2, a toxic component of the venom. The weak antivenom activity of *Paullinia pinnata* may be attributed to the absence of flavonoids in the extract.

One of the symptoms being presented by patients of *Echis carinatus* evenomation is intense pain at the site of the bite (Warrell, 1976; Warrell and Arnett, 1974; Warrell *et al*., 1977). The conventional anti-snake venom employed in the treatment of snakebite patient does not always alleviate the symptom (Warrell *et al*., 1977, Mahanta and Mukherjee, 2001). The use of medicinal plant extract with analgesic effect will obviously resolve this problem in snakebite patients. The

analgesic activity *Detarium microcarpum* extract therefore correctly suggests that certain chemical compounds are responsible for the inhibition of the writhing movements in the animals. The analgesic effect of *Detarium microcarpum* extract could be exhibited through the inhibition of the enzyme lipooxygenase or cyclooxygenase pathway of arachidonic acid metabolism (Venkatrao *et al*, 2007). This is because flavonoids are well known inhibitors of cyclooxygenase and lipooxygenase, as well as phospholipase A_2 (Welton *et al*, 1988). The analgesic superiority of the extract of *Detarium microcarpum* over *Paullinia pinnata* extract may be due to the presence of flavonoids in the extract of *Detarium microcarpum*.

The victims of *Echis carinatus* envenomation also present fever as one of the symptoms of envenomation (Warrell *et al*., 1977; Warrell, 1976). Antipyretic drug substances are of immense help in reducing elevated body temperature in patients. The use of medicinal plants with antipyretic activity might be the basis for the application of traditional medicine for the “cooling” of the body during treatment of snakebite patients. The plants with this property are valuable in the management of fever arising from envenomation from this species of snake. The potent antipyretic effect of the extract of *Detarium microcarpum* may be attributed to the presence of the phytochemical compounds with antipyretic effect, which the extract of *Paullinia pinnata* may not possess.

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasma fluids and blood cells. Albumin-induced rat paw oedema has been used as an inflammation model in order to investigate the anti-inflammatory effect of a drug (Gupta et al, 2003). The oedema is known to be mediated by histamine, serotonin, prostaglandins and bradykinins. Swelling at the site of *Echis carinatus* bite is one of the local effects of carpet viper bite envenomation. This study shows that the extract of *Detarium microcarpum* (600mg/kg) dose dependently suppressed the albumin-induced oedema produced by the chemical mediators. It is not significantly different from that produced effect by indomethacin. These results therefore indicate that the extract of *Detarium microcarpum* exhibits its anti-inflammatory action by means of either inhibiting the synthesis, release or action of inflammatory mediators. A drug developed from the extract could be useful in the management of oedema to improve the quality life of snakebite patient.

Spontaneous bleeding and coagulation disturbances are some of the haematological effects of *Echis carinatus* venom in patients of carpet viper bites (Warrell *et al*., 1977, and Warrell, 1976). The fundamental difference between blood bleeding and clotting determinants is that bleeding is associated with the integrity of blood vessels, while clotting is a function of clotting factors deficiency. The capacity of plasma to form thrombin is also relevant in the blood coagulation system. All these blood

characteristics are affected by the toxic components of *Echis carinatus* venom (Denson *et al*, 1972).

The use of medicinal plants, which could prevent the coagulation disturbances and establish haemostasis, will be beneficial in the management of carpet viper bite patients. The *Echis carinatus* venom has been noted to be one of the coagulant venoms producing disseminated intravascular coagulation (DIC). This effect is produced through the conversion of prothrombin to thrombin. The venom injection produces intravascular minute fibrin clots (through the conversion of Prothrombin to Thrombin) followed by incoagulability of the blood (through the activation of fibrinolysin enzyme, which causes the dissolution of fibrin clots) resulting to marked prolongation of the clotting time (Mohamed *et al*., 1969).

