PHARMACOGNOSTIC AND ANALGESIC STUDIES OF*DESMODIUM VELUTINUM* (P.BEAUV.) DC.(PAPILIONACEAE)

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BY

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A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATESTUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER DEGREE IN PHARMACOGNOSY

DEPARTMENTOF PHARMACOGNOSY AND DRUG DEVELOPMENT,FACULTY OF PHARMACEUTICAL SCIENCES,

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NIGERIA

JULY,2014

DECLARATION

I declare that the work in this thesis entitled "PHARMACOGNOSTIC AND ANALGESIC STUDIES OF *DESMODIUM VELUTINUM* (P.BEAUV.) DC. (PAPILIONACEAE)" has been carried out by me in the Department of Pharmacognosy and Drug Development. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other university.

Isah Otaru, AUDU		
Name of Student	signature	Date

CERTIFICATION

This thesis entitled "PHARMACOGNOSTIC AND ANALGESIC STUDIES OF *DESMODIUM VELUTINUM* (P.BEAUV.) DC. (PAPILIONACEAE)" by Isah Otaru AUDU meets the regulations governing the award of MasterDegree in Pharmacognosy of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to Allah for giving me good health, physical and mental strength and to my late father and also my mother who supported me financially and morally, my siblings, my wife and friends whose support during the period of this study cannot be quantified.

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ABSTRACT

Pain is an unpleasant sensation often associated with disease and/ or injury. The people in Lokoja use traditional medicine especially medicinal plants in the treatment and management of pain.The use of orthodox medicine for example, pentazocine, in managing pain is associated with some problems such as dizziness, stomach upset, impaired breathing and mental function. Orthodox medicine is more expensive, less accessible by local people and less acceptable to the body in contrast to traditional medicine. The broad aim of this research is to evaluate traditional pain management or treatment in Lokoja.An ethnomedical survey on medicinal plants/recipes used in pain management was conducted between March and August, 2012 in Lokoja, Kogi State, Nigeria. Information on indigenous medicinal plant wealth of the people was obtained using a scientifically structured questionnaire with key people who are recognized in alternative medicine. The investigation yielded a total of 23 medicinal plant species represented by 23 genera from 17 families cited by 52 informants. The vernacular names of the plants were recorded in Igala, Ebira and Okun (the major local languages in Lokoja). From the ethnomedical survey result,ASTERACEAE is most represented (17%),most of the plants are wild (65%) and shrub has the largest representation (39%). The mode of administration (mostly oral or topical) and dosage-regimen were also reported. Out of the 23 surveyed plants, 5 which are more frequently used traditionally as analgesic agents by the people and have minimal scientific information were subjected to analgesic activity test out of whichone of the most potent and has the least scientific information (*Desmodium velutinum*)(P. Beauv.) DC. (PAPILIONACEAE) was selected for pharmacognostic studies. The LD_{50} was greater than 3000mg/kg. The analgesic activity test in animal models showed that the extract at dose of a 100mg/kg exhibited 45.32%

inhibition, which is comparable to the standard drug (pentazocine) with 49.07% at 20mg/kg. These findings help to explain the analgesic property of *D. velutinum* commonly used in Lokoja, Kogi State, Nigeria for pain management.Macroscopy shows that the leaf has entire margin, symmetrical base, blunt apex, and hairy lower and upper epidermis. Microscopy indicates the presence of unicellular non glandular trichomes. The thin layer chromatography(TLC) of the leaf powder revealed the presence of tannins, steroids, flavonoids and terpenes.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Statement of the Research Problem

Pain is an unpleasant sensation often associated with disease and/ or injury. The use of orthodox medicine for example, pentazocine in managing pain is associated with some problems such as dizziness, nausea, impaired breathing, stomach upset, itching and rashes. Orthodox method of treatment is more expensive, less accessible to the majority of local people and less acceptable to the body in contrast to traditional method (Sofowora, 2008). Different communities in Lokoja town have been using plant parts for management and treatment of pain without scientific proof of their efficacy and safety.

1.2 Justification of the Research Problem

Although it has been stated that the long historical use of plants in pain management has demonstrated its efficacy and safety (Aiyelero*et al*., 2009) and people in Lokoja, Kogi State, Nigeria have been using plants and their recipes formanaging pain because it is cheaper and easily accessible. This research, therefore surveyed the plants used for treatment and management of pain by the people of Lokoja, Kogi State, Nigeria, conducted analgesic test on some of the plantsand investigated the phytochemical constituents which may be responsible for the analgesic properties.

1.3 Aim of the Research

To conduct an ethnomedical survey on plants used for treating/managing pain in Lokoja, Kogi State, Nigeria.

1.4 Statement of the Research Specific Objectives

1. To conduct an ethnomedical survey of traditional pain management/treatment using medicinal plants in Lokoja, Kogi State, Nigeria.

2. To collect some of the selected plants, identify the mode of preparation and administration.

3. To conduct pharmacognostic evaluation of *D. velutinum*(P. Beauv.) DC.

4. To test for the analgesic activities of the methanolic leaf extract of *D. velutinum* using hot plate and acetic acid induced writhing test methods.

1.5 Statement of the Research Hypothesis

Medicinal plants used in Lokoja, Kogi State, Nigeria for pain management/treatment do have analgesic properties.

1.6 Backgroundofthe Study

Pain is a general health problem that may be caused by diseases and/ or injuries. It has been managed locally by various cultures and communities using traditional/plant recipes. People in Lokoja, Kogi State,Nigeria use plant materials/recipes for managing/treating pain. Pain is called *'oyizi'*, '*uwo*' and '*irora',*in *Ebira,Igala* and *Okun*languages/dialects respectively. The*Ebira,Igala*and *Okun*are the dominant tribes living in Lokoja town where the survey was conducted.

Plants with valuable contributions in the field of medicine include *Catharanthus roseus* from which vincristine and vinblastine used for cancer treatment are derived;*Cinchona succirubra* from which quinine and quinidine used as antimalarial are derived;*Arthemesia*

anua from which arthemisinin used as antimalarial is derived; *Digitalis purpurea* from which digitalis used as cardiotonic is derived;*Physostigma venenosome*from which physostigmine used as cholinergic agent is derived. The research into plants with folkloric use as pain relievers is definitely a fruitful and logical research strategy in the search for new analgesic drugs.

1.6.1 Location of Lokoja/study site

Lokoja is located within the western senatorial district of Kogi State, Nigeria. It lies between latitude 7^0 N and longitude 6^0 E in the north central savannah region of Nigeria (Wikipedia encyclopedia, 2012). The area which is largely hilly is bounded southward by Ajaokuta, eastward by part of River Niger and Koton-Karfi, northwards by the upper part of River Niger and Jamata, and westward by Adavi and Kabba lands.The entire land mass of Lokoja is 3,180 $km^{2.}$ It is close to the point where River Niger and River Benue join. This confluence of the two rivers in the State gives it name 'Confluence State'. Lokoja land has been highly reduced by River Niger and its streams as well as the mountainous nature of the city. This terrain is probably by far the greatest threat to the development of the city. The presence of hills, mountains and rivers in Lokoja makes the city undulating in nature.The city is cosmopolitan and serves as home to people of different cultures and tribes likeEbira, Igala, Okun, Yoruba, Hausa, Igbo, Nupe, Bassa and other ethnic groups in Nigeria as well as some foreigners (Wikipedia Encyclopedia, 2012).

1.6.2 Pain

Pain is an unpleasant sensation that is often associated with actual or potential tissue damage that is caused by disease and/ or injury (Coda and Bonica, 2001). According to the

International Association for the Study of Pain (IASP), there is a difference between pain and nociception. The word “pain” comes from the latin word “peona” meaning a fine or penalty (Wikipedia encyclopedia). The term pain is a subjective feeling that is accompanied by nociception, but can also arise without any stimulus, and thus includes the emotional response (Coda and Bonica, 2001). Based on the causes, pain is classified into nociceptive pain (which is due to peripheral nerve fibre stimulation, inflammatory pain (that is associated with tissue damage) and pathologic pain (that is associated with disease) (Coda and Bonica, 2001).

