COMPARATIVE ANTIBACTERIAL, ANTI-VENOM AND PHYTOCHEMICAL STUDIES OF *INDIGOFERA CAPITATA* KOTSCHY AND *INDIGOFERA CONFERTA* GILLETT IN ALBINO RATS

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COMPARATIVE, ANTIBACTERIAL, ANTI-VENOM AND HYTOCHEMICAL STUDIES OF *INDIGOFERA CAPITATA* KOTSCHY AND *INDIGOFERA CONFERTA* GILLETT IN ALBINO RATS

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DECLARATION

I declare that the work in the thesis entitled "Comparative Antibacterial, Antivenom and Phytochemical Studies of *Indigofera capitata* Kotschy and *Indigofera conferta* Gillett in albino Rats" has been performed by me in the Department of Biological Sciences. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree at any University.

______________________	______________________	______________________
Name of Student	Signature	Date

CERTIFICATION

This thesis entitled "COMPARATIVE ANTIBACTERIAL, ANTIVENOM AND PHYTO-CHEMICAL STUDIES OF *INDIGOFERA CAPITATA* KOTSCHY AND *INDIGOFERA CONFERTA* GILLETT IN ALBINO RATS" by Saidu KADIRI meets the regulations governing the Award of the Doctor of Philosophy Degree in Botany of Ahmadu Bello University, Zaria, Nigeria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my late Father Abdulkadir, my late Mother Hauwa, My Uncle Ibrahim Adamu and all members of my family.

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ABSTRACT

This study was conducted to establish and compare anti-bacterial, anti-venom and phytochemical properties of *Indigofera capitata* and *I. conferta* in albino rats. The two species of plants belong to the family Fabaceae. They are found growing on red soil or old farm land. The two plant materials were collected, dried, grounded and successively extracted using maceration with petroleum ether, ethanol and aqueous. The extract of each solvent of the two plants was used for acute toxicity studies, *in-vitro* anti-bacterial and *in-vivo* anti-venom studies: Oedema forming activity, bleeding and clotting time and histopathological examination of liver, kidney and heart were also carried out. The most active extracts (ethanol and aqueous) were further subjected to thin layer chromatographic (TLC) studies. Antibacterial activities of all the extracts (petroleum ether, ethanol and aqueous) of both plants showed significant effects (P<0.05) against the growth of *Staphylococcus aureus* and *Salmonella typhi. Indigofera conferta* petroleum ether extract had MIC of 6.2 mg/ml and 12.5 mg/ml and MBC of 12.5 mg/ml and 25.0 mg/ml, ethanol extract showed MIC of 12.5 mg/ml and MBC of 25.0 mg/ml and aqueous extract showed MIC of 6.25 mg/ml and 25.0 mg/ml and MBC of 12.5 mg/ml and 50.0 mg/ml while *Indigofera capitata* petroleum ether had MIC of 3.125 mg/ml and 50.0 mg/ml and MBC of 6.25 mg/ml and >50.0 mg/ml, ethanol extract MIC of 12.5 mg/ml and 25.0 mg/ml and MBC of 25.0 mg/ml and 50.0 mg/ml, aqueous extract had MIC of 3.125 mg/ml and 25.0 mg/ml and MBC of 6.25 mg/ml and 50.0 mg/ml against the two bacterial strains respsctively. The results of bleeding and clotting time showed that, all the extracts of both plants showed significant (P<0.05) reduction in the bleeding and clotting time of envenomed rats. Ethanol and aqueous extracts of

Indigofera capitata were more effective at dose of 300 mg/kg with lowest clotting time of 174±3.67 second and 1000 mg/kg with lowest bleeding time of 228±3.00 second while in the case of *Indigofera conferta* at high dose of 1000 mg/kg with lowest clotting time of 173±5.61 second (ethanol extract) and lowest bleeding time of 234±7.64 second (aqueous extract) compared with the positive control. Oedema forming activity was also inhibited by ethanol and aqueous extracts of the two plants and the effects were more effective at higher dose of 300 mg/kg(ethanol extract) and 1000 mg/kg (aqueous extract) with lowest oedema forming activity of 108.80±1.90 and 102.00±1.90 (%mm) respectively of *Indigofera capitata* while *Indigofera conferta* at dose of 250 mg/kg, 500 mg/kg and 1000 mg/kg of aqueous extract with the lowest oedema forming activities of 100.8±1.89, 100.20±1.90 and 100.60±1.90 (percentage millimeter) respectively compared with the positive control. The histopathological examination of the kidney, heart and liver showed that ethanol and aqueous extracts of both plants were found to have more tissues protective effects at doses of 300 mg/kg (ethanol extract) and 1000 mg/kg (aqueous extract of *Indigofera capitata* while *Indigofera conferta* at doses 500 mg/kg and 1000 mg/kg of both ethanol and aqueous extracts against the *N. nigricollis* envenomed rats. Thin layer chromatographic profile of the most active extracts (ethanol and aqueous) indicated that, both plants *contained* flavonoids, phenolic compounds, steroids, triterpenes, anthraquinone and alkaloids. The present studies had established that, the two plant species had antibacterial and antivenom activities especially against *Naja nigricollis* as claimed by some traditional medical practitioners and therefore isolation of the active constitutes in the two plants should be carried out.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 PLANT RESOURCES AS POTENTIAL TRADITIONAL MEDICINE

Many natural products isolated from plants serve as “template” or lead molecules for the design and generation of completely new drugs. The potential of higher plants as sources of new drugs is still largely unexplored. Among all the different species of plants in the world very few have been investigated phytochemically and the fraction subjected to biological or pharmacological screening (Aida *et al.,* 2001).

Traditional medicine is the most ancient art of medical practices on which many of rural populations of developing countries rely for their health care, due to economic accessibility and the belief that natural products are more acceptable to the body and as such have fewer side effects than synthetic drugs (Aida *et al.,* 2001). Despite the fact that traditional medical practices are limited by lack of precise diagnosis, standardization, hygiene, ethics in dosaging and sometime claims or exaggerated claims which cannot be proved scientifically; traditional medicine still enjoys a wide acceptability among the citizens of developing countries. Most bioactive substances from plants are discovered as a result of scientific investigations of the plants used in traditional medicine (Aida *et al., 2001).*

Most plants have a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial

effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. At least 12,000 of such compounds have been isolated so far; a number estimated to be less than 10 % of the total (Aida *et al.,* 2001).These phytochemicals are divided into primary metabolites such as sugars and fats, which are found in all plants; and secondary metabolites, compounds which are found in a smaller range of plants, serving specific functions. For example, some secondary metabolites are toxins used to defend the plants and others are used to attract insects for pollination. Secondary metabolites have therapeutic actions in humans and can be refined to produce drugs. Chemical compounds in plants mediate their effects on the human body through processes similar to those chemical compounds used in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects (Mesfin *et al*., 2009).

In all countries of the world there exists traditional knowledge related to the health of humans and animals. The definition of traditional medicine may be summarized as the sum total of all the knowledge and practice, whether explicable or not, used in the diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing. Traditional medicine might also be considered as a solid amalgamation of dynamic medical known-how and ancestral experience. The interest in traditional knowledge is more and more widely recognized in policies, the media and sciences (Mesfin *et al*., 2009). In Africa, traditional healers and

remedies made from plants play an important role in the health of millions of people. Traditional medicine is one of the surest means to achieve total health care coverage of the world's population (Techacondo *et al*., 2011)

1.2 DESCRIPTION AND DISTRIBUTION OF *INDIGOFERA* SPECIES

Indigofera is the generic name given to some group of plants that belongs to the family Fabaceae. The generic name was derived from the colour dye called Indigo produced by member of the species. About 76 species of *Indigofera* are found in tropical West Africa out of which 62 species are found in Nigeria. In the Northern Nigeria among the Hausa – Fulani tribes the generic name given to all members of *Indigofera* species is called "Baba". Members of *Indigofera* species are annual or perennial herbs or shrubs ranging from 1 to 5 feet high depending on the environmental conditions. Flowers are usually in axillary racemes, less often in open or sub-capitata panicles which appear single in the leaf axil or denser axillary cluster. Corolla is usually red or pink. Fruits (pods) are usually dehiscent; possess one seed by abortion or two to many seeds. The shape of the pod may be cylindrical, tetragonal or flattened. Calyx is about 6–8 mm long. Endocarp often spotted forming septa between the seeds in some species (Tamilveslvi *et al.,* 2011).

1.3 STAPHYLOCOCCI

Staphylococci are gram–positive, spherical or slightly oval, non-motile, non-capsulated, non-sporing micro-organism of about 0.5 to 1µm in diameter. They have the tendency to grow in cluster. Their shape is coccoid and the cells are in cluster form with golden

yellow colour. There are two species of staphylococci i.e. *S. epidermidis* and *S. aureus*. *Staphylococcus aureus* is the most important pathogenic microbe from the medical points of view because it is one of the most frequent microbes associated with human disease (Hamdan *et al.,* 2010) and may cause disease in any part of the body of human such as skin, gastro-intestinal, urinary tract and cardio-vascular system. The organism grows well on ordinary media like media containing high concentration of sodium chloride and blood agar (Hamdan *et al.,* 2010).

1.4 *SALMONELLA*

Salmonella are gram-negative short rod-organisms, motile, non-sporing, non-capsulated *Bacilli.* They are rod-shape and cells arrangement in circular form with pale or colourless colour. The cells form colony and are pale or colourless on mac-conkey agar medium. They grow readily on nutrient agar over a wide temperature range with an optimum of 37°C. The colonies are characteristically circular in outline with a smooth glossy surface (Hatta *et al.,* 2002).There are two major common species of *Salmonella* i.e. *S. typhi* and *S. enteritidis*. The S. *typhi* was named after the disease typhoid fever. *Salmonella typhi* is responsible for the disease typhoid fever, the disease occurred as a result of ingestion of water or food contaminated by this pathogen (Hatta *et al.,* 2002). About 2 - 4 percent of typhoid, patients become chronic carriers of the infection. In view of the higher incidence in typhoid carriers infection of the bile-duct with liver fluke may be a factor responsible for the persistence of *S. typhi* within the liver (Den *et al.,* 2003). *Salmonella* is a highly infectious organism, rampant in places where public sanitation and health measures are inadequate. It is often found endemic along with dysentery and

cholera in parts of the world, such as Africa, Asia, and Latin America where *Salmonella* infection is directly related to the contamination of water, milk and food. The symptoms of the disease are headache, abdominal discomfort, joint pain, constipation or less commonly diarrhea, vomiting, cough and backache are the most frequent symptoms (Den *et al.,* 2003).

1.5 CLASSIFICATION AND MORPHOLOGY OF SNAKES

Snakes are cold blooded vertebrate animals of the class Reptilia and order Squamata, which include lizards, alligators, crocodiles, turtles and tortoises. About 3000 snake species are known worldwide and most of them are non-venomous. Some snake species have special glands that produce venom which are mostly derived from salivary gland. The venom performed several functions which include rapid immobilization and predigesting of prey. Snakes arose during the createaceous era (100-120 million years ago). The poisonous forms are more recent, existed during the Miocene era, (30 million years ago). Snakes can be divided into families and the groupings were made based on the presence of fangs (Shine *et al.,* 2007). Physical characteristics are used to identify a snake species. The characters observed are used to ease identification of poisonous snakes and non poisonous snakes. These characters are body shape, head and neck shape, length, colour pattern, scale texture, eye pupil shape, tail scales and plate division. Geographical location, habit and habitat also provide information of identifying snake. Eye pupil shape is a reliable indicator for determining whether a snake is poisonous or not. Snakes with elliptical vertical eye pupils resembling the eye of a cat are poisonous while those with round shape eye pupils that resemble the eye of

man are snakes belonging to a category of nonpoisonous. Most poisonous snakes have a single row of scales under the tail beginning at the vent while most non-poisonous snakes have a double row of scales running the length of the underneath of the tail (Shine *et al.,* 2007).

1.6 GENERAL DESCRIPTION OF SPECIES OF COBRA

Snakes are considered to be the second most special group of reptiles consisting of over 2,900 species. One particular snake that is unique and well- adaptive in its character and defense behaviour is the spitting cobra (*Naja nigricollis*). Spitting cobra belongs to the family Elapidae; consist of several other species such as members of coral snakes, Tiponds and Kraits (Luiselli *et al.,* 2002). *Naja nigricollis* are documented to belong to the most diverse and wide spread genus of cobras. They are medium sized, less than two meters and black in colouration. *Naja nigricollis* belongs to the most venomous and widely distributed snake family. *Naja migricollis* has become one species of interest due to its ecological success in dietary type of prey and size in an altered habitat (Luiselli *et al.,* 2002). Spitting cobras are categorized as generalist predators. They are well equipped to prey on a wide variety of small vertebrates (Luiselli *et al.,* 2002). They have an adaptive capability in which they could prey on several different species of other animals when exposed to different microhabitats. When spitting cobra feels threatened it exhibits a defensive behaviour. *Naja nigricollis* defend themselves by spitting their venom in the face of their harassers and predators, thus given the common name spitting cobra. The venom of the spitting cobra is harmful to the eyes of victims (Luiselli *et al.,* 2002).

1.6.1 TAXONOMY OF COBRA SNAKES

The common name "Cobra" is applied to about thirty (30) species of snakes in seven (7) genera (*Aspidalaps, Boulengerina, Hemachatus, Naja, Ophiophagus, Pseudohaje and Walkerinnesia*) within the family Elapidae (Shine *et al.,* 2007). The term 'Cobra' is abbreviated from the Portuguese "Cobra de pello", which means snake with hood. Hence, cobra refers to any species in the family Elapidae that can produce a hood when threatened (Shine *et al.,* 2007).

1.6.2 SPECIES OF COBRA AND THEIR COMMON NAMES

Cobras are grouped into seven, based on their generic and common names (Shine *et al.,* 2007).

1. Genus: *Aspidelaps* (common name Shield nose Cobra)
2. Genus: *Boulengerina* (common name water cobra)
3. Genus: *Hemachatus* (common name spitting cobra)
4. Genus: *Naja* (common name Black cobra)
5. Genus*: Ophiophagus* (common name King cobra)
6. Genus: *Aseudohaje (*common name Forest cobra)
7. Genus: *Walter inesia* (common name Black desert cobra)

The genus *Naja* is comprised of five (5) species. These include; *Naja naje, Naja naja, Naja melanoleuca, Naja nigricollis* and *Naja nivea*.

1.7 *NAJA* SPECIES (SPITTING COBRAS) FAMILY ELAPIDAE

Many species of *Naja* are found in Africa with variable colours. The most common ones found in Nigeria are *N. nigricollis, Naja melanoleuca*, *Naja goldii* and *Naja nigricollis* (black spitting cobra or black necked cobra) is black with pink across of varying width alternating with the black colour. The spitting cobra is not usually aggressive but when molested it will attack seriously and persistently. *Naja melanoleuca* (the black and white cobra) has a similar appearance with the spitting cobra but usually has white or yellowish marking on the back. *Naja goldii* which is usually found in trees and low shrubs, is rather not hooded and attacks without been molested. *Naja haje* is an Egyptian cobra but occasionally found in the semi-desert areas of Northern Nigeria particularly in Sokoto. It is hooded, brown coloured with a dark band on the neck (Luiselli *et al.,* 2002).

The most common cobras found in Nigeria are *N. nigricollis*, *Naja katiensis* and *Naja mossambica*. *Naja katiensis* is rare but mostly found in Northern Nigeria especially around the Katsina and Malunfashi axis (Luiselli *et al.,* 2002). All species of *Naja* (cobras) are fast moving animals that are very active in the night and may have grooved fangs which when provoked will spit, squirting a stream of extremely painful venom to a distance of 2 meters from their fangs. All spitting cobras are highly secretive and more commonly found in and around a quite environment in villages. The venom is highly poisonous and a bite can cause a lot of tissue destruction if untreated. Cobra venom contains large quantities of acetyl cholinesterase which has the effect of lowering the acetylcholine levels at the synaptic cleft and thus has a co-operative effect with the neurotoxins in blocking signal transduction (Luiselli *et al.,* 2002).

Plate 1.1: *Naja nigricollis* (Black spitting Cobra

1.8 ANTI- SNAKE VENOM AND ITS LIMITATIONS

Anti-venom for ophidian bite is a suspension of antibodies prepared mainly by injecting the venom into the body of animal e.g. horse. Such animals like horses are hyper-immuinized against the venom of a given species of snake (monovalent) or venom from several different species of snakes at the same time (polyvalent). The animals injected with the venom undergo an immune response to the venom, thereby produced antibodies against the venoms active molecule which can then be harvested from the animal's blood and used to treat envenomation and regarded as monovalent or polyvalent. Infusion of these anti-snake venoms may lead to adverse reaction ranging from early reaction to potentially fatal anaphylaxis (Bawaskar, 2004). According to Bawaskar (2004), the incidence of these reactions varies from 5 to 80 %. There are also pyrogens reactions (rise in temperature in the victim body) due to endotoxin contamination and sickness may also develop in certain cases (Mahadeswaraswany *et al.,* 2009). The production and supply of anti-venom is associated with logistical, marketing, storage and economic difficulties. The development is costly time-consuming process requiring ideal storage conditions. The liquid form of antisnake venom has a half-life of two (2) years. Storage at 0-4^{0}C is necessary, otherwise rapid deterioration set in rendering it unfit for use (Mahadeswaraswany *et al.,* 2009).

1.9 CLASSIFICATION OF ANTI-VENOM OF SNAKE

Anti-venom is classified into two types i.e. (a) mono-valent anti-venom which is very effective against given species venom (b) Polyvalent which are effective against a range of species (Goswami *et al.,* 2014).

1.10 STATEMENT OF THE RESEARCH PROBLEMS

i. *Indigofera capitata* and *I. conferta* have some diagnostic phytochemical and biological properties that have not been established.

ii. Snake bites mostly of the poisonous ones such as the spitting Cobra is most common in rural areas where access to hospital is difficult; orthodox treatment is quite expensive and complicated, hence alternative mode of treatment from medicinal plants need to be searched, thus to help rural dwellers on how to overcome such problems of snake bite

iii. Snake bite till date remains a public health hazard in tropical countries. Snake venom is the most complex of all poisons being a mixture of enzymatic and none enzymatic toxic compounds as well as other non-toxic proteins, non-protein including carbohydrate and metals that are stored in poison glands (Antony *et al.,* 2010).

iv. The only available treatment against snake bite is the usage of anti-venom. The first anti-venom was developed by Alberte calmette against Cobra (Goswami *et al.,* 2014). The anti-venom is made by immunization of mammals such as horse etc. with particular snake venom and specific immunglobins are isolated from the blood.

The animals injected with venom undergo an immune response to the venom, there by produced antibodies against the venom's active molecules which can then be harvested from the animal's blood and use to treat envenomation.

v. Most antibiotics used for treatment of bacterial infections are unavailable and expensive to a common man, therefore the use of plants extracts as a substitute may be of greater value for effective control of some of these bacterial diseases

vi. The growing demand for herbal products has led to high increase in plant materials traded across the world and their uses in raw and boiled forms has been known and accepted by all nations as the first treatment available to man in most cities and rural areas. (Ahmad and Beg, 2001).

vii. Many plant species harbour inexhaustible active ingredients that are often valuable in the management of many diseases and almost all plants are of medicinal value. However, these active components need to be extracted from all parts of the plants, quantified and processed for therapeutic purposes (Ahmad and Beg, 2001).

1.11 JUSTIFICATION OF THE STUDIES

i. The use of *Indigofera capitata* and *I. conferta* ethnomedically in treatment of some diseases and snake bites make it very important and thus the need to establish the phytochemical, anti-bacterial and antivenom potentials of the two plant species.

ii. The acute toxicity studies of the two *Indigofera* species will help in providing information regarding their safety or otherwise to the users.

1.12 AIM AND OBJECTIVES

1.12.1 AIM

To compare the anti-bacterial, anti-venom and phytochemical properties of *Indigofera capitata* and *I. conferta* with a view to either support or debunk the traditional claims of using the two plants in ethnomedicine as antibacterial agents and in the management of snake bites.

1.12.2 OBJECTIVES

The objectives of the present studies are to evaluate and compare:

i. The antibacterial activity of the petroleum ether, ethanol and aqueous extracts of the two *Indigofera* species

ii. The effects of three extracts of the two plant species in *Naja nigricollis* envenomed rats in terms of bleeding and clotting time.

iii. The effects of the three extracts of the two plant species in *Naja nigricollis* envenomed rats in terms of oedema forming activity.

iv. The effects of the three extracts of the two *Indigofera* species in *Naja nigricollis* envenomed rats in terms of histopathological lesions.

v. The Thin layer chromatographic profile of the phytochemical constituents of the most active extracts of the two plant species.

1.13 HYPOTHESIS

i. There is no significant difference between the antibacterial activities of the various extracts of both *Indigofera* species.

ii. There is no significant difference between the effects of various extracts of the two *Indigofera* species in *Naja nigricollis* envenomed rats in terms of bleeding and clotting time.

iii. There is no significant difference between the effects of various extracts of the two plant species in *Naja nigric*ollis envenomed rats in term of oedema forming activity.

iv. There is no significant difference between the effects of the various extracts of both *Indigofera* species on the histopathological lesions in *Naja nigricollis* envenomed rats.

v. There is no significant difference between the thin layer chromatographic profiles of the phytochemical constituents of the most active extracts of the two *Indigofera* species.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1MORPHOLOGICALFEATURES AND CLASSIFICATION OF *INDIGOFERA CAPITATA* AND *INDIGOFERA CONFERTA*

The name *capitata* was derived from the shape of the flower (i.e. *capitata* means head-like flower) and the plant can be found on bushy area and old farmland. It is perennial herbs, 1 to 3 feet high, flower is pink and terminal leaves are linear-compound pinnate while *I. conferta* is commonly found growing in red soil and it is an annual herb with sticky hairy leaves, branches and flowers, 1 to 2 feet high. The leaves also possess hairs (Tamilveslvi *et al.,* 2011).

The two plant species are classified as:

Kingdom: Plantae

Division: Spermatophyta

Subdivision: Angiospermae

Class: Dicotyledonae

Order: Papillionales

Family: Fabaceae

Genus: *Indigofera*

Species: *capitata* and *conferta*

2.2 PHYTOCHEMICAL CONSTITUENTS OF *INDIGOFERA* SPECIES

Report documented by Natural Product Alert (NAPRALERT, 2013) showed the phytochemical screening of some *Indigofera* species. These include *Indigofera spinosa* plant extract that contained alkaloids, flavonoids, tannins, coumarins, sterol or

triterpene and saponins while *Indigofera argentia* aerial part extract contained coumarins, carotene and saponins. The root and the leaves extracts contained leucoanthocyanins, alkaloids, flavonoids and saponins.

2.3 BIOLOGICAL ACTIVITIES OF SOME *INDIGOFERA* SPECIES

The researches carried out on the biological activities of some *Indigofera* species were as follows: In Nigeria, the leaves of *Indigofera dendroids* were used for anti-microbial activities using chromatography fraction 14.0 mg/ disc against *Salmonella typhimuriun*, *Bacillus subtilis* and *Escherichia coli* cultured in different agar plate. The result obtained was very effective against *Salmonella typimuriun* and not effective against *Bacillus subtilis* and *Escherichia coli* (Adiwu and Muko, 1999). The biological activities of *Indigofera pulchra* was carried out to validate traditional claims of usefulness of the plant in management of poisonous snake bites. The result showed the extract of *Indigofera pulchra* gave 33.3 % protection against LD_{99} *Naja nigricollis* venom in treated mice, gross pathologic symptoms of envenomation were alleviated (Abubakar, *et al*., 2000). *Indigofera pulchra* was found to affect the plasma recalcification, haemolytic effect and phospholipase activity of crude venom. The study also showed *Indigofera pulchra* is useful in some pathologic effects of *Naja nigraricollis* venom which provides some scientific basis for the use of the plants in management of poisonous snake bites (Abubakar *et al*., 2000). In Sudan, the dried fruits of *Indigofera arrecta* were used for molluscidal activity against *Bulinus trancatus* and *Biomphalaria pleifferi,* using ethanol extract, while the hot water extract of the dried leaves was used for insulin release stimulation in rat and the results obtained from the two were found

very effective (Nyorka and Addy,1998). In Rwanda, the methanolic extract of the dried leaves of *Indigofera arrecta* was used for anti-bacterial activities against *Bacillus subtilis* and *Staphylococcus aureus* cultured on agar media, the concentration used was 50 mg/ml and the result obtained was very effective. The leaves and flowers of *Indigofera aspalathoides* are used for various types of rashes in skin and the root is used in preparing medicated oil for treatment of scabies and leprosy. The medicated oil mixed with the powder of the whole plant is used for treatment of dermatitis and various ulcers (Sonia *et al.,* 2006). In East Africa, the methanolic extract of the dried root bark of *Indigofera circinella* was used for anti-bacterial activities against *Bacillus subtilis* and *Escherichia coli* cultured on agar media (Rosy *et al.,* 2010).

Indigofera hebepetala is rich in organic and fatty acids and some flavonoids. Chemical investigation of *Indigofera tinctoria* has revealed the presence of carotenoids, coumarins and flavonoids as the major biologically active constituents (Saurabh *et al.,* 2010). In an investigation carried out by Sarawathi *et al.* (2012) isolation of two, new 2-ary1-3 methylbenzofurans from leaves of *Indigofera microcarpa* was made. The investigation also revealed the presence of 3,5-digalactoside in the leaves of *Indigofera hirsuta*, P-hydroxyl benzoic acid, apigenin 7-diglucoside, apigenin 7-rhamnoglucoside and kaempferol 3-neohesperidoside from the leaves of *Indigofera mysorensis* while a phytochemical investigation of *Indigofera heterantha* has led to the characterization some leaf flavonoids, aglycones and phenolic acid. Also, a nitropropanoyl glucopyranoside has been recorded from *Indigofera suffructicosa*.

Glycosides were isolated from the methanolic plant extract by chromatographic fraction. Acid hydrolysis of each glycoside with hydrochloric acid, gave kaempferol as the only aglycone, the sugar of the monoglucoside was identified as callose by thin layer chromatography in five solvent systems compared with the standard sugar samples and the position of attachment of the sugar was determined by UV spectral analysis. The glycosylation site was found to be at carbon seven of the aglycone (Sarawathi *et al.,* 2012). On alkaline hydrolysis, it remained unchanged and no organic acid was detected, showing it was not acetylated, which indicates the presence of kaempferol 7-alloside. Sugars obtained after acid hydrolysis of diglycosides were identified by co-chromatography as arabinose. In the above research, soluble compound of *Indigofera hebepetala* were exhaustively extracted from 1kg air-dried leaves of the plant with 85 % methanol and defatted with tri-chloro methame ($CHCl_3$). Identification of glycoside, kaempferol 7-alloside showed dull yellow colour under UV and changing to yellow with ammonia (NH_3). Kaempferol 3, 7- diarabinoside showed dark purple colour under UV changing to yellow with NH_3 (Sarawathi *et al.,* 2012).

2.4 *INDIGOFERA* SPECIES USED AGAINST BACTERIA

The antibacterial activity of *Indigofera glandulosa* leaf extracts was carried out against some species of bacterial (*Alcaligenes* species and *Aeromonas liquefacians*). The acetone leaf extract exhibited moderate antibacterial activity with inhibitory zone of 9 mm against *Alcaligenes* species and 13 mm against *Aeromones liquefaciens*. The study also revealed that, the aqueous extract showed maximum zone of inhibitions of 14 mm against *A. liquetaciens*, 13 mm against *Pseudomonas aeruginosa* and 15 mm against

Alcaligenes species (Probakaran, 2011). The investigation was comparable to the finding of antibacterial activity of *Indigofera longeracemosa* which was tested against selected pathogenic bacteria (Thangadurai *et al.,* 2002).

Rosy *et al.*(2010) reported that, the chloroform extract of *Indigofera aspalathoides* exhibited very promising antibacterial activity against *Staphylococcus aureus*, *Salmonella typhi*, *Vibrio cholera*e, *Bacillus subtilis*, *Staphylococcus epidermis Escherichia coli*, *Klebsiella pneumonae*, *Enterobacteria* species, *Proteus vulgaris* and *Proteus mirabilis.* Various organic and aqueous extracts of *Indigofera suffruticosa* leaves obtained by infusion and maceration were screened for their antibacterial and antisfungal activities. The extracts were tested against five (5) different species of human pathogenic bacteria and seventeen (17) fungal strains by the agar-solid diffusion method. Most of the extracts were devoid of antifungal and antibacterial activity except the aqueous extract of leaves of *I. Suffruticosa* obtained by infusion, which showed strong inhibitory activity against the gram-positive bacteria *Staphylococcus aureus* with a minimum inhibitory concentration (MIC) of 5000 mg/ml while that of dermatophyte strains (*Triclophytom ribrum* and *Microsporum canis)* was 2500 mg/ml (Sonia *et al.,* 2006).

2.5 TRADITIONAL USES OF SOME *INDIGOFERA* SPECIES

Indigofera tinctoria is used in constipation, liver disease and heart palpitation .The roots, stems and leaves are bitter, thermogenic, laxative, trichsogenous, expectorant, anthelmiknthic, tonic, naturopathy, splenomegaly, echolalia, cardiopathy, chronic

bronchitis, asthma, ulcers, skin diseases, diuretic and for promoting hair growth (Saraswathi *et al.,* 2012). The juice expressed from the leaves is useful in the treatment of hydrophobia. An extract of the plant is good for epilepsy and neuropathy (Saraswathi *et al.,* 2012). *Indigofera tinctoria* possesses anti-toxic property. The plant is stimulant alternative, deobstruent and purgative.

Indigo is antiseptic and astringent, the juice of the leaves and Indigo in powder is used mixed with honey in enlargement of liver, spleen, epilepsy and other nervous infections. The juice is also used for treatment of whooping cough, palpitation of heart, lung disease and kidney complaints. Decoction of the root and juice of young branches of the stem of *Indigofera tinctoria* mixed with honey are used as medication against poisoning by arsenic (Saraswathi *et al.,* 2012). Powdered dried leaves of *I. tinctoria* were mixed with castol oil and applied to the navel of children to promote or stimulate bladder for retention of urine. These leaves also improved renal creatine clearance and reduced renal total protein lost and nephroprotective properties (Saraswathi *et al.,* 2012).