The effect of the extract of *Paullinia pinnata* correcting these occurrences may be an indication of therapeutic potentials of the plant in correcting blood clotting disturbances in snakebite envenomation. The present result establishes the fact that treatment of the animals with extract/venom mixture abolishes the blood incoagulability. The prevention of blood incoagulability (clot failure) by the extract may be considered as evidence of neutralization of the clot disturbance effect of the venom. The results suggest that extract of *Paullinia pinnata* has haemostatic effect, which is in accord with the traditional use of the plant.

Carpet viper bite envenomation also causes disturbances of haematological values in the patients. Clinical effects of *Echis carinatus* venom poisoning depress platelet counts and increase polymorph leukocytosis (Warrell *et al*., 1977, and Theakston and Reid, 1983). The increase in PCV in *Detarium microcarpum* treated animals may be as a result of dehydration due to diuretic effect of the extract. Leucocytosis occurred in venom treated rats possibly because of tissue necrosis and inflammatory reactions by the venom. The two extracts abolished these effects due in part to anti-inflammatory effect of *Detarium microcarpum* and antihistaminic effect of *Paullinia pinnata*. The overall results indicate that the extracts of *Paullinia pinnata* and *Detarium microcarpum* are protective against the blood cells from being adversely affected by *Echis carinatus* venom.

Histamine is a well known potent vasodilator substance and increases the vascular permeability (Gupta et al, 2003). Shock, which arises from increased vascular permeability, is the main cause of death after viper bite envenomation (Reid and Theakston, 1993; Warrell *et al*., 1977). The condition occurs as a result of the release of pharmacologically active substances such as histamine from the tissues by the venom leading to peripheral vasodilatation. The increase in capillary permeability by histamine causes the outward passage of plasma proteins and fluid into extracellular spaces and formation of oedema. This ultimately results into loss of plasma volume, and eventually

circulatory collapse and irreversible shock (Bowman and Rand, 1983). The study show that the venom increased capillary permeability in the rabbits and this was annulled by the extract of *Paullinia pinnata.* However, this effect was not observed in animals treated with the mixture of venom and *Detarium microcarpum* extract. The extract of *Paullinia pinnata* impressively antagonized the effect of *Echis carinatus* venom comparable to that of the standard anti-histamine, promethazine. This result indicates that the extract of *Paullinia pinnata* exhibits its action by means of inhibiting the release or action of the mediator. This outcome supports the findings of Osman and Gumaa (1974) which states that capillary permeability is antagonized by pretreatment of the animal with promethazine.

The study also demonstrates that an aqueous extract of *Detarium microcarpum* (400ml/kg, i.p) produces significant increase in urinary excretion of water and electrolytes, while *Paullinia pinnata* extract not perform. This finding suggests that the extract of *Detarium microcarpum* contains compounds that possess diuretic effect. However, the extract of *Paullinia pinnata*, which could not increase urinary excretion of water and electrolytes, might have anti-diuretic effect or the kidney function impaired. The clinical implication is that the extract of *Detarium microcarpum* may be useful in suppressing oedema which often accompanies snake venom poisoning.

Echis carinatus venom has been implicated in blood glucose disturbance. It produces hyperglycemia as well as loss of liver and muscle glycogen (Mohamed *et al*., 1963). The hyperglycemia occurs as a result of mobilization of glycogen in liver and muscle which may arise from the direct action of the *Echis carinatus* venom in either inhibiting the activity of glucokinase or indirectly stimulating the release of adrenaline (Greene and Harris, 1996; Lullmann *et al*., 2000; Mohamed *et al*., 1963; Osman and Gumaa, 1994). The results of this work reveal that aqueous extract of *Paullinia pinnata* does not relieve the hyperglycaemia produced by the venom. The *Detarium microcarpum* extract however, aborts the hyperglycaemic effect of the venom. The result therefore suggests that extract of *Detarium microcarpum* exerts hypoglycaemic activity in venom-induced hyperglycaemia and this activity seems to occur through the same pathway by which the *Echis carinatus* venom produces hyperglycaemia. The action of the extract probably occurs by protecting the enzyme glucokinase or blocking the release of adrenaline.