1.6.3 Analgesics

Throughout history, man has used many different forms of therapy for the relief of pain. Among them, medicinal herbs are highlighted due to their wide popular use. Examples include *Papaver somniferum* from which morphine and codeine were isolated and *Cannabis sativa* from which cannabinoid was isolated. Morphine is regarded as the prototype of opiate analgesic drugs.In the relief of pain, opiates are generally considered to act on the central nervous system (CNS) exercising their effects through opioid receptors(Klawe and Maschke, 2009).Such drugs are especially important for the treatment of chronic pain. Although morphine has reigned for centuries as the king of painkillers, its rule has not been totally benign (Anonymous, 2009).

CHAPTER TWO

2.0LITERATURE REVIEW

2.1Historical Background of Some Medicinal Plants

The use of plants for the management of pain is well known in several parts of the world. The age-old materials of herbalists must be tapped. Plants have served as useful starting materials for drug development. Examples include; morphine and codeine from opium capsules and leaves of *Papaver somniferum,* cannabinoids from *Cannabis sativum*(Hassan and Ahmad, 2008).Medicinal plants are the main sources of chemical substances with potential therapeutic effects. The use of medicinal plants for the treatment of pain and other related conditions is associated with folk medicine from different parts of the world (Klawe and Maschke, 2009). Naturally occurring compounds from plants, fungi and microbes are still used in pharmaceutical preparations in pure or extracted form for treating diseases. The contribution of medicinal plants in health care is well established. Today, plants provide about 25% of drugs used in orthodox medicine and are normally the first choice of material for treating diseases(Almeida *et al*., 2001).

Medicinal plants are used in the treatment and prevention of various health problems, from simple to complex disease situations both in rural and urban populations globally, thereby improving the quality of life. Many reasons have been advanced for the increase in the use of herbal medicine. The inaccessibility and non-affordability of modern drugs among the rural populations of tropical Africa have made a large proportion of rural people to depend on traditional herbal drugs (Oladele *et al*., 2011). In sub saharan Africa, modern health care delivery is beyond the reach of people living in rural areas, hence they greatly rely on

readily available medicinal plants in their neighbourhoods. It is estimated that over 70% of people in the developing countries of Africa are peasant farmers, who live in rural areas and use medicinal plants for their health care needs (Birhan *et al*., 2011). Plants' use for medicine varies among different ethnic and cultural groups (Mussema, 2006).

2.2.1 Traditional medicine

Man has used plant drugs for health care delivery over the centuries; disease remedies from plants sources for mankind are as old as human history and are still in use to date. It is estimated that about 75% of useful bioactive plant derived pharmaceuticals used globally are discovered by systemic investigation of leads from traditional medicines (Tomoko *et al*., 2002).

Traditional medicine is defined by the World Health Organization (WHO) as "the total combination of methods and practice, whether explicable or inexplicable, used in the diagnosis, prevention or elimination of mental, physical or even social diseases (WHO, 2001). Traditional medicine is an aspect of ethnomedicine and ethnobotany. It is a practice by means of which diseases are treated or managed with the use of local materials. Ethnobothany is the study of how the people of a particular culture and region make use of indigenous plants. Ethnomedicine is a subfield of ethnobotany which is based on the medicinal use of plants and epidemiology (Anilkumar, 2010). The use of traditional medicine in various therapies by indigenous populations all over the word cannot be over emphasized as 80% of the world's population depends on it (WHO, 2002). With the poor economic status of many underdeveloped countries, this form of medical practice is worthy of consideration. This is because it is cheaper, more affordable, more acceptable and easily accessible when compared to orthodox form (Sofowora, 2008).However, it has the

following challenges in the form of criticisms, shortcomings and inadequacies leveled against traditional medicine such as bad record keeping as well as its vulnerability to medical error in the areas of diagnosis and prescription as stated by the orthodox practitioners (Elujoba, 2003).Many herbal preparations are being prescribed as analgesic in traditional literature.

The search for new analgesic agents from the huge array of medicinal plants is intensifying. Such taxa may hold potential for the discovery of novel therapeutic agents capable of suppressing, reducing or relieving pain (Anilkumar, 2010). In Africa, most herbalists keep no records and information is usually passed on orally, from generation to generation. Since oral information may not be accurate as was told to the recipient, a whole library of herbal information is being buried gradually with every practicing herbalist that dies (Sofowora, 2008). The need for a systematic approach to the use of the locally available medicinal plants as tools in drug development has been recognized. It is obvious that the plant kingdom offers a better opportunity of providing useful medicinal compounds (Gill, 1992).

In modern times, the active ingredients and curative actions of medicinal plants were first investigated through the use of European scientific methods. The most important ingredients present in plants include alkaloids, terpenoids, steroids, phenols, glycosides, tannins etc (Iauk *et al*., 1993). Sofowora (2006) earlier opined that in every developing country, it is necessary that documentation of medicinal plants be treated as a matter of extreme urgency.

A survey by the United Nations Commission for Trade and Development (UNCTAD) indicated that about 33% of drugs produced in the developed countries are derived from plants and, that if micro-organisms are added, 60% of medicinal products would be of

natural origin. In recent years, there has been tremendous increase in research into Nigeria medicinal plants, with higher plants attracting much research attention (Sofowora, 2006).

2.2.2 Traditional medicine practice in Lokoja, Kogi State, Nigeria

Like any other typical form of traditional medicine practice all over the world (such as Ayurveda in India and Acupuncture in China), the people of Lokoja have similar practice. Traditional medicine practice involves diagnoses (using signs and symptoms) observed on patients, treatment by using various modes of drug preparations and administrations. The techniques normally used include fasting, dieting, herb selling, bone setting, traditional birth attendance, thermotherapy, cupping and phytotherapy (Personal communication, 2012).

2.3.1 Ethnomedical survey of Nigeria and some other countries

Ethnobotany is a discipline that deals with how people of a particular culture and tradition make use of their locally available plants. One of the fathers of ethnobotany, Richard Evans Skultes, defined the discipline as "the study of human evaluation and manipulation of plant materials, substances and phenomenon, including relevant concepts, in primitive society". The most widely known definition of ethnobotany is the use of plants in primitive societies (Manzoor *et al*, 2006). This line of research has proved very resourceful and invaluable in the selection of phytomedicine which has most often served as lead to drug discovery and development.

In the selection of plants for pharmacological examination, five approaches could be used, namely: the random approach, phytochemical targeting, chemotaxonomic approach, morphological screening (active part e.g. leaf, bark, root or seed), and ethnobotanical survey approach. However, of all the approaches mentioned, it has been reported that plant

sampling based on ethnobotanical survey approach showed a greater percentage yield of bioactive useful medicinal compounds over the other methods (Khafagi and Deweder, 2000). Typical evaluation study of Northern Nigeria has revealed that some plant species from the savanna region have been selected based on their ethnomedical usage and literature survey by the Hausa people (Adoum *et. al*., 1997).

Ethnobotanical research conducted in the last few decades have revealed the analgesic properties of plants cited in traditional literature. An ethnobotanical survey conducted into plants and plant recipes used in the treatment of inflammatory diseases in Ogun State through the use of semi-structured questionnaire revealed that 33 of the plants collected are therapeutic to rheumatoid arthritis, 23 to asthma, 28 to pruritus, 12 to furuncle (boil) and 4 to gout. This ethnobotanical survey has revealed some of the local uses of these plants in specific areas of West Africa as well as the type of remedies used for the treatment of certain inflammatory diseases (Omonike *etal*, 2010).

In a study conducted on *Ficus ingens*, the analgesic activity was evaluated using acetic acid-induced writhing test and hot plate in mice. The result showed that itpossesses significant analgesic properties which supported the folkloric claim for the use of the plant in the management of haemorrhoids. The phytochemical screening revealed the presence of alkaloids, flavonoids, saponins and tannins which might be responsible for the observed analgesic activity (Aiyelero *et al*, 2009).