2.6 MEDICINAL USES OF SOME *INDIGOFERA* SPECIES

Renukadevi and Suhani (2011) studied the antibacterial, anti-oxidant and cytotoxic activities of *Indigofera tinctoria* leaves extract. The antibacterial activity was carried out *in vitro* on lung cancer cells. The leaves extract effectively inhibited the growth of gram positive bacteria namely *Staphylococcus aureus, Bacillus punilis* and *Streptococcus pyrogens.* Zone of inhibition was observed to increase up to 17 mm, while

in gram negative, the growth of bacteria like *Escherichia coli* and *Pseudomonas aeruginosa* were inhibited.

The cytotoxic effect of *I. tinctoria* leaves extract on lung cancer cells was studied. The result showed that the percentage cell viability of the cells decreased with increase in the concentration of the extracts. Verma *et al.*(2010) estimated the percentage reduction in blood glucose level of diabetes induced rabbits at different time intervals after administration of *Indigofera tinctoria* leaf extract and the result showed a significant difference in blood glucose reduction between different group of diabetes induced rabbbits. Gunase *et al.* (2009) investigated the anthelmintic activity of *Indigofera tinctoria* against *Pheretima posthuma*. The extracts of the plant were tested in the assay, which involved the determination of paralysis time and death time of the earth worms. The methanolic extract of *I. tinctoria* exhibited to maximum antihelminatic activity comparable to standard drug piperazine citrate while petroleum ether and chloroform extracts exhibited a similar activity. Tanko *et al.*(2009) investigated the behavoural effect of Hydromethanolic crude extract of aerial part of *Indigofera pulchra* in mice and the result revealed that the extracts significantly and dose-independently reduced the onset and prolonged the duration of sleep induced by diazepam. By potentiating the diazepam-induced sleep, the extract possesses sleep inducing properties.

Preliminary gastrointestinal studies of methanolic extract of *Indigofera pulchra* was carried out in rodents to investigate the effects of the extract on gastrointestinal smooth muscles so as to validate the use of the plant in traditional medicine for treatment of

diarrhoea and other gastrointesnal disturbances. The result obtained showed that, the extract possess antidiarrhoeal activities. The extract exhibited significant and dose-dependent anti-diarrhoeal activity against castor oil-induced diarrhoea in mice (Musa *et al.,* 2008). The behavioural effects of hydromethanolic crude extract of aerial part of *Indigofera pulchra* were also investigated in mice, the result showed that, hydromethanolic extract of plant contained biologically active constituents that are stimulative in nature and of ethnomedical uses (Tanko *el al., 2009).*

2.7 PLANT CONSTITUENTS AS CRUDE DRUGS

Crude drugs contain natural components of plants, animal or minerals origin which has not undergone any processing other than collection and drying or are drugs that has not been advanced or improved by any physical or chemical treatment. Plants have been used as a source of traditional drugs and pharmaceutical preparation for man and other animals. According to a survey by the United Nations Commission for Trade and Development (UNCTAD), more than 33 % of modern drugs and medicinal products are derived from plants. The chemical substances present in plants are regarded as chemical constituents. Not all the chemical compounds produced by plants have the same activities, but the so called active constituents frequently used as crude drugs are alkaloids, glycosides, flavonoids coumarins, tannins, anthraquinone, steroidal saponins and volatile oil (Antony *et al.,* 2010).

2.8 ETHNOMEDICAL USES OF SOME *INDIGOFERA* SPECIES

The ethnomedical information on *Indigofera* species shows that, most of the species are highly valuable in traditional medicine. In Mexico, the root of *I. soffruticosa* is used to overcome nerve sickness, jaundice and lice. The dried leaves are used for treatment of ulcer and urinary diseases (Leite *et al.,* 2004). In central Africa, the roots of *I. simplicifolia* are used to induce abortion when taken with hot water by pregnant women while the entire plant of *I. spicata* is used to cause abortion in pregnant grazing animal like cow, when administered orally and the dried roots are used to treat schistosomiasis in human adult. The dried leaves of *I. arrecta* are used for treatment of diabetes, respiratory ailment, leprosy, malaria, scabies, sores, eczema and worms, while the dried roots are used for stomach conditions; alleviate labour pain and tuberculosis (Saurabh *et al*., 2010). In Somalia, the root of *I. schimperi* is used for the cure of temporary impotent when taken orally by human adult male. The leaves of *I. oblongifolia* Forsk are used for treatment of urinary tract, cough and skin infection and anti-microbial activity. A methanolic extract of the whole plant showed strong toxicity in brine shrimp lethality test (Ezaz *et al*., 2005).

2.9 EVALUATION OF PLANT CRUDE DRUGS

Evaluation of a drug means confirmation of its identity and determination of its quality, purity and detection of nature of adulteration. This is necessary because of the biochemical variation in the drug, deterioration due to treatment and storage. Adulteration at times occurred as a result of carelessness and ignorance (Kokate *et al*., 2002).

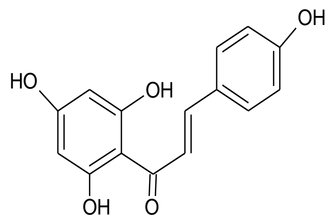
Physical and chemical techniques involving isolation and characterization of phytoconstituents are highly significant in quality control of vegetable drugs. The quality control of herbal drugs and their bio-constituents is very important in justifying their acceptability in modern system of medicine. Knowledge of the biological activities and chemical constituents of plants is desirable not only for the discovery of new therapeutic agents but also because such information may be of value in disclosing new sources of economic materials for the synthesis of complex substance (Saurabh *et al*., 2010).

Phytochemical screening and biological evaluation is the study of chemical constituents of plants and their biological activities on animals. The extraction of bioactive agents from plants is one of the most intensive areas of natural product research today. A reason for screening plants for bioactive agent is to use the extracted compound for pharmaceutical purposes (Mors *et al.,* 2000). The activity of any extracted compound has been shown to be due to a particular chemical compound present and this has made most of the pharmaceutical studies possible to achieve their goals. It is not always easy to isolate the bioactive agent in a plant, but such attempt should continue as characterization of the active agent, structure and activities of related studies carried out that will lead to the synthesis of more potent drugs with reduced toxicity. The active component of any part of plant that can produce biological effect depend on the extraction of the active principle with a correct solvent. A better formulation into more appropriate dosage may lead to a better understanding of the diseases. The isolation of bioactive agent from plant can be grouped into two broad fundamental procedure:

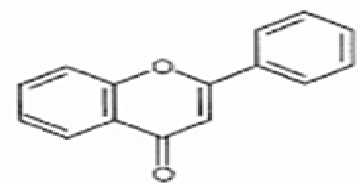
phytochemical screening i.e searching for the compound itself and biological screening i.e. searching for the physiological effect which the plant can produce (Mors *et al.,* 2000).

2.10 FLAVONOIDS AND THEIR CLASSIFICATION

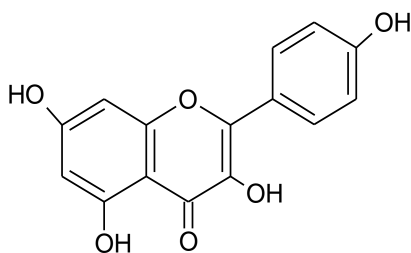
The flavonoids are polyphenolic compounds possessing fifteen carbon atoms, two benzene rights joint by a linear three carbon chain. Flavonoids constitute one of the most characteristic classes of compounds in higher plants; many flavonoids are easily recognized as flower pigment in most angiosperm families (flowering plants). However, their occurrence is not restricted to flowers but include all part of the plants (Hausteen, 2002). The chemical structure of flavonoids was based on a C_{15} skeleton which can be represented as C_6-C_3-C_6 system (Hausteen, 2002). The various subgroups of flavonoids are classified according to the substitution patterns of the ring. Both the oxidation state of the heterocyclic ring and the position of the ring played important role in classification of flavonoids and some major subgroups are recognized (Hausteen, 2002). According to Hausteen (2002) chalcone is derived from three acetates and cinnamic acid. Anthocyanidin is an extended conjugation made up of the aglycone of the glycoside anthocyanins. Anthocyanins are the most important group of plant pigments visible to the human eye. Chalcones and flavanones are efficient precursors of isoflavonoids. A good example of biologically active isoflavonoid is Rotenone which may be obtained from most of the family of Leguminosae (now Fabaceae). Some flavonoids may be named directly after the unique plants that contain them. Ginkgetin is an example of flavonoid from the ginkgo tree and Tangeretin is a flavonoid from the tangerine (Hausteen, 2002).



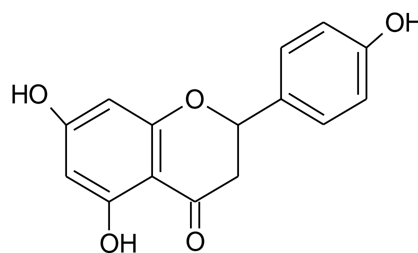
(i) Chalcones



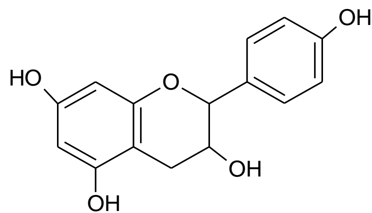
(ii) Flavone



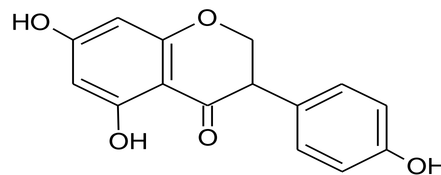
(iii) Flavonol



(iv) Flavanone



(v) Anthocyanins



(vi) Isoflavone

Figure 2.1: Basic Structures of Flavonoids (Hausteen, 2002)

2.10.1 MEDICINAL USES OF FLAVONOIDS

Most flavonoids function in the human body as antioxidants. They helped to neutralize reactive oxygen-containing molecules and prevent the reactive molecules from damaging part of cells. Plants flavonoids have been used for centuries in conjunction with their antioxidant and protective properties. Flavonoids have been shown to have antibacterial, anti-inflammatory, antiallergic, antiviral, antineoplastic, anti-thrombotic and vasodilatory activity. In some cases, flavonoids can act directly as antibiotic by disrupting the function of microorganisms like virus or bacteria. The antiviral function of flavonoids has been demonstrated with the HIV virus and also with HIV-1, a herpes simplest virus (Middleton *et al.,* 2000). Flavonoids deficiency in the body of human can lead to nose bleeding, swelling after injury and haemorrihoids. Generally, weakened immune function and cold or infections can also be a sign of inadequate dietary intake of flavonoid. Fruits, vegetables, herbs and spices contain a wide variety of flavonoids. Poor intake of fruits and vegetables or routine intake of high processed fruits and vegetables are common contributing factors to flavonoid deficiency. Flavonoids played a role in the prevention and treatment of Asthma, candida infection, Diabetes, muscular degeneraton, stomach ulcer and Atopic dermatitis (Harborne and William, 2000).

2.10.2 TOXICITY OF FLAVONOIDS

It has been suggested that because flavonoids are widely distributed in edible plants and beverages and have previously been used in traditional medicine, they are likely to have minimal toxicity. Flavonoids are family of compounds that has a diverse range of activities in mammalian cells and in vivo conformation of their side effects would be

necessary for a full evaluation of their practical usefulness in the field of modern medicine (Middleton *et al*., 2000). The selectivity of flavonoids for eukaryotic enzymes appears to vary from compound to compound; such a study would need to assess the toxicity of these phytochemicals on an individual basis (Middleton *et al*., 2000).

2.11 SAPONINS

Saponins are a diverse class of natural surfactants or detergents, found in many plants, but they are most abundant in the desert plants yucca and Quilaja. Extracts from these plants are commonly used as foaming agents for beverages. Saponins also have commercial applications in industrial and mining operations and are useful in products such as photographic emulsions, cosmetics and shampoos (Edeoga and Okwu, 2005). Several classes of phytochemical saponins exhibit a wide spectrum of biological activity as antifungal and antibacterial agents. Saponin-rich herbs may also contribute to lowering blood cholesterol and inhibition of cancer cell growth. Some saponins affect the heart and have been used for over hundred years to treat heart conditions. *Digitalis* is one of such saponin and is derived from the common garden plants and used to strengthen the contractions of the heart muscle which could be beneficial for those with heart disease. *Digitalis*-type saponins can be toxic in high doses and have been used as arrow and spear poisons by African and South American native (Edeoga and Okwu, 2005).

2.12 SNAKE VENOMS

Snake venoms are secretion of venomous snake which are synthesized and are stored in venomous gland. The glands which secrete the zootoxin is a modification of the salivary gland and are situated on each-side of the head below and behind the eye encapsulated in muscular sheath. The glands have large alveoli in which the venom is stored before being conveyed by the duct to that tubular fang, through which it is released. Snake venom is a combination of many different proteins, peptides and enzyme. Snake venoms are complex mixture of enzymatic and toxic proteins which include phospholipase A2 (PLA2), myotoxin, haemorrhage metalloproteinase and other proteolytic enzymes, coagulant components, cardiotoxins, cytotoxin and neurotoxins (Goswami *et al.,* 2014).

2.12.1 COMPOSITION OF SNAKE VENOM

Snake venom consists of protein, enzymes, neurotoxins coagulants, anticoagulants and substances with cytotoxin effects. It has acidic P^H. Specific gravity is low and is water soluble. Phospholipase A2 causes haemolysis by lysing cell membrane of red blood cells. Oxidases and proteases are used for digestion. Snake venom contains inorganic cations such as sodium, potassium, magnesium and small amount of zinc, nickel iron and cobalt. Zinc is necessary for anticholinesterase activity (Goswami *et al.,* 2014)

2.12.2 TYPES OF SNAKE VENOM

Different species of snakes have different types of venom which depends upon its species geographical location, its habitat, climate, age etc. There are three types of venom according to their effects,

(a) Haemotoxic venoms which affect cardiovascular system

(b) Cytotoxic venoms which targets specific cellular sites.

(c) Neuro-toxic venoms which harm nervous system of human body.

Enzymes present in snake venom hydrolyze protein and membrane components which lead to tissue necrosis (Debnath *et al.,* 2007).

2.12.3 THERAPEUTIC ROLE OF SNAKE VENOM

Many toxins from snake venom are investigated and formulated into drugs for the treatment of conditions such as cancer, hypertension and thrombosis. Snake venom significantly lowers the blood pressure in human victim and experimental animals (Goswani *et al.,* 2014).

2.12.4 FIBRINOLYTIC ACTIVITY OF SNAKE VENOM

Snake venom enzymes remove fibrinogen from the circulation without converting it to fibrin. Venoms with anti-coagulant properties are extensively studied for medical application but a drug called tirobifan (Aggrastat) was developed from a compound obtained in the venom of the saw-sealed viper and issued as an anti-platelet drug (Goswani *et al.,* 2014).

2.12.5 CARDIOTONIC ACTIVITY OF SNAKE VENOM

Venom obtained from most poisonous snake has blood thinning properties and could be very effective in treating stroke patients. Venom obtained from cobra has been investigated for the treatment of cancer in mice. In case of *in vitro* study, cobra venom showed potent cytotoxic and apoptogenic effect on human Leykemic cells by reducing cell proliferation rate and produced morphological alteration (Jain and Kumar, 2012).

2.12.6 MUSCULAR DEPOLARIZATION AND HAEMOLYSIS OF SNAKE VENOM

Cytotoxin or cardiotoxins are polypeptide of 60-70 amino acid residues found in snake of elapid family having various pharmacological alterations or effect. These include: - Muscular depolarization of muscle and haemolysis, anaphylactic reaction difficulty in breathing, reddening of skin, swelling of eyes and face, fever etc. Pyrogen reaction due to the action of high concentration of non-humanoglubulin proteins inflammation of joints and enlargement of lymph gland (Goswani *et al.,* 2014).

2.13 *NAJA NIGRICOLLIS* VENOM AND ITS SITE EFFECT TO MAN

Black-necked spitting cobra (*N.nigricollis*) is one of the most venomous snakes distributed in the tropics. In Africa, snake bites more especially of *N. nigricollis* causes more than one thousand death each year and thousands of cases of permanent physical disability (Theakston *et al.,* 2003). Biochemical characterization of the venom of a snake from a particular geographical location is of great importance because, pathogenesis developing after a bite is dependent on the qualitative composition of the venom and on the quantitative distribution of different components in particular venom

(Theakston *et al.,* 2003). Venoms contain a variety of enzymes, non-enzymatic polypeptide toxins and non-toxic proteins. Toxicity of venom depends on the qualitative and quantitative distribution of different enzymes and toxins in the venom. The phospholipase A_2 (PLA_2) consist of a broad range of enzymes defined by their ability to hydrolyze glycerophospholipid (water insoluble substrate phospholipids) at the position of the glycerol back bone. Phospholipase A_2 (PLA_2) from *N. nigricollis* contains multiple forms of enzymes (Kini, 2011). Phospholipase A2 from *N. nigricollis* may exhibit a specific pharmacological effect such as pre-synaptic or post-synaptic neurotoxicity, cardiotoxicity, myotoxicity, platelet aggregation, Oedema formation, haemolysis, anticoagulation, convulsion and hypertension (Zon *et al*., 2012).

2.14 BLEEDING AND CLOTTING TIME

Bleeding time is the time taken for bleeding to stop or is the time interval from oozing of blood after a cut or injury till arrest of bleeding while clotting time is the time interval from oozing of blood after a cut or injury till the formation of clot (Omale *et al.,* 2012).

2.15 OEDEMA FORMING ACTIVITY

The oedema forming activity of *Naja nigricrollis* in rats is the effect of venom in the blood vessels which result in the swelling of the legs or feets due to excess fluid strapped in the body's tissues that occurs when tiny blood vessels in the body (capillaries) leak fluid. The fluid builds up in surrounding tissues leading to swelling of arms, feets, ankles and legs (Juliana *et al.,* 2011).

2.16 MEDICINAL PLANTS ACTIVE AGAINST SNAKE BITES.

Medicinal plants are the local heritage with global importance. Various plants that were used against snake bite were reported to possess compounds with antisnake venom activities and most of these plants have no scientific validation (Antony *et al.,* 2010). Antivenoms in most countries over the world are costly and may be in limited supply. Anti-venoms for therapeutic use are often preserved freeze-dried ampoules but some available only in liquid form and may be kept refrigerated. In almost all part of the world where venomous snakes occur, numerous plants species are used as folk medicine to treat snake bite (Antomy *et al.,* 2010). Generally, an aqueous, methanol or ethanol extract is prepared out of the plant parts. Topical application of the plant or its sap onto the bitten area, chewing leaves or bark or drinking plant extracts decoctions or injecting the extracts helped to counteract the snake venom activity (Antomy *et al.,* 2010).

The roots of plants: *Ophiorrhiza mungo, Peristrophe bicalyculata, Gymnema sylvestris, Gloriosa superba, Cucumis colosynthis, Alangium saluifolrum,* leaves of *Enicostemma axillare, Calycopteris floribunda, Calotropis gigantea, Aristolochia indica, Abrus precatorious, Azadirachta indica, Caeseria sylvestris and Parkia biglobosa* are used as drugs to treat snake bite (Asuzu and Ode, 2006). Tea made from the leaves of *Cecropia peltata* is used as a cure for a wide variety of ailments including snake bite. *Achyranthes aspera* is used in treatment of bleeding, renal complications; scorpion and snake bite (Asuzu and Harvey, 2003). Aqueous extracts of the bark of *Schunanniophyta magnificicum* and the leaves of *Mucuna pruriens*, *Strophanthus gratus* and *Strophanthus hispidus* have the ability to prolong the clotting time when administered long with a

standardized dose of *Echis carinatus* venom. Partial protection of haemorrhage was also shown by *Aristolochia grandiflora*, *Columnea kalbreyeriana, Sida acuta, Selaginella* species, rhizomes of *Renealmia alpinia*, stem of *Strychnos xunovensis,* leaves, branches and stem of *Hyptis capitata, Ipomoea cairica, Neurolaena lobata, Ocimum micranthum, Piper pulchrum, Siporuna theraphora, Castilla elastica, Allamanda cathartica*, the macerated fruits of *Capsicum frutescens*, unripe fruits of *Crescentia cujete,* leaves and branches of *Piper arboretum* and *Passiflora quadrangularis* (Oserio *et al.,* 2000).

The stem barks of *Brownea rosademante*, *Tabebuia rosea* and rhizome of *Renealmia alpinia*, *Haliconia curtispatha*; the whole plants of *Pleopeltis percussa*, *Trichomanes elegans* and ripe fruits of *Citrus lemon* showed hundred percent neutralizing capacity of snake (*Bothrops atrax*) venom within forty eight (48) hours. Partial protection was also shown by the leaves, branches and stems of *Castus lasius*, the whole plant of *Sida acuta,* rhizome of *Dracontium croatii*, leaves and branches of *Bixa orellena* and *Struthanthus orbicularis* (Oserio *et al.,* 2000). *Mucuna pruriens* seeds aqueous extrct significantly inhibited the *Echis carinatus* venom induced myotoxic, cytotoxic and coagulation activities in experimental animals (Aguiyi *et al.,* 2001).

Aqueous and alcoholic extracts of dried roots of *Mimosa pudica* inhibited lethality myotoxicity and toxic enzymes of *Naja kaouthia* venom. Oral administration of garlic could be used as a prophylactic tool against cobra venom induced histological and histochemical patterns of the gastric and hepatic tissue changes in rats (Rahny and Hemmaid, 2001). Phospholyses activity of *Rotalus durissus* venom showed only partial

inhibition of *Bothrops* venoms sow (Biondo *et al.,* 2003). The extract of the Brazilian plant *Marsypianthes chanaedrys* inhibited fibrinogen clotting induced by several Brazilian snake venoms indicating that, it affects thrombin-like enzymes (Castro *et. al.,* 2003).

Mandevilla illustris inhibited phospholipase activity of *Crotalus durissus* snake venom along with prolongation of survival time and a decrease in lethality (Izidoro *et al.,* 2003) *Mimosa pudica* and *Eclipta prostrata* partially inhibited the haemorrhagic activity but displayed very low anti-phospholipase A_2 activity and did not inhibit proteolytic activity of viper's venom (Girish *et al.,* 2004). Oedema, defibrination and coagulation effect of *Bothrops asper* venom were neutralized by the leaves and branches of *Bica orellena*, *Ficus nymphaeifolia*, *Struthanthus orbicularis* and *Gonzalagunia panamensis*, the stem barks of *Brownea rosademonte* and *Tabebuia rosea*, the whole plant of *Pleopeltis percussa* and *Trichomanes elegans,* rhizomes of *Renealmia alpinia, Haliconia curtispatha* and *Dracontium creatii* and the ripe fruit of citrus lemon (Nunez *et al.,* 2004).

The aqueous extract of *Tabemaemo catherinensis* inhibited the lethal activity of *Crotalus durissus* snake venom (Almeida *et al.,* 2004). The ethanol root extract of *Acalypha indica* possess potent snake venom neutralizing properties (Shirwaikar *et al.,* 2004). The methanol extract of the roots of *Annona senegalensis* caused reduction in the *N. nigricollis* venom induced hyperthemia in Rats (Adzu *et al.,* 2005). *Pentaclethra maeroloba* exhibited full inhibition of haemorrhagic and nucleolytic activities induced

by several snake venoms along with partial inhibitions of myotoxic, lethal, phosphohpase and edema activities. It totally inhibited *Bothrops jararacussu* metalloprotease induced haemorrhage *in vivo* model (Silva *et al.,* 2005). Aqueous extracts of fresh roots, stems or leaves of *Mikania glomerata* efficiently neutralized different toxic, pharmacological and enzymatic effects induced by venoms from *Bothrops* and *Crotalus* snakes (Maiorano *et al.,* 2005). Aqueous extracts of *Croton urucurana* antagonized the haemorrhagic activity of *Bothrops jamraca* venom and pronthocyanidins were involved in this activity (Esmeraldino *et al.,* 2005). *Cerdia verbenacae* inhibited paw oedema induced by *Bothrops jararacussu* snake venom (Ticli *et al.,* 2006).

Aqueous extract of aerial parts of *Bauhinia forticata* is a promising source of natural inhibitors, proteases that is involved in blood clotting disturbances induced by *Bothrops* and *Crotalus* crude venoms (Olivera *et al.,* 2005). The methanol bulb extract of *Crinum jagus* significantly protected mice from death, myonecrosis and haemorrhage induced by the lethal effects of *Echis ocellatus*, *Bitis arietans* and *N. nigricollis* venoms (Ticli *et al.,* 2005). Tamarind seed extract inhibited the PLA2, protease, hyaluronidase, 1–amino acid oxidase and 5–nucleotidase enzyme (major hydrolytic enzymes) activities of *Vipera ruselli* venom in a dose-dependent manner, the extract also neutralized the degradation of the B-beta chain of human fibrinogen and indirect haemolysis caused by venom. The extract exerted a moderate effect on the clothing time, edema, haemorrhage and myotoxic effects. Lethal induced by venom were neutralized significantly when different doses of the extract were preincubated with venom before injected to the

experimental animals. On the other hand, animals that received extract ten (10) minutes after the injection of venom were protected from venom induced toxicity (Ushanandini *et al.,* 2006).

Dichloromethane extract of leaves of *Artemisia campestris* neutralized the venom induced actions of *Macrovipera lebetina* (Memmi *et al.,* 2007). Edema, haemorrhage, myonecrosis and coagulation induced by *Echis carinatus* (Saw-scabbed viper) venom were neutralized by the methanol seed extract of *Vitis vinifera* (Mahadeswaraswamy *et al.,* 2008). The aqueous extracts of *Schizolobium parahyba* showed potent anti-snake venom activity (Mendes *et al.,* 2008).

The active fraction of *Aristolochia indica*, *Hemidesmius indicus, Gloriosa superba*, *Eclipta prostrata* and *Andrographis pamiculata* neutralized rattle snake venom induced action (Samy *et al.,* 2008). The methanol root extracts of *Vitex negundo* and *Embilica officinalis* significantly neutralized the *Vipera russelli* and *N. kaouthia* venom induced lethal activity *in vivo* studies, *Vipera russelli* venom-induced haemorrhage, coagulant defibrinogenating and inflammatory activity were significantly neutralized by both plant extracts (Alarm *et al.,* 2003). *Hemidesmus indicus* root extracts effectively neutralized viper venom-induced lethal, haemorrhage, coagulant, anticoagulant and inflammatory activities (An active compound from the *Strychmus nux* whole seed extract, neutralized *Daboia russelli* venom induced lethality, haeomorrhage, defibrinogenating PLA_2 enzyme activity and *N. kaouthia* venom induced lethality, cardiotoxicity, neurotoxicity

PLA_2 enzyme activity and also inhibited *Viper* venom induced lipid peroxidation in experimental animals (Chatterjee *et al.,* 2004).

Anti-venoms are usually hyper immune sera collected from animals which bind and inactivate venom components. Anti-serum development in animals is time consuming, expensive and requires ideal storage condition. Over the years, many attempts have been made for the development of snake venom antagonists especially from plants sources (Bawaskar, 2004). The use of plants against the effect of snakes bite has been long recognized and more scientific attention has been given for over twenty years ago (Santosh *et al.,* 2004). Extracts from plants have been used among traditional healer especially in tropical areas where there are plentiful sources, as therapy for snake bite for a long time. Several medicinal plants which are used to produce oily drugs or which have been passed on by oral tradition are believed to have snake bite antidotes. In modern science, there have been many attempts to study many plants that are of medicinal value against snake bite in order to clarify their effectiveness (Alarm *et al.,* 2003).

Many plants with medicinal value were recommended for the treatment of snakebite. The methanolic root extracts of *Vitex negundo* and *Emblifera officinalis* were tested for antisnake venom activity. Both plant extracts significantly neutralized the *Vipera russellii* and *Naja kaouthia* venom induced lethal activity both *in vitro* and *in vivo* studies. *Vipera russellii* venom-induced haemorrhage, anticoagulant, defibrinogenating and inflammatory activity were significantly neutralized by both plant extracts (Alarm *et*

al., 2003). *Hemidesmus indicus* root extracts effectively neutralized viper venom-induced lethal, haemorrhagic, coagulant, anticoagulant and inflammatory activity. The butanolic extract of *Eclipta prostrata* partially inhibited the haemorrhagic activity but displayed very low anti phospholipase A_2 activity and did not inhibit proteolytic activity of viper venom (Pimolpan *et al.,* 2004).

Lupeol acetate from *Tamarindus indica* significantly neutralizes lethality, haemorrhage, and defibrinogenation, oedema, and PLA_2 activity induced by *Daboia russellii* venom. It also neutralized *Naja kaouthia* venom induced lethality, cardiotoxicity, neurotoxicity and respiratory changes in experimental animals (Chatterjee *et al.,* 2006). Beta-sitosterol and stigmosterol isolated from the root extract of *Pluchea indica* (Astercaceae) may play an important role, along with anti-serums in neutralizing snake venom-induced actions. Several plant constitutents like flavonoids qunonoid, xanthene, polyphenols and terpenoids possessed protein binding and enzyme inhibiting properties and also inhibit snake venom phospholipase A_2 activities of both viper and cobra venom (Chatterjee *et al.,* 2006).

Traditional healers are the first line defense against illnesses. The success of their activities is vaguely understood and direct testimony from victim of snake bite confirms success of their treatment. These traditional healers serve more snake bite accident victims than modern practioners (Sanni *et al.,* 2012). Traditional healers attest to the usage of *Bridelia ferruginea* leaves in the treatment of snake bites (Sanni *et al.,* 2012).

Medicinal plants have been used as folk medicine for treatment of snake bites. Reliance on medicinal plants is primarily due to their safety, effectiveness, cultural preferences, inexpensive nature and their availability (Silva *et al*., 2008).

Traditional healers are practicing herbal medicine to cure snake envenomation, the practice is not really recognized by modern medicine and the numbers of studies evaluating the pharmacologically active principles against snake bites are few (Silva *et al*., 2008). Screening of plants used in traditional medicine and determination of their active principle and different activities is very importance. The active principles isolated from plant has the potential of being associated with the various pharmacological properties and may provide a substantial contribution to the modern therapeutics of snake bites (Chatterjee *et al*., 2006).

Investigation of therapeutic potential of plants used for snake bites show the presence of different phytochemical constituents. Lupeol acetate from roots of *Hemidesmus inducus* was reported to significantly neutralize oedema, induced by Russell's viper in experimental animals and the plant also neutralize cardiotoxicity, neurotoxicity and respiratory changes induced by *Naja kaouthia* venom (Chatterjee *et al*., 2006). Ethanolic extracts of *Bixa orellena, Brownea latifolia*, *Dracontium croatii, Struthanthus obincularis, Gonzalagunia panamensis* and *Trichomanes elegans* were reported to inhibit edema due to *Bothrops asper* venom. Decrease in oedema formation with aqueous extracts of *Caeseria sylvestris* has been noted in rats injected with lethal doses of *Bothrops asper* venom (Silva *et al*., 2008).