Echis carinatus envenomation is known to produce local swelling (oedema) and tissue necrosis at the site of bite (Warrell *et al*., 1977). The reactions may be due to either the presence of proteolytic enzyme component of the venom or the release of histamine from tissues poisoned by the venom. In the course of the treatment of snakebite poisoning, application of adjuvant therapeutic remedies are necessary to prevent local tissue damage (Reid and Theakston, 1983). The extract of

Paullinia pinnata antagonized the oedema formation at the site of venom injection. The antihistamine, promethazine, also inhibited the development of local reactions by the venom at site of injection. These results suggest that extract of *Paullinia pinnata* antagonizes the release of histamine from the tissues by inhibiting the proteolytic enzyme of the venom at the site of injection. These findings also concur with the experimental works of Mohamed *et al* (1968), and Mohamed *et al* (1971). However, the extract of *Detarium microcarpum* did not affect the local effects of the venom. This therefore suggests that the extract may not have effect on the proteolytic enzyme of the venom which releases tissue histamine.

The aqueous extracts of *Paullinia pinnata* (30µg/kg) and *Detarium microcarpum* (15µg/kg) in sodium thiopental anaesthetized cats depressed the blood pressure. The hypotensive effect of *Detarium microcarpum* was neither blocked nor potentiated by cyproheptadine. However, the hypotensive effect of *Paullinia pinnata* was potentiated by cyproheptadine indicating the involvement of serotonin receptors. The pretreatment of animals with indomethacin, markedly blocked the hypotensive effect of *Detarium microcarpum* but not the hypotensive effect of *Paullinia pinnata*. This result also indicates that the extract of *Detarium microcarpum* released prostaglandins which caused the decrease in the blood pressure. However, promethazine (2.5mg/kg) did not affect the hypotensive activity of the two extracts. This means that

histamine was not involved. The pretreatment of the cats with adrenaline, markedly blocked the hypotensive effect of *Paullinia pinnata* but no effect on the blood pressure of the animals treated with *Detarium microcarpum*. These findings suggest that the extract of *Paullinia pinnata* probably elicits its hypotensive effect through action at β–adrenergic receptors, while the effect of *Detarium microcarpum* may be through arachidonic metabolic pathways in which the prostaglandins are involved.

Echis carinatus venom is known to produce hypotension (Osman and Gumaa, 1974; Ismail *et al*., 1972; and Reid and Theakston, 1983). The pretreatment of the cats with small dose of *Paullinia pinnata* blocked the hypotensive effect of the venom (5µg/kg). However, higher dose of the venom (10µg/kg) after the extract, very markedly reduced the B.P in one of the animals which could not be reversed by the extract of *Paullinia pinnata* and adrenaline, and the animal died. Although the cause of the death in venom/extract treated animal is not clear, haematotoxicity and cardiotoxicity may not be ruled out.

Snake venom neurotoxins bind to acetylcholine (Ach) receptors at the motor end plate (Tu 1977). In skeletal muscles, the venom interrupts neuroeffector transmission mainly by a post-junctional mechanism and is of the non-depolarizing type of blockade. The venom binds selectively and irreversibly to acetylcholine receptors with the skeletal muscle relaxation followed by total spastic paralysis of the diaphragm muscles with loss of respiration (Bowman and Rand, 1983). The venom exhibits

neuromuscular mode of action by interfering with transmission of neuromuscular impulses at neuromuscular junction by inhibiting the enzyme acetylcholinesterase (AChE) which is responsible for inactivating the neurotransmitter at the synapse (Venkatrao *et al*, 2007). Plant extracts with direct anti-venom activity would involve complexation of the active compounds with venom constituents, thus rendering them unable to act on the receptors; or to act by competitive blocking of the receptors (Lans et al, 2001).