Flavonoid and phenol fractions showed significant analgesic activities as tested against pain caused by acetic acid-induced abdominal constriction (Zahida *et al*., 2007). The study of Sankaradoss *et al.*, (2012) on the tannin and steroid fractions showed potent analgesic activities when tested against pain initiated by hot plate. Phytochemical screening of *D.velutinum* by Anowi *et al.*, (2012), revealed the presence of resins, tannins, flavonoids and saponins. The ethanolic extract showed significant anti-pyretic activity on the experimental rats used.The phytochemical

screening of species belonging to the Fabaceae showed that they possess tannins, flavonoids, phenols and steroids in common (Frank and Buckingham, 2009 and Bojaxa *et al*. 2010). Study using aqueous extract of *Hyptis suaveolens* leaves for the antinociceptive property in chemical and thermal models of nociception in mice showed, it dose-dependently reduced the number of writhings induced by acetic acid, decreased the licking activity of the early phase in formalin test and increased the reaction time in hot-plate test (Santos *et al*, 2007).

A study carried out in the South-Eastern region of Nigeria revealed that the medicinal plant wealth resources of the region was facing rapid depletion due to current focus on herbal traditional medicine as an alternative source of drugs. Most medicinal plants are collected from wild sources without recourse to cultivation. The common plants used by the indigenous people of this region were catalogued based on their ethno therapeutic indications. Ways of exploring short and long term strategies of conservation were also among the highlights of the study conducted (Obute, 2005).Fatope *et al*., (1993) showed from typical tests that significant correlation often exists between the uses of plants in traditional medicine and the observed bioeffect of the extractives. The leaves and bark of *Azadirachta indica*, *Morinda lucida* and *Mangifera indica*, leaves of *Uvaria chamae*, *Aspiliaafricana*, *Carica papaya* and the stem bark of *Parkia biglobosa*, were among the evaluated plants with significant analgesic activity (Almeida *et al*., 2001).

2.3.2Ethnomedicine of Lokoja

In order to study the ethnomedicine of a particular region, its phytogeography is considered alongside. Phytogeography is the study of the distribution of both wild and cultivated plants among which some are medicinal (Gilmore, 2005). It would be appropriate, while determining the phytogeography of Lokoja, to consider its history which includes: location,

ecology, archeological and ethnological evidences of the past activities of Lokoja people. In this way, ethnomedicinal uses of plants in this region can be understood and such investigation from an ethnomedical survey may give fascinating information that can be applied in enlarging the list of plants that are useful to man in the area of medicine.

2.4 Flora and Fauna of Lokoja

From the ethnogeographical survey, Lokoja land is endowed with rivers for fishing and land with vegetation for cultivation and hunting (plate I). The area which lies in the southern guinea savanna belt region of Nigeria gets adequate rainfall during rainy season but experiences high temperature. Thus, apart from the main crops, e.g yam, cassava, sugarcane, tomatoes, pepper and onions cultivated by farmers of Lokoja, great importance is attached to various kinds of trees, edible fruits and wild plants. These include mangoes (*Mangifera indica*), pawpaw (*Carica papaya*), oranges (*Citrus sinensis*), palm kernel,*Desmodium velutinum*, *Crossopteryx febrifuga*, neem (*Azadirachta indica*) for use as food, medicines and in particular management of pain. These are commonly found and constitute significantly, the dominant vegetation of the area (personal communication, 2012).

The huge mountains and hills of Lokoja are rich sources of various species of animals. Night owl, cattle eglet and water bird are among the common species of bird, while species like mud fish, cat fish, etc thrive well in various streams, river Niger as well as its tributaries that are found in Lokoja and its environs. Among the common domestic animals are chicken, goats, sheep, cattle, cats. The surrounding mountains of the region stand out as a relief tourist site and a rich natural game reserve. This is exploited by the people for hunting, expedition as well as collection of medicinally useful plants.

2.5 Pain and its Classification

Pain is an unpleasant sensation that is associated with disease and/ or injury. Based onduration, pain can be classified into two types:**Acute Pain:** This isa short term kind of pain whose symptoms can be easily identified. It is sharp and fast. It is the body's indication of present damage or disease. It responds well to medication (Turk and Okifuji, 2001).**Chronic Pain**: This type of pain can last for a long time. In medicine, the distinction between acute and a chronic pain has traditionally been determined by an arbitrary interval of time since onset. The commonly used marker is 3 months and 6 months since onset (Turk and Okifuji, 2001). Pain may also be divided based on causes; into **nociceptive pain**(that is caused by activation of nociceptors) and **neuropathic pain**(which is caused by damage to or malfunctioning of the nervous system).Nociceptive pain may be "superficial" or "deep". The **deep pain** is further divided into "deep" somatic and "deep" viscera. **Superficial pain** is initiated by the activation of nociceptors in the skin or superficial tissues. **Deep somatic pain** is initiated by activation of nociceptors in ligaments, tendons, bones, blood vessel, fasciae and muscles and is dull, aching and poorly localized. **Deep visceral pain** originates in the visceral structures (Turk and Okifuji,2001).

2.6 Analgesic and its Types

Analgesics are medications used to relieve pain without reducing the consciousness of the patient. They work by reducing the amount of pain felt and this is generally achieved by interfering with the way the pain message is transmitted by the nerves. Analgesics do not treat the cause of the pain but they will provide temporary relief from pain symptoms (Klawe and Maschke, 2009). There are concerns about addictive properties and side effects

some morphine like analgesics, which include respiratory depression, drowsiness, decreased gastrointestinal motility, nausea and several alterations of the endocrine and autonomic nervous systems. Therefore, the search for morphine like - substances with improved analgesic activity devoid of the side effects of morphine and related drugs is a necessity(Klawe and Maschke, 2009).There are three main categories of analgesics. The first is the **opioid analgesics** which are prescriptions or medicines that are very potent, being chemically related to morphine. The second is the **non-opioid analgesics**. Non-opioid analgesics work by affecting the prostaglandin system, which is the system within the body responsible for producing pain. This category includes non-steroidal anti-inflammatory drugs or NSAIDs, such as aspirin, ketoprofen and ibuprofen. The last category is the **adjuvant analgesics**, which are medicines typically used for purposes other than pain relief. This includes some antidepressants e.g orphenadrine that may also help to relieve pain in specific circumstances (Klawe and Maschke, 2009).

2.7 Relationship between Ethnomedicine and Ethnopharmacology as Interdisciplinary Research Fields

The term ethnobotany was first introduced by the American botanist John Harshberger in 1896 as "the study of plant use by humans". Ethnobotany studies the relationship between humans and plants in all its complexity and is generally based on a detailed observation and study of the use a society makes of plants, including all the beliefs and cultural practices associated with this use(Heinrich*et al*., 1998). It is useful for ethnobotanists to live with indigenous people, to share the everyday life of their community and, of course, to respect the underlying cultures. Ethnobotanists have a responsibility both to the scientific community and to the indigenous cultures. The discipline of ethnobotany is a much broader

one which is interested in all studies about the relationship between people and plants. Ethnobotany focuses not only on medicinal plants, but also on other natural products derived from nature, such as food, plants used in rituals, colouring agents, fibre plants, poisons, fertilizers, building materials for houses, household items, boat, etc. (Heinrich *et al.*, 2004). Because plants play an important role in almost every aspect of human activity, ethnobotany encompasses many fields including botany, biochemistry, pharmacognosy, toxicology, medicine, nutrition, agriculture, ecology, evolution,comparative religion, sociology, anthropology, linguistics, cognitive studies, history and archeology. The multidisciplinary nature of ethnobotany allows for a wide array of approaches and applications and leaves the way open for many scientists to study the plant uses in different ways (Alexiades*et al*., 2006). Medicinal plants have always been the main research interests of ethnobotany and the study of these resources has also made significant contributions to the theoretical development of the field (Heinrich *et al.*, 2004).

Ethnopharmacology is defined as "the interdisciplinary scientific exploration of biologically active agents traditionally employed or observed by man" (Elufioye *et al*., 2012). Ethnobotany and ethnonopharmacology research processes are closely related to the extent that it is most often impossible to distinguish between their methodologies. Heinrich *et al*., (2004) expressed an opinion that the interdisciplinary nature of ethnopharmacology is largely dependent on ethnobotany, which studies the traditional practices and uses of plants in primitive societies. The use of these plant medications singly or in combination with other non-plant materials as recipes in all part of the study comprises medical ethnobotany. What then happens to the individual on consumption of such crude medicinal preparations and how this is related to some other biochemical and epidemiological findings between the

local man and his environment are studies undertaken by the ethnopharmacologist. These two fields of study encompass anthropology, botany, clinical research, pharmacognosy, pharmacology, natural product chemistry and toxicology. To express this furthermore, Adesanya (2005) noted that drugs are 'preparations' developed to aid the recovery of man from a state of chemical in-balance.