Methanolic extract of seeds of *Vitis vinifera* has shown promise for the treatment of local effects of *Vipera russelli* bites; the extract neutralized edema-inducing property of the venom (Mahadeswaraswany *et al.,* 2009).

Aqueous seeds extracts of *Tamarindus indica* of different doses preincubated with venom before assays significantly neutralized edema– inducing property of viper venom (Ushanandini *et al.,* 2006). *Ancardium occidentale* bark extract has also been shown to neutralize edema induced by viper venom (Ushanandini *et al.,* 2009).

Flavonoids have been attributed to the antihaemorrhagic potential. Quercetin is a potent Lipoxygenase inhibitor (Nishijima *et al.,* 2009). *Tamarindus indica* seed extract has neutralized haemorrhage, indirect haemolysis and degradation of beta chain of human fibrinogen caused by viper venom in experimental animals (Ushanandini *et al.,* 2006). The aqueous extract of leaves of *Schizolobium parahyba* significantly inhibited the coagulant, haemorrhagic and fibrigenolytic activities induced by *Bothrops pauloensis* and *Crotalus durissus* venoms and their isolated toxins after preincubation with venoms and toxins before assays (Mendes *et al.,* 2008).

In-vivo tests with polyphenols of *Areca catechu* and *Quercus infectoria* showed inhibition of the haemorrhage activity of *Calloselasma rhodostoma* venom and dermonecrotic activity of *Naja kauothia* venom (Leanpolchareanchai *et al.,*2007). Inhibition of fibrinocoagulation activity induced by *Bothrops jamraca* venom was reported with the extracts of *Marsypianthes chanaedrys* (Castro *et al.,* 2003). The organic acid from root extract of *Hemidesmus indicus* significantly antagonized

haemorrhage, coagulant and anticoagulant activities in experimental rodents, induced with viper venom.

Lupeol acetate from *Hemidesmus indicus* has neutralization potential against haemorrhagic and defibrinogen induced by *Russell's viper* (Chatterjee *et al.,* 2006). Inhibition of haemorrhage and dermonecrotic activities of venom *in vivo* was reported with methanolic extracts of leaves of *Camellia sinensis*. The action has been attributed to the various compounds present in the methanolic extracts against venom proteins (Pithayanukul *et al.,* 2010).

The aqueous extract of *Pantaclethra prostrata* has shown to have neutralization potential against haemorrhagic and myotoxin activity of *Bothrops jamraca* venom. The butanolic extract of the same plant showed partial inhibition of haemorrhagic activity of *B. jamraca* venom (Pithayanukul *et al.,* 2004). Glycyrrhizin (thrombin inhibitor) from the roots of *Glycyrrhizin glabra* has shown anti-thrombotic properties *in vivo* prevention of venom-induced changes in haemostasis, both *in vivo* and *in vitro* (Assafim, 2006). Neutralization of haemorrhagic, fibrinolytic and proteolytic activities of metalloprotease from *Bothrops* snake venoms was reported with triterpenoids and saponin isolated from *Pentaclethra maeroloba* (Silva *et al.,* 2007). Artumerone is a compound derived from *Curcuma longa* roots which has shown neutralization of the lethal effect of *Crotatus durissus* and *Crotalus adomenteus* venom (Januario *et al.,* 2004). *Bacharis trimera* has yielded clerodone diterpenoid which possessed anti-haemorrhagic properties against

snake venom. Neutralization of haemorrhagic due to viper venom was documented with seed extract of *Strychnos nex-vomica* (Chatterjee *et al.,* 2004).

Enzyme inhibition and protein binding properties have been associated with chemically active compounds of flavonoids, polyphenols, terpenoids and xanthene. These phytochemical also inhibit phospholipase (PLA_2) activities of viper and cobra venoms (Lans *et al.,* 2001). Inhibition of enzymatic activity was reported with extracts of *Caeseria sylvestris* in experimental animals injected with lethal doses of *Bothrops* venoms (Silva *et al.,* 2008).

Neutralization of *Vipera russelli* venom enzymes namely phospholipase, protease and hyaluronidase were reported by the bark extract of *Ancardium occidentale* which showed a dose–dependent manner (Ushanandini *et al.,* 2009). Lupeol acetate from the roots of *Hemidesmus indicus* significantly neutralized phospholipase (PLA2) activity induced by *Russell's viper* venom (Chatterjee *et al.,* 2006). Anti hyaluronidase activity is reported with *Mimosa pudica* against *Naja nigricollis, Vipera russelli* and *Echis carinatus* Venoms (Girish *et al.,* 2004). Methanolic leaf extract of *Azadirachta indica* has shown significant inhibition of PLA_2 enzymes of Cobra and *Russell's viper* venoms (Mukherjee *et al.,* 2008). Plant extracts of *Andrographis pamiculata* and *Aristolochia indica* effectively inhibited the main toxic enzymatic effects of *Echis carinatus* venom. Inhibition of PLA_2 and neutralization of procoagulant activity was also observed with both plants extracts (Meanatchisuderam *et al.,* 2009).

Aristolochic acid from *Aristolochia radix* was reported to inhibit the enzymatic and pharmacological activities of PLA_2 induced by *Vipera russellii* venom (Soares *et al.,* 2005). *In -vitro* test with polyphenols from *Areca catechu* and *Quercus infectoria* showed inhibition of PLA_2, proteases, hyaluronidase and Lamino acid oxidase of *Naja naja* and *Calloselasma rhodostoma* venoms (Leanpolchareanchai *et al.,* 2007). Methanolic extract of fresh leaves of *Camellia sinensis* showed inhibition of PLA_2 hyaluronidase, L-amino acid oxidase in venoms of *Naja naja* and *Calloselasma rhodostoma* by *in vitro* neutralization (Pithayanukul *et al.,* 2010). Pentacyclic triterpenes betulin and batulinic acid extracted from *Betula alba* have demonstrated activity against PLA_2 (Soares *et al.,* 2005).

A triterpenoid saponin from *Gymnema sylvester*, potassium salt of gymnemic acid has inhibited ATPase induced by *Naja naja* venom (Soares *et al.,* 2005). Ellagic acid from *Caeseria sylvestris* aqueous extract has shown inhibition of myotoxic activity in rats when tested against effects, from both total venom and PLA_2 from genus *Bothrops* (Silva *et al.,* 2008). Significant inhibition of myotoxicity induced by *Bothrops pauloensis* and *Crotalus durissus* venoms and their isolated toxins by aqueous extract of leaves of *Schizolobium parahyba* has been documented (Mendes *et al.,* 2008).

Methanolic extract of *Vitis vinifera* seed has shown neutralization of myonecrotic properties of viper's venom (Mahadeswaraswamy *et al.,* 2009). Myotoxic induced by *Bothrops jararacussu* snake venom and its main PLA_2 homologues was reported to be inhibited with *Cerdia verescens* extract (Ticli *et al.,* 2005). The aqueous extract of

Eclipta prostrata has shown antimyotoxic activity against *Bothrops jamraca* and *Bothrops jararacussu* venoms and two isolated myotoxins bothropetoxin and crotoxin (Mahadeswaraswany *et al.,* 2009). The extracts of genetically modified *Eclipta alba* inhibited myotoxic induced by PLA_2 from the venoms of *Crotalus durissus* and *Bothrops jararacussu*. Dried root extracts of *Mimosa pudica* have inhibited the myotoxic activity due to *Naja kaouthia* venom. Partial inhibition of myotoxic activity has been reported with *Pentaclethra macroloba* against *Naja naja* venom (Silva *et al.,* 2005). Many plants have been conserved and used as antidotes for snake envenomations. Increase in survival rates of rats has been observed with *Casearia sylvestris* extract (Silva *et al.,* 2008).

A glycoprotein with functional oligosaccharide chains isolated from the plant is said to be responsible for the neutralization of venom-induced actions. The seeds of the plant have been used as oral prophylactics for snake bite in Nigeria. Experimental studies on rats pretreated with extract and challenged with different snake venoms were done to investigate the effectiveness of *Mucuna pruriens* in neutralizing the toxicity of snake venoms *in vitro*. It was observed that, pretreatment provided effective protection against lethality of venoms of *Naja sputatrix* and moderate protection against *Colloselasma rhodostma* showing involvement of immunological neutralization. Significant neutralizing capacity against *Macrovipera lebetina* venom has been observed with the dichloromethane extract of *Artemisia compestris* (Memmi *et al.,* 2007). The 12-hydroxy-4-methylvoa chalotime isolated from *Tabernaemontana catherinensis* has inhibited the lethal activity of crotoxin, the main toxin of *Crotalus durisssus* (Almeida *et*

al., 2004). Leaf extract of *Guiera senegalensis* detoxified venom of *Echis carinatus* and *Naja nigricollis in vitro.* Albino mice given reconstituted venom incubated with the extract, intraperitoneally, showed reduction in mortality when compared with those given venom alone (Abubakar *et al.,* 2000). Lipid peroxidation induced by viper venom in experimental animals is reported to be inhibited with *Strychnos xenovensis* seeds extract. The plant effectively neutralized *viper* venom lethality. It contains coffeic acid and monomeric caffeic acid, an antidote for snake bites (Chatterjee *et al.,* 2004). Stem bark extract of *Parkia biglobosa* has shown neutralization of venom of *Naja nigricollis* and *Echis carinatus* in experimental models (Asuzu and Harvey, 2003). Snake venom neutralization has also been associated with leaf extract of *Acalypha indica* (Shirwaikar *et al.,* 2004).

Cordia ecalyculata and *Echinodorus grandiflora* were reportedly used in Brazil for various condition including snake bites (Silva *et al.,* 2010). Pentacyclic triterpenes or glycoside shown to possess antisnake venom activity found in *Alstonia scholaris*, *Aegle marmelos*, *Centipeda minima*, *Aloe vera* and *Elephentopus scaber* (Mors *et al.,* 2000). Plant extract of *Aristolochia indica* (terponoid) *Hemidesmus indica* (phenols), *Achyranthes aspera* (glycosides), *Andrographis pamiculata* (terpenoids), *Gloriosa superba* (esters), *Strychnos nuxvomica* (Phenols), *Rauwolfia serpentine* (alkaloids) and *Eclipta prostrata* (wedelolactane) have been shown potent venom neutralizing effect. The plant extracts and partially purified fractions were administered orally to rats envenomed with rattle snake venom. Significant protection against venom-induced changes in serum was seen after administration of purified fraction (Samy *et al.,* 2008).

Trichosanthes tricuspidata is used for snake bites. The plant mainly contains pharmacologically important phytochemical trichotetral cucurbitane glycoside and cucurbitacins (Bhandari *et al.,* 2008). Inhibition of lethality, myotoxicity and toxic enzymes of *Naja kaouthia* venom has been reported with aqueous and alcoholic roof extracts of *Mimosa pudica* (Mahanta and Mukherjee, 2001). Plants used for snake bite include *Amaranthus spinosa* (roots and stem), *Amaranthus viridis* (stem and leaves), *Argemone mexicana* (Leaves), *Bryophyllum pinnatum* (Leaves) *Commelina benghalenesis* (roots), *Pouzolzia indica* (roots), *Senna tora* (roots), *Eclipta alba* (whole plant), *Moringa oleifera* (roots and stem bark) *Rauwolfia serpentina* (roots) and *Tephrosia purpurea* (roots). Most of them proved excellent potential in the treatment of snake bites (Skaria *et al.,* 2005).

The Zingiberaceae family comprising of rhizomatous medicine plants is characterized by the presence of volatile oils and oleoresins. *Curcuma aromatica* and *Curcuma longa* have been used as antidotes in snake bites (Skaria *et al.,* 2005). Among the various other medicinal plants used for treatment of snake bites are *Vitex negundo* and *Embilica officinalis* which significantly neutralized *Vipera russellii* and *Naja kaouthia* venom-induced effect both *in vitro* and *in vivo* studies (Skaria *et al.,* 2005). Triterpenoids from the root extract of *Embilica officinalis* and *Vitex negundo* significantly neutralize anti-snake activity of *Vipera russellii* and *Naja kauothia* (Gomes *et al.,* 2007).

Steroids form complexes with venom held together by hydrophobic forces (Gomes *et, al.,* 2007).Viper venom induced lipid peroxidation is reported to be inhibited in

experimental animals by *Strychnos nuxvomica* seeds extract (Chatterjee *et al.,* 2004). *Costus speciosus* roots which contain diosgenin and starch in the rhizome have been used for snake bites (Vasagam *et al.,* 2010). *Ipomoea digitata* contains triterpenoids, phenolic compound and flavonoids is used in snake bites. The root extract of the plant has shown antioxidant activity (Vasagam *et al.,* 2010). *Acorus calamus* (Stem), *Buchanania ionzan* (Stem and bark), *Moringa oleifera* (stem and leaves), *Achyranthes aspera* (rhizome), *Gynandropsis gynandra* (rhizome) and *Bombax ceiba* (stem) were known to possess an antidote for snake bite and scorpion sting (Vasagam *et al.,* 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 PLANT COLLECTION AND IDENTIFICATION

The collection of *Indigofera capitata* and *I. conferta* was carried out in Awon village in Kachia Local Government area (Lat: 9^0 45ʹ N, Lon: 70 58ʹ E, Al: 615m), Kaduna State. They were identified and authenticated by a taxonomist in the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria. Voucher specimen numbers of 1757 and 1084 respectively were assigned to them and herbarium specimens were deposited at the herbarium section for future reference.

3.2 PREPARATION OF PLANT MATERIALS

The collected samples of the two plants were air dried in the laboratory. They were powdered by using pestle and mortar. The powdered whole plants materials of both plants were kept in clean air tight containers and they were subsequently macerated with various solvents.

3.3 EXTRACTION OF THE *INDIGOFERA CAPITATA* AND *INDIGOFERA CONFERTA* WHOLE PLANTS

This was aimed at obtaining the various solvent extracts of each of the two whole plants by successive solvents maceration method by using the modified method cited by Anas *et al.* (2010). Powdered whole plant material (160 g) of each of the two plants was successively macerated with petroleum ether, ethanol and aqueous (2.5 litres each) for three days and each of the extracts was filtered. The filtrate of each solvent extract of the two plants was evaporated to dryness on water bath and the yield of each extract of the

two plants was calculated in percentage. The three different extracts of each of the two plants were subsequently used for acute toxicity and biological studies.

3.4 EXPERIMENTAL ANIMALS

A total of one hundred and sixty seven (167) albino rats weight 130g to 190g of different sex(male and female) were used for each plant species, out of which 39 for acute toxicity, 18 for histopathological lesions, 55 for bleeding and clotting time and 55 for oedema forming activities of *N.nigricollis* venom. All the animals were acclimatized for seven days and allowed free asset to water and food.

3.5 ANTI-BACTERIAL ACTIVITIES OF CRUDE EXTRACTS OF *INDIGOFERA CAPITATA* AND *INDIGOFERA CONFERTA*

Two species of Bacteria: *Salmonella typhi* and *Staphylococcus aureus* were obtained from the Department of Microbiology Ahmadu Bello University Zaria. The two bacteria species were isolated, culture and used for the research. A modified method described by Nweze *et al*. (2004) and Akter *et al.* (2010) were adopted. Standard inocula of the bacterial isolate were streaked on sterilized Mueller Hinton agar plates with the aid of a sterile swab sticks. Five wells were punched on each inoculated agar plate with a sterile cork borer (8 mm).The wells were properly labeled according to different concentrations of the different extracts prepared, which were 200, 100, 50, 25, and 12.5 mg/ml (serial dilution). Each well was filled up with 0.2 ml of the different extracts of *I. capitata* and *I. conferta* plant.

The inoculated plates with the different extracts were allowed to stay on the bench for about one hour, to enable the extracts to diffuse on the agar. The plates were then

incubated at 37^0C for 24 hours. At the end of incubation period, the plates were observed for any evidence of inhibition which will appear as a clear zone that was completely devoid of growth around the wells (zones of inhibition).The diameter of the zones of inhibition for each concentration of each extract of the two plants were measured using a transparent ruler calibrated in millimeter and the results obtained were recorded.

3.6 ACUTE TOXICITY STUDIES OF *INDIGOFERA CAPITATA* AND *INDIGOFERA CONFERTA*

The method described by Lorke (1983) was adopted. A pilot study was carried out with various extracts of the two plants on albino rats, by using nine rats of three groups (three rats per each group). Each group was administered with 10, 100 and 1000 mg respectively. From the result of the pilot studies suitable dose intervals was chosen and use to conduct the final toxicity study. Four rats of four groups were used for final toxicity of each extract.

3.7 *IN- VIVO* EVALUATION OF THE CRUDE EXTRACTS OF *INDIGOFERA CAPITATA* AND *INDIGOFERA CONFERTA* AGAINST *NAJA NIGRICOLLIS* ENVENOMATED

3.7.1 CAPTURING OF SNAKE AND COLLECTION OF VENOM

One *Naja nigricollis* was captured around Zaria villages. The captured snake was identified by a taxonomist in the museum Department Biological Sciences, Ahmadu Bello University, Zaria. The venom was collected by the milking method used by Theakston *et al.* (2003). Using this method, *Naja nigricollis* was held at the neck region and a conical flask with the mouth covered by black polythene was brought close to the

mouth of the snake in such a way any bite on the black polythene by the snake allowed collection of the venom into the flask. The venom was then stored at - 4°C until required. This was considered as the crude venom.

3.7.2 DETERMINATION OF MINIMUM LETHAL DOSE (LD_{99}) OF *NAJA NIGRICOLLIS* VENOM ON MICE.

The method used by Theakston *et al.* (2003) was adopted. Using this method, four groups of the rats (n=5) were injected intraperitoneally with 0.4 ml of different doses of the reconstituted venom (0.02, 0.04, 0.08 and 0.16 mg). The control group (n = 5) received only normal saline (0.4 ml), for each group the average time of death and number of death was recorded to determine the minimum lethal dose of *Naja nigricollis* venom (LD_{99}) using statistical method of determining dose-response relationships (probit analysis).

3.7.3 DETERMINATION OF BLEEDING AND CLOTTING TIME OF RATS TREATED WITH DIFFERENT PLANT EXTRACTS OF *INDIGOFERA CAPITATA* AND *INDIGOFERA CONFERTA* AGAINST *NAJA NIGRICOLLIS* ENVENOMED RATS.

The modified method of Omale *et al.* (2012) was adopted. The albino rats used for the research were allowed to acclimatize for one week with access to clean water and animal feeds to ensure good health. The albino rats were then divided into five (5) groups as follows:-

Group 1: Received normal saline as negative control (n=.5)

Group 2: Received LD_{99} *Naja nigricolis* venom as positive control (n=5).

Group 3: Received plant extracts and LD_{99} *Naja nigricollis* venom (n=5).

Group 4: Received plant extracts and LD_{99} *Naja nigricollis* venom (n=5).

Group 5: Received plant extracts and LD_{99} *Naja nigricollis* venom (n=5).

All the albino rats used were injected intraperitoneally *(ip)*. Rats in Group 3, 4 and 5 were injected with each solvent extract of each plant based on the results of acute toxicity studied thus; 50, 100 and 200 mg/kg petroleum ether extract, 75, 150 and 300 mg/kg ethanol extract and 250, 500 and 1000 mg/kg aqueous extract respectively of *Indigofera capitata* while 250, 500 and 1000 mg/kg petroleum ether, ethanol and aqueous extracts respectively of *Indigofera conferta,* one hour (1 hr) after LD_{99} *Naja nigricollis* venom was administered intraperitoneally (*i.p*)..

Bleeding and clotting time were determined two hours (2 hrs) after the treatment of the animals. For bleeding time, the tip of the tail of each rat was cut to cause bleeding. A stopwatch was started as soon as animal began to bleed. A blotting paper was used to wipe off blood every 15 seconds. As soon as bleeding ceased the stop watch was stopped and the time recorded as bleeding time for that particular animal.

For the clotting time a drop of blood from the cut tail of each rat was placed on a clean glass slide and a stopwatch was started at the same time. A pin was passed across the drop of blood once every 15 seconds. As soon as thread of fibrin was noticed, the stopwatch was stopped and the time recorded as the clotting time for that particular rat.

3.7.4 DETERMINATION OF OEDEMA FORMING ACTIVITY ON THE EFFECT OF DIFFERENT PLANT EXTRACTS OF *INDIGOFERA CAPITATA* AND *INDIGOFERA CONFERTA* AGAINST *NAJA NIGERICOLLIS* ENVENOMED RATS.

The effect of the different extracts of both plants on edema forming activity of *Naja nigricollis* in Rats were carried out using the modified method of Diganta *et al. (*2013). In this method, fifty five (55) albino rats were used and divided into five groups accordingly.

Group 1: Received normal saline as negative control (n=.5)

Group 2: Received LD_{99} *Naja nigricolis* venom as positive control (n=5).

Group 3: Received plant extracts and LD_{99} *Naja nigricollis* venom (n=5).

Group 4: Received plant extracts and LD_{99} *Naja nigricollis* venom (n=5).

Group 5: Received plant extracts and LD_{99} *Naja nigricollis* venom (n=5).

Group one received only normal saline and group two LD_{99} *N. nigricollis* venom subcutaneously. Albino rats in group 3, 4 and 5 were injected with each solvent extract of each plant based on the results of acute toxicity studied thus; 50, 100 and 200 mg/kg petroleum ether extract, 75, 150 and 300 mg/kg ethanol extract and 250, 500 and 1000 mg/kg aqueous extract respectively of *Indigofera capitata* while 250, 500 and 1000 mg/kg petroleum ether, ethanol and aqueous extracts respectively of *Indigofera conferta (i.p*) based on the results of acute toxicity studied. After 1hr they were injected subcutaneously in the right hind foot pad with LD_{99} *N. nigricollis* venom. Two hours (2 hrs) later the thickness of the individual rat hind foot pads, (right and left) were measured with a small thread and transparent ruler calibrated in millimeter (mm).

The increase in thickness due to oedema was expressed as ratio of the thickness of oedematous right hind foot pad to the thickness of left hind foot pad (control) in percentage.

3.8 HISTOPATHOLOGICAL STUDIES ON THE EFFECT OF EXTRACTS OF *INDIGOFERA CAPITATA* AND *INDIGOFERA CONFERTA* AGAINST *NAJA NIGRICOLLIS* ENVENOMED RATS.

The method described by Sanni (2012) was adopted. In this method five groups of Wister rats comprise of five rats per group were treated as follows:-

Group 1 were administered with normal saline (negative control)

Group 2 were administered with LD_{99} *N. nigricollis* venom (positive control)

Group 3 were administered with plant extracts and LD_{99} *Naja nigricollis* venom.

Group 4 were administered with plant extracts and LD_{99} *Naja nigricollis* venom.

Group5 were administered with plant extracts and LD_{99} *Naja nigricollis* venom.

All the albino rats used were injected intraperitoneally (*ip*). Rats in Group 3, 4 and 5 were injected with each solvent extract of each plant based on the results of acute toxicity studied thus; 50, 100 and 200mg/kg petroleum ether extract, 75, 150 and 300 mg/kg ethanol extract and 250, 500 and 1000 mg/kg aqueous extract respectively of *Indigofera capitata* while 250, 500 and 1000 mg/kg petroleum, ethanol and aqueous extracts respectively of *Indigofera conferta* based on the results of acute toxicity studied. 1hour later LD99 *N. nigricollis* venom (0.4ml). After a period of 2 hours the animals were sacrificed and liver, kidney and heart were removed, fixed with 10% formal saline, dehydrated with ascending graded alcohol (70, 80, 90 and 100 % absolute), cleared with toluene, infiltrated with molten paraffin wax using a

Leica automatic tissue processor. Sectioning the tissues was achieved by using Hertz rotary microtome (Cambridge model). Tiny sections obtained were stained in haematoxylin and Eosin using Leica automatic stained machine (Auwioro, 2010). The stained tissues were observed under Leica microscope and photographs of the observed tissues of rats treated with different extracts at different doses of both plants against *N. nigricollis* venom were taken and compared with that of control groups (Sanni, 2012).

3.9 THIN LAYER CHROMATOGRAPHY OF THE MOST ACTIVE EXTRACTS OF *INDIGOFERA CAPITATA* AND *INDIGOFERA CONFERTA*

The most active extracts (ethanol and aqueous) were subjected to further phytochemical analysis and the method used by Heinz *et al. (*2001) was adopted. In this method the most active extracts (ethanol and aqueous) of the two plant species were dissolved in methanol before spotting on chromatographic plates. TLC plates were cut and a line was drawn on each plate at the lower end. A capillary tube was used for spotting the extracts**:** ethanol and aqueous at a point interval along the straight line. TLC profile was then carried out by using two different solvent systems in order to determine the best one to be used for separation of the various active principles from the two different extracts of the two plants. After the TLC profile, the solvent with best finger prints was used and detecting reagents were used to spray the separated active constituents. The detecting reagents such as P-anisaldehyde/H_2SO_4 acid (for general), Ferric chloride (Phenolics), Aluminum chloride/UV light (Flavonoids), Liberman-burchard (Steroids & Triterpenes), Borntrager's (Anthraquinone) and Dragendorff's (Alkaloids).

3.10 STATISTICAL ANALYSIS

For the data obtained from the *in vivo* anti-venon activities, analysis of variance (ANOVA) was used. Least significant difference (LSD) was also used to analyse and compare the results that were obtained with that of the positive control group.

CHAPTER FOUR

4.0 RESULTS

4.1 EXTRACTIVE YIELD OF *INDIGOFERA CAPITATA* AND *INDIGOFERA CONFERTA*

The average yielding extracts (percentage) of *I. capitata* are shown in Figure 4.1 and the average extractive values obtained are petroleum ether (3.16 %), ethanol (4.21 %) and aqueous (5.42 %) whereas for *I. conferta,* the average yield extracts (percentage) were petroleum ether (4.21 %), ethanol (5.31 %) and aqueous (6.21 %). From the various results obtained; *I. conferta* yielded more plant extracts than *I. capitata* as shown in Figure 4.1 and Appendix I.

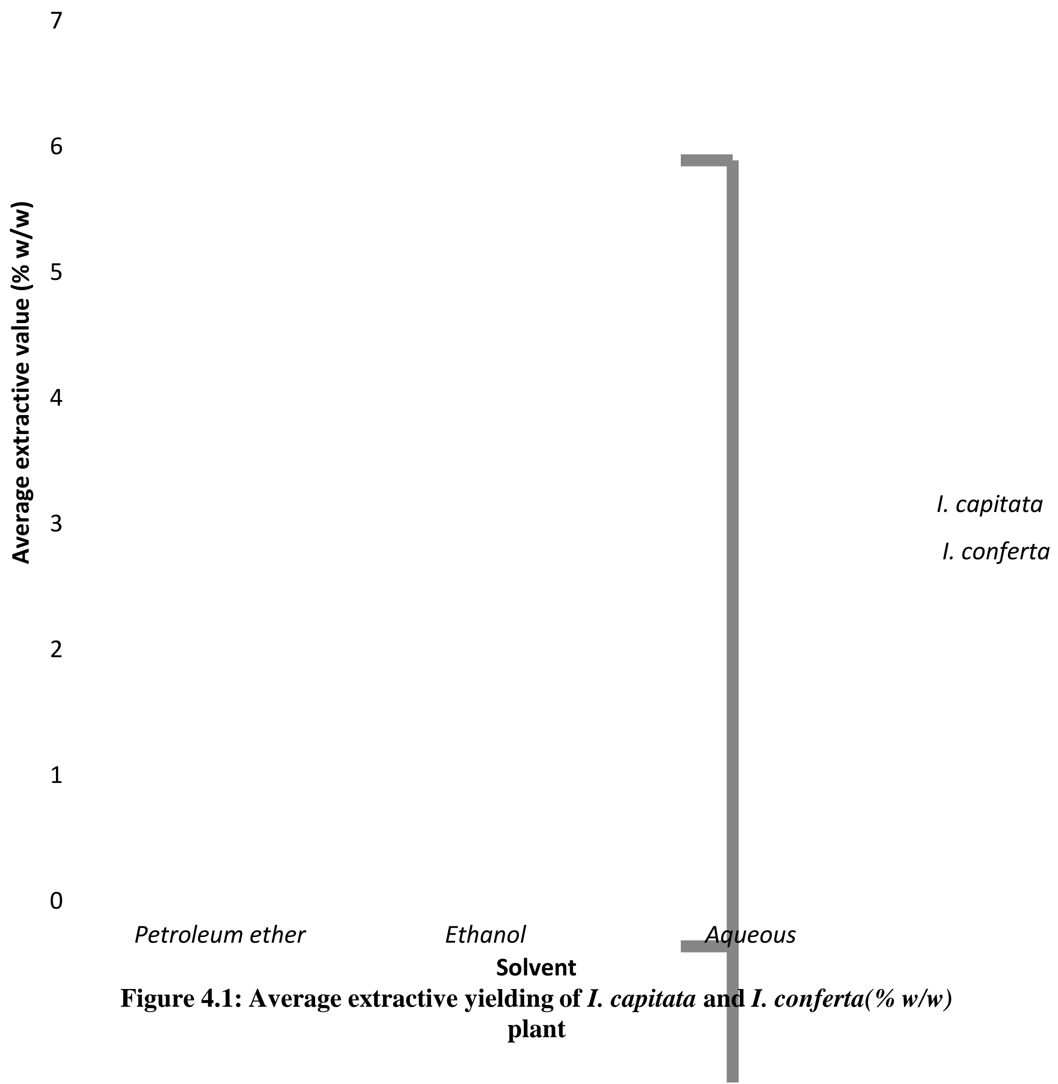


Figure 4.1: Average extractive yielding of *I. capitata* and *I. conferta(% w/w)* plant

4.2: ACUTE TOXICITY STUDIES OF *INDIGOFERA CAPITATA* AND *INDIGOFERA CONFERTA* IN ALBINO RATS

The results of acute toxicity studies (LD_{50}) of different extracts of the two plant species are tabulated in table 4.1-4.6. Table 4.1 showed the result of acute toxicity of petroleum ether extract of *I. capitata* with LD_{50} value of 774.6mg/kg, this value was very low, indicated the extract was relatively toxic and choice of dosage for *in-vivo* activity of the extract has to be low while Table 4.2 showed the result of acute toxicity of petroleum ether extract of *I. conferta* with LD_{50} value of 3807.8mg/kg , this value was very high, indicated the extract was relatively safe and choice of dosage for *in-vivo* activity must be higher than that of *I.capitata*. The result of acute toxicity of ethanol extract of *I. capitata* had LD_{50} value of 1131.4mg/kg which indicated the extract had significant level of toxic effect in the treated rats while that of *I. conferta* had LD_{50} of 3807.8mg/kg , this also indicated the extract was relatively safe. The result of acute toxicity of aqueous extracts of *I. capitata* and *I. conferta* showed both plants had LD50 of 3807.8mg/kg, these indicated that the aqueous extracts of the two the plants were relatively safe and choice of dosage for *in-vivo* must be higher (Table4.1).