The effect of the aqueous extracts of the two plants was evaluated on skeletal muscle preparations of rat phrenic nerve hemi-diaphragm. It was performed to demonstrate whether or not the crude extracts exhibit activity on skeletal muscle (Tu, 1977). The phrenic nerve hemi-diaphragm preparation allows observation of nerve-mediated muscle contraction and its modification by the extracts. The extracts of *Detarium microcarpum* and *Paullinia pinnata* increased contraction of the nerve stimulated diaphragm muscle. The increase was 55% and 45% more than that produced by the control, respectively. The extracts of *Paullinia pinnata* and *Detarium microcarpum* were found to produce a dose dependent increase in the amplitude of the nerve stimulated twitch of the rat diaphragm-phrenic nerve preparation. The addition of *d*-tubocurarine solution to the organ bath failed to reverse the increase in contraction of the phrenic nerve diaphragm by leaf extract of *Detarium microcarpum* (Figure 3 iii). The muscle contractions by the extract of *Paullinia pinnata*

were significantly depressed by *d*-tubocurarine (a competitive Ach receptor blocker) as compared to the controls (Figure 3 iii). These data suggest that the increase in contraction by *Detarium microcarpum* may occur through a mechanism other than blocking Ach receptors. Whereas, the action of *Paullinia pinnata* is mediated through Ach receptors since the depressant action of *d*-tubocurarine in the presence of *Paullinia pinnata* was effectively reversed by neostigmine.

The extracts of *Paullinia pinnata* and *Detarium microcarpum* also exhibited agonist effect in isolated rabbit jejunum by depressing the spontaneous contractions of tissue in a manner similar to that of adrenaline. The spontaneous contractile activity of the tissue was reduced significantly ($p < 0.05$) by *Paullinia pinnata*, exhibiting potent β-sympathomimetic activity, and was effectively blocked by atenolol, a β_1 adrenergic receptor blocker. The agonist effect of *Detarium microcarpum* was however blocked by prazosin, suggesting an α-sympathomimetic activity. The extracts of *Paullinia pinnata* and *Detarium microcarpum* were found to block specific receptors (cholinergic and histamine) via which acetylcholine and histamine had previously initiated contractions in guinea pig ileum. *Paullinia pinnata* aqueous extract depressed the histamine and Ach-induced contractions. The extract, therefore, may have histamine, as well as cholinergic blocking activity. The strong blocking effect against histamine-induced contractions supports the effect of this extract against local effects of

Echis carinatus venom in the animals. The extract of *Detarium microcarpum* inhibited contractions produced by acetylcholine on the guinea pig ileum. However, this effect was not observed in contractions produced by histamine. The extract of this plant may have some active compounds that possess anti-cholinergic effect. The overwhelming evidence may therefore point to interference with cholinergic transmission. Possibly, the extract contains anti-cholinergic agent(s) that could block the autonomic cholinoceptors.

In conclusion, the results of the present study indicate the presence of anti-carpet viper (*Echis carinatus*) venom compounds in the roots of *Paullinia pinnata* Linn and leaves of *Detarium microcarpum* Guill and Perr which can neutralize the effects of the venom. The extracts have also been found to possess multiple pharmacological properties such as antibacterial, analgesic, antipyretic, and anti-inflammatory activities which can alleviate symptoms accompanying envenomation. The potent action of the extracts of *Paullinia pinnata* and *Detarium microcarpum* in reducing morbidity and mortality in animals provides some scientific support for their use in the traditional management of snake venom poisoning. There may be need therefore for more studies to assess, isolate and characterize compounds responsible for the antivenom activity as well as the pharmacological properties of the extracts of the two plants.