Consequently, the discovery of these preparations from nature involved many steps which in context include the historical medical practice to research into active plants and their co-genetic species and subsequently, the identification of their effects in animal and man and their management and rational uses in medical practice. These, therefore, indicate the interrelationship existing between these two disciplines and explicitly seek the need for ethnobotanists and ethnopharmacologists to work closely together for an effective research work, data and productive result oriented findings.

2.8Botanical Description of *D. velutinum*

Desmodium velutinum(P. Beauv)DCis from the family PAPILIONACEAE (Anowi *et al.*, 2012). Itis a perennial, erect shrub, up to 3m high; branches dark red, yellow-brown when young.Leaves are ovate with blunt apex, 4-20 cm long and 2.5-13 cm wide, coriaceous, upper and lower surfaces velutinous; inflorescence often dense, terminal or auxiliary, 4-2cm long, with 2-5 flowers at each node; flowers purple to pink. Pods are narrowly oblong, 1-2.5 cm long, 2-3 mm wide. Seeds are ovate, flat, 1.3-1.6mm x 1.8-2.5mm and yellow when ripe.

2.9Geographical Distribution of *Desmodium velutinum*

It is distributed in sub-tropical Asia (China, Taiwan, India, Indonesia, Laos, Malaysia, Myanmar, Sri Lanka, Thailand) and tropical African countries including Nigeria (Anowi *et al.*, 2012).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1Experimental Design

This research is in five parts. The first part is the survey, which includes the collection of information from informants and the procurement of plants used for pain management in Lokoja, Kogi State, Nigeria followed by the identification, preparation of herbarium samples and subsequent compilation of data. The second andthird partsfocus on acute toxicity and analgesic activity studies of the most frequently mentioned plants from the ethnomedical survey with little or no scientific information from the collections. Thefourth and fifth parts focus on thin layer chromatography and pharmacognostic evaluation.

3.2 Study Design

The survey for gathering information about plants used for analgesia in Lokoja involved visits to traditional medical practitioners, herbalists, herb sellers and individuals knowledgeable in the use of medicinal plants using a scientifically structured questionnaire (see appendix I). The interview was conducted in local languages with the questionnaire as a guide, using a tape recorder and/ or camera where applicable. Interpreters were incorporated where necessary.Ethnobotanical study mainly involves a field research work. Hence a survey research approach becomes the adopted strategy.

3.3 Study Site The areas covered include Felele, International market, Old market, Adankolo, Lokongoma, Sarkin-Noma and Gadumo. A selected sample of (at least) ten interviews were administered in each area.

3.4 Study Population

The sample population is male and female inclusive within the age bracket of 30 t0 70 years old. It was made up of traditional medicine practitioners (TMPs), herbalists, herb sellers, elderly people with reputable knowledge on medicinal plants and their traditional uses.

3.5 Survey of Medicinal Plants used for Pain Management

The survey which aimed at identifying and documenting the medicinal plants used in pain management in Lokoja, Kogi State, Nigeria was conducted between March and August, 2012 in the seven focal areas listed above (study site).

3.6 Preparation of Herbarium Specimen

The method described by Francis (2005) was adopted. The plant samples were collected by the person who normally prepares the herbal remedy/informant, so as to avoid the collection of wrong specimens through variation in local names. The collected plants were then prepared according to standard procedures of herbarium specimen preparation and preservation as follows; both flowers and fruits (if available) were left intact, clean cut of the stem was made using a secateur, every specimen was tagged using Jeweller's tag, name or initials and a unique collection number on one side and date and site number on the other side were recorded, unnecessary twiggy shoots were cut away, the specimens were flattened out using a press and straps, the site, habitat, habit and flower colour were recorded, the samples were made pressed and dried quickly; change in flower colour (if occurred) was noted.

Photographs of some of the collected plant species were also made so as to facilitate their identification process. Final identification, authentication with voucher's numbers were made at the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria with the help of the Taxonomists of the unit, Mr U.S. Gallah and Mr Musa Muhammad. Voucher specimens were deposited for future reference. The plant parts prescribed for therapy were collected in considerable quantity for preliminary screening and biological studies.

3.7 Data Collection

A tape recorder and a camera were employed during the interview session to record the information from the informants. A structured questionnaire to furnish data was used as a supplementary tool. The structured questionnaire was adopted from that designed by Sofowora (2006). The method employed by Betti (2004) was adopted as a guide for the selection of useful medicinal plants. In this method, enquiry was made as to what ailments were treated by which plant species rather than which plant was used to treat which ailment. This ensures good data selection/collection; whereas the plant-ailment method (that is asking what plant is used to treat what ailment) may lead to wrong data as the healer may always find a treatment for any plant indicated. Data acquisition was successively collected using the communicable dialects within the area in line with standard enquiry procedure and with the aid of research instruments. On the basis of the opinion put forward by Cordell (1995), data collection procedure for the expedition of this study was conducted taking into consideration the following measures:

a. The usage of plants in pain management in the communities

b. Traditional medicine practice of the people inpain management i.e the medicinal plants, method of preparation, mode of application, dosage and duration of treatment, years of practice, sources of knowledge, extent of patronage, gender of the informants, and the level of success with the used prescription/recipes.
c. Information on culture such as beliefs with regards to modern method of pain management, socio-cultural values, social norms, etc.
d. Ecological findings sought information on the activity of the people and its effects on medicinal wild flora conservation and extinction of medicinal plant flora and questions such as how readily the informants part with the information they possess as well as the experiences ofthe people with medicinal plants knowledge were asked.

3.8 Data Analysis

The method described by Akuezilo, (1993) was adopted. It involved techniques whereby the investigator extracts information from raw data that was not originally there before and which would enable a summary description of the subject studied to be made. Hence the descriptive statistical tools; tables were utilized to prove the validity of the research questions raised for the realization of the aims and objectives of the study.

3.9 Plant Screening for Laboratory Investigation

The collected plants were screened based on the number of informants who mentioned them and the number of scientific information on them (that is those that have the highest frequency of occurrence from the ethnomedical survey and fewest scientific information from literature survey were selected for laboratory investigation).

3.10 Pharmacological Tests of *D. velutinum* Leaf

3.10.1 Extraction for analgesic activity

Theplant was air-dried and powdered using pestle and mortar. About 250g of the powdered material were macerated in 500ml of 70% methanol for 24 hours. The extract was concentrated on a water-bath.

3.10.2 Experimental animals

Swiss albino mice (18-20g) of both sexes were used and maintained on standard animal feed (Excel Feed$^{(R)}$, Ilorin-Nigeria) and water *ad libitum* in the Animal House Facility of the Department of Pharmacology and Toxicology, Ahmadu Bello University, Zaria.

3.10.3 Acute toxicity studies

The method described by (Lorke, 1983) was adopted. Thirteen mice were used for the plant extract. In the first phase, 3 different doses (10, 100, 1000 mg/kg) were administered to 3 groups each containing 3 mice. In the second phase, more specific doses of 1600, 2900 and 5000 mg/kg were administered to 3 or 4 groups, each containing one mouse based on the number of deaths recorded in the first phase. The median lethal dose (LD_{50}) value was obtained as a geometric mean of the highest non-lethal dose and the lowest lethal dose.

3.10.4 Tests for analgesia

3.10.4.1 *Hot plate method*

The method as described by Raquibul *et al.*,(2010) was adopted. Five groups each containing 5 mice were used. The three different doses used in this procedure were determined from the acute toxicity studies. The first group served as **negative** control and received normal saline of 10mg/kg. The second group was administered with morphine as the standard drug (20mg/kg) which served as **positive** control while the other 3 groups

received extract of 3 different doses 100, 200, 300mg/kg respectively as **test** doses. All administrations were made via the intra-peritoneal route. The tests were carried out using Eddy's hot plate apparatus. The temperature was set at 55±1°C. Each mouse was placed on the hot plate and the reaction time in seconds for flicking of hind paw or jumping at 0 second, 30 minutes, 60minutes, 90 minutes and 120 minutes with cut off time of 15seconds was recorded. The reaction time following the administration of the test extracts, reference standard drug, and control saline vehicle were recorded.