Table 4.1:LD$_{50}$ values of *Indigofera capita* and *Indigofera conferta* extract in albino rats

Solvent extracts	**LD$_{50}$ *I. capitata* (mg/kg)**	**LD$_{50}$ *I. conferta* (mg/kg)**
Petroleum extract	774.6	3807.8
Ethanolic extract	1131.4	3807.8
Aqueous extract	3807.8	3807.9

4.3 ANTIBATECRIAL ACTIVITIES OF SUCCESSIVE EXTRACTS OF *INDIGOFERA CAPITATA* AND *I. CONFERTA*

The results of the antibacterial activities of various crude extracts (Pet. Ether, Ethanol and Aqueous) of *I. capitata* and *I. conferta* whole plant against *Staphylococcus aureus* (Gram-positive) and *Salmonella typhi* (Gram-negative) are presented in figure 4.2-4.4 & appendix II-IV. The zone of inhibition measured in millimeter is the representation of antibacterial activity exhibited by each crude extracts against the tested bacteria (*S. aureus* and *S. typhi*). The results of the Pet. Ether extracts of *I. capitata* showed no antibacterial activity at concentration of 12.5mg/ml, 25mg/ml and 50mg/ml. against *S.typhi* while in the case of *I. conferta* at concentration of 12.5mg/ml and 25mg/ml. Highest zone of inhibition was observed in *I. capitata* Pet. ether extract against *S.aureus* with zone of inhibition of 18mm as shown figure 4.2 and Appendix II.

The Ethanolic extracts of *I. capitata* and *I. conferta* showed no effect at concentration of 12.5 mg/ml against *S. typhi* and the highest zone of inhibition was observed in *I. conferta* against *S. typhi* with inhibition zone of 17.5 mm at concentration of 200 mg/ml in both the two plant species, the zone of inhibition increase with an increase in the concentration of ethanolic extract (Figure 4.3 & Appendix III). The aqueous extracts of *I. capitata* and *I. conferta* showed zone of inhibition at all concentration (12.5, 25, 50, 100 and 200 mg/ml) against *S. aureus*. The highest zone of inhibition was observed in both *I. capitata* and *I. conferta* against *S. aureus* with inhibition zone of 17.5 mm at concentration of 200 mg/ml while against *S. typhi* the highest zone of inhibition was observed in *I. capitata* with inhibition zone of 16.0 mm (Figure 4.4 and Appendix IV).

In the control group Ciprofloxacin was used against the two species of bacterial (*S. aureus* and *S. typhi*), the standard drug provide zone of inhibition of 49.5 mm against *S. aureus* and zone of inhibition of 40.0 mm against *S. typhi*.

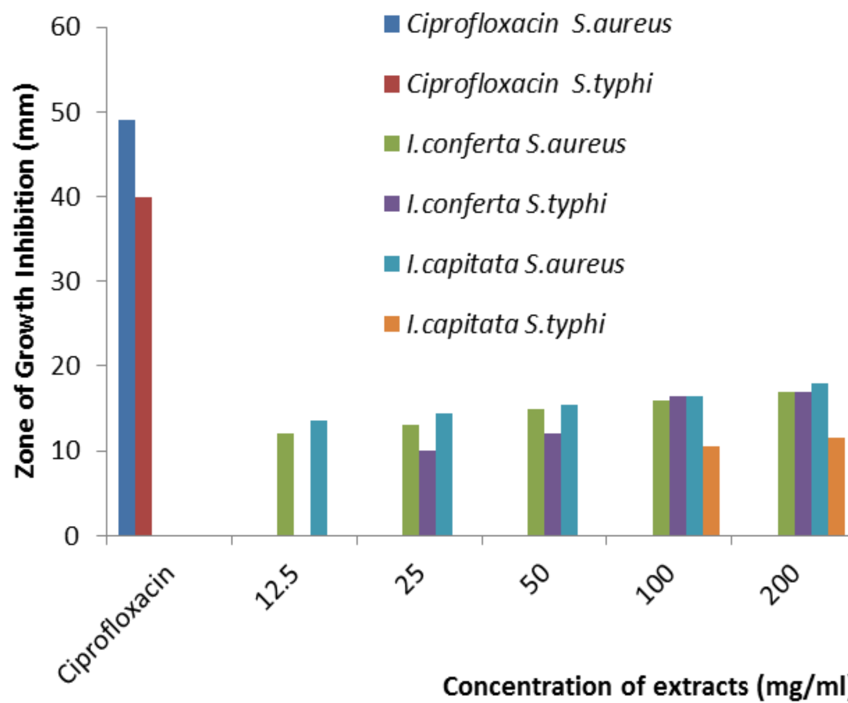


Figure 4.2: Zone of inhibitions (mm) of Petroleum ether extracts of *I. conferta* and *I. capitata* plant against *S. aureus* and *S. typhi*

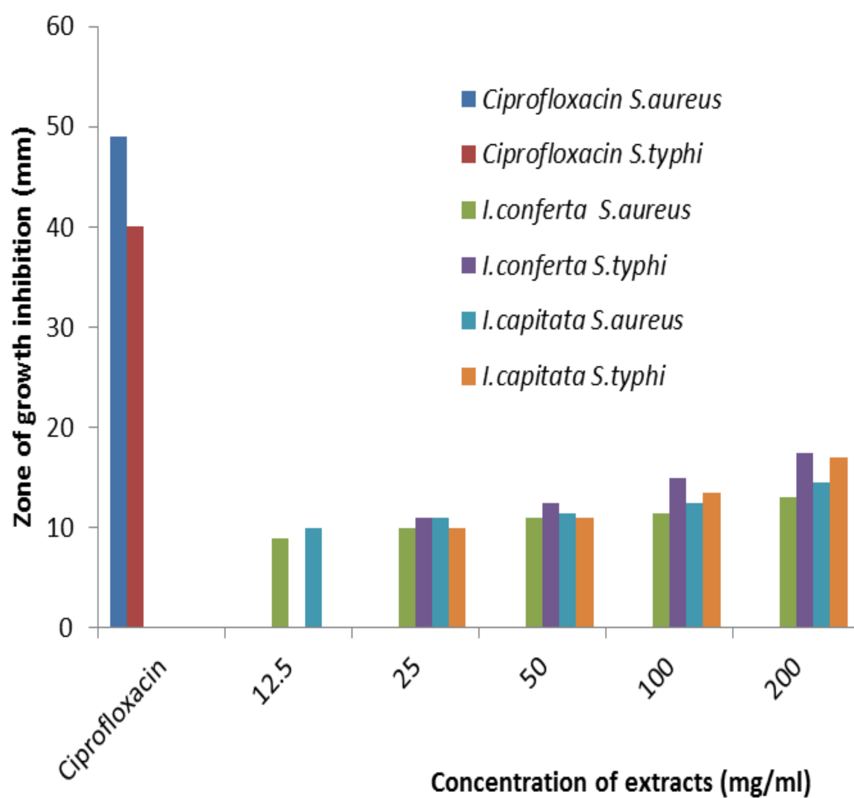


Figure 4.3: Zone of inhibition (mm) of ethanol extracts of *I. conferta* and *I. capitata* plant against *S. aureus* and *S. typhi*

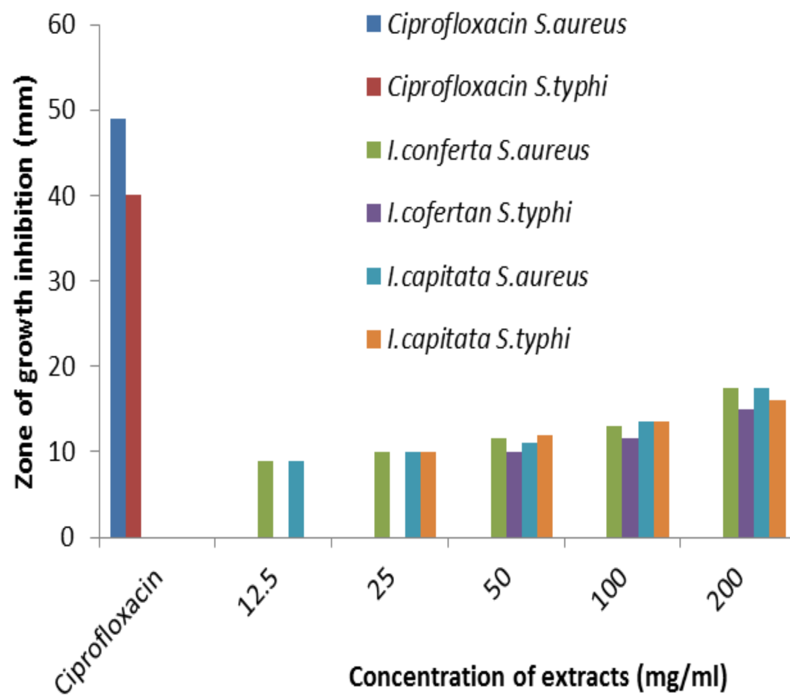


Figure 4.4: Zone of inhibition (mm) of aqueous extracts of *I. conferta* and *I. capitata* plant against *S. aureus* and *S. typhi*

The results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the various extracts of *I. conferta* and *I. capitata* against *S. aureus* are presented in table 4.2 and table 4.3. The MIC and MBC of the extracts against *S.aureus* showed ethanol extracts of *I. conferta* with MIC of 12.5 mg/ml and MBC 25 mg/ml. Petroleum ether and aqueous extracts of *I.conferta* with MIC of 6.25 mg/ml and MBC of 12.5 mg/ml while that of *I.capitata* with MIC of 3.12 mg/ml and MBC of 6.25 mg/ml while ethanol and aqueous of *I. capitata* have MIC and MBC value of 25 mg/ml and 50mg/ml respectively. Petroleum ether extract of *I. capitata* showed MIC value of 50mg/ml and MBC above 50mg/ml. ethanolic extract of *I.capitata* showed MIC of 25 mg/ml and MBC of 50 mg/ml.

Table 4.2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of successive extracts of *Indigofera conferta* and *Indigofera capitata* plant against *Staphylococcus aureus*.

Plant extracts	*I. conferta*		*I. capitata*	
	MIC(mg/ml)	MBC (mg/ml)	MIC (mg/ml)	MBC (mg/ml)
Petroleum ether extract	6.25	12.50	3.13	6.25
Ethanolic extract	12.50	25.00	12.50	25.00
Aqueous extract	6.25	12.50	3.13	6.25

Table 4.3: Minimum inhibitory concentration (MIC) and minimum bactericidal (MBC) of successive extracts of *I. conferta* and *I. capitata* plant against *S. typhi*

Plant extract	***I. conferta***		***I. capitata***	
	MIC (mg/ml)	**MBC (mg/ml)**	**MIC (mg/ml)**	**MBC (mg/ml)**
Petroleum ether extract	12.50	25.00	50.00	>50.00
Ethanolic extract	12.50	25.00	25.00	50.00
Aqueous extract	25.00	50.00	25.00	50.00

4.4 BLEEDING AND CLOTTING TIME OF ALBINO RATS TREATED WITH SUCCESSIVE EXTRACTS OF *INDIGOFERA CAPITATA* AGAINST LD_{99} *NAJA NIGRICOLIS* VENOM

The results of different extracts (i.e. petroleum. ether, ethanol and aqueous) of *I. capitata* against LD_{99} were analyzed as shown in Table 4.4-4.6 and Appendix XII-XIII. Table4.4 showed the results of bleeding and clotting time of albino rats treated with different doses of petroleum ether extract against LD_{99} *N.nigricollis* venom, the effect of the extract in reducing the bleeding and clotting time was very small, the extract has very little protection against the venom. The results of ethanol and aqueous extracts were found to be very effective against the venom more especially at higher doses of the two extracts and increases in doses of extracts also increase the protection or neutralization potential against the LD_{99} *N. nigricollis* venom as shown in Table 4.5 and 4.6.

Table 4.4: Bleeding and Clotting time of albino rats treated with different doses of Petroleum Ether extract of *Indigofera capitata* plant against LD_{99} *Naja nigricollis* venom.

Treatment group	**Bleeding time(second)**	**Clotting time(second)**
Group 1 Received no treatment	237±5.61	171±3.67
Group 2 Received LD_{99} venom	**336±7.65**	**225±4.74**
Group 3 50mg/kg+LD_{99} venom	327 ± 5.61^{a}	216 ± 3.67^{d}
Group 4 100mg/kg+LD_{99} venom	330 ± 4.74^{b}	216 ± 3.07^{e}
Group 5 200mg/kg + LD_{99} venom	321 ± 3.67^{c}	207 ± 3.00^{f}

Results in mean ± SEM (n= 5); Values in the same column with the same superscript are considered significant (P<0.05) when compared with group2 (positive control).

Table 4.5: Bleeding and Clotting time of albino rats treated with different doses of Ethanol extract of *Indigofera capitata* plant against LD_{99} *Naja nigricollis* venom.

Treatment group	Bleeding time(second)	Clotting time(second)
Group 1 Received no treatment	237±5.61	171±3.67
Group 2 Received LD_{99} venom	**336±7.65**	**225±4.74**
Group 3 75mg/kg+LD_{99} venom	$240±4.74^{a}$	$189±3.61^{c}$
Group 4 150mg/kg+LD_{99} venom	$231±3.67^{b}$	$185±3.67^{d}$
Group 5 300mg/kg + LD_{99} venom	$231±3.67^{b}$	$174±3.67^{e}$

Results in mean ± SEM (n= 5); Values in the same column with the same superscript are considered significant (P<0.05) when compared with group2 (positive control).

Table 4.6: Bleeding and Clotting time of albino rats treated with different doses of Aqueous extract of *Indigofera capitata* plant against LD_{99} *Naja nigricollis* venom.

Treatment group	Bleeding time(second)	Clotting time(second)
Group 1 Received no treatment	237±5.61	171±3.67
Group 2 Received LD_{99} venom	**336±7.65**	**225±4.74**
Group 3 250mg/kg+LD_{99} venom	237±5.61[a]	183±5.61[d]
Group 4 500mg/kg+LD_{99} venom	231±3.67[b]	183±5.61[d]
Group 5 1000mg/kg + LD_{99} venom	228±3.00[c]	177±5.61[e]

Results in mean ± SEM (n= 5); Values in the same column with the same superscript are considered significant ($P<0.05$) when compared with group2 (positive control).

4.5 BLEEDING AND CLOTTING TIME OF ALBINO RATS TREATED WITH SUCCESSIVE EXTRACTS OF *INDIGOFERA CONFERTA* AGAINST *NAJA NIGFRICOLLIS* VENOM

The results of the different extracts of *I. conferta* against LD_{99} *N. nigricollis* venom in rats were analyzed and tabulated as shown in Table 4.7-4.9 and Appendix XII and XIII. The result obtained from petroleum ether, extract was found to show a significant difference ($P<0.05$) when compare with the positive control as shown in Table4.7.

The significant difference observed showed little difference and the values obtained were found to have a little control on the venom activities. Table 4.8 and 4.9 showed results of ethanol and aqueous extracts of *I. conferta*. The results of these two extracts showed a high significant difference in the neutralization potential of the extracts against the LD_{99} *N.nigricollis* venom in the treated rats. The reduction in bleeding and clotting time shown by these extracts against the venom were higher enough to show a complete elimination of the venom induce activities. The result of ethanol and aqueous has drastically reduce the bleeding and clotting time. The bleeding and clotting times are almost closer or the same range with that of negative group that received no treatment as shown in Table 4.8 and 4.9.

Table 4.7: Bleeding and Clotting time of albino rats treated with different doses of Petroleum ether extract of *Indigofera conferta* plant against LD_{99} *Naja nigricollis* venom.

Treatment group	Bleeding time(second)	Clotting time(second)
Group 1 Received no treatment	237±5.61	171±3.67
Group 2 Received LD_{99} venom	**336±7.65**	**225±4.74**
Group 3 250mg/kg+LD_{99} venom	333±8.79[a]	216±4.94[d]
Group 4 500mg/kg+LD_{99} venom	335±7.65[b]	210±5.73[e]
Group 5 1000mg/kg + LD_{99} venom	324±8.79[c]	192±4.73[f]

Results in mean ± SEM (n= 5); Values in the same column with the same superscript are considered significant (P<0.05) when compared with group2 (positive control).

Table 4.8: Bleeding and Clotting time of albino rats treated with different doses of Ethanol extract of *Indigofera conferta* plant against LD_{99} *Naja nigricollis* venom.

Treatment group	Bleeding time(second)	Clotting time(second)
Group 1 Received no treatment	237±5.61	171±3.67
Group 2 Received LD_{99} venom	**336±7.65**	**225±4.74**
Group 3 520mg/kg+LD_{99} venom	$258±8.79^{a}$	$180±4.92^{d}$
Group 4 500mg/kg+LD_{99} venom	$237±7.65^{b}$	$198±4.92^{e}$
Group 5 1000mg/kg + LD_{99} venom	$243±8.79^{c}$	$173±5.61^{f}$

Results in mean ± SEM (n= 5); Values in the same column with the same superscript are considered significant (P<0.05) when compared with group2 (positive control).

Table 4.9: Bleeding and Clotting time of albino rats treated with different doses of aqueous extract of *Indigofera conferta* plant against LD_{99} *Naja nigricollis* venom.

Treatment group	Bleeding time(second)	Clotting time(second)
Group 1 Received no treatment	237±5.61	171±3.67
Group 2 Received LD_{99} venom	**336±7.65**	**225±4.74**
Group 3 250mg/kg+LD_{99} venom	241±8.79[a]	183±4.92[d]
Group 4 500mg/kg+LD_{99} venom	240±8.79[b]	180±3.67[e]
Group 5 1000mg/kg + LD_{99} venom	234±7.64[c]	175±4.92[f]

Results in mean ± SEM (n= 5); Values in the same column with the same superscript are considered significant (P<0.05) when compared with group2 (positive control).

4.6 OEDEMA FORMING ACTIVITIES IN ALBINO RATS TREATED WITH SUCCESSIVE EXTRACTS OF *INDIGOFERA CAPITATA* AGAINST LD_{99} *NAJA NIGRICOLLIS* VENOM

The results obtained were analyzed and tabulated as shown in Table 4.10-4.12 & Appendix XIV. The petroleum ether extracts of *I.capitata* was found to showed little significant different when compare with the positive control. Although the effect of these extracts against the venom was not that higher enough to show complete protection against *N. nigricollis* venom in the treated rats. This may be as a result of the the pet. ether extracts of the plant do not have much antivenin active principles against *N. nigricollis* as shown in Table 4.10. Table 4.11 and 4.12 showed results of the neutralization potential of ethanol and aqueous whole plant extracts of *I.capitata* against *N.nigricollis*. The results obtained compared with the positive control showed remarkable differences. This signify that the extracted compounds of these two plants has active principles that neutralized or against *N. nigricollis* venom and the effects were more in higher doses of the two extracts (ethanol and aqueous).

Table 4.10: Oedema forming activity in albino rats treated with different doses of Petroleum ether extract of *Indigofera capitata* plant against LD_{99} *Naja nigricollis* venom.

Treatment group	Oedema forming activity (millimetre)
Group 1 Received no treatment	100.00±1.90
Group 2 Received LD_{99} Venom	**125.20±2.40**
Group 3 50mg/kg + LD_{99} venom	122.40±1.90[a]
Group 4 100mg/kg+LD_{99} Venom	122.20±2.30[b]
Group 5 200mg/kg+LD_{99} venom	122.00±1.80[c]

Results in mean ± SEM (n=5); Values in the same column with the same superscript are considered significant ($P<0.05$) when compared with group2 (positive control).

Table 4.11: Oedema forming activity in albino rats treated with different doses of Ethanol extract of *I. capitata* plant against LD_{99} *N. nigricollis* venom.

Treatment group	Oedema forming activity (millimetre)
Group 1 Received no treatment	100.00±1.90
Group 2 Received LD_{99} Venom	**125.20±1.40**
Group 3 75mg/kg + LD_{99} venom	120.20±1.80[a]
Group 4 150mg/kg+LD_{99} Venom	113.40±1.90[b]
Group 5 300mg/kg+LD_{99} venom	108.80±1.90[c]

Results in mean ± SEM (n = 5); Values in the same column with the same superscript are considered significant ($P<0.05$) when compared with group2 (positive control).

Table 4.12: Oedema forming activity in albino rats treated with different doses of aqueous extract of *Indigofera capitata* plant against LD_{99} *Naja Nigricollis* venom.

Treatment group	**Oedema forming activity (millimetre)**
Group 1 Received no treatment	100.00±1.90
Group 2 Received LD_{99} Venom	**125.20±1.40**
Group 3 250mg/kg + LD_{99} venom	112.80±2.10[a]
Group 4 500mg/kg+LD_{99} Venom	105.40±1.90[b]
Group 5 1000mg/kg+LD_{99} venom	102.00±1.89[c]

Results in mean ± SEM (n = 5); Values in the same column with the same superscript are considered significant (P<0.05) when compared with group2 (positive control).

4.7 OEDEMA FORMING ACTIVITIES IN ALBINO RATS TREATED WITH DIFFERENT EXTRACTS OF *INDIGOFERA CONFERTA* AGAINST LD_{99} *NAJA NIGRICOLLIS* VENOM

The results obtained were tabulated and analyzed as shown in Table 4.13-4.15 and Appendix XIV. The petroleum ether extract of *I. conferta* was found to showed significance change in oedema forming activity of treated rats, the slight change is an indication that the extracts has little protection against the venom of *N.nigricollis* as shown in Table 4.13. In the case of ethanol and aqueous extracts, the results obtained are highly significant difference compared with the positive control as shown in Table 4.14 and 4.15. The significant difference may likely be as a result of the active principles extracted by the two solvents from *I. conferta*.

Table4.13: Oedema forming activity in albino rats treated with different doses of Petroleum ether extract of *Indigofera conferta* plant against LD_{99} *Naja nigricollis venom.*

Treatment group	Oedema forming activity (millimetre)
Group 1 Received no treatment	100.00±1.90
Group 2 Received LD_{99} Venom	**125.20±1.40**
Group 3 250mg/kg + LD_{99} venom	114.40±1.89[a]
Group 4 500mg/kg+LD_{99} Venom	124.20±1.90[b]
Group 5 1000mg/kg+LD_{99} venom	123.60±1.89[c]

Results in mean ± SEM (n = 5); Values in the same column with the same superscript are considered significant ($P<0.05$) when compared with group 2 (positive control).

Table 4.14: Oedema forming activity in albino rats treated with different doses of Ethanol extract of *Indigofera conferta* plant against LD_{99} *Naja nigricollis* venom.

Treatment group	Oedema forming activity (millimetre)
Group 1 Received no treatment	100.00±1.90
Group 2 Received LD_{99} Venom	**125.20±1.40**
Group 3 250mg/kg + LD_{99} venom	105.20±1.89[a]
Group 4 500mg/kg+LD_{99} Venom	101.20±1.90[b]
Group 5 1000mg/kg+LD_{99} venom	101.60±1.89[c]

Results in mean ± SEM (n = 5); Values in the same column with the same superscript are considered significant ($P<0.05$) when compared with group2 (positive control).

Table 4.15: Oedema forming activity in albino rats treated with different doses of aqueous extract of *Indigofera conferta* plant against LD_{99} *Naja nigricollis* venom.

Treatment group	**Oedema forming activity (millimetre)**
Group 1 Received no treatment	100.00±1.90
Group 2 Received LD_{99} Venom	**125.20±1.40**
Group 3 250mg/kg + LD_{99} venom	100.80±1.89[a]
Group 4 500mg/kg+LD_{99} Venom	100.20±1.90[b]
Group 5 1000mg/kg+LD_{99} venom	100.60±1.90[c]

Results in mean ± SEM (n = 5); Values in the same column with the same superscript are considered significant ($P<0.05$) when compared with group2 (positive control).

4.8 HISTOPATHOLOGICAL INVESTIGATION OF THE EFFECT OF DIFFERENT EXTRACTS OF *INDIGOFERA CAPITATA* AND *INDGOFERA CONFERTA* AGAINST LD_{99} *NAJA NIGRICOLLIS* ENVENOMED RATS

The different extracts of the two plants were subject to histopathological studies to determine the effects of the two plants against *N. nigricollis* (LD_{99}) envenomed rats. The results obtained from normal rats (negative control) are shown in Plates 4.1 – 4.3 while that of Albino rats treated with only LD_{99} *N. nigricollis* venom (positive control) are shown in Plates 4.4–4.6. Both extracts of plants showed remarkable protection in the liver, kidney and heart against lethal dose (LD_{99}) of *N. nigricollis* venom in rats. The protection of different extracts of the two plant species are more effective in rats treated with ethanol and aqueous extracts.

The effect of the different extracts and their neutralization potential increases with increase in doses of the extracts of the two plants .Higher doses of ethanol and aqueous extracts of both plants showed higher protections against pathological lesions in *N. nigricollis* envenomed rats compared to the positive and negative control (rats received venom and rats that received normal saline)where the tissues of the organs (liver, kidney and heart) were normal in terms of architecture and morphology. However, rats that served as positive control (rats received LD_{99} *N.nigricollis* venom) there are some pathological lesions caused by *N. nigricollis* venom namely tissues damage, vascular congestion, haemorrhage and necrosis as shown in Plates 4.4 –4. 6.

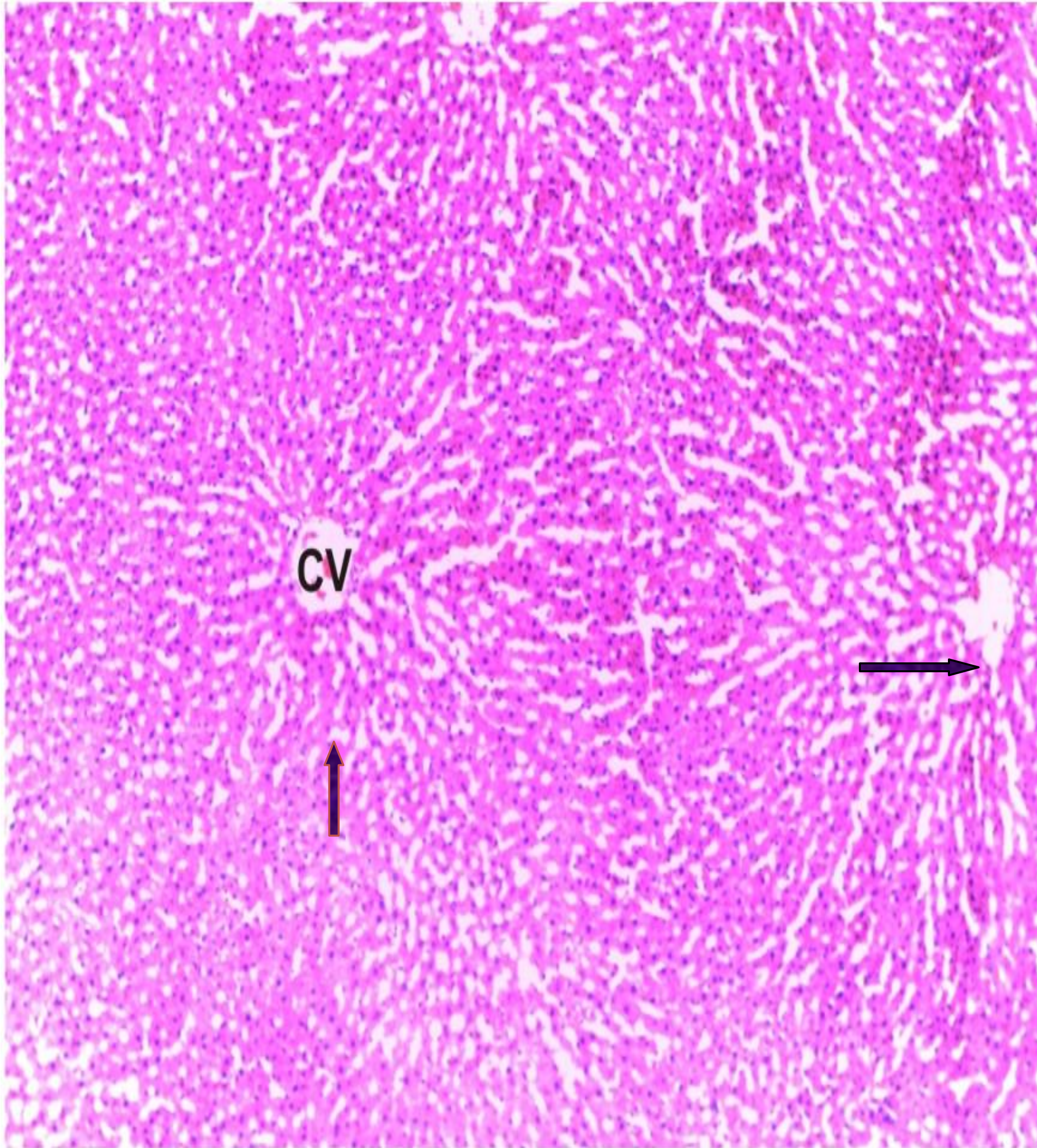


PLATE 4.1: T S of liver from Albino rats (negative control group) that received no treatment, shows normal hepatocytes arranged in polygonal units (→ containing a central vein (cv). (Haematoxylin and Eosin stained, Mg x100).