5.2 **SUMMARY OF FINDINGS**

Paullinia pinnata Linn and *Detarium microcarpum* Guill and Perr are West African savannah plants used in traditional medical practice for the treatment of various disease conditions including snakebite poisoning. The effects of *Paullinia pinnata* and *Detarium microcarpum* on *Echis carinatus* venom were investigated in animals and on some isolated tissues. Findings from phytochemical screening indicate that the aqueous extract of *Paullinia pinnata* root bark contains carbohydrates, saponins, steroids, and tannins, while *Detarium microcarpum* has anthraquinones, flavonoids, saponins, steroids, and tannins. The trace metals of iron, calcium, zinc, and lead are present in the two extracts. The aqueous extracts of *Paullinia pinnata* root bark and *Detarium microcarpum* leaves given to mice significantly reduced mortality in *Echis carinatus* venom treated animals. The extracts of the plants also exhibited antibacterial activities against wound causing bacteria of *Staphylococcus aureus, Proteus mirabilis,* and *Pseudomonas aeruginosa*. Extract of *Paullinia pinnata* inhibited growth of all test organisms while *Detarium microcarpum* was active against all test organisms except *Staphylococcus aureus*. *Detarium microcarpum* was only active against *Proteus mirabilis* in higher concentration. The extract of *Paullinia pinnata* also significantly (1) restored blood coagulability in rats treated with the venom; (2) annulled local effects of the venom in rabbits; (3) raised blood glucose level in venom treated rabbits; (4) exhibited antidiuretic effect in

rats; (5) inhibited capillary permeability increasing properties of *Echis carinatus* venom in rabbits; and (6) counteracted effects of venom on blood cell indices in rats. The extract, however, did not exhibit analgesic, antipyretic, or anti-inflammatory effect in mice or rats respectively. The extract of *Detarium microcarpum* exhibited analgesic, antipyretic, anti-inflammatory, diuretic, and hypoglycaemic effects in animals; the properties of which extract of *Paullinia pinnata* does not possess. The extracts of the two plants lowered blood pressure of anaesthetized cats even in the presence of standard antagonists like cyproheptadine, and promethazine. This effect was annulled by indomethacin and adrenaline, indicating the involvement of arachidonic metabolic and adrenergic pathways. *Paullinia pinnata* and *Detarium microcarpum* extracts enhanced phrenic nerve-stimulated contraction of hemi-diaphragm muscle preparations. The effect of *Paullinia pinnata* on the tissue was blocked by *d*-tubocurarine but neostigmine restored the contraction. The extracts also depressed the spontaneous contractions of isolated rabbit jejunum. The effect of *Paullinia pinnata* extract on this tissue was blocked by atenolol but not prazosin, while effect of *Detarium microcarpum* was blocked by prazosin only. The extract of *Paullinia pinnata* blocked the effect of acetylcholine and histamine on guinea pig ileum. The extract of *Detarium microcarpum* also blocked effect of acetylcholine on the same tissue but did not affect the effect of histamine. The overwhelming evidence that the extracts of the two plants can reduce morbidity and mortality in venom treated animals, suggests that the medicinal plants contain active principles that can be useful in the

treatment of venom toxic effects and lethality arising from *Echis carinatus* (carpet viper) bite. The results of this study have therefore provided some scientific support for their use in the traditional management of snakebite patients.

5.3 **CONTRIBUTION TO KNOWLEDGE**

The concept of testing medicinal plants for pharmacological activity is underlined by the fact that some traditional herbal medicines have proved, on investigation, to be of value in orthodox medicine. It is on this basis that useful drugs have been developed from plants, some of which were originally used in traditional medicine. The study therefore is innovative because it has increased awareness in the development and use of herbal medicine for the treatment of snakebite patients.

The evaluation of pharmacological properties of *Paullinia pinnata* and *Detarium microcarpum* for antivenom activity has made significant contribution to knowledge creation in scientific world. This is because the research work has been able to identify strengths and gaps in the use of conventional antivenoms and came up with new methodologies of developing antivenoms from plants to meet the challenges.