3.10.4.2 *Acetic acid-induced writhing method*

The method as described by Raquibul *et al*., (2010) was adopted. Five groups each containing 5 mice were used. One group,the negative control, received normal saline (10mg/kg). Another group, the positive control received pentazocine as the standard drug (20mg/kg). The remaining 3 groups, the test groups received 3 different doses (100, 200, 300mg/kg) of extract as determined from the toxicity studies. Each group was treated 30 minutes before the administration of 0.6% acetic acid. After an interval of 5mins, the mice were observed for abdominal contraction referred to as writhing for the next 10 minutes.

3.10.5 Statistical analysis

The results obtained from the analgesic tests were statistically analyzed using analysis of variance (ANOVA).

3.11Macroscopical/Microscopical Examinationof *D. velutinum* Leaf

3.11.1 Macroscopical examination of the leaves

The following features were used for the macroscopic identification of the leaf composition; leaf base, lamina, shape, venation, margin, apex, surface, texture, colour and taste in the case of powdered sample.

3.11.2Microscopical examination of leaf

The powdered samples, transverse and longitudinal sections of the leaves of *Desmodiumvelutinum* were used for this study. Both quantitative and qualitative studies were carried out on the leaves using standard methods.

3.11.2.1 *Qualitative leaf microscopy*

Thin longitudinal and transverse sections of the leaf were cut and cleared using chloral hydrate solution. The section was placed on a clean slide. A few drops of dilute glycerol was added and covered with a clean cover slip. The slide was observed under a compound microscope. The nature of epidermal cells, types of stomata, veins and trichomes as well as the arrangement of the various plant tissues were noted (Evans, 2009).

3.11.2.2 *Quantitative leaf microscopy*

Quantitative microscopy was carried out on the anatomical section and powdered sample of the leaves based on the method outlined in Evans (2009).

Stomatal number: Fragments of leaf from the middle of the lamina were cleared with chloral hydrate solution. Using a camera lucida and a stage micrometer, a clean white paper was divided into squares of 1mm x 1mm using (x10) objective. The stage micrometer was then removed and replaced by the cleared preparation of the leaf. The number of stomata was counted by indicating it on the paper by a small cross. The total number of stomata in one square of 1mmx1mm was then determined (Evans, 2009).

Stomatal index: Pieces of leaf were cleared with chloral hydrate solution, mounted in dilute glycerol and viewed under the microscope using 4mm (x40) objective. The number of stomata divided by summation of epidermal cells and stomata multiplied by 100% gives the stomata index.

Veinlet number, Veinlet termination number, Veinislet number: Fragments of leaf from the middle of the lamina were cleared with chloral hydrate solution. Using a camera lucida and a stage micrometer, a clean white paper was divided into squares of 1mm x 1mm using (x10) objective. The stage micrometer was then removed and replaced by the slide of the cleared preparation of the leaf. The number of veinlet, veinlet termination and veinislet were counted and recorded accordingly.

3.12Chemomicroscopic Examination

The leaf powder of *Desmodium velutinum* was cleared using chloral hydrate and little quantity of it was mounted on a clean slide using dilute glycerol and observed under the compound microscope for the presence of cell inclusions such as cellulose, starch, oil, tannins, and calcium oxalate crystals according to the method outlined in Evans (2009).

Test for cellulose**:** A small quantity of the cleared sample of leaf was mounted in iodine solution followed by 66% sulphuric acid. A blue colour indicates the presence of cellulose.

Test for Tannins: A small quantity of the cleared powdered sample of leaf was mounted in ferric chloride solution on a clean slide. A dark blue or dark green colour indicates the presence of tannins.

Test for Starch: A small quantity of the powdered sample of leaf was mounted in iodine solution. A blue colour indicates the presence of starch.

Test for Calcium Oxalate Crystals: A small quantity of thecleared sample of leaf was mounted on a clean slide using dilute glycerol. Calcium oxalate crystals, if present, are seen as bright structures of definite shapes and sizes. After addition of a few drops of concentrated hydrochloric acid and viewing under the microscope, the disappearance of the crystals without effervescence confirms the presence of calcium oxalate crystals.

Test for Oil: A small quantity of the powdered sample of leaf was mounted in Sudan (IV) reagent on a slide and warmed. A pink colour in any of the structures indicates the presence of oils.

3.13Determination of Physical Constants of *D. velutinum* Leaf

3.13.1 Determination of moisture content ofthe powdered sample by "loss on drying method"

An evaporating dish was heated to a constant weight, allowed to cool in a desicator and weight noted. The powdered drug (3g) was accurately weighed into the dish. The dish with its content was weighed(W_1) and placed in an oven at 105^0C and dried to constant weight (W_2) (this can be achieved by checking the weight at 30 minute intervals after an initial drying of one hour, two consecutive constant weights confirmed a constant weight (W_2). The total loss in weight (i.e. the weight of the moisture) was found by subtracting the final weight of the dish and powder from the initial weight of the dish and powder. The percentage of the moisture content with reference to the initial weight of the drug powder was calculated as followed: W_1-W_2/3 X 100%, where W_1 is the weight of the evaporating

dish and powdered drug before heating and W_2 is the weight of the dish and powdered drug after heating (Evans, 2009).

3.13.2 Determination of ash value

A nickel crucible was heated at 105^0C to a constant weight (W_1),cooled in a desicator. About 2g of the powdered drug was weighed into the crucible and total weight (W_2) was noted. The crucible was heated gently with its content until it was moisture-free and completely charred.

The heat was gradually increased until most of the carbon (black residue) had been vapourized and finally heated strongly until the residue was free from carbon (i.e almost white). The crucible was cooled and weighed(W_3). The heating and cooling continued until the weight of the residue (ash) was constant. The weight of the ash was found by subtracting the weight of the crucible from the final weight (i.e the weight of the crucible and residue or ash). The ash value (in percentage) of the powdered drug with reference to its initial weight was calculated as followed: W_3-W_1/2 X 100% (Evans, 2009).

3.13.3 Determination of acid-insoluble ash value

The crucible with the ashfrom the above experiment was washed using25ml of dilute hydrochloric acid and the washings passed through the filter paper into a beaker. The washing was repeated three times, every-time passing the washings through the filter paper in such a manner that the residue was collected in the tip of the cone of the filter paper. The funnel was dried along with the filter paper in an oven at 105^0C. The weight of a clean and heated nickel crucible was accurately determined(W_1). The filter paper with the residue was folded into a small cone and transferred to the tarred crucible. The crucible was gently

heated until the filter paper was completely charred and then heated again strongly for a few minutes. The crucible with its content was cooled, weighed and the final total weight noted (W_2). The weight of the residue (the acid insoluble ash) was found by subtracting the weight of the crucible from the final weight. The Acid-insoluble ash value (in percentage) with reference to the initial weight of the powdered drug (in previous experiment) was calculated as followed: $W_2 - W_1/_2$ X 100 (Evans, 2009).

3.13.4 Alcohol – soluble extractive values

The powdered vegetable drug (5g) was accurately weighed into a 250ml stopper conical flask. Ethanol (90%) of 100ml was added and the stopper was replaced firmly.The flask was shaken on a mechanical shaker for 6 hours and then allowed to stand for 18 hours. The extract was filtered quickly. The accurate weight of a clean and heated evaporating dish was determined(W_1). The filtrate (20ml) was put into an evaporating dish and weighed(W_2). The evaporating dish was heated on water-bath to evaporate the filtrate to dryness on a hot plate. The residue was dried to constant weight at 105^{o}C in an oven and the final total weight was noted (W_3). The weight of the residue obtained from the 20ml extract was found by subtracting the weight of the evaporating dish (W_1) from the final weight (W_3). The alcohol-soluble extractive value (in percentage) with reference to the initial weight of the powdered drug was calculated: $W_3 – W_1/5$ X 100% (Evans, 2009)

3.13.5 Water- soluble extractive value

The above procedure was repeated using distilled water in place of ethanol as the extracting solvent.The water soluble extractive value (in percentage) was calculated as done for the alcohol soluble extractive value (Evans, 2009).