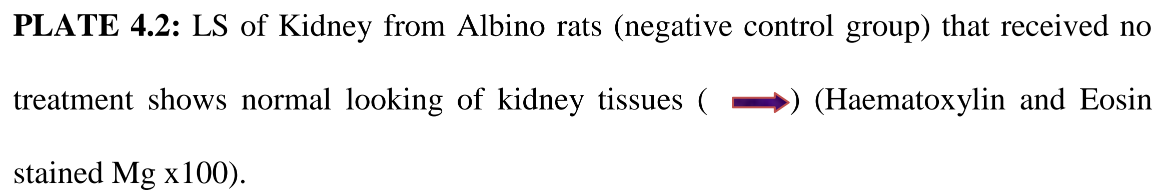
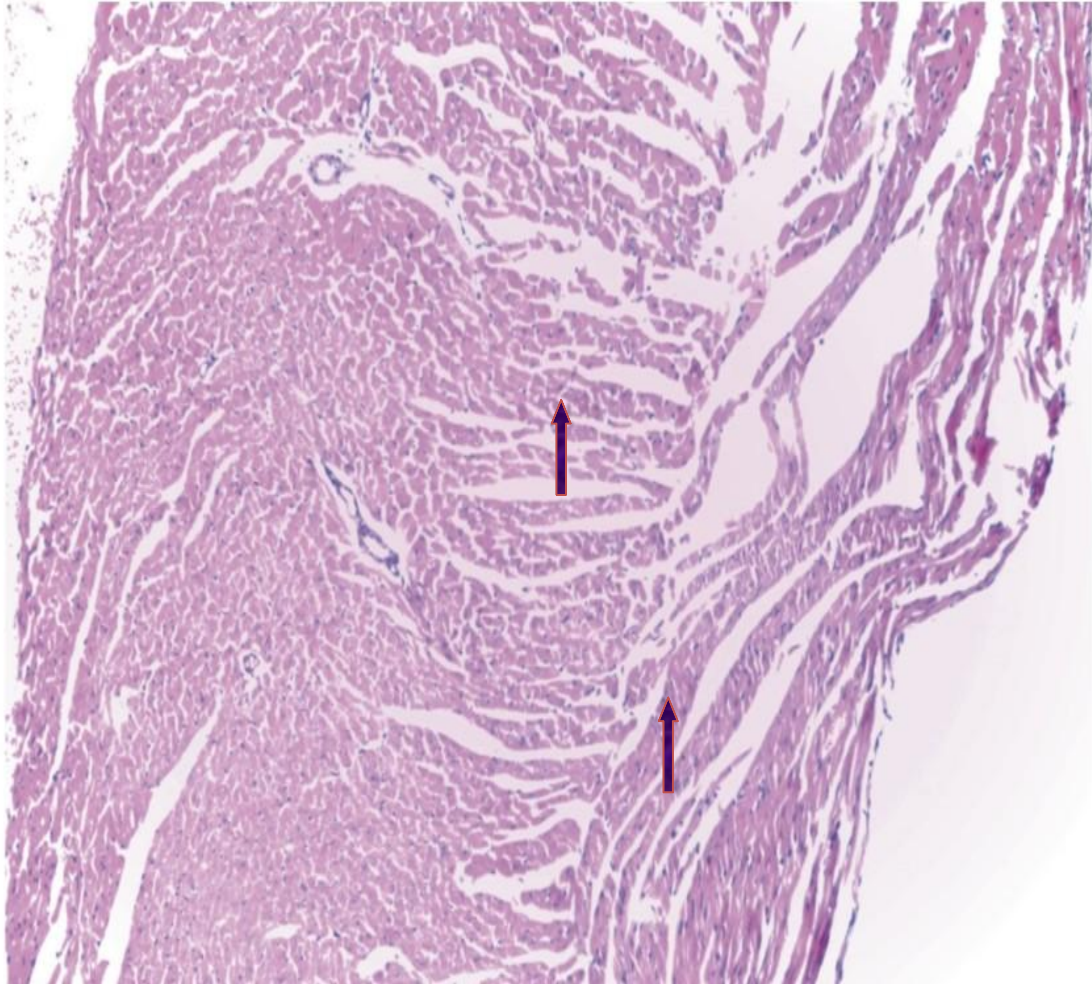


PLATE 4.2: LS of Kidney from Albino rats (negative control group) that received no treatment shows normal looking of kidney tissues ( ) (Haematoxylin and Eosin stained Mg x100).



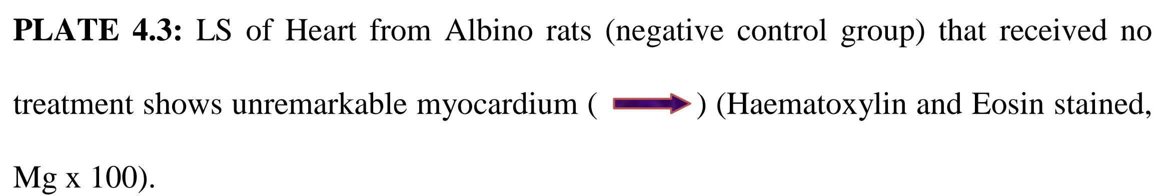


PLATE 4.3: LS of Heart from Albino rats (negative control group) that received no treatment shows unremarkable myocardium (→) (Haematoxylin and Eosin stained, Mg x 100).

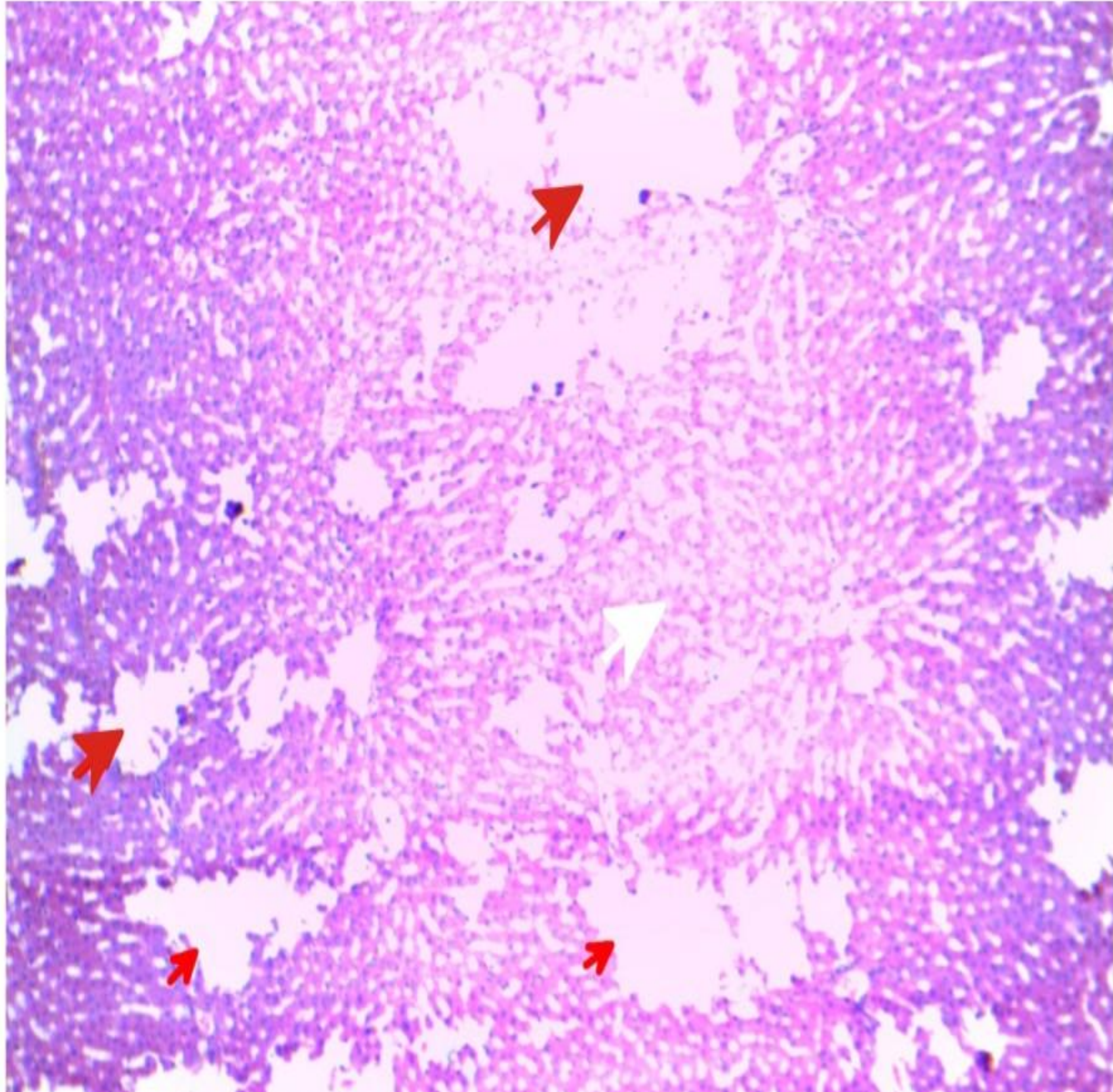


PLATE 4.4: TS of Liver from Albino rats (positive control group) that received only LD_{99} *N. nigricollis* venom shows liver damage (red arrow) and with area of necrosis (blue arrow). (Haematoxylin and Eosin stained Mg x 100).

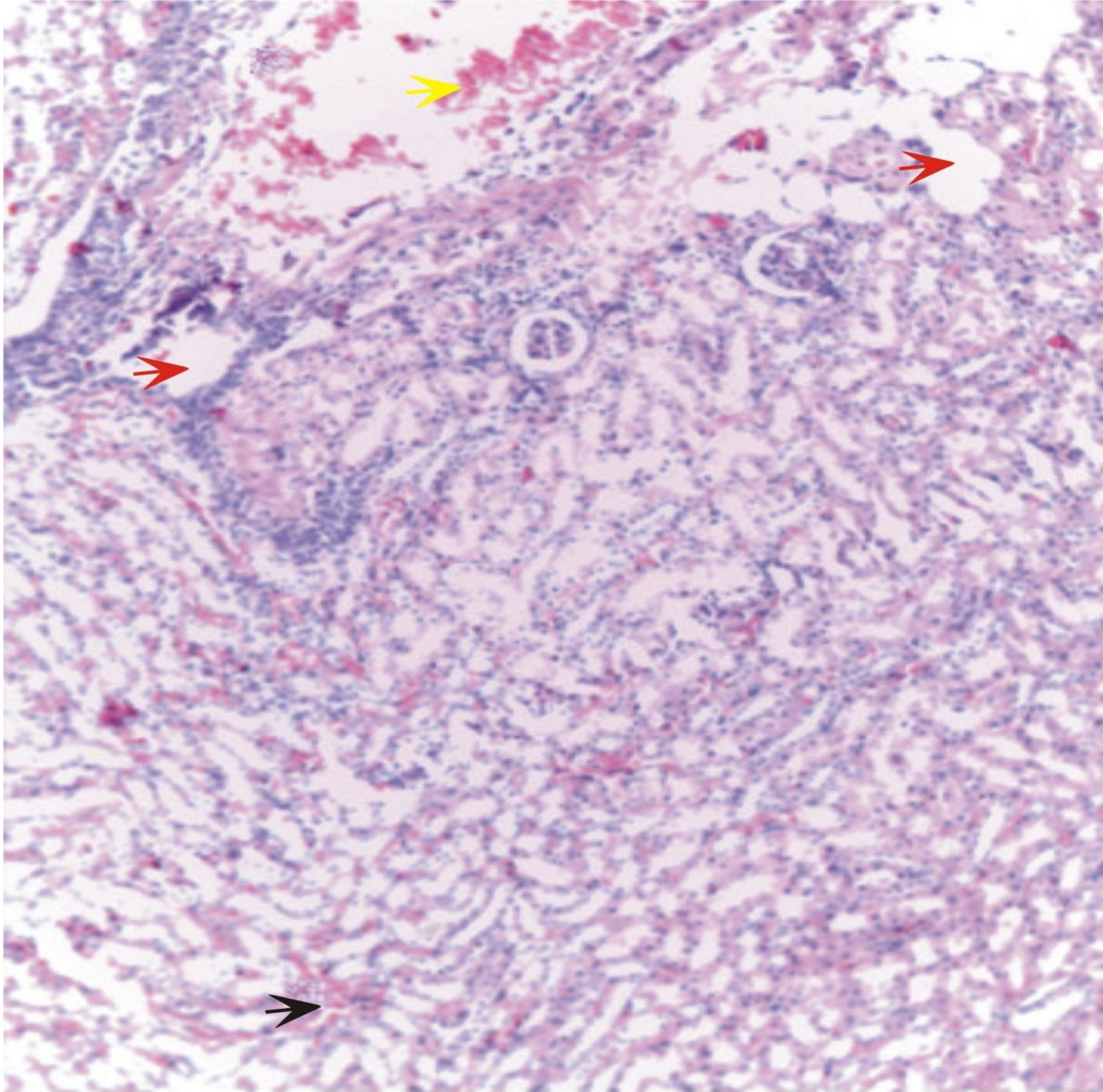


PLATE 4.5: LS of Kidney from Albino rats (positive control group) that received only LD_{99} *N. nigricollis* venom, shows vascular congestion (→), haemorrhage (→) and tissue damage (→), (Haematoxylin and Eosin stained, Mg x 100).

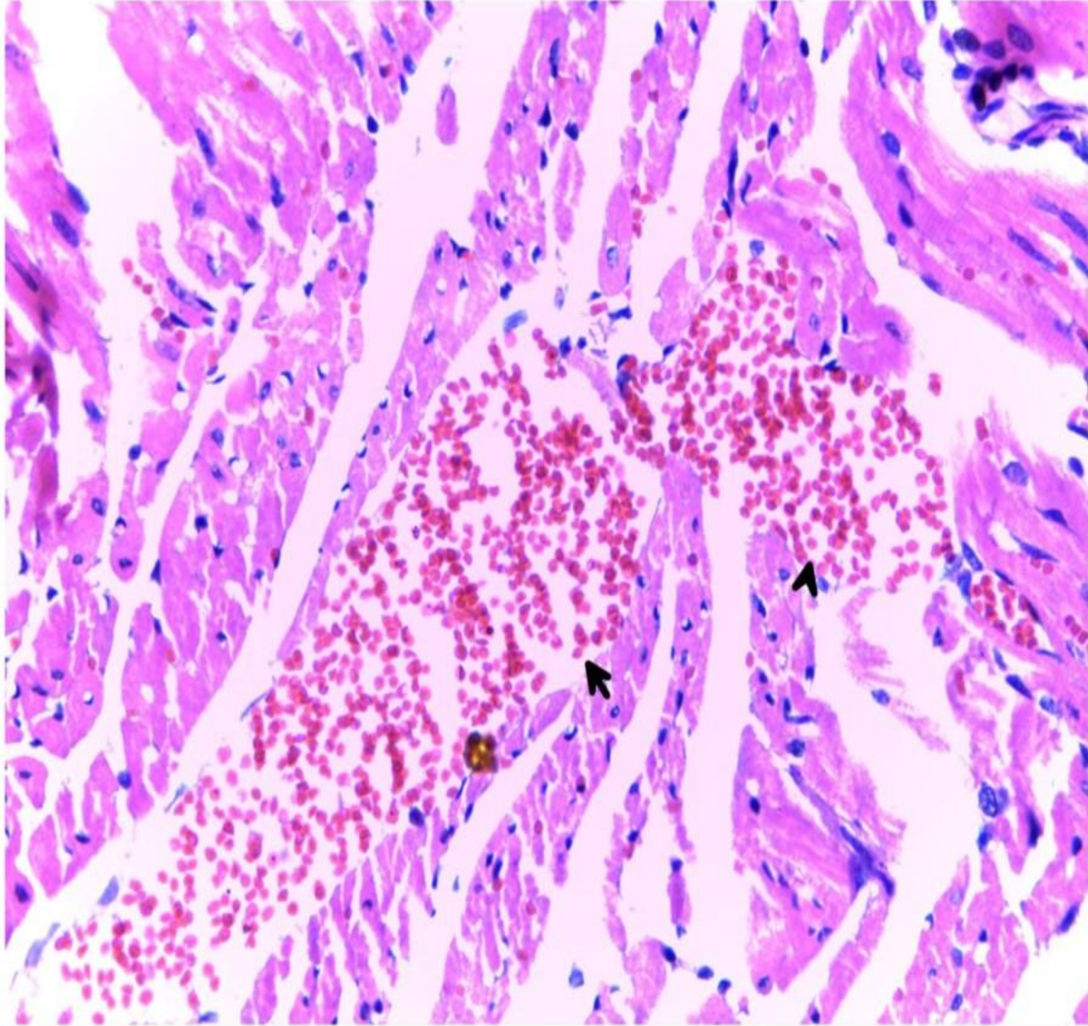


PLATE 4.6: LS of Heart from Albino rats (positive control group) that received only LD_{99} *N. nigricollis* venom, shows areas of haemorrhage (➔), (Haematoxylin and Eosin stained, Mg x 100)

4.8.1 Histopathological Responses of Liver, Kidney and Heart in Albino Rats treated with different doses of petroleum ether extracts of *Indigofera capitata* plant against LD_{99} *Naja nigricollis* Venom

The results of the histopathological responses of the liver, kidney and heart in treated rats with petroleum ether extract of *I. capitata* against LD_{99} *N. nigricollis* venom are presented in Plates 4.7-4.9. Using Leica microscope (x100Mg), the histopathological changes were observed in all rats treated with different doses (50 mg,100 mg and 200 mg/kg) of Petroleum ether extract of *I. capitata* against LD_{99} *N. nigricollis*. The changes include tissue damage, vascular congestion, haemorrhage, inflammation and necrosis. These occurred in all rats treated with different doses even at high dose(200mg/kg) of the plant extracts as shown in Plates 4. 7 – 4.9.

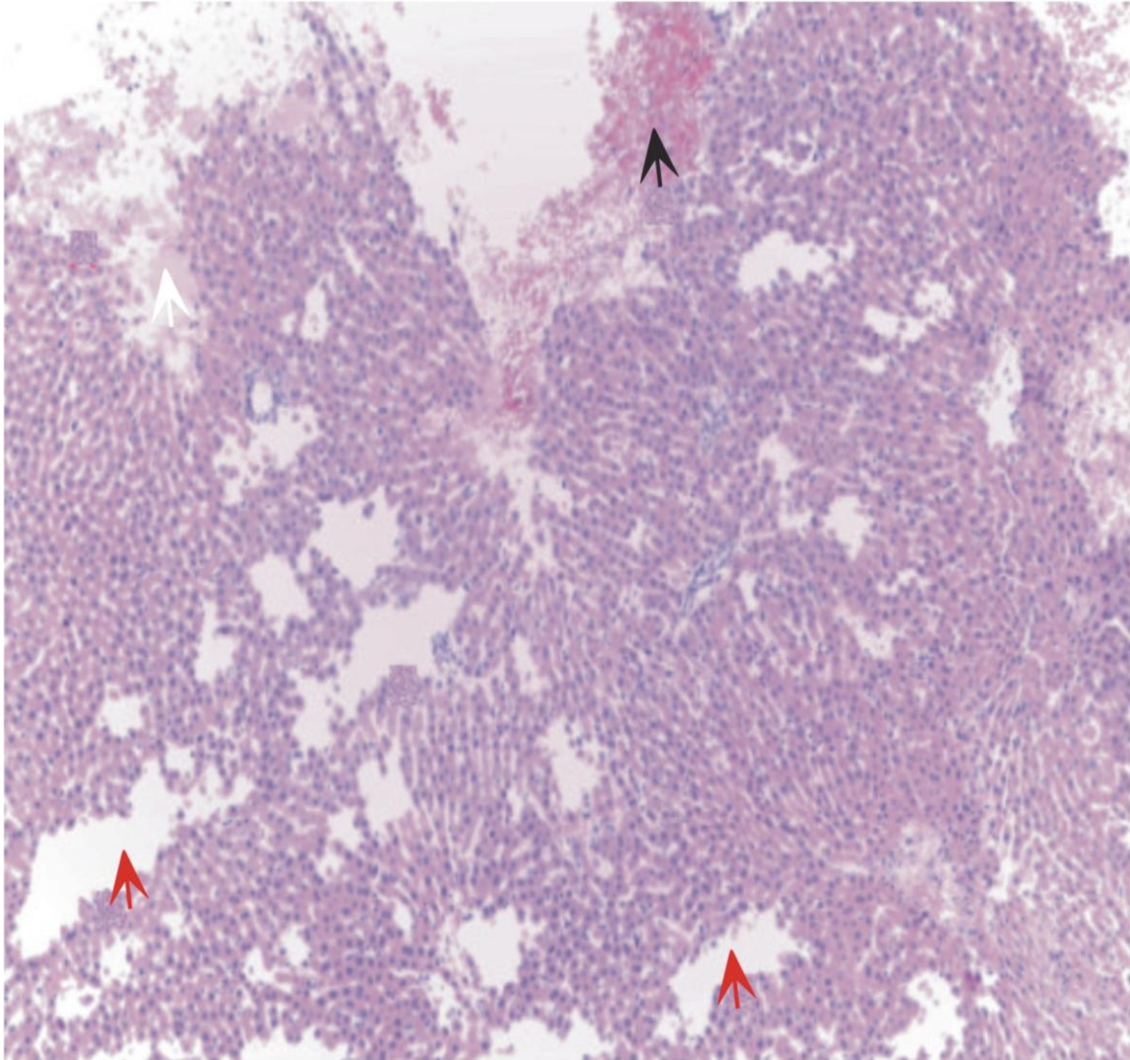


PLATE 4.7: TS of Liver from Albino rats treated with 200 mg/kg of petroleum ether extract of *I. capitata* plant and LD_{99} *N. nigricollis* venom, shows areas of haemorrhage (➔) and Liver damage (➔).(Haematoxylin and Eosin stained Mg x 100).

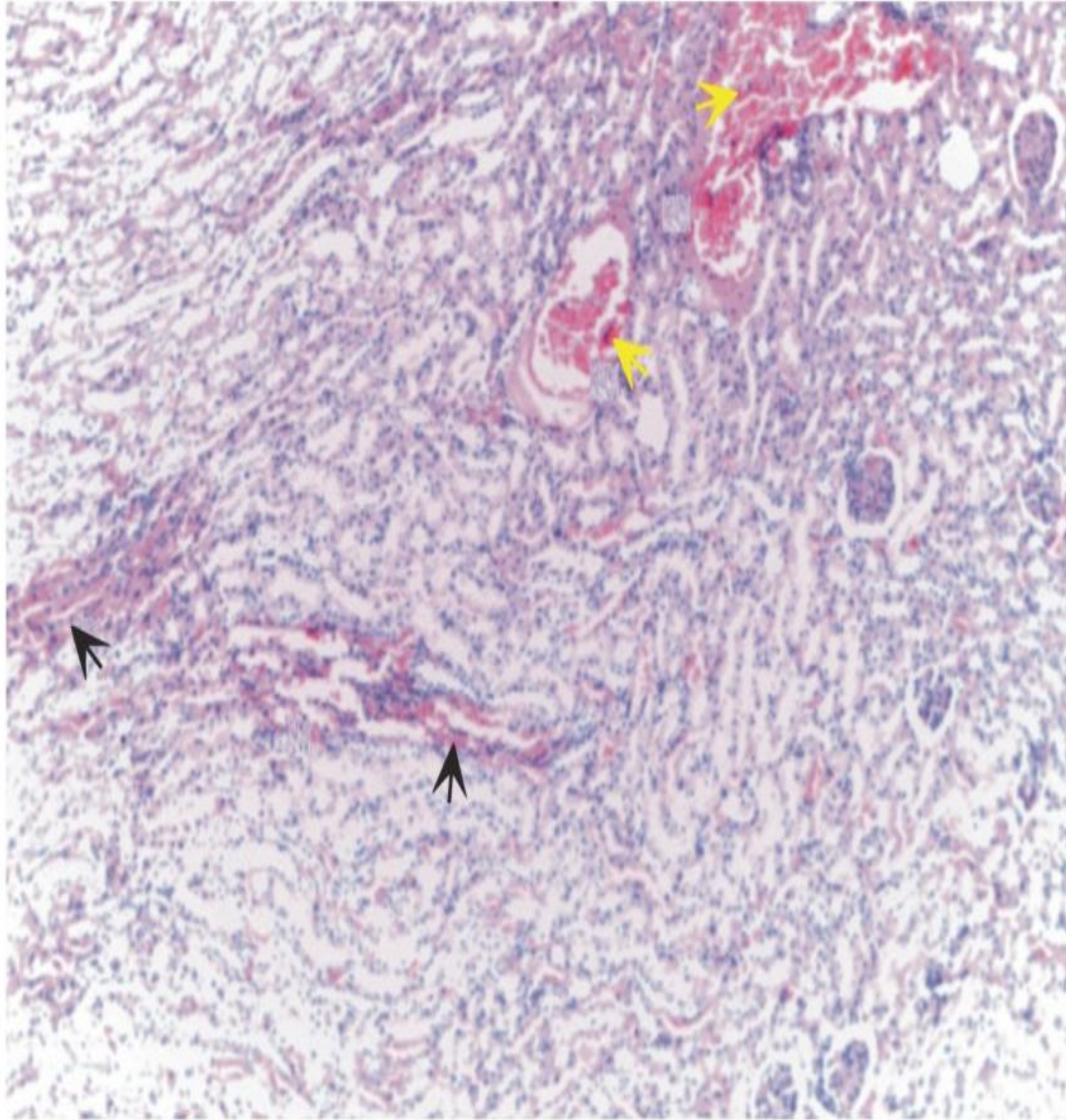


PLATE 4.8: LS of Kidney from Albino rats treated with 200 mg/kg of petroleum ether extract of *I. capitata* plant and LD_{99} *N. nigricollis* venom, shows areas of vascular congestion (yellow arrow) and haemorrhage (➔). (Haematoxylin and Eosin stained, Mg x 100).

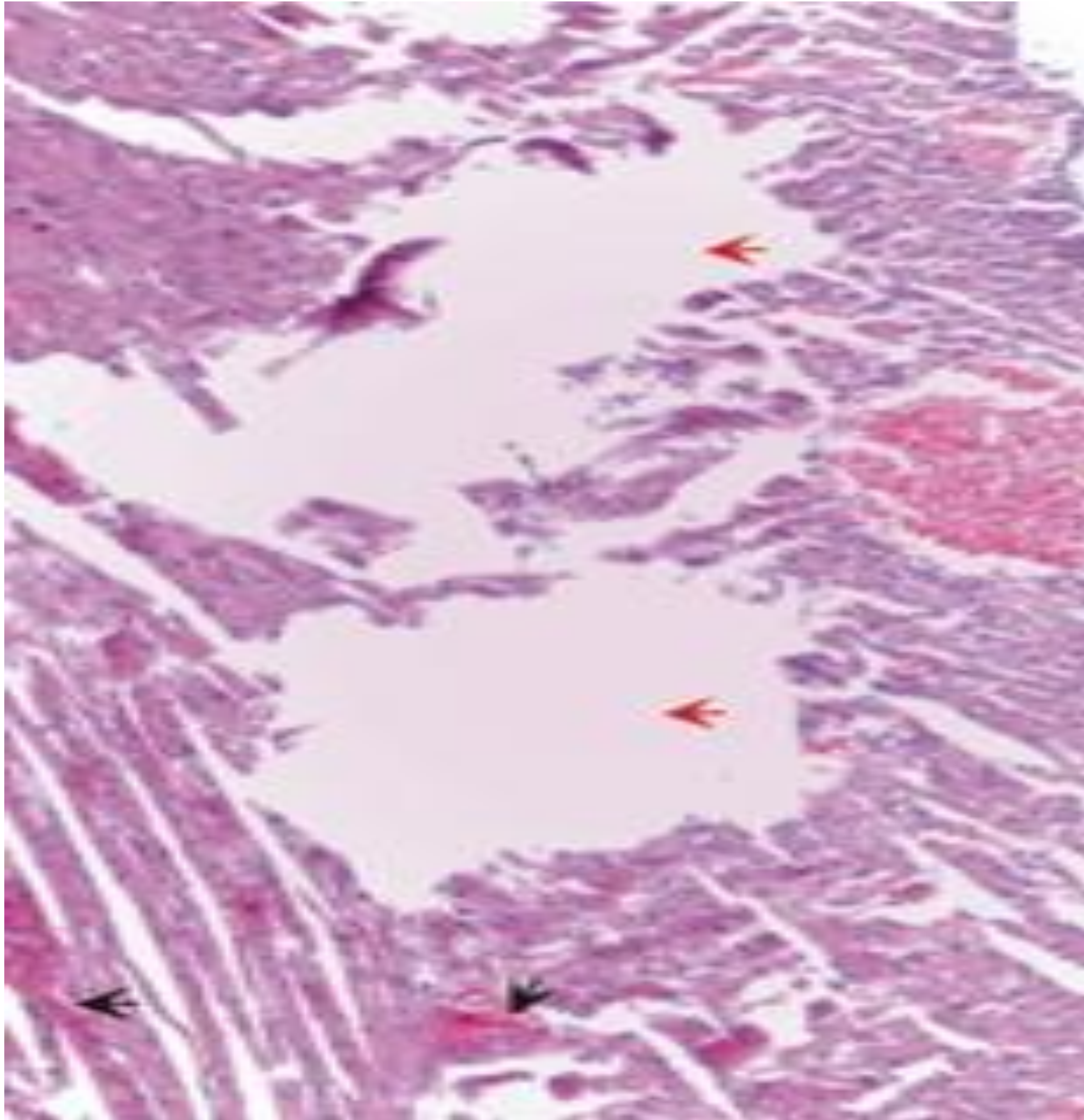


PLATE 4.9: LS of Heart from Albino rats treated with 200 mg/kg of petroleum ether extract of *I. capitata* plant and LD_{99} *N. nigricollis* venom, shows area of haemorrhage (➔)and heart tissues damage (➔),(Haematoxylin and Eosin stained Mg x 100)

4.8.2 Histopathological Responses of Liver, Kidney and Heart in Albino Rats treated with different doses of ethanolic extracts of *Indigofera capitata* plant against LD_{99} *Naja nigricollis* venom

The results of histopathological responses of liver, kidney and heart in Albino rats treated with different doses of Ethanol extracts of *I.capitata* against LD_{99} *N.nigricollis* venom were observed using Leica microscope (x100Mg), the responses of the liver, kidney and heart observed are mild vascular congestion, necrosis, haemorrhage dilation of Bowman's capsule, tissues damage at low doses (75mg and 150mg/kg) but at higher dose (300mg/kg), the extract showed protection against the venom as shown in Plates 4.10, 4.11and 4.12. These signify that the venom has no effect in the experimental animals that were treated with high dose of the extract.