The extracts of *Paullinia pinnata* and *Detarium microcarpum* have significantly reduced morbidity and mortality without deleterious effects in animals. The results of this study suggest that the roots of *Paullinia pinnata* and leaves of *Detarium microcarpum* contain compounds with better anti-carpet viper (*Echis carinatus*) venom properties. The findings

have therefore provided an insight into the possibility of sourcing from plants cheap and effective antivenoms suitable for emergency treatment of snakebite poisoning.

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GLOSSARY OF TECHNICAL TERMS AND ABBREVIATIONS

A6	The extract of *Paullinia pinnata*; a medicinal plant used by traditional healers in the treatment of various disease conditions including snakebite poisoning.
A11	The extract of *Detarium microcarpum*; a medicinal plant used by traditional healers in the treatment of various disease conditions including snakebite poisoning.
Bitis arietans	Puff adder
B.P	Blood pressure or British pharmacopoeia
Causus masculatus	Night adder
Dispholidus typus	Boomslang
DBP	Diastolic blood pressure
Ecarin	Procoagulant enzyme contained in viper venom that activates prothrombin to thrombin
Echis	The genus which encompasses all species of the snake, carpet viper

Echis carinatus	The species of carpet viper or saw-scaled viper that causes severe morbidity and fatalities in human populations. It is commonly found in sub-Saharan Africa.
Echis carinatus ocellatus	The sub-species of carpet viper that occurs in Nigeria; and indeed West Africa
ECV	*Echis carinatus* venom; venom of the carpet viper
ED_{50}	Effective dose 50%; the dose that cures by 50% *in vivo*. The lower the ED_{50}, the more potent is the drug.
In vitro	In the test tube, as opposed to experiments in animals
In vivo	In living being (animal)
i.p	Intraperitoneally; injected into the peritoneum
Haemorrhagin	Blood vessel wall damaging substance contained in viper venom
LD_{50}	Lethal dose 50%; the dose that will kill 50% of target animals: the lower the LD_{50}, the more toxic is the substance.
MAP or MABP	Mean arterial blood pressure
MBC	The minimum concentration of antimicrobial agent needed to yield a 99.9% reduction in viable colony forming units of a bacterial or fungal suspension.

MIC	The minimum concentration of antimicrobial agent needed to prevent visually discernible growth of a bacterial or fungal suspension.
MLD	Minimum dose which kills all the untreated animals in a group
Naja species	Cobras
Naja haje	Egyptian cobra
Naja melanoleuca	Black cobra, forest cobra
Naja nigricolis	Spitting cobra, black-necked cobra
Ophiophagus hannah *(Naja hannah)*	King cobra
p.o	Per os (by mouth)
ppm	parts per million
SBP	Systolic blood pressure
TI	Therapeutic index, the ratio of the dose of drug needed to cause a toxic effect in or kill, a certain proportion of the population (lethal dose or LD) to the dose needed to effect cure in a certain proportion of the population (effective dose or ED).