3.14 Thin Layer Chromatography (TLC) of *D. velutinum* Leaf

3.14.1 Extraction of the leaf of *D. velutinum* for chromatographic analysis

The method described by Eloff, (1998) was adopted.The leaf powder of the plant (0.5g) was extracted using 5ml methanol under vigorous shaking and the insoluble residue was removed by centrifugation for five minutes. The procedure was repeated twice and the methanol was removed from the combined extracts under air stream at room temperature. This fresh extract was used for thin layer chromatographic (TLC) analysis.

3.14.2Solvent systems and chromatography

Enough solvent system was prepared and poured into a glass tank and allowed to equilibrate for 1-2 hours (Kotz and Eloff, 2002). Benzene - ethanol - ammonia (9:1:0.1)

Chloroform - ethylacetate - formic acid (5:4:1)

Ethylacetate - methanol - water (10:1.35:1).

The second solvent system gave the best separation, and so was finally used.On a prepared silica gel plate, the methanol extract was spotted at about 1cm from the bottom end of the plate using a capillary tube. The plates were developed in the glass tank while closed.Then, the plates were removed, the solvent front marked and dried in an oven at 105^0C for about 3mins. Each chamber contained plates, some for ultraviolet examination and others sprayed with different reagents, observed, and sometimes heated before observation.

3.14.3 Spraying

The methods described by Touchstone and Wiley (1992) and Harborne (1998) were adopted. Some plates were viewed under 360nm ultraviolet light the different colours that

developed were observed.spraying reagents for visualizing components were also prepared fresh. chromatograms were carefully heated at 105^0C until optimal colour development.Recording chromatogram under UV light was done with the aid of a digital camera.

1. Test for alkaloids:

   The chromatograms were sprayed with Dragendorff's reagent (0.11g KI+0.18g Bismuth subnitrate dissolved in 20ml acetic acid, made up to 100ml with distilled water). Appearance of a red or orange colour indicates the presence of alkaloids.

2. Test for tannins:

   (a) The chromatogramswere sprayed with ferric chloride solution. Appearance of a dark blue or dark green colour indicates the presence of phenolic compounds including tannins.

   (b) The chromatograms were sprayed with bromine water. A dull yellow spot indicates the presence of tannins.

3. Test for steroids:

   The chromatograms were sprayed with 0.5g vanillin in 20ml ethanol and 80ml sulphuric acid. Presence of steroids would be indicated by a purple colour.

4. Test for flavonoids:

   The chromatograms were sprayed with 1% ethanolic solution of aluminum chloride and viewed under ultraviolet (UV) light (360nm). The presence of flavonoids would be indicated by a yellow fluorescence.

5. Test for terpenes:

   The chromatograms were sprayed with 0.5ml P-anisaldehyde in 50ml ethanol and 1ml sulphuric acid. The appearance of a green colour would comfirm the presence of terpenes.

CHAPTER FOUR

4.0 RESULTS

4.1 Experimental Design

The survey was conducted, data collected from the informants on plants used for pain management in Lokoja, Kogi State, Nigeria, the plant samples procured and identified, herbarium specimens prepared and subsequently data compiled. Acute toxicity and analgesic activity were studied on the five most frequently mentioned plants from the ethnomedical survey with little or no scientific information. The thin layer chromatography and pharmacognostic evaluation were conducted on one of the most potent with the fewest scientific information (*D. velutinum*).

4.2 Study Design

The interview was conducted in local languages with the questionnaire as a guide, using a tape recorder and/ or camera where applicable with the incorporation of interpreters where necessary onmedicinal plants used in Lokoja for pain management using a scientifically structured questionnaire.

4.3 Study Site

Sarkin-Noma is a settlement just opposite International market at a ‘T’ junction of three roads; one from Abuja,one to Lokoja town on the left and the other continued from Abuja road to Okene. Next to Sarkin-Noma along Okene road is Felele (the seat of Kogi State

Polytechnic). Along the road to Papalander Square in Lokoja town is Old market which is about 400m from the International market. Gadumo is located along the road to Ajaokuta in between Ganaja junction and Ganaja village. Adankolo covered an area from Federal Medical Centre to Ganaja junction. Lokongoma extends from Lokongoma market to the second gate of phase II along the metropolitan road that also linked to Okene road(plate I).



Plate I: Location of the Seven Surveyed Areas in Lokoja, Kogi State, Nigeria

4.4 Study Population

The studies comprised of forty traditional medical practitioners, nine herbalists/herb sellers and three elderly people.

4.5 Ethnomedical Survey

Of the 70 interactive sessions targeted for the seven areas 10 for each area under study in Lokoja, 52 were successful, indicating an overall response rate of 78% (table 4.1). These 52 informants cut across several tribes which include Igala, Ebira, Okun as the major ones. Age range is 30 to 70. The population of Male (35), female (17), Herbalists (9), traditional medical healers (40), others (3). The twenty three plant species and other data are shown in (Tables 4.1 – 4.4).

4.6 Preparation of Herbarium Specimen

The plant samples were collected by the persons who normally prepare the herbal remedy/informant. The collected plants were then prepared and preserved. Both flowers and fruits (where available) were left intact and clean cut of the stems were made. The site, habitat, habit, flower colour and other necessary data were recorded in the questionnaire, the samples were made pressed and dried quickly; change in flower colour (where occurred) was noted. Photographs of some of the collected plant species were also made. Final identification, authentication with voucher's numbers was made.

4.7 Data Collection

Table 4.1: Collected Plants, Their Identities and Local Names in Ebira, Igala and Okun Languages

S/N	Scientific Name	Family	Voucher No.	Local Names		
				Ebira	**Igala**	**Okun**
1.	*Acanthospermum hispidum*Schrank	ASTERACEAE	900051	*Ovareyikoza*	*Ashaga*	*Dawgomugo*
2.	*Ananas comosus*(Merr) L.	BROMELIACEAE	032310	*Epoyivo*	*Penapulu*	*Kuku*
3.	*Annona senegalensis* Pers	ANNONACEAE	190	*Ochiku*	*Aloko*	*Eye abo*
4.	*Aspilia africana* C.D Adams	ASTERACEAE	1146	*Owozunava*	*Abikdo*	*Yunyun*
5.	*Azadirachta indica* A. Juss	MILIACEAE	0900151	*Ochitoyivo*	*Dogoyaro*	*Dogoyaro*
6.	*Calotropis procera*W. T. Aiton	ASCLEIADACEAE	060723	*Omunu*	*Bombom*	*Bomibom*
7.	*Carica papaya* Linn	CARICACEAE	230510	*Irenwa*	*Echibakpa*	*Ibekpe*
8.	*Chromolaena odorata* Linn	ASTERACEAE	102306	*Awo*	*Awo*	*Akitola*
9.	*Cissus ibuensis*Hook	VITACEAE	230410	-	-	*Eyila*

10.	*Cochlospermum planchonii* Hook	COCHLOSPERMACEAE	2759	*Evaze*	-	-
11.	*Crossopteryx febrifuga* Afzell	RUBIACEAE	062307	*Umarupa*	*Omupapa*	-
12.	*Desmodium velutinum* (P.Beauv.) DC	FABACEAE	1553	*Ema*	*Ema*	*Ege*
13.	*Euphorbia hirta* Linn	EUPHORBIACEAE	583	*Irevi uku*	*Omiakede*	*Orisa ode*
14.	*Ficus capensis* Thunb	MORACEAE	1019	*Ebankoro*	-	-
15.	*Hyptis suaveolens* Poit	LAMIACEAE	012310	*Avi opari*	*rojaanabo*	*Effon*
16.	*Ipomea asarifolia* Linn	CONVOLVULACEAE	062408	*Etana*	*Bebenesho*	*Bibehi*
17.	*Mangifera indica* Linn	ANNACARDIACEAE	1944	*Mangoro*	*Umangolo*	*Mangoro*
18.	*Momordica charantia*Schum& Thonn	CUCURBITACEAE	022310	*Avi ehi*	-	-
19.	*Morinda lucida* Benth	RUBIACEAE	1862	*Oguro*	-	*Oliturare*