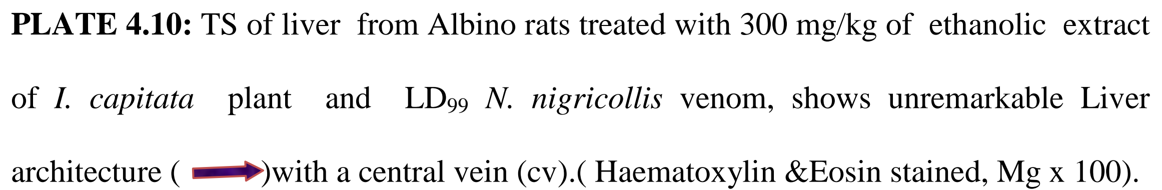


PLATE 4.10: TS of liver from Albino rats treated with 300 mg/kg of ethanolic extract of *I. capitata* plant and LD_{99} *N. nigricollis* venom, shows unremarkable Liver architecture (→)with a central vein (cv).(Haematoxylin &Eosin stained, Mg x 100).

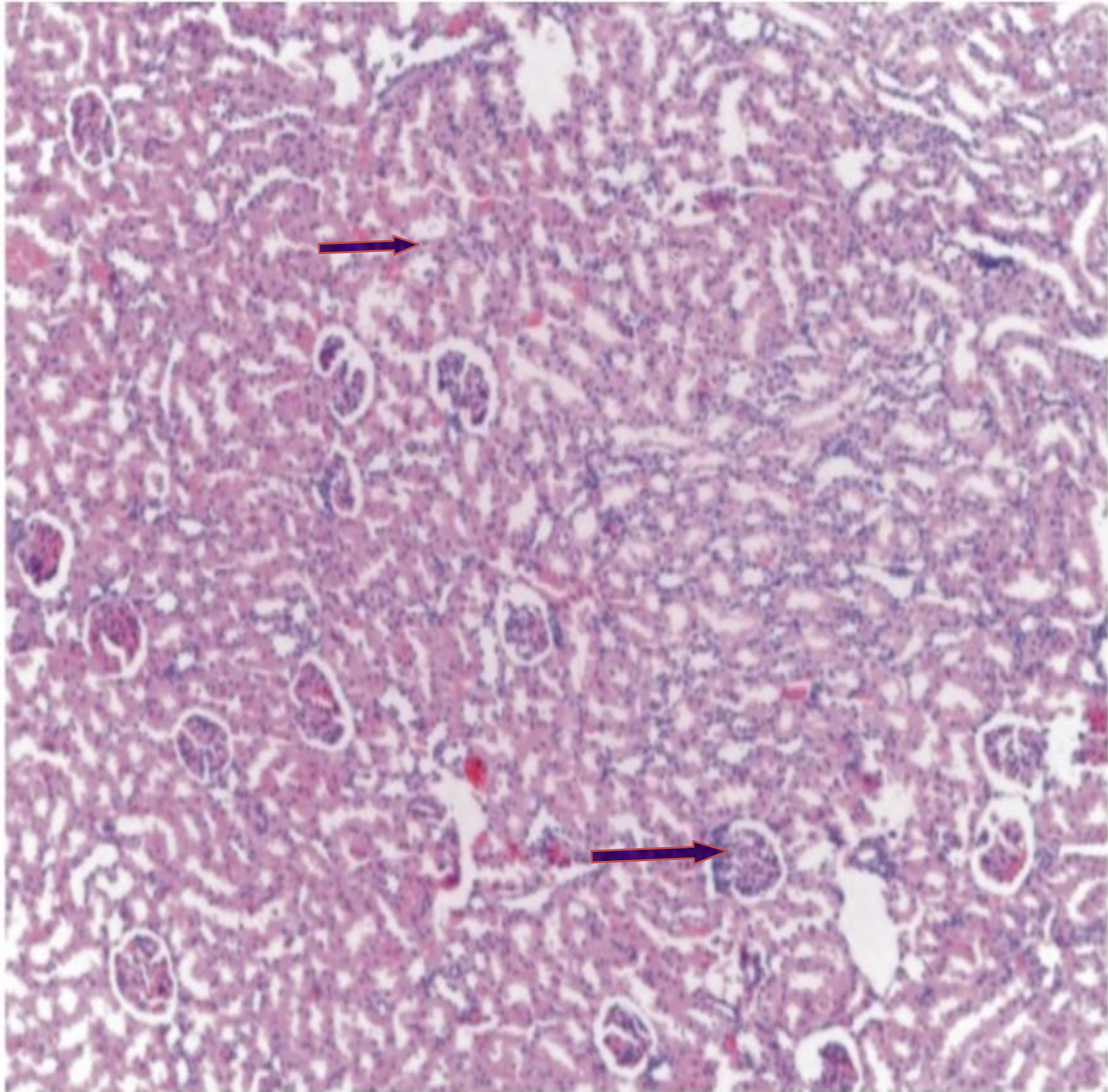


PLATE 4.11: LS of kidney from Albino rats treated with 300 mg/kg of ethanolic extract of *I. capitata* plant and LD_{99} *N. nigricollis* venom, shows normal looking of kidney(→) tissues and convoluted tubules. (Haematoxylin and Eosin stained, Mg x 100).

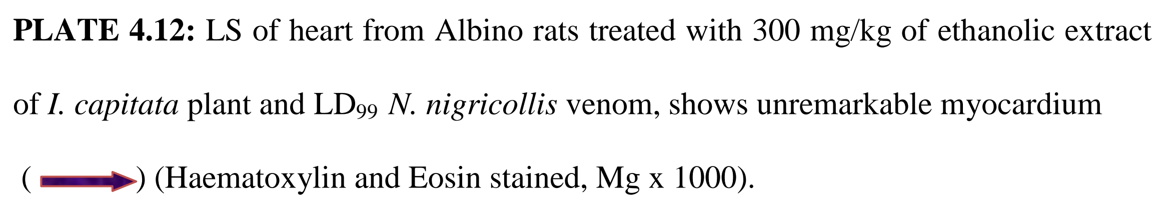


PLATE 4.12: LS of heart from Albino rats treated with 300 mg/kg of ethanolic extract of *I. capitata* plant and LD_{99} *N. nigricollis* venom, shows unremarkable myocardium (→) (Haematoxylin and Eosin stained, Mg x 1000).

4.8.3 Histopathological Responses of Liver and Kidney and Heart in Albino Rats treated with different doses of aqueous extract of *Indigofera capitata* plant against LD_{99} *Naja nigricollis* venom.

The results of the histopathological responses of liver, kidney and heart in rats treated with different concentration of aqueous extract of *I.capitata* against LD_{99} *Naja nigricollis* venom were observed using Leica microscope (x100Mg). At low dose of 250mg/kg and 500mg/kg, areas of histopathological lesions were observed in some organs of the experimental animals, such as mild vascular congestion and haemorrhage. At high dose of 1000mg/kg, the plant aqueous extract was shown to have protection against the effect of the venom as shown in plates 4.13-4.15

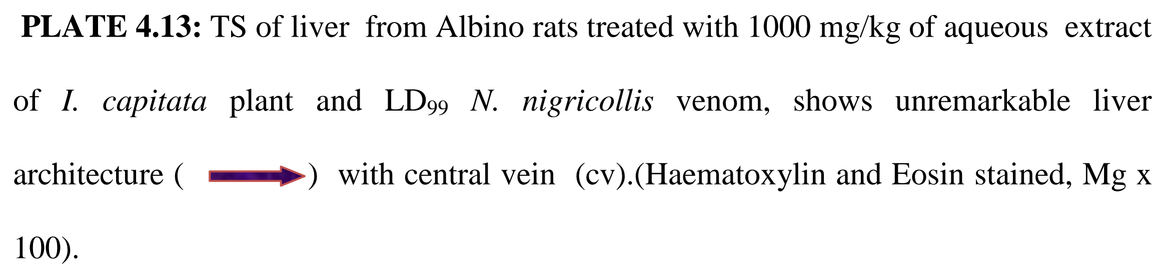


PLATE 4.13: TS of liver from Albino rats treated with 1000 mg/kg of aqueous extract of *I. capitata* plant and LD_{99} *N. nigricollis* venom, shows unremarkable liver architecture (→) with central vein (cv).(Haematoxylin and Eosin stained, Mg x 100).

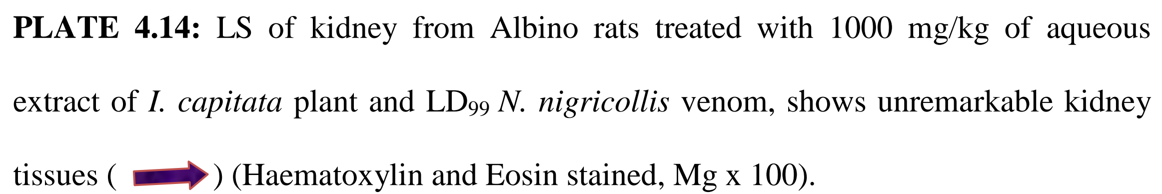


PLATE 4.14: LS of kidney from Albino rats treated with 1000 mg/kg of aqueous extract of *I. capitata* plant and LD_{99} *N. nigricollis* venom, shows unremarkable kidney tissues ( ) (Haematoxylin and Eosin stained, Mg x 100).

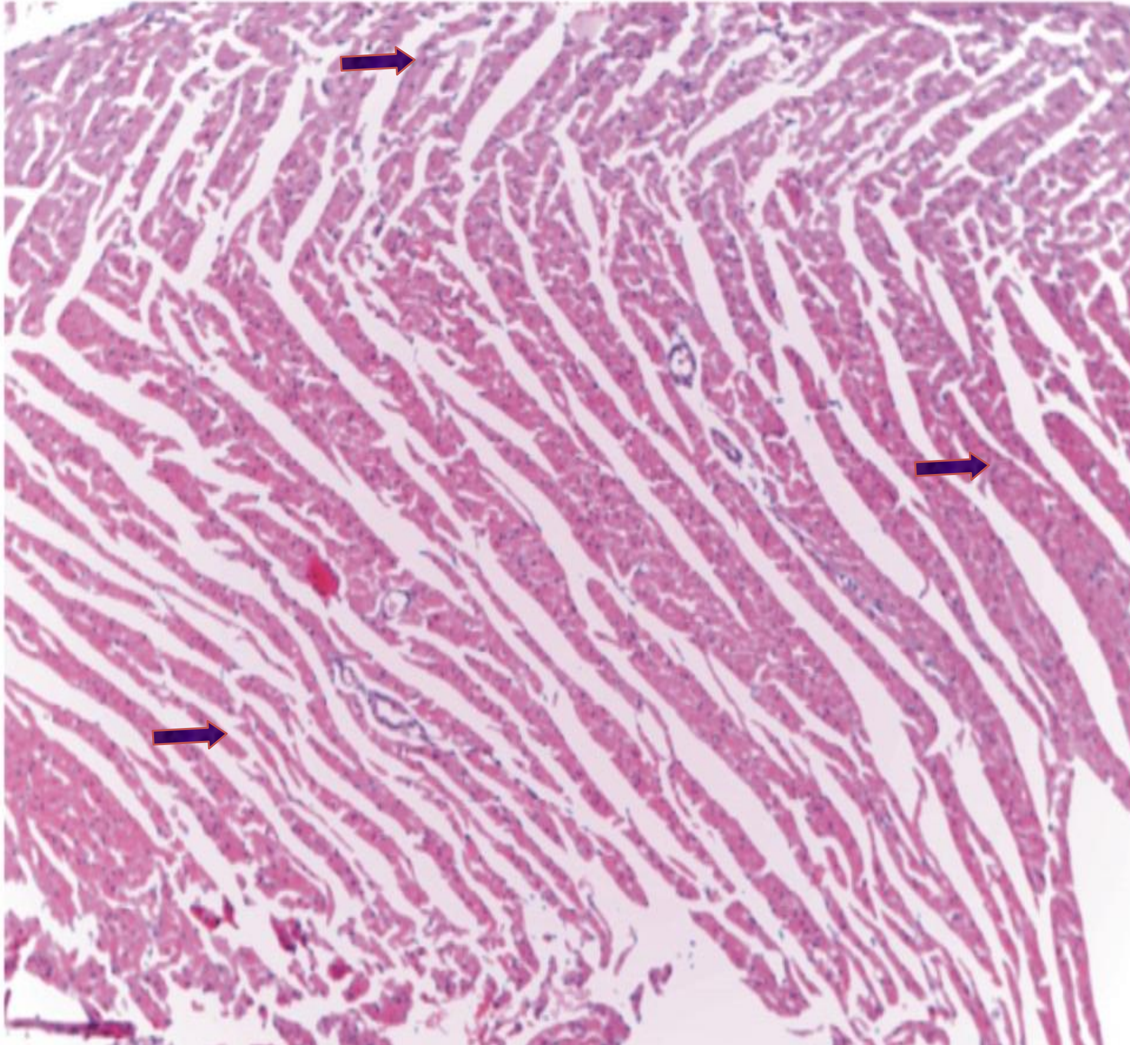


PLATE 4.15: LS of heart from Albino rats treated with 1000 mg/kg of aqueous extract of *Indigofera capitata* plant and LD_{99} *N. nigricollis* venom, shows unremarkable myocardium(➡) (Haematoxylin and Eosin stained, Mg x 100).

4.8.4 Histopathological Responses of Liver, Kidney and Heart in Albino Rats treated with different doses of petroleum ether extract of *Indigofera conferta* plant against *Naja nigricollis* venom.

The results of the histopathological responses of liver, kidney and heart in rats treated with Pet. ether whole plant extract of *I. conferta* are shown in Plates 4.16- 4.18, using Leica microscope (x100Mg). The petroleum ether extract of the plant has no effect on the venom activities in all the treated rats with the plant (*I. conferta*) extract. In all the doses of the petroleum ether extract of the plant (250 mg, 500 mg and 1000 mg/kg), the organs showed histopathological lesions such haemorrhage and vascular congestion even at high dose of 1000 mg/kg as shown in Plates 4.16- 4.18.

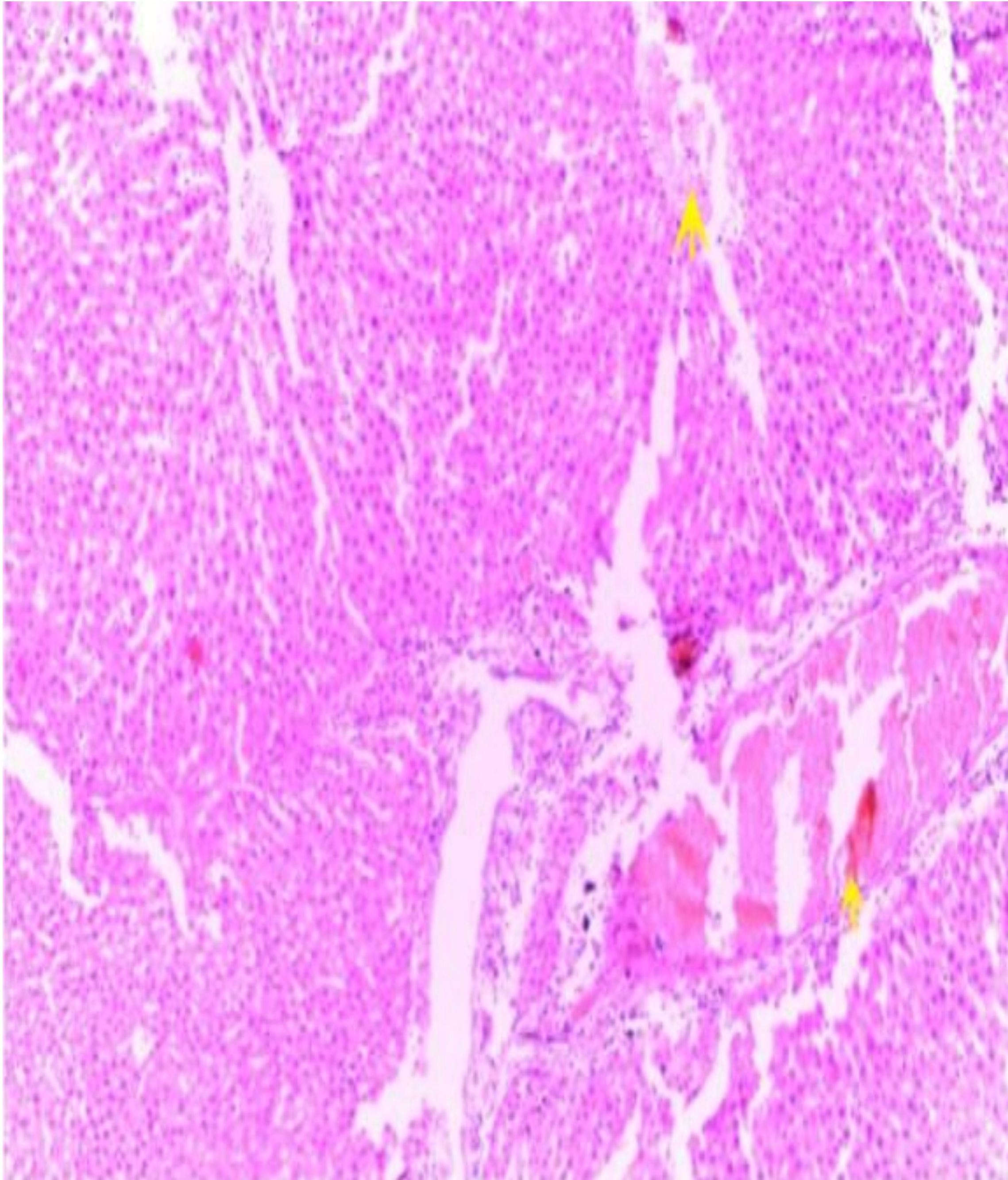


PLATE 4.16: TS of liver from Albino rats treated with 1000 mg/kg of petroleum ether extract of *I. conferta* plant and LD_{99} *N. nigricollis* venom, shows areas of mild vascular congestion (➔). (Haematoxylin and Eosin stained, Mg x 100).

Mg x 100).

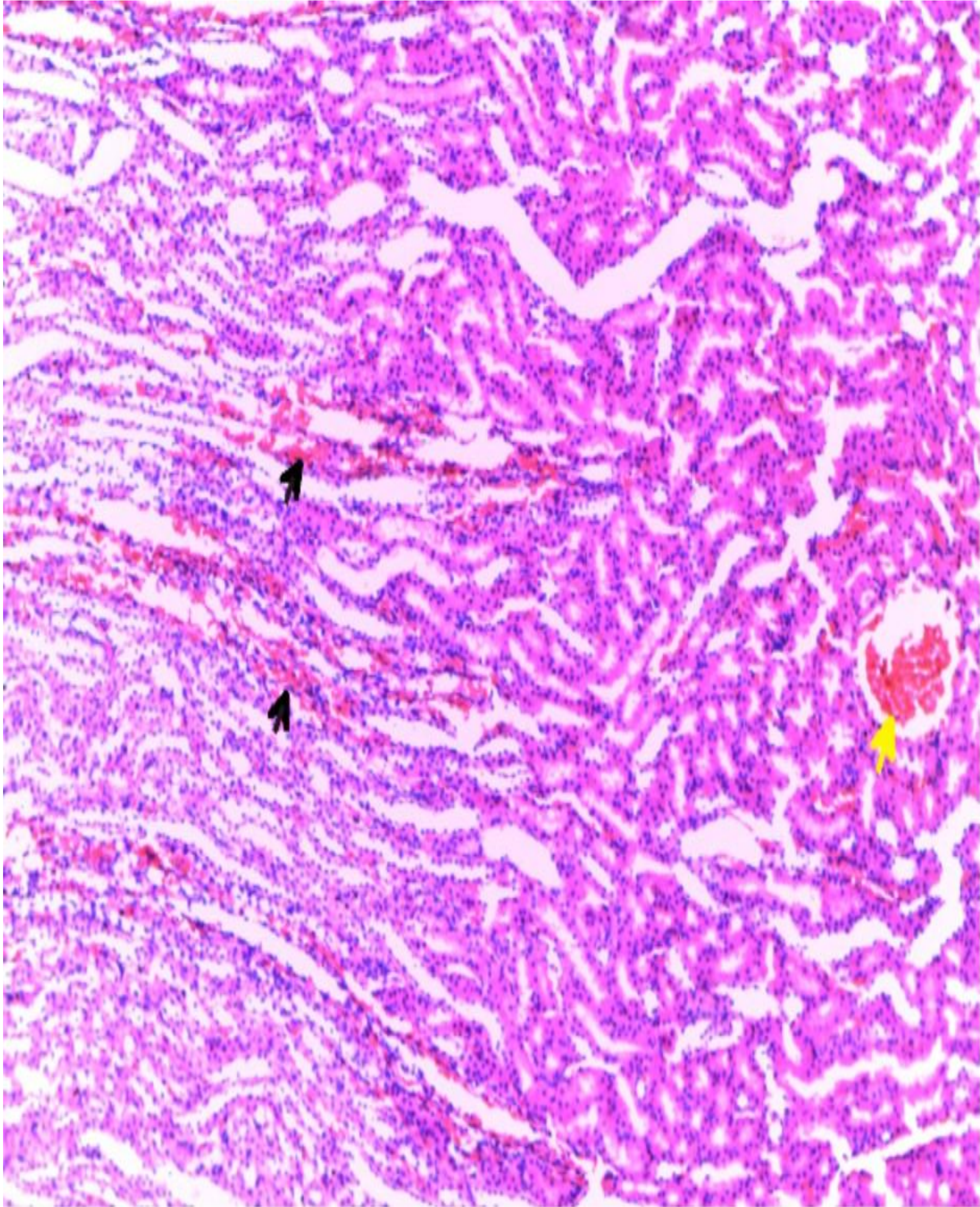


PLATE 4.17: LS of kidney from Albino rats treated with 1000 mg/kg of petroleum ether extract of *I. conferta* plant and LD_{99} *N. nigricollis* venom, shows areas of mild vascular congestion (→), and mild haemorrhage (→).(Haematoxylin and Eosin stained, Mg x 100).

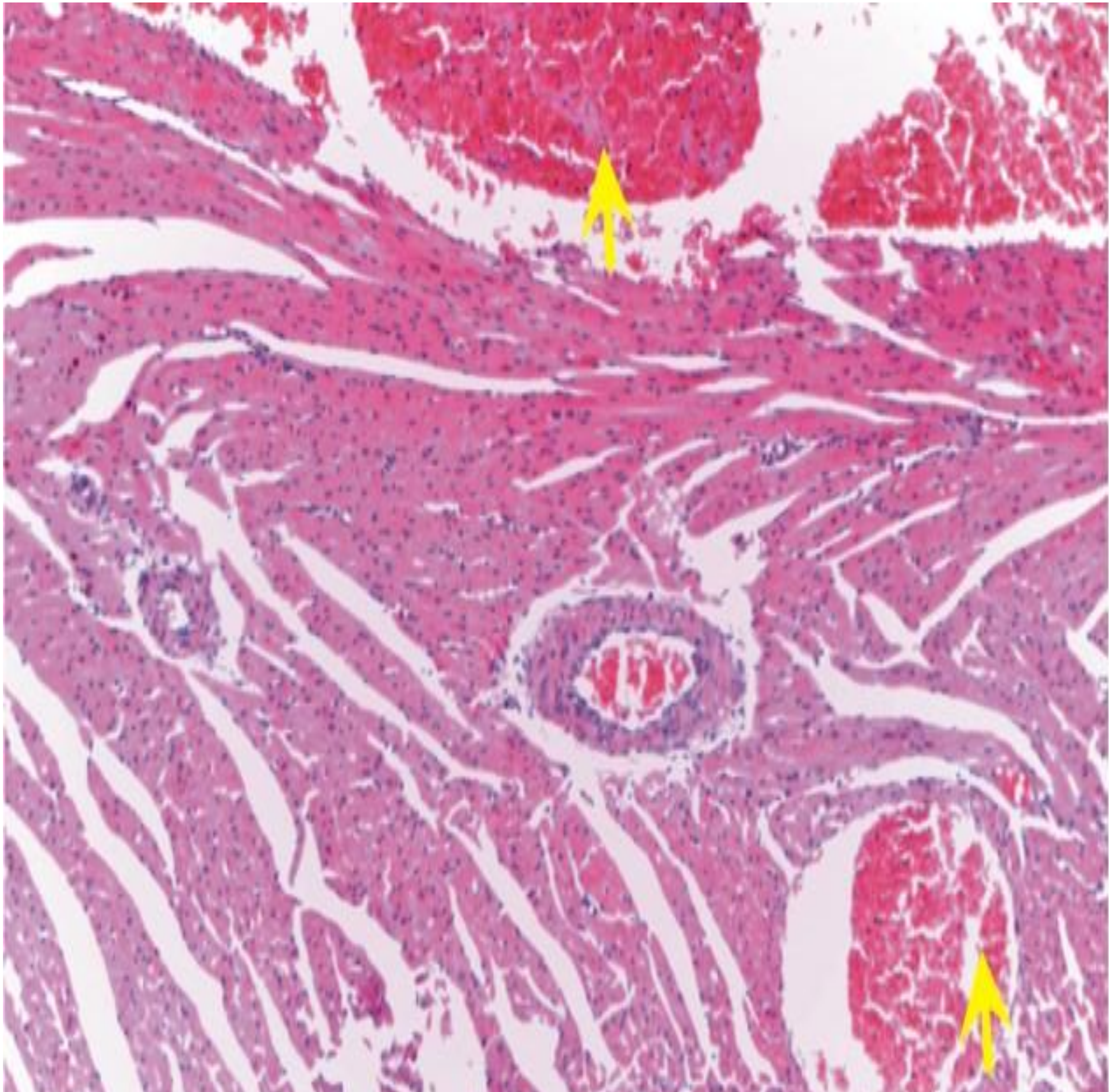


PLATE 4.18: LS of heart from Albino rats treated with 1000 mg/kg of petroleum ether extract of *I. cconferta* plant and LD_{99} *N. nigricollis* venom, shows areas of vascular congestion (➔).(Haematoxylin and Eosin stained, Mg x 100).

4.8.5 Histopathological Responses of Liver, Kidney and heart in Albino Rats treated with Different doses of ethanolic extract of *Indigofera conferta* plant against LD_{99} *Naja nigricollis* venom.

The results of histopathological responses of Lives Kidney and Heart in rats treated with different doses (250, 500 and 1000mg/kg) of Ethanolic extract of *I. conferta* against LD_{99} *N. nigricollis* venom are shown in Plates 4.19–4.21, using Leica microscope (x100mg). The responses of the three organs (Liver, Kidney and Heart) observed due to the effect of the venom (*N. nigricollis*) in the presence of *I. conferta* ethanolic extract of different doses are tissue damages, haemorrhage and vascular congestion in low dose of the plant extract (250 mg/kg) but as the dose increase to 500 mg/kg and 1000 mg/kg, the effect of the venom was completely controlled by the two doses of the ethanolic extract as observed in Plates 4.20 and 4. 21.

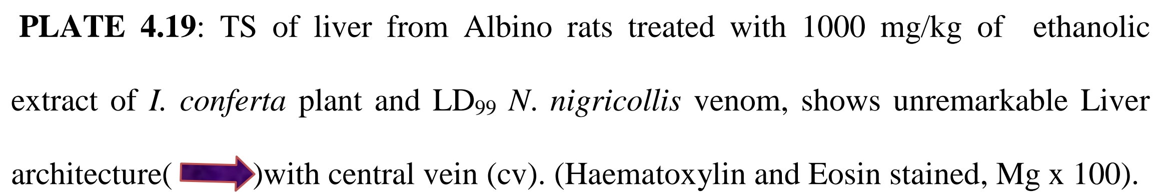


PLATE 4.19: TS of liver from Albino rats treated with 1000 mg/kg of ethanolic extract of *I. conferta* plant and LD_{99} *N. nigricollis* venom, shows unremarkable Liver architecture( )with central vein (cv). (Haematoxylin and Eosin stained, Mg x 100).

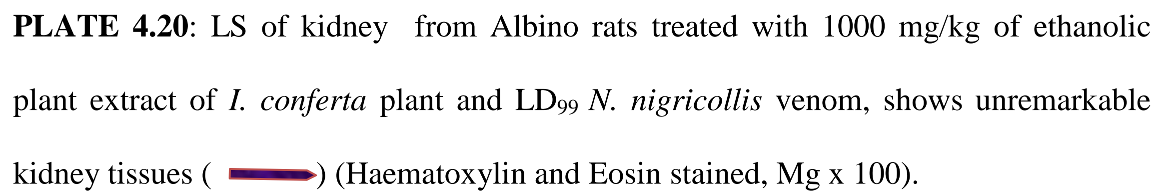


PLATE 4.20: LS of kidney from Albino rats treated with 1000 mg/kg of ethanolic plant extract of *I. conferta* plant and LD_{99} *N. nigricollis* venom, shows unremarkable kidney tissues (➡) (Haematoxylin and Eosin stained, Mg x 100).