APPENDIX A

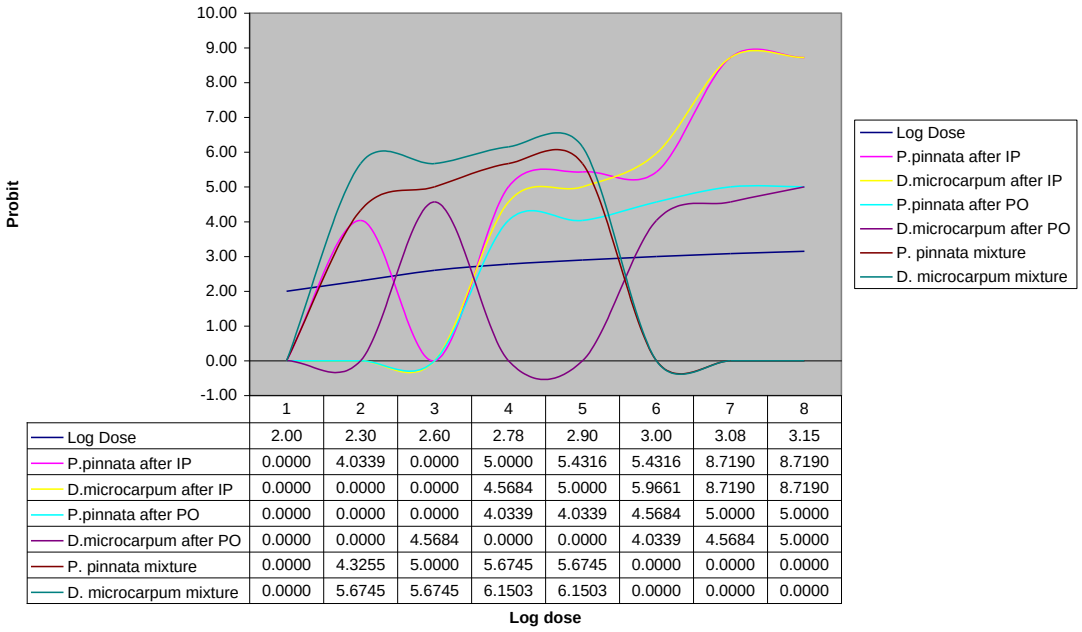
COMPOSITION OF PHYSIOLOGICAL SALT SOLUTIONS COMMONLY USED IN ISOLATED TISSUE EXPERIMENTS (g/l)

Composition	Frog Ringer	Krebs	Ringer Locke	De Jalon	Mc Ewens	Tyrode's
NaCl	6.5	6.9	9.0	9.0	7.6	8.0
KCl	0.75	0.35	0.42	0.42	0.42	0.2
$CaCl_2$	1.0	0.28	0.24	0.06	0.3	0.2
$NaHO_3$	0.4	2.1	0.5	0.5	2.1	1.0
NaH_2PO_4	-	-	-	-	0.14	0.05
$MgCl_2$	-	-	-	-	-	0.1
$MgSO_47H_2O$	-	0.29	-	-	-	-
Glucose	-	2.0	1.0	0.15	20.	1.0
Sucrose	-	-	-	-	4.5	-
KH_2PO_4	-	0.16	-	-	-	-
Aeration	Air	$O_{2\ (95\%)}+CO_{2(5\%)}$	O_2	$O_{2\ (95\%)}$ + $CO_{2\ (5\%)}$	$O_{2\ (95\%)}$ +$CO_{2\ (5\%)}$	O_2/Air
Typical tissue	Amphib-ian	Mammalian + Avian Skeletal muscle	Heart muscle	Uterus	Urinary tract tissues	Intestin-al tissues

Courtesy: Pharmacology Laboratory, University of Jos, Nigeria

APPENDIX B

THE GRAPH OF LOG DOSE OF PAULLINIA PINNATA AND DETARIUM MICROCARPUM EXTRACTS AGAINST PROBIT OF ANIMAL SURVIVAL



	1	2	3	4	5	6	7	8
Log Dose	2.00	2.30	2.60	2.78	2.90	3.00	3.08	3.15
P.pinnata after IP	0.0000	4.0339	0.0000	5.0000	5.4316	5.4316	8.7190	8.7190
D.microcarpum after IP	0.0000	0.0000	0.0000	4.5684	5.0000	5.9661	8.7190	8.7190
P.pinnata after PO	0.0000	0.0000	0.0000	4.0339	4.0339	4.5684	5.0000	5.0000
D.microcarpum after PO	0.0000	0.0000	4.5684	0.0000	0.0000	4.0339	4.5684	5.0000
P. pinnata mixture	0.0000	4.3255	5.0000	5.6745	5.6745	0.0000	0.0000	0.0000
D. microcarpum mixture	0.0000	5.6745	5.6745	6.1503	6.1503	0.0000	0.0000	0.0000