20.	*Mucuna pruriens* Linn	FABACEAE	1588	*Idaku*	*Awabaru*	*Ehihi*
21.	*Nauclea latifolia* Smith	RUBIACEAE	1268	*Obedu*	-	-
22.	*Spilanthes filicaulis*Schum&Thonn	ASTERACEAE	534	*Osete*	*Oliturare*	-
23.	*Uvaria chamae* P.Beauv	ANNONACEAE	3129	*Epochi oji*	-	-

Table 4.2: Habit, Number of Informants Citing the Plant and Parts Used for Therapy

S/N	Scientific Name	Habit/Habitat	Informants	Part Used
1.	*Acanthospermum hispidum*	herb/wild	2	whole plant
2.	*Ananas comosus*	shrub/cultivated	5	fruit peel
3.	*Annona senegalensis*	shrub/wild	3	Leaf
4.	*Aspilia africana*	herb/wild	4	Leaf
5.	*Azadirachta indica*	tree/cultivated	6	leaf, bark
6.	*Calotropis procera*	shrub/wild	4	leaf
7.	*Carica papaya*	shrub/cultivated	6	leaf, fruit peel
8.	*Chromolaena odorata*	shrub/wild	3	leaf
9.	*Cissus ibuensis*	shrub/wild	3	leaf
10.	*Cochlospermum planchonii*	shrub/wild	3	leaf
11.	*Crossopteryx febrifuga*	shrub/wild	5	leaf
12.	*Desmodium velutinum*	shrub/wild	5	leaf
13.	*Euphorbia hirta*	herb/wild	5	whole plant
14.	*Ficus capensis*	tree/wild	3	leaf, bark
15.	*Hyptis suaveolens*	herb/wild	4	whole plant
16.	*Ipomea asarifolia*	climber/wild	4	whole plant
17.	*Mangifera indica*	tree/cultivated	6	leaf, bark
18.	*Momordica charantia*	climber/ wild	5	leaf
19.	*Morinda lucida*	shrub/wild	5	leaf
20.	*Mucuna pruriens*	climber/wild	3	whole plant

21.	*Nauclea latifolia*	shrub/wild	3	leaf, bark
22.	*Spilanthes filicaulis*	shrub/wild	5	leaf
23.	*Uvaria chamae*	shrub/wild	4	leaf

Table 4.3: Collected Plants, Mode of Preparation, Dose-Regimen and Route of Administration

S/N	Plant	Preparation	Dosage- regimen	Admin. Route
1.	*Acanthospermum hispidum*	Alcohol maceration is made from the leaf powder and mixed with garden egg.	2 small cup, twice daily,	Oral
2.	*Ananas comosus*	An infusion is prepared by boiling	1 small cup, twice daily	Oral
3.	*Annona senegalensis*	Prepared into soup with dried fish	2 spoon, twice daily	Oral
4.	*Aspilia africana*	Infusion is prepared and taken.	1 cup, twice daily,	Oral
5.	*Azadirachta indica*	Concocted with pine apple fruit peel	twice daily	Oral
6.	*Calotropis procera*	Fresh leaf macerated, rubbed on part	1small cup, 3times daily	Topical
7.	*Carica papaya*	Concocted with pine apple fruit peel	twice daily	Oral
8.	*Chromolaena odorata*	Fresh leaves rubbed on the painful area	Daily	Topical
9.	*Cissus ibuensis*	Fresh leaves rubbed on the painful area	Daily	Topical
10.	*Cochlospermum planchonii*	Aqueous concoction with neem leaf	1 small cup daily	Oral

11.	*Crossopteryx febrifuga*	Leaves are eaten fresh	twice daily	Oral
12.	*Desmodium velutinum*	Prepared into decoction	2small cup, 3times daily	Oral
13.	*Euphorbia hirta*	Prepared into soup with salt, red oil, and dried fish.	once daily	Oral
14.	*Ficus capensis*	Paste prepared with red oil and rubbed on the painful area.	twice daily	Topical
15.	*Hyptis suaveolens*	Prepared into soup using red oil and dried fish.	twice daily	Oral
16.	*Ipomea asarifolia*	Prepared into paste and rubbed on painful fractured or dislocated area.	twice daily	Topical
17.	*Mangifera indica*	Concoction made with pine apple peel, lemon	small cup, 3 times daily	Oral
18.	*Momordica charantia*	Decoction and taken warm	Once daily	Oral
19.	*Morinda lucida*	Decoction prepared	1small cup twice daily	Oral
20.	*Mucuna pruriens*	Prepared into paste, rubbed on painful part	Twice daily	Topical
21.	*Nauclea latifolia*	Decocted leaf extract is mixed with red potash	1 small cup, twice daily	Oral

22.	*Spilanthes filicaulis*	Soup prepared with dried fish	1small spoon, 3times daily	Oral
23	*Uvaria chamae*	Decoction prepared with morinda lucida and azadirachta indica	1small cup twice	Oral

Table 4.4: Collected Plants, Class of Phytochemical Constituents Present, Therapeutic Indication and Reported Pharmacological Evaluation

S/N	Plant	Diseases	Therapy
1.	*Acanthospermum hispidum*	meningitis, diarrhoea,cholera, typhoid	anti-diarrhoea, anticholera
2.	*Ananas comosus*	fever, headache	analgesic, antipyretic
3.	*Annona senegalensis*	malaria ever, pain	analgesic, antipyretic
4.	*Aspilia africana*	haemorrinage,	wound healing, haemorrhage cure
5.	*Azadirachta indica*	fever, pain	antiinflammatory,
6.	*Calotropis procera*	inflammation, malaria, leprosy	antimicrobial, analgesic, antipyretic
7.	*Carica papaya*	fever	antiinflammatory
8	*Chromolaena odorata*	leprosy	antiseptic
9	*Cissus ibuensis*	convulsion	anticovulsant
10.	*Cochlospermum planchonii*	fever, jaundice,backache	analgesic, antipyretic
11.	*Crossopteryx febrifuga*	pain, pyrexia, malaria.	antipyretic, Analgesic, anti-inflammatory.
12.	*Desmodium velutinum*	fever, pain	antipyretic
13.	*Euphorbia hirta*	dysentery	antibacteria, analgesic
14.	*Ficus capensis*	asthma, inflammation, fever	antipyretic,anti-plasmodic.

15.	*Hyptis suaveolens*	Typhoid	antibacteriae
16.	*Ipomea asarifolia*	fever	antipyretic
17.	*Mangifera indica*	Joint pain	analgesic
18.	*Momordica charantia*	fever, pain	analgesic,antipyretic
19.	*Morinda lucida*	diabetes, mellitus	antihyperglycaemia.
20.	*Mucuna pruriens*	parkinson's disease	antisplasmodic, anticancer.
21.	*Nauclea latifolia*	diabetes, cancer	analgesic,laxative
22.	*Spilanthes filicaulis*	infant diarrhoea	antihelminthic,antimicrobial, antipyretic
23.	*Uvaria chamae*	toothache, stomache stomache upset,	analgesic, antipyretic, antibacterial,antiplasmodial

4.8 Data Analysis

Table 4: Ethnobotanical Survey and Traditional Healers in Lokoja Town

Site of Survey	Respondents		Response Rate	
	Traditional Healers			
	Male	Female		
	Age:30-70	**Age:40-70**	**Total**	**%**
Felele				
No. of Interviews	4	3	7	
No. of Collection	**3**	**3**	**6**	**86**
International Market				
No. of Interviews	7	6	13	
No. of Collection	**6**	**4**	**10**	**77**
Old Market				
No. of Interviews	6	5	11	
No. of Collection	**5**	**4**	**9**	**82**
Adankolo				
No. of Interviews	6	-	6	
No. of Collection	**4**	**-**	**4**	**67**
Lokongoma				
No. of Interviews	4	2	6	
No. of collection	**2**	**2**	**4**	**67**
Gadumo				
No. of Interviews	5	1	6	
No. of collection	**5**	**1**	**6**	**100**
Sarkin-Noma				
No. of Interviews	3	-	3	
No. of collection	**2**	**-**	**2**	**67**
Total	**35**	**17**	**52**	**78**

4.9 Plant Screening for Laboratory Investigation

Desmodium velutinum, Aspilia africana, Hyptis suaveolens, Crossopteryx febrifuga, Mucuna pruriens were selected and tested for analgesic activity because they were most frequently mentioned during the ethnomedical survey and have the fewest scientific information. However, further laboratory works were conducted on *D. velutinum*because it has the least scientific information (for instance, no scientific information on analgesic activity) and is one of the most potent plants from the results of analgesic tests. The photographs of the five plants which were subjected to analgesic activity tests are shown in (Plates II - XI).