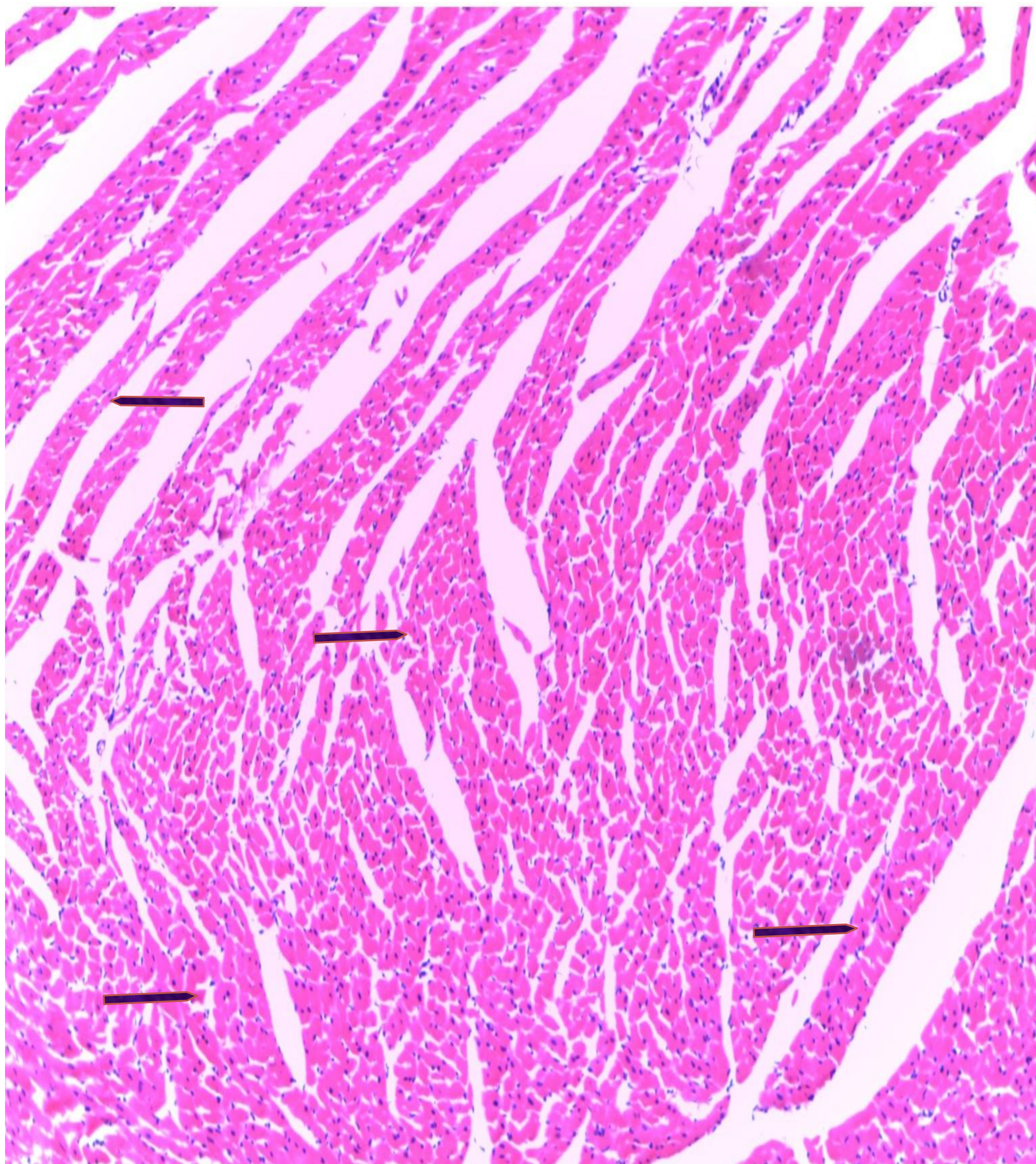


PLATE 4.21: LS of heart from Albino rats treated with 1000 mg/kg of ethanolic extract of *I. conferta* plant and LD_{99} *N. nigricollis* venom, shows unremarkable myocardium( ), (Haematoxylin and Eosin stained, Mg x 100).

4.8.6 Histopathological Responses of Liver, Kidney and Heart in Albino Rats treated with different doses of aqueous extract of *Indigofera conferta* plant against LD_{99} *Naja nigricollis* venom.

The results of histopathological responses of Liver Kidney and Heart in treated Albino rats with different doses (250, 500 and 1000mg/kg) of aqueous whole plant extract of *I.conferta* against LD_{99} *N.nigricollis* venom are shown in Plates 4.22–4.24 using Leica microscope (x100). The organs were affected by the venom even in the presence of aqueous extract of *I.conferta* at dose of 250 mg/kg but increase the dose the extract to 500 mg/kg and 1000 mg/kg, the aqueous extract of the plant (*I.conferta*) neutralized the effect of the venom and no organs responded as shown in Plates 4.22 - 4.24.

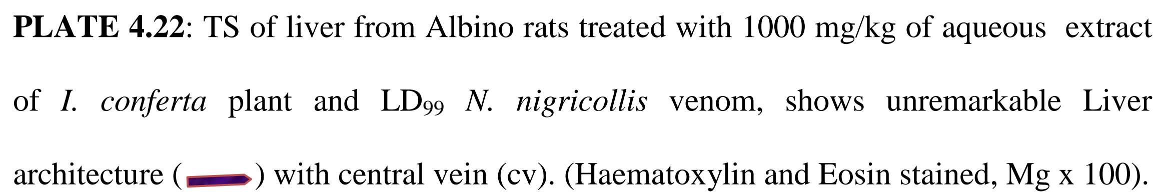


PLATE 4.22: TS of liver from Albino rats treated with 1000 mg/kg of aqueous extract of *I. conferta* plant and LD_{99} *N. nigricollis* venom, shows unremarkable Liver architecture ( ) with central vein (cv). (Haematoxylin and Eosin stained, Mg x 100).

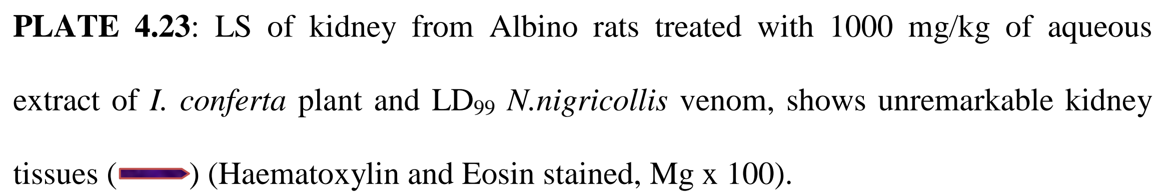


PLATE 4.23: LS of kidney from Albino rats treated with 1000 mg/kg of aqueous extract of *I. conferta* plant and LD_{99} *N.nigricollis* venom, shows unremarkable kidney tissues ( ) (Haematoxylin and Eosin stained, Mg x 100).

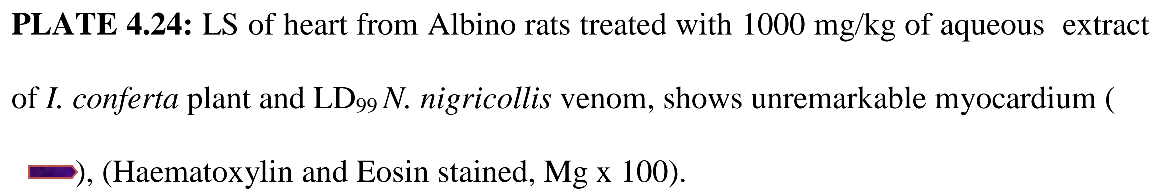


PLATE 4.24: LS of heart from Albino rats treated with 1000 mg/kg of aqueous extract of *I. conferta* plant and LD_{99} *N. nigricollis* venom, shows unremarkable myocardium (➡), (Haematoxylin and Eosin stained, Mg x 100).

4.9 THIN LAYER CHROMATOGRAPHY OF THE MOST ACTIVE EXTRACTS OF *INDIGOFERA CAPITATA* AND *INDIGOFERA CONFERTA*

The results of TLC of the most active extracts (aqueous and ethanol)of the two plants showed Butanol, Acetic acid and Water (BAW) in the raio of 8:1:1(v/v) as the best TLC solvent used for seperation of active principles in the two extracts as shown in Appendix XVII. The general detection of the active principles of the two extracts (aqueous and ethanol) Showed the appearance of different coloured compounds at different spots these indicated the nunber of active ingredients found in the two plant species (Appendix XVII –XXII). The results of Ferric chloride spray indicated the presence of Phenolic compounds in both the two extracts of the two plants(Appendix XVIII). The results of Dragendorff spray indicated the presence of Alkaloids in the extracts of the two plants(Appendix XIX). The results of Borntrager spray indicated the presence of Anthraquinones in both the two extracts(Appendix XX). The results of Liberman buchard spray indicated the presence of Steroids and Triterpenes in both plant extracts(Appendix XXI) and the results of Aluminium chloride spray which later subjected to UV flourescence indicated the presence of Flanoids in both extracts of the two plant species(Appendix XXII). Rf values were determined for each colour spot of *Indigofera conferta* and *I.capitata* ethanol and aqueous extracts as shown in Appendix XXIII-XXVII.

CHAPTER FIVE

5.0 DISCUSSION

Medicinal plants are the local heritage with global importance. Crude drugs contain natural drug of plants, animal or minerals origin which has not undergone any treatment other than collection and drying or are drugs that has not been advanced or improved by any physical or chemical treatment. Plants have been used as a source of traditional drugs and pharmaceutical preparation for man and other animals. According to a survey by the United Nations Commission for Trade and Development (UNCTAD), more than 33 % of modern drugs and medicinal products are derived from plants. The chemical substances present in plants are regarded as chemical constituents. Not all the chemical compounds produced by plants have the same activities but the so called active constituents frequently used as crude drugs are alkaloid, glycosides, flavonoids coumarins, tannins, anthraquinone, steroidal saponins and volatile oil. Various plants that were used against snake bite were reported to possess compounds with antisnake venom activities and most of these plants have no scientific validation (Antony *et. al.,* 2010).

Anti-venoms in most countries over the world are costly and may be in limited supply. Anti-venoms for therapeutic use are often preserved freeze-dried ampoules but some available only in liquid form and may be kept refrigerated. In almost all parts of the world where venomous snakes occur, numerous plants species are used as folk medicine to treat snake bite (Antomy et al., 2010).

Generally, an aqueous, methanol or ethanol extract is prepared out of the plant parts. Topical application of the plant or its sap onto the bitten area, chewing leaves or bark or drinking plant extracts decoctions or injecting the extracts helped to counteract the snake venom activity (Antomy *et al.,* 2010).

The quality control of herbal drugs and their bio-constituents is very important in justifying their acceptability in modern system of medicine. Knowledge of the biological activities and chemical constituents of plants is desirable not only for the discovery of new therapeutic agents but also because such information may be of value in disclosing new sources of economic materials for the synthesis of complex substance (Saurabh *et al*., 2010). Phytochemical screening and biological evaluation is the study of chemical constituents of plants and their biological activities on animals. The extraction of bioactive agents from plants is one of the most intensive areas of natural product research today. A reason for screening plants for bioactive agent is to use the extracted compound for pharmaceutical purposes (Mors *et al.,* 2000). The activity of any extracted compound has been shown to be due to a particular chemical compound present and this has made most of the pharmaceutical studies possible to achieve their goals. It is not always easy to isolate the bioactive agent in a plant, but such attempt should continue as characterization of the active agent, structure and activities of related studies carried out that will lead to the synthesis of more potent drugs with reduced toxicity. The activities of any part of plant that can produce biological effect depend on the extraction of the active principle with a correct solvent. A better formulation into more appropriate dosage may lead to a better understanding of the diseases.

The isolation of bioactive agent from plant can be grouped into two broad fundamental procedures: phytochemical screening i.e. searching for the compound itself and biological screening i.e. searching for the physiological effect which the plant can produce (Mors *et al.,* 2000).

Many natural products isolated from plants serve as "template" or lead molecules for the design and generation of completely new drugs. The potential of higher plants as sources of new drugs is still largely unexplored. Among all the different species of plants in the world very few have been investigated phytochemically and the fraction subjected to biological or pharmacological screening (Aida *et al.,* 2001).

Traditional medicine is the most ancient art of medical practices on which many of rural populations of developing countries rely for their health care, due to economic accessibility and the belief that natural products are more acceptable to the body and as such have fewer side effects than synthetic drugs (Aida *et al.,* 2001). Despite the fact that traditional medical practices are limited by lack of precise diagnosis, standardization, hygiene, ethics in dosaging and sometime claims or exaggerated claims which cannot be proved scientifically; traditional medicine still enjoys a wide acceptability among the citizens of developing countries. Most bioactive substances from plants are discovered as a result of scientific investigations of the plants used in traditional medicine (Aida *et al., 2001).*

Most plants have a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as

insects, fungi and herbivorous mammals. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. At least 12,000 of such compounds have been isolated so far; a number estimated to be less than 10% of the total (Aida *et al.,* 2001).These phytochemicals are divided into primary metabolites such as sugars and fats, which are found in all plants; and secondary metabolites, compounds which are found in a smaller range of plants, serving specific functions. For example, some secondary metabolites are toxins used to defend the plants and others are used to attract insects for pollination. Secondary metabolites have therapeutic actions in humans and can be refined to produce drugs. Chemical compounds in plants mediate their effects on the human body through processes similar to those chemical compounds used in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects (Mesfin *et al*., 2009).

From the results of the present research, the powdered of *Indigofera capitata* and *Indigofera conferta* were successively extracted with petroleum ether, ethanol and aqueous. The yielding extracts of these solvent extractions indicated that aqueous extraction of both the two plant species yielded the highest value then followed by ethanol extraction in both the two plants, this shows aqueous and ethanol would be regarded as the best solvents that could be used for extraction of the two plant powdered materials. The results obtained is in conformity with the finding of Saurabh *et al.*(2010) that showed *Indigofera tinctoria* after extraction with petroleum ether and

methanol yield 7.85% and 21% respectively which implies that the methanol is the best solvent to be used for the extraction of its powder material. The successive solvents extraction method was always used to determine the phytochemical profile of the various plant extracts. High extractive values of any plant would serve as a useful tools or gauge in standardization of the plant material and isolation of medicinally plants (Ajazuddin and Shaidender (2010).

The results of antibacterial activities of various extracts of *I.capitata* and *I.conferta* revealed that all the extracts of the two plant species has inhibitory effect against *S. aureus* and *S. typhi.* This inhibitory effects were observed as concentration depended which implies that as the concentration of the various extracts (pet either, ethanol and aqueous) increases the inhibitory effect also increases. This agreed with other finding which showed related species of *Indigofera* has antibacterial activities with many extracts. The study of *Indigofera glandulosa*, aqueous extract showed maximum zone of inhibition of 14mm against *A. liquefaciens,* 13mm against *Pseudomonas aeruginosa* (Prabakaram, 2011). Thangadurai *et al*.(2002) investigated the antibacterial activity *of Indigofera longeracemosa* and *Indigofera caendea* aqueous, hexane, chloroform and methanol leaves extracts against *Escherichia coli, Klebsiella Pneumonae*, *Vibrio cholerae, Bacillus subtilis* and *Streptococcus pneumonae*. Rosy *et al.* (2010) also reported that, the chloroform extract of *Indigofera aspalathoides* exhibited very promising antibacterial activity against *Staphylococcus aureus*, *Salmonella typhi*, *Vibrio cholera*e, *Bacillus subtilis*, *Staphylococcus epidermis Escherichia coli*, *Klebsiella pneumonae*, *Enterobacteria* species, *Proteus vulgaris* and *Proteus mirabilis.* In another

related finding by Sonia *et al.* (2006) showed various organic and aqueous extracts of *Indigofera suffruticosa* leaves which obtained by infusion and maceration were screened for their antibacterial and antisfungal activities. The extracts were tested against five (5) different species of human pathogenic bacteria and seventeen (17) fungal strains by the agar-solid diffusion method. Most of the extracts were devoid of antifungal and antibacterial activity except the aqueous extract of leaves of *I. Suffruticosa* obtained by infusion, showed strong inhibitory activity against the gram-positive bacteria *Staphylococcus aureus* with a minimum inhibitory concentration (MIC) of 5000 mg/ml while that of dermatophyte strains (*Triclophytom ribrum* and *Microsporum canis)* was 2500 mg/ml.

Renukadevi and Suhani (2011) also studied the antibacterial, anti-oxidant and cytotoxic activities of *Indigofera tinctoria* leaves extract. The antibacterial activity was carried out on *in vitro* lung cancer cells. The leaves extract effectively in inhibited the growth of gram positive bacteria namely *Staphylococcus aureus, Bacillus punilis* and *Streptococcus pyrogens.* Zone of inhibition was observed to increase up to 17 mm. while in gram negative, the growth of bacteria like *Escherichia coli* and *Pseudomonas aeruginosa* were inhibited.

The results of acute toxicity studies of the various extracts of the two plants(*I.capitata* and *I.conferta*) indicated that aqueous extracts of both the plants has an LD_{50} value higher enough to classified it as less toxic. In this research, *I. conferta* can be regarded as the less toxic plant (LD_{50} of 3807.9 in petroleum ether, ethanol and aqueous extracts)

than *I. capitata* (LD_{50} of 3807.9 in aqueous extract, 774.6 in pet. ether and 1131.4 in ethanol extracts). However, LD_{50}values in petroleum ether (774.6) and ethanol (1131.4) were found to below which implies that these extracts if they are used as drugs are likely to have toxic effect. Extracts with high LD_{50} value when used as drugs may likely to have low toxic effect. This finding was supported by Lorke (1983) which indicated that less value obtained in the acute toxicity study is an indication of the plant extract that when used as drugs to have high toxic effect and the higher the value obtained is an indication of the plant extract when used as drugs to have low toxic effect.

Bleeding and clotting time provide enough information about platelet activation and function and may serve as a means of accessing clinical conditions such as intravascular coagulation, thrombocytopemy and end stage liver failure (hematological abnormalities) (Saliu *et al.,* 2012).

The results of bleeding and clotting, time indicated that both plant species of *Indigofera* showed significantly different at $P<0.05$ as they reduced the bleeding and clotting time compared with that of the positive control. The effect of the various extracts of the two plants proved to be more effective in ethanol and aqueous extracts and the dose of the extracts played vital roles in the protection of the effect of venom on bleeding and clotting time of treated rats. The bleeding time is determined and associated with integrity of blood vessels and hence the effects of the venom in the positive control group caused pathological disturbances leading to incoagulability of blood. The anticoagulant activity of venom in positive control group which also prevent blood from clotting it may be due to the effect of the venom fribronolysis or fibrinogenolysis or

action of phospholipase in plate lets or plasma phospholipids. This can be supported by the finding of Ushanandini *et al,* (2006) which showed aqueous extract of aerial parts of *Bauhinia forticata* is a promising source of natural inhibitors, proteases that is involved in blood clotting disturbances induced by *Bothrops* and *Crotalus* crude venoms.

Ticli *et al.* (2005) showed the methanol bulb extract of *Crinum jagus* significantly protected mice from death, myonecrosis and haemorrhage induced by the lethal effects of *Echis ocellatus*, *Bitis arietans* and *N. nigricollis* venoms. He also showed that the seed extract of *Tamarindus* inhibited the PLA_2, protease, hyaluronidase, 1–amino acid oxidase and 5–nucleotidase enzyme (major hydrolytic enzymes) activities of *Vipera ruselli* venom in a dose-dependent manner, the extract also neutralized the degradation of the B-beta chain of human fibrinogen and indirect hemolysis caused by venom. The extract exerted a moderate effect on the clothing time, edema, hemorrhage and myotoxic effects. Lethal induced by venom were neutralized significantly when different doses of the extract were preincubated with venom before injected to the experimental animals. On the other hand, animals that received extract ten (10) minutes after the injection of venom were protected from venom induced toxicity.

The results of oedema forming activities in treated rats with the various extracts of *I. capitata* and *I. conferta* against LD_{99} *N. nigricollis* venom revealed that some of the extracts have little protection and some has high protection against the LD_{99} *N. nigricollis*. The results of different extracts with different doses analyzed showed a significant effect in the oedema forming activity of *N. nigricollis* venom in treated rats

(P<0.05). The neutralization potential of the various extracts was observed in ethanol and aqueous extracts of the two plant species which the effect increases with an increase in the doses of the extracts. The significant effect was found to be more effective at high dose 500mg/kg and 1000mg/kg of aqueous extracts of the two plants. The oedema forming activity of *N. nigricollis* venom in positive control rats was formed as a result of the effect of the venom in the blood vessels which result into the swelling of right foot when compared with left hind food that received normal saline. The swelling was due excess fluid trapped in the body's tissues that occurred as a result of accumulated tissues fluid in the surrounding tissues. This agreed with the finding of Abubakar *et al*. (2006), reported that *I. pulchra* showed inhibitory effect against the anticoangulant, hemolytic and PhospholipaseA2 activities of *N.nigricollis* venom by methanolic extract of the plant. The results obtained can also be supported by the finding of Chatterjee *et al*., (2006) which showed phytochemical constituents from the roots of *Hemidesmus inducus,* significantly neutralize oedema induced by Russell's viper in experimental animals and the plant also neutralize cardiotoxicity, neurotoxicity and respiratory changes induced by *Naja kaouthia* venom. Ethanolic extracts of *Bixa orellena, Brownea latifolia*, *Dracontium croatii, Struthanthus obincularis, Gonzalagunia panamensis* and *Trichomanes elegans* were reported to inhibit oedema due to *Bothrops asper* venom. Decrease in oedema formation with aqueous extracts of *Caeseria sylvestris* has also been noted in rats injected with lethal doses of *Bothrops asper* venom (Silva *et al*., 2008). Methanolic extract of seeds of *Vitis vinifera* has shown promise for the treatment of local effects of *Vipera russelli* bites; the extract neutralized oedema-inducing property of the venom (Mahadeswaraswany *et al.,* 2009).

Aqueous seeds extracts of *Tamarindus indica* of different doses preincubated with venom before assays significantly neutralized oedema– inducing property of viper venom (Ushanandini *et al.,* 2006). *Ancardium occidentale* bark extract has also been shown to neutralize oedema induced by viper venom (Ushanandini *et al.,* 2009).

The results of the various extracts of *I. capitata* and *I. conferta* on the venom activities in the tissues of the envenomed rats showed significant protection against the LD_{99} *N.nigricollis* venom. In both plant species aqueous extract showed significant protection against LD_{99} *N. nigricollis* venom. The results of the negative and positive control showed remarkable differences between the two groups. Group of rats treated with LD_{99} of *N. nigricollis* shows tissues damaged, haemorrhage, vascular congestion, necrosis and inflammation. Petroleum ether extracts of the two plants were found to have little or no protection against the histopathological lesions even with increase in the dose of the extracts. However ethanol and aqueous of the two plants were found to play a significant protective role against the tissues damage caused by the venom even though ethanol extracts have been found to have little or no effect on the venom, some organs responded even at high dose. In the case of *I. conferta* both ethanol and aqueous extracts were found very effective in preventing effect of the venom as there was no observe lesions in organs of the treated rats. This signified that *I. conferta* extracts are more effective in the neutralization potential of lesions caused by *N. nigricollis* venom than *I.capitata* and it may be as a result of the types of active principle found in the plant extracts.

The results obtained is in conformity with the finding of Samy *et al.* (2008) which showed the active fraction of *Aristolochia indica*, *Hemidesmius indicus, Gloriosa superba*, *Eclipta prostrata* and *Andrographis pamiculata* neutralized rattle snake venom induced action. The methanol root extracts of *Vitex negundo* and *Embilica officinalis* significantly neutralized the *Vipera russelli* and *N. kaouthia* venom induced lethal activity *in vivo* studies, *Vipera russelli* venom-induced haemorrhage, coagulant defibrinogenating and inflammatory activity were significantly neutralized by both plant extracts (Alarm *et al.,* 2003).

The unremarkable appearance of liver, kidney and heart in rats treated with different doses of ethanol and aqueous extracts of *I. conferta* is an indication that *I. conferta* may offer the maximum protection against *N. nigricollis* venom than *I. capitata*. The result obtained agreed with finding of Abubakar *et al.* (2006) which showed *I. pulchra* offered the highest protection against lethal dose (LD_{99}) venom of *N. nigricollis*. His finding also had shown that the effect of extract of *I. pulchra* against the lethal dose (LD_{99}) of *N. nigricollis* venom increase by an increase in the dose of the extract. Aqueous and alcoholic extracts of dried roots of *Mimosa pudica* inhibited lethality myotoxicity and toxic enzymes of *Naja kaouthia* venom. Oral administration of garlic could be used as a prophylactic tool against cobra venom induced histological and histochemical patterns of the gastric and hepatic tissue changes in rats (Rahny and Hemmaid, 2001). *Mandevilla illustris* inhibited phospholipase activity of *Crotalus durissus* snake venom along with prolongation of survival time and a decrease in lethality (Izidoro *et al.,* 2003).

The TLC results revealed the presence of Phenolic compounds, anthraquinone, flavonoids, alkaloids, steroids and triterpenes. These phytochemical might likely be responsible for antibacterial, and tissues protective effects of rats envenomed with *N. nigricollis* of the two plants. This can be supported by Assafin, (2006) who reported that triterpenes helped in neutralizing potential of haemorrhagic, fibrinolytic activities of metalloprotease from Bothrops snake venom. Lans *et al.(*2001) also revealed flavonoids, polyphenols and terpenoids are responsible for inhibition of phospholipase(PLA2) activities of Viper and Cobra venoms. Flavonoid has been reported to have antihaemorrhagic potential against cobra venom (Soares *et al.,* 2005).

The finding was also supported by NAPRALERT(2013) report which indicated that related species of *Indigofera* like *I. spinosa* which contained alkaloids, flavonoids, tannins,coumarins,sterol or triterpenes and saponins and *I. argentia* which contained coumarins, carotene. The root and the leave extract contained leucoanthocyanins, alkaloids, flavonoids and saponins. *Indigofera hebepetala* is rich in organic and fatty acids and some flavonoids. Chemical investigation of *Indigofera tinctoria* also carried out by Saurabh *et al.* (2010) revealed the presence of carotenoids, coumarins and flavonoids as the major biologically active constituents. 3,5-digalactoside in the leaves of *Indigofera hirsuta*, P-hydroxyl benzoic acid, apigenin 7-diglucoside, apigenin 7-rhamnoglucoside and kaempferol 3-neohesperidoside from the leaves of *Indigofera mysorensis* and flavonoids aglycones, phenolic acid and nitropropanoyl glucopyranoside from *Indigofera suffructicosa*.

Triterpenoids from the root extract of *Embilica officinalis* and *Vitex negundo* significantly neutralize anti-snake activity of *Vipera russellii* and *Naja kauothia* (Gomes *et al.,* 2007). *Costus speciosus* roots which contain diosgenin and starch in the rhizome have been used for snake bites and *Ipomoea digitata* contains triterpenoids, phenolic compound and flavonoids used against snake bites (Vasagam *et al.,* 2010).

Glycosides were isolated from the methanolic plant extract by chromatographic fraction. Acid hydrolysis of each glycoside with hydrochloric acid, gave kaempferol as the only aglycone, the sugar of the monoglucoside was identified as callose by thin layer chromatography in five solvent systems compared with the standard sugar samples and the position of attachment of the sugar was determined by UV spectral analysis. The glycosylation site was found to be at carbon seven of the aglycone (Sarawathi *et al.,* 2012). On alkaline hydrolysis, it remained unchanged and no organic acid was detected, showing it was not acetylated, which indicate the presence of kaempferol 7-alloside. Sugars obtained after acid hydrolysis of diglycosides were identified by co-chromatography as arabinose. In the above research, soluble compound of *Indigofera hebepetala* were exhaustively extracted from 1kg air-dried leaves of the plant with 85 % methanol and defatted with tri-chloro methame ($CHCL_3$). Identification of glycoside, kaempferol 7-alloside showed dull yellow colour under UV and changing to yellow with ammonia (NH_3). Kaempferol 3, 7- diarabinoside showed dark purple under UV changing to yellow with NH_3 (Sarawathi *et al.,* 2012).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

The antibacterial activities of the various extracts of the two plants revealed the effect of active components presence in the two plant species that may help for the treatment of some bacterial diseases by traditionally herbalist. The acute toxicity study of the different extracts of the two plants helped to provide the safety dose to be used for in vivo activities of the various extracts of the two plants. It also helped to know the safeties of the various extracts of the two plants if medically used as drugs. The bleeding and clotting time of treated rats with different extracts of *I.capitata* and *I. conferta* against *N.nigricollis* venom revealed that the two plant different extracts significantly reduce the bleeding and clotting time when compare with the positive control group at ($P<0.05$) and the neutralization potential of the various extracts of the two plants were found to be dose dependent. The effect of the various extracts of the two plant against *N. nigricollis* venom on edema forming activities showed the extracts of the two plants significantly reduce edema induce activities of LD_{99} *N. nigricollis* venom in envenomed rats. The histopathological studies revealed the respond of organs and the effect of the various extracts of *I capitata* and *I. conferta* against LD_{99} *N. nigricollis* venom. Petroleum ether extracts of the two plant were found not effective against the venom activities in envenomed rats even if the dose of the extras increased to the higher level the venom was observed to still very strong in destroying the organs of the treated rats but in the case of ethanol and aqueous extracts of the two plants, aqueous extra of *I. capitata* was found to play a significant role against the venom activities while the

ethanol extracts had been found to show little or no effect on the venom activities, as some organs of the treated rats responded to the venom activities even at high dose of the extracts. In the case of *I. conferta* both ethanol and aqueous extracts were found very effective in controlling the venom activities, this signifies *I. conferta* is more effective than *I.capitata* and have the neutralization potential against *N. nigricollis* venom which may be as a result of the concentration of active principles found in the plant extracts.

6.2 CONCLUSION

The present studies had established:

i. that the petroleum ether, ethanol and aqueous extracts of both plant species had significant ($p<0.05$) and concentration-dependent antibacterial activity. Petroleum ether extract of *Indigofera conferta* had MIC and MBC of 6.2 and 12.5; 12.5 and 25.0, ethanol extract: 12.5 and 25.0; 12.5 and 25.0, and aqueous extract: 6.25 and 25.0; 12.5 and 50.0 mg/ml while petroleum ether of *Indigofera capitata* had 3.13 and 50.0; 6.25 and >50.0, ethanol extract: 12.5 and 25.0; 25.0 and 50.0 and aqueous extract: 3.13 and 25.0; 6.25 and 50.0 mg/ml against *Staphylococcus aureus* and *Salmonella typhi* respectively.

ii. that the ethanol and the aqueous extracts of the two plants had significant ($p<0.05$) and dose-dependent activity against LD_{99} *Naja nigricollis* venom, the extracts of the two plants reduced the bleeding time and clotting time in envenomed rats and the effects were more effective at higher dose of 300mg/kg with lowest clotting time (second) of 174±3.67 (ethanol extract) and 1000mg/kg with lowest bleeding time

(second) of 228±3.00 (aqueous extract) of *Indigofera capitata* while in the case of *Indigofera conferta* at higher dose of 1000mg/kg with lowest clotting time (second) of 173±5.61(ethanol extract) and lowest bleeding time (second) of 234±7.64 (aqueous extract) Compared with the positive control group.

iii. that the ethanol and the aqueous extracts of the two plants had significant ($p<0.05$) and dose-dependent edema forming activity against LD_{99} *Naja nigricollis* envenomed rats. In case of *I. capitata* extracts at the doses of 300mg/kg (ethanol) and 1000 mg/kg (aqueous) had the lowest edema forming activity of 108.80±1.90 and 102.00±1.89 (% mm) respectively while aqueous extract *I. conferta* at doses of 250mg/k, 500mg/kg and 1000mg/kg gave the lowest edema forming activities (%mm) of 100.80±1.89, 100.20±1.90 and 100.60±1.90 respectively better than the other two extracts compared with the positive control group.

iv. that the ethanol and aqueous extracts of the two plants were very effective against histopathological lesions of the liver, kidney and heart in the envenomed rats. In the case of *Indigofera capitata* both the ethanol and aqueous extracts had effect only at higher doses while for *Indigofera conferta* the effects were observed both in lower and higher doses and the lesions were drastically reduced with increase in doses.

V. that the Thin layer chromatographic profiles of the active principles identified in the most active extracts (ethanol and aqueous) of the two plants were flavonoids, anthraquinone, alkaloids and steroids/triterpenes which might be responsible for the antibacterial and antivenom activities of the two plants.

6.3 RECOMMENDATION

More research should be carried out to further validate the use of these two *Indigofera* species in ethnomedicine as antibacterial and antivenom agents.

This will go along with putting more efforts toward isolation and characterization of the phytochemical constituents in the ethanolic and aqueous extracts of the two plant species in order to establish their identities which could lead to discovery of the compounds having the antibacterial and antivenom activities from these *Indigofera* species.

The choice of time for administering the plant extracts for *in vivo* antivenom activities should be one hour or less before LD_{99} *Naja nigricollis* venom. Isolation and characterization of the separated active principles should be carried out to determine the structure of the various compounds and the isolated compounds should be investigated for antibacterial activities and anti-venom activities such as bleeding and clotting time, oedema forming activity and histopathological lesions.

The isolated compounds should also be subjected to other activities of *N. nigricollis* venom such as phospholipase A_2, proteolytic myotoxic, neurotoxic and haemorrhagic.

Other related species of *Indigofera* should be investigated for antibacterial and antivenin activities.

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APPENDIX I

Average extractive yield of the various solvent extracts of *Indigofera capitata* and *Indigofera conferta* (% w/w)

Extracts	Colour of the yield extracts	Averaged extractive yield (%w/w)	
		I.capitata	*I.conferta*
Petroleum ether	Dark green (sticky)	3.16	4.21
Ethanol	Dark green (non sticky)	4.21	5.31
Aqueous	Dark green (non sticky)	5.42	6.21

APPENDIX II

Zone of inhibition (mm) of Petroleum ether extracts of *Indigofera conferta* and *Indigofera capitata* plant against the tested bacterial strains.

Concentration (mg/ml)	*I. conferta*		*I. capitata*	
	S. aureus	*S. typhi*	*S. aureus*	*S. typhi*
200	17.0	17.0	18.0	11.5
100	16.0	16.5	16.5	10.5
50	15.0	12.0	15.5	0.0
25	13.0	10.0	14.5	0.0
12.5	12.0	0.0	13.5	0.0

APPENDIX III

Zone of inhibition (mm) of Ethanol extracts of *Indigofera conferta* and *Indigofera capitata* plant against the tested bacterial strains.

Concentration of extracts (mg/l)	*I. conferta*		*I. capitata*	
	S. aureus	*S. typhi*	*S. aureus*	*S. typhi*
200	13.0	17.5	14.5	17.0
100	11.5	15.0	12.5	13.5
50	11.0	12.5	11.5	11.0
25	10.0	11.0	11.0	10.0
12.5	9.0	0.0	10.0	0.0

APPENDIX IV

Zone of inhibition (mm) aqueous extracts of *Indigofera. conferta* and *Indigofera capitata* plant against the tested bacterial strains.

Concentration of extract (mg/l)	*I. conferta*		*I. capitata*	
	S. aureus	*S. typhi*	*S. aureus*	*S. typhi*
200	17.5	15.0	17.5	16.0
100	13.0	11.5	13.5	13.5
50	11.5	10.0	11.0	12.0
25	10.0	0.0	10.0	10.0
12.5	9.0	0.0	9.0	0.0

APPENDIX V

LD_{50} value of *Indigofera capitata* petroleum ether extract on Albino rats.

Phase of toxicity testy	**Dose (mg/kg body weight)**	**Number of death**
Phase I	1 0	0/3
	100	0/3
	1000	1/3
Phase II	600	0/1
	1000	1/1
	1600	1/1
	2900	1/1

$$LD_{50} = \sqrt{600 \times 1000} = 774.6 mg/kg$$

The petroleum ether extract of *Indigofera capitata* had LD_{50} value of 774mg/kg which indicated that the LD_{50} value obtained was very low and the extract may be regarded as highly toxic.

APPENDIX VI

LD_{50} value of *Indigofera conferta* petroleum ether extract on Albino rats

Phase of toxicity test	Dose (mg/kg body weight)	Number of death
Phase I	10	0/3
	100	0/3
	1000	0/3
Phase II	1200	0/1
	1600	0/1
	2900	0/1
	5000	1/1

$$LD_{50} = \sqrt{2900 \times 5000} = 3807.8 mg/kg$$

The petroleum ether extract of *Indigofera conferta* had LD_{50} value of 3807.8 mg/kg which indicated that the LD_{50} value obtained was very high and the extract may be regarded as having less toxic effect.

APPENDIX VII

LD $_{50}$ value of *Indigofera capitata* ethanol extract on Albino rats

Phase of toxicity test	Dose (mg/kg body weight)	Number of death
Phase I	10	0/3
	100	0/3
	1000	2/3
Phase II	200	0/1
	400	0/1
	800	0/1
	1600	1/1

$LD_{50} = \sqrt{800 \times 1600} = 1131.4 mg/kg$

The petroleum ether extract of *Indigofera capitata* had LD50 value of 1131.4 mg/kg which indicated that the LD_{50} value obtained was low and the extract may be regarded to have toxic effect.

APPENDIX VIII

LD $_{50}$ value of *Indigofera conferta* ethanol extract on Albino rats

Phase of toxicity test	Dose (mg/kg body weight)	Number of death
Phase I	10	0/3
	100	0/3
	1000	0/3
Phase II	1200	0/1
	1600	0/1
	2900	0/1
	5000	1/1

$LD_{50} = \sqrt{2900 \times 5000} = 3807.8 mg/kg$

The *Indigofera conferta* ethanol extract had LD_{50} value of 3807.8 mg/kg which indicated that the LD_{50} value obtained was very high and the extract may be regarded to have less toxic effect.

APPENDIX IX

LD_{50} value of *Indigofera capitata* aqueous extract in Rats

Phase of toxicity test	Dose (mg/kg body weight)	Number of death
Phase I	10	0/3
	100	0/3
	1000	0/3
Phase II	1200	0/1
	1600	0/1
	2900	0/1
	5000	1/1

$$LD_{50} = \sqrt{2900 \times 5000} = 3807.8mg$$

The *Indigofera capitata* aqueous extract had LD_{50} value of 3807.8 mg/kg which indicated that the LD_{50} value obtained was very high and the extract may be regarded to have less toxic effect.

APPENDIX X

LD $_{50}$ value of *Indigofera conferta* aqueous extract in Rats

Phase of toxicity test	Dose (mg/kg body weight)	Number of death
Phase I	10	0/3
	100	0/3
	1000	0/3
Phase II	1200	0/1
	1600	0/1
	2900	0/1
	5000	1/1

$LD_{50} = \sqrt{2900 \times 5000} = 3807.8 mg/kg$

The *Indigofera conferta* aqueous extract had LD_{50} value of 3807.8 mg/kg which indicated that the LD_{50} value obtained was very high and the extract may be regarded to have less toxic effect.

APPENDIX XI

ANALYSIS OF RESULTS

The statistical analysis of the data obtained on the venom versus extracts showed that both *I.capitata* and *I. confreta* have significant effect on the bleeding and clotting time and oedema forming activities of *N. nigricollis* venom as shown in Appendix XII-XIII

APPENDIX XII

One way ANOVA of the data on the bleeding time of the albino rats treated with two plant species extracts against LD_{99} *Naja nigricollis* venom

Source of variance	DF	MS *I. capitata*	MS (*I. conferta*)
Between group	10	4342.5*	3841.8*
Within group	44	293.5	173.7
Total	54		

*Significant at P<0.05

MS= Mean Square

APPENDIX XIII

One way ANOVA of the data on the clotting time of albino rats treated with two plant species different extracts against LD_{99} *N. nigrocollis* venom

Source of variance	DF	MS(*I. capitata)*	MS (*I. conferta*)
Between group	10	743.1*	674.0*
Within group	44	120.2	107.3
Total	54		

*Significant at P<0.05

MS = Mean Square

APPENDIX XIV

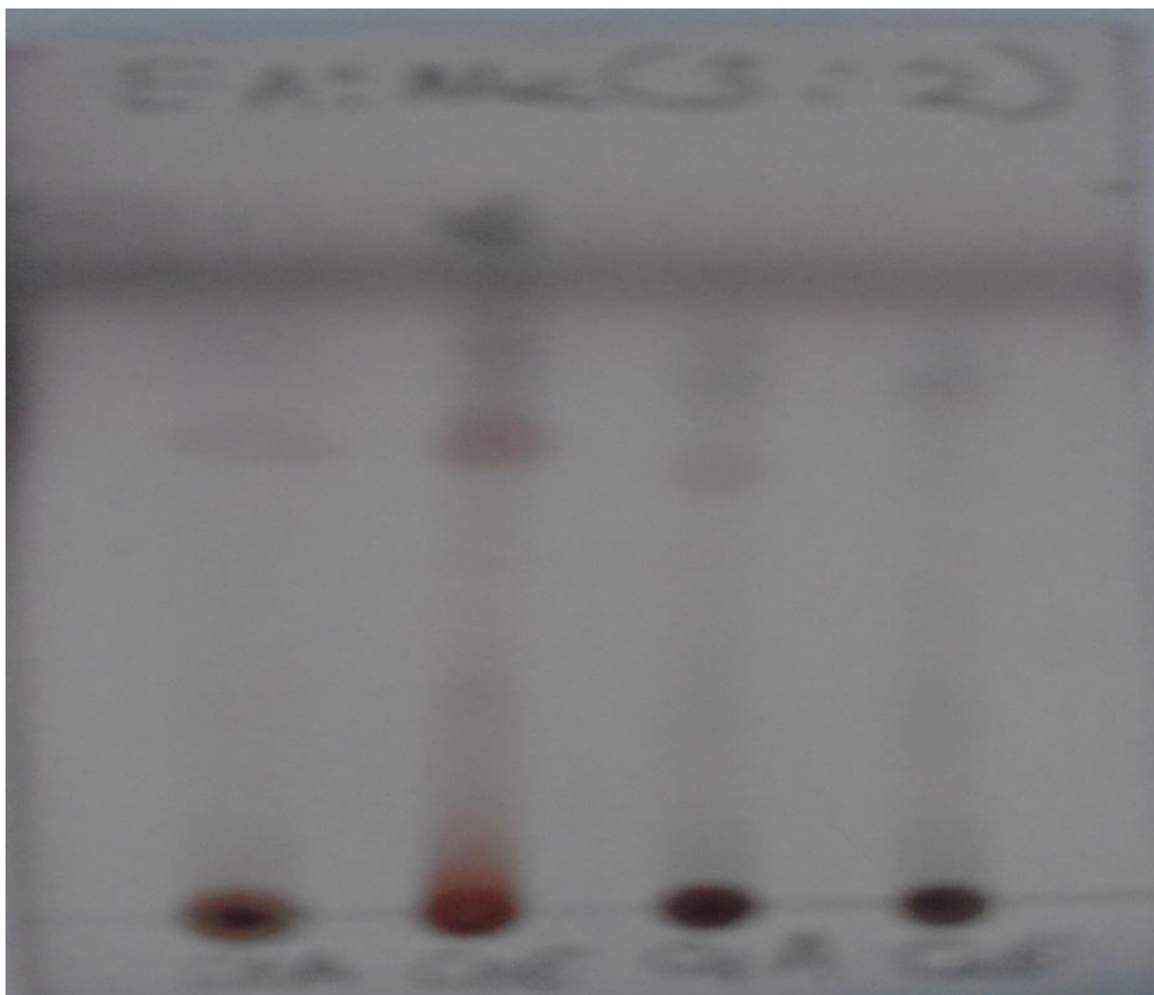
One way ANOVA of the data on the oedema forming activity albino of the albino rats treated with two plant species different extracts against LD_{99} *Naja nigricallis* venom

Source of variance	DF	MS *I. capitata*	MS(*I.conferta*)
Between group	10	748.2*	648.5*
Within group	44	118.3	103.2
Total	54S		

*Significant at P<0.05

MS = Mean Square

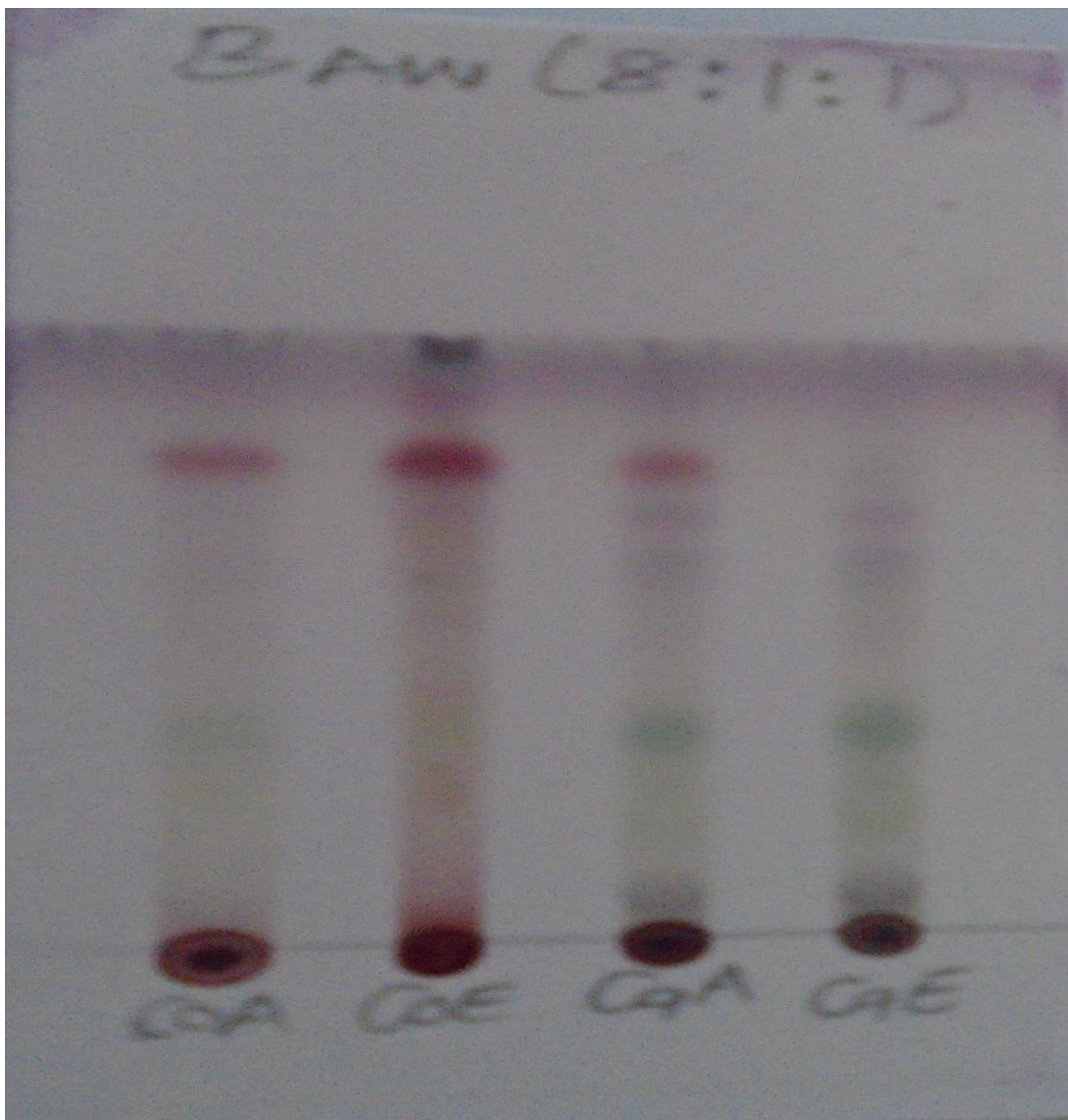
APPENDIX XV



CoA CoE CaA CaE

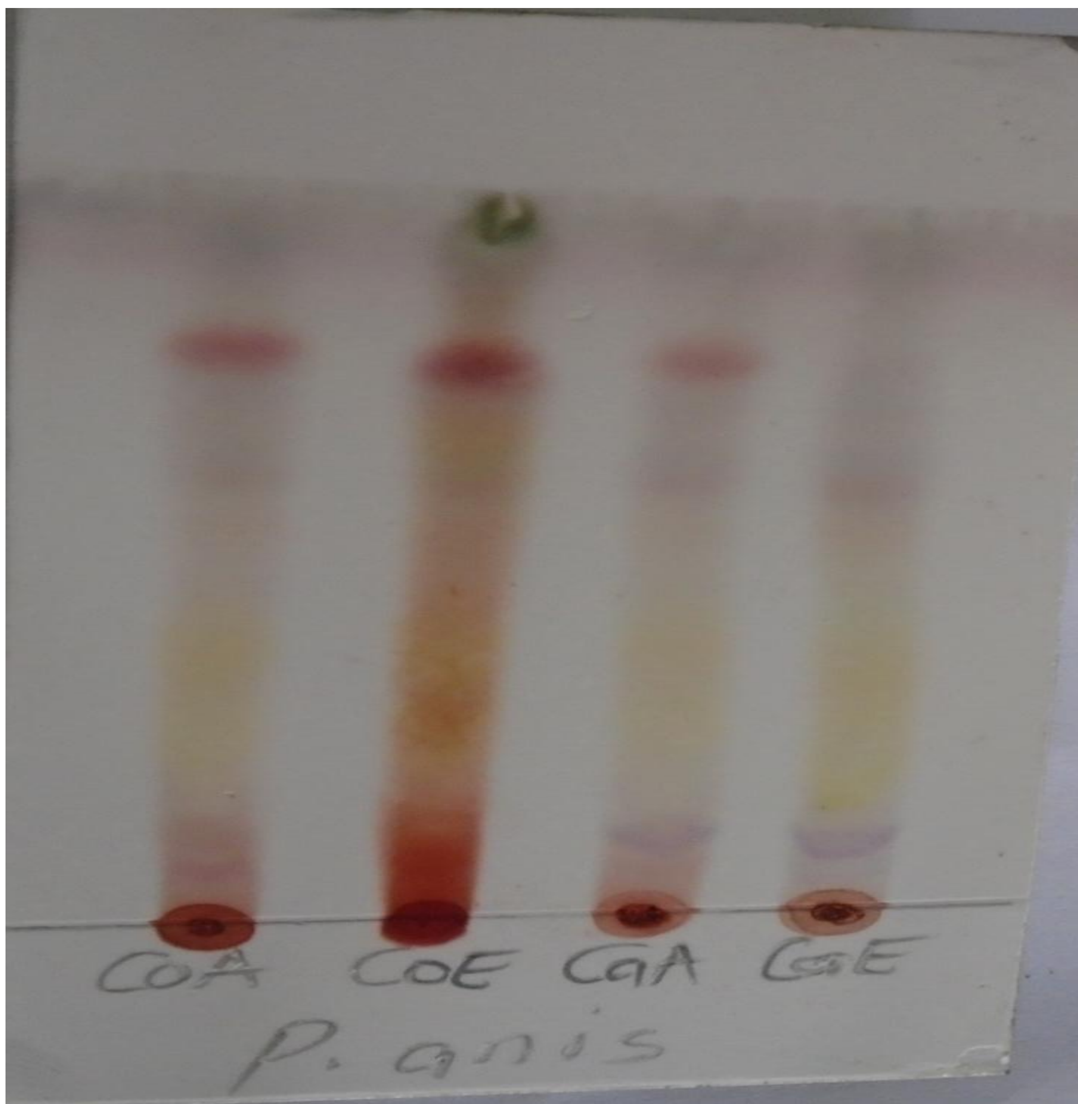
TLC finger print for seperation of active constituents in aqueous and ethanol extracts *of Indigofera conferta* and *Indigofera capitata* develop in Ethyl acetate and Methanol(3:2) visualized by using day light. Where CoA=Aqueous extract of *I. conferta*, CoE = Ethanol extract of *I. conferta*, CaA =Aqueous extract of *I. capitata* and CaE= Ethanol extract of *I. capitata*

APPENDIX XVI



TLC finger print for seperation of active constituents in aqueous and ethanol extracts of *Indigofera conferta* and *Indigofera capitata* develop in Butanol Acetic acid :Water(8:1:1) as visualized by using day light. Where CoA=Aqueous extract of *I. conferta*, CoE = Ethanol extract of *I. conferta*, CaA =Aqueous extract of *I. capitata* and CaE= Ethanol extract of *I. capitata*

APPENDIX XVII



TLC finger print *of Indigofera conferta and Indigofera capitata* using P-anisaldehyde sulphuric acid spray for general detection of active principles. Where CoA=Aqueous extract of *I. conferta*, CoE = Ethanol extract of *I. conferta*, CaA=Aqueous extract of *I. capitata* and CaE= Ethanol extract of *I. capitata*

APPENDIX XVIII

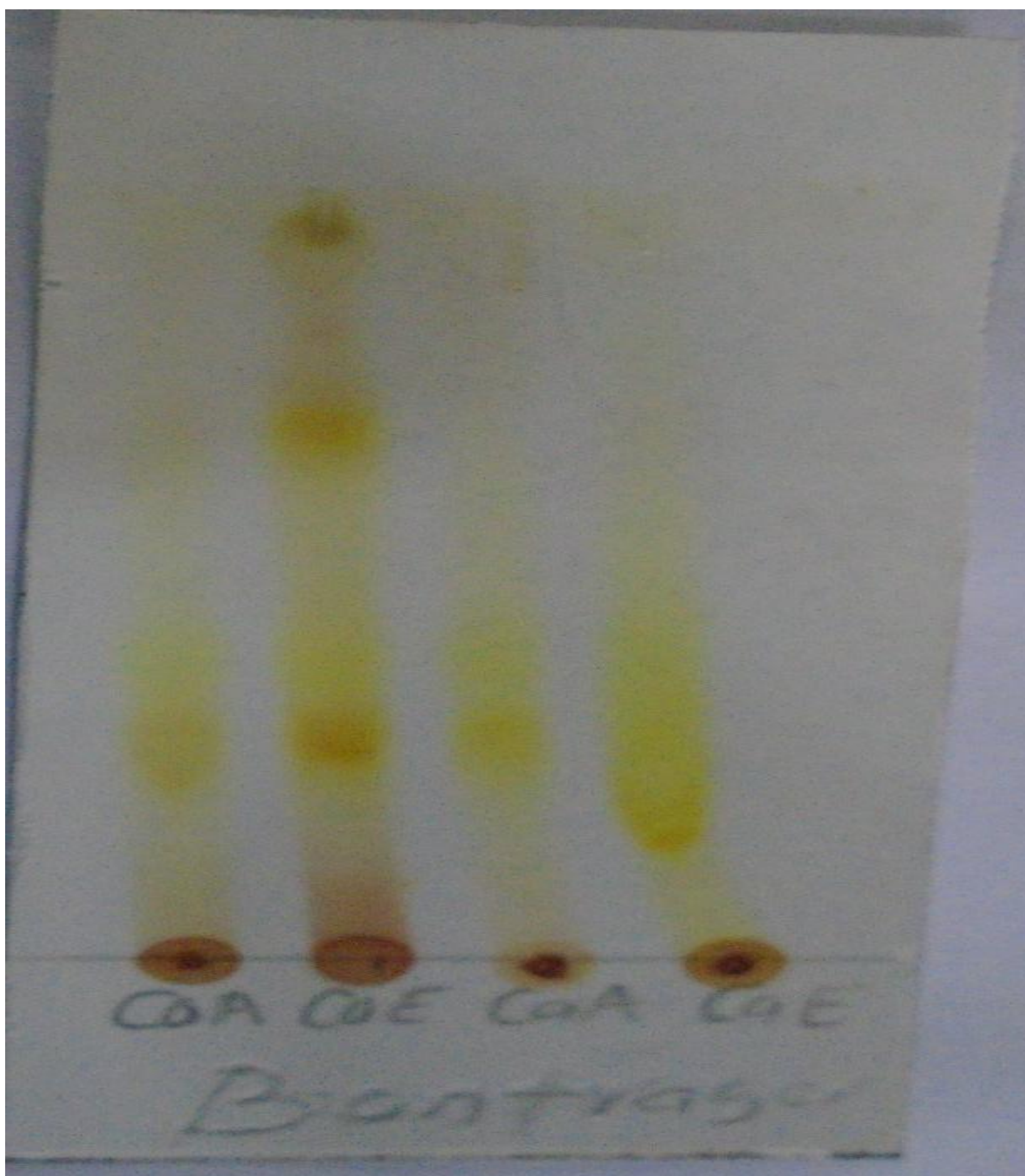
TLC finger print of ethanol and aqueous extracts of *Indigofera conferta* and *Indigofera capitata* using Ferric chloride spray which indicated the presence of phenolic compounds. Where CoA =Aqueous extract of *I. conferta*, CoE = Ethanol extract of *I. conferta*, CaA =Aqueous extract of *I. capitata* and CaE = Ethanol extract of *I. capitat*

APPENDIX XIX

CoA CoE CaA CaE

TLC finger print of ethanol and aqueous extracts of *Indigofera conferta* and *Indigofera capitata* using Dragendorff spray which indicated the presence of Alkaloids. Where CoA =Aqueous extract of *I. conferta*, CoE = Ethanol extract of *I. conferta*, CaA =Aqueous extract of *I. capitata* and CaE = Ethanol extract of *I. capitata*

APPENDIX XX



TLC finger print of ethanol and aqueous extracts of *Indigofera conferta and Indigofera capitata using* Borntragers spray which indicated the presence of Anthraquinone. Where CoA =Aqueous extract of *I. conferta*, CoE = Ethanol extract of *I. conferta*, CaA =Aqueous extract of *I. capitata* and CaE = Ethanol extract of *I. capitata*

APPENDIX XXI

TLC finger print of ethanol and aqueous extracts of *Indigofera conferta* and *Indigofera capitata* using Liberman Buchard spray which indicated the presence of Steroids and Triterpenes. Where CoA =Aqueous extract of *I. conferta*, CoE = Ethanol extract of *I. conferta*, CaA =Aqueous extract of *I. capitata* and CaE = Ethanol extract of *I. capitata*

APPENDIX XXII

CoA CoE CaA CaE

TLC finger print of ethanol and aqueous extracts of *conferta* and *Indigofera Indigofera capitata* using Aluminiun chloride spray/UV which indicated the presence Flavonoids. Where CoA =Aqueous extract of *I. conferta*, CoE = Ethanol extract of *I. conferta*, CaA =Aqueous extract of *I. capitata* and CaE = Ethanol extract of *I. capitata*

APPENDIX XXIII

Spot Colour and Rf Values of finger print for seperated active constituents of *Indigofera conferta* and *Indigofera capitatata* plant

Spot Colours				R_f Values			
CoA	CoE	CaA	CaE	CoA	CoE	CaA	CaE
Blue	Brown	Brown	Brown	0.34	0.35	0.36	0.32
Brown	Brown	Blue	Blue	0.52	0.57	0.56	0.56
Brown	Brown	Brown	Brown	0.64	0.65	0.67	0.65
-	Brown	Brown	-	-	0.78	0.74	-

Where CoA =Aqueous extract of *I. conferta*, CoE = Ethanol extract of *I. conferta*, CaA =Aqueous extract of *I. capitata* and CaE = Ethanol extract of *I. capitata*

APPENDIX XXIV

Spot Colour and Rf Values of detected compound Sprayed with P-anisaldehyde Sulphuric Acid (General)

Spot Colours				R_f Values			
CoA	CoE	CaA	CaE	CoA	CoE	CaA	CaE
Brown	Brown	Purple	Purple	0.36	0.35	0.34	0.34
Yellow	Brown	Yellow	Yellow	0.47	0.45	0.46	0.47
Brown	Brown	Brown	Brown	0.63	0.64	0.63	0.62
Brown	Brown	Brown	Brown	0.72	0.71	0.73	0.72
-	Green	-	-	-	0.98	-	-

Where CoA =Aqueous extract of *I. conferta*, CoE = Ethanol extract of *I. conferta*, CaA =Aqueous extract of *I. capitata* and CaE = Ethanol extract of *I. capitata*

APPENDIX XXV

Spot Colours and Rf Values of active constituents Sprayed with Dragendorff's Reagent (Specific)

Spot colour				Rf Values			
CoA	CoE	CaA	CaE	CoA	CoE	CaA	CaE
Yellow	Yellow	Yellow	Yellow	0.45	0.46	0.45	0.45
_	Brown	_	_	_	0.95	_	_

Where CoA =Aqueous extract of *I. conferta*, CoE = Ethanol extract of *I. conferta*, CaA =Aqueous extract of *I. capitata* and CaE = Ethanol extract of *I. capitata*

APPENDIX XXVI

Spot Colours and Rf Values of active constituents Sprayed with Borntrager's Reagent (Specific)

Spot Colours				Rf Values			
CoA	CoE	CaA	CaE	CoA	CoE	CaA	CaE
Yellow	Yellow	Yellow	Yellow	0.45	0.46	0.45	0.46
Yellow	Yellow	-	-	0.67	0.68	-	-
-	Yellow	-	-	-	0.87	-	-

Where CoA =Aqueous extract of *I. conferta*, CoE = Ethanol extract of *I. conferta*, CaA =Aqueous extract of *I. capitata* and CaE = Ethanol extract of *I. capitata*

APPENDIX XXVII

Spot Colours and Rf Values of active constituents Sprayed with Liberman Buchard's Reagent (Specific)

Spot Colours				Rf Values			
CoA	CoE	CaA	CaE	CoA	CoE	CaA	CaE
Brown	Brown	Brown	Brown	0.43	0.43	0.43	0.43
Yellow	Yellow	Yellow	Yellow	0.52	0.54	0.56	0.56
Yellow	-	Brown	Brown	0.67	-	0.65	0.64
Brown	Brown	Brown	Brown	0.73	0.74	0.74	0.71
-	Brown	-	-	-	0.97	-	-

Where CoA =Aqueous extract of *I. conferta*, CoE = Ethanol extract of *I. conferta*, CaA =Aqueous extract of *I. capitata* and CaE = Ethanol extract of *I. capitata*

APPENDIX XXVIII

Picture of *Indigofera capitata* plant

APPENDIX XXIX

Picture of *Indigofera conferta* plant