Photographs of the Five Plants Subjected to Analgesic Activity Test



PlateII:***Aspilia africana*** **CD Adams (ASTERACEAE)Obtained from the Side of Meme River.**



Plate III: ***Crossopteryx febrifuga*** **Afzell (RUBIACEAE)Obtained from Mountain Pati**



Plate IV: ***Desmodium velutinum*** **(P. Beauv.) DC (PAPILIONACEAE) Obtained from Kogi State Polytechnic Site**



Plate V:***Hyptis suaveolens*** **Poit (LAMIACEAE) Obtained from Otokiti village**



Plate VI: ***Mucuna pruriens*** **Linn (PAPILIONACEAE) Obtained from the Side of Meme River**

4.10Pharmacological Tests of of *D. velutinum* Leaf Methanolic Extracts

4.10.1 Acute toxicity studies

Mice treated with doses of 10,100 and 1000 mg/kg for the phase 1 of the experiment did not show any sign of toxicity or death after 24 hours. From the second phrase, the mouse which was administered with a dose of 5000mg/kg died after 24 hours. This showed that the crude drug is to some extent toxic. The LD_{50} was the taken as the geometric mean of the lowest lethal dose and the highest non lethal dose. LD_{50} is 3807.

4.10.2 Tests for analgesia

4.10.2.1*Acetic acid-induced writhing test*

Table 4.6: Effects of Methanolic Extract (ME) of the Leaf of *D. velutinum* on Acetic Acid Induced Writhing in Mice

S/N	**Treatment (mg/kg)**	**Mean number of Writhings**	**% Inhibition**
1	Normal saline (10)	42.80±3.96	0.00
2	Pentazocine (20)	21.80±1.27	49.07
3	ME (100)	23.40± 2.15	45.32
4	ME (200)	24.60± 2.71	42.52
5	ME (300)	27.20± 3.34	36.62

n = 5

4.10.2.2*Hot plate method*

Table 4.7: Effect of Methanolic Extract (ME) of the Leaf of *D. velutinum* on Hot Plate Induced Pain in Mice

S/N	**Treatment in mg/kg**	**0 min**	**30min**	**60min**	**90min**	**120min**
1	Normal saline (10)	1.4	1.4	1.6	1.2	1.4
2	Morphine (20)	2.2	4.6	3.6	3.4	3.0
3	ME (100)	2.0	3.4	3.2	2.4	1.4
4	ME (200)	1.4	2.8	2.2	1.6	1.4
5	ME (300)	1.4	3.0	2.2	1.4	1.2

n = 5

The results of the analgesic tests of the remaining four plants selected;*A. Africana*, *C. febrifuga*, *H. suaveolens* and *M. pruriens* are in (appendix II)

4.10.3 Statistical analysis for the analgesic results of *D. velutinum*

Results were expressed as mean ± standard error of mean (SEM). Analysis of Variance test was used to analyze the data.

Table 4.8Analysis of Variance Test

Source of Variation	**d*f***	**Definition (SS)**	**Mean**	**F**
Treatment	4	11.48	2.89	5.66
Error	19	9.68	0.51	
Total	23			

Where d*f* is degree of freedom, SS is sum of square. Tabular value is 2.89 while the calculated value is 5.66. Since the calculated value (5.66) is greater than the tabular value Comparison between control and drug treated groups were considered to be significant ($P<0.05$)

4.11Macroscopical and Microscopical Examination of *D. velutinum* Leaf

4.11.1Macroscopical examination

Itis an annual, erect shrub, up to 3m high, branches dark red, yellow-brown when young. Leaf - ovate, 4-20 cm long and 2.5-13 cm wide, coriaceous, upper and lower surfaces densely velutinous, flowers purple to pink. The leaf margin is entire, base is symmetrical,apex is blunt.

4.11.2Microscopic examination

4.11.2.1*Qualitative leaf microscopy*

The microscopic examination of the leaf powder and section of the leaf revealed various anatomical features of the plant. The epidermal cells are cuboidal forms. Anomocytic type of stomata ranging from 10-12µm long and 6-8µm wide were observed only on the lower epidermis (plates VII). Calcium oxalate crystals were observed in between veins in fresh leaf and numerous unicellular covering trichomes were observed all over the epidermis (plate VIII).

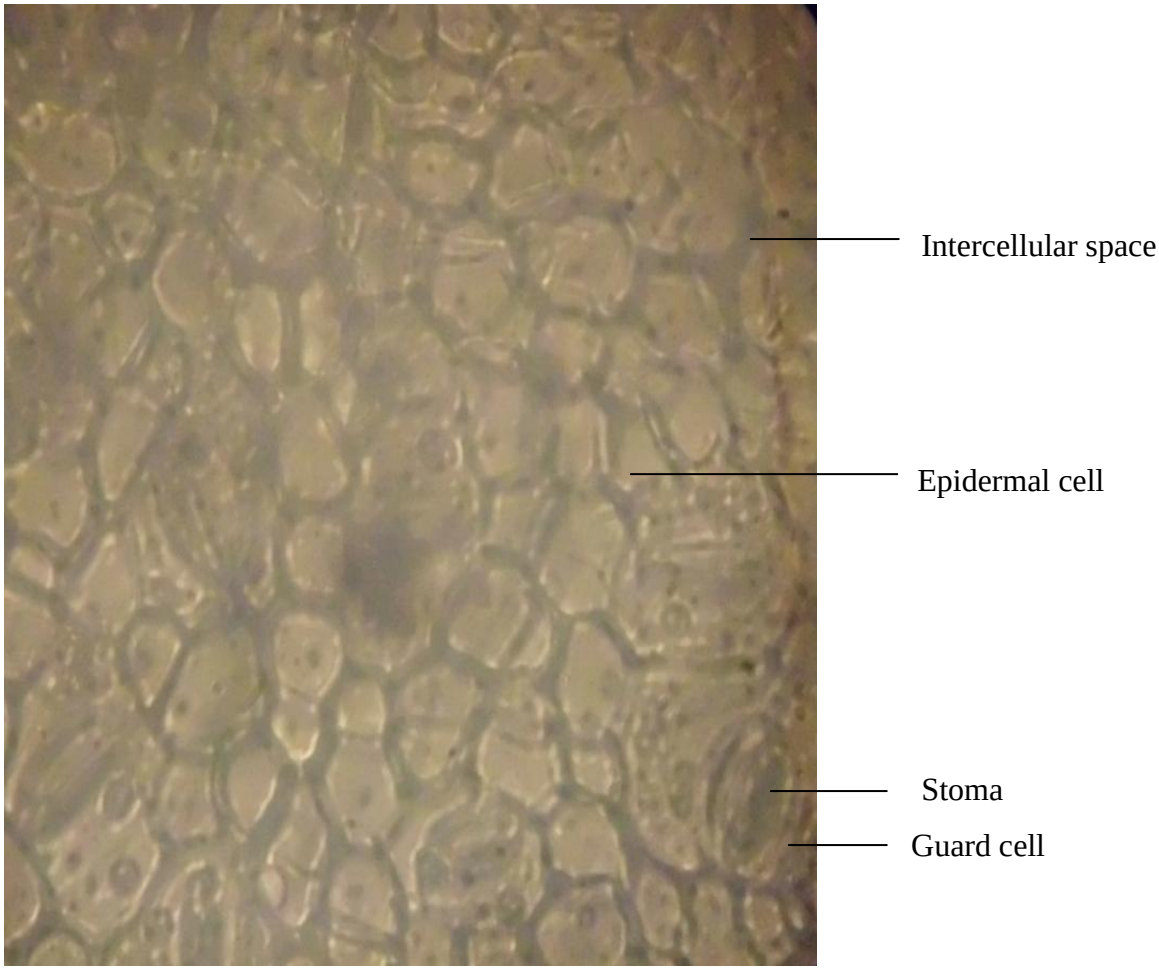


Plate VII:**Cross section of the Lower epidermis of *D. velutinum* leaf viewed under compound microscope (X40)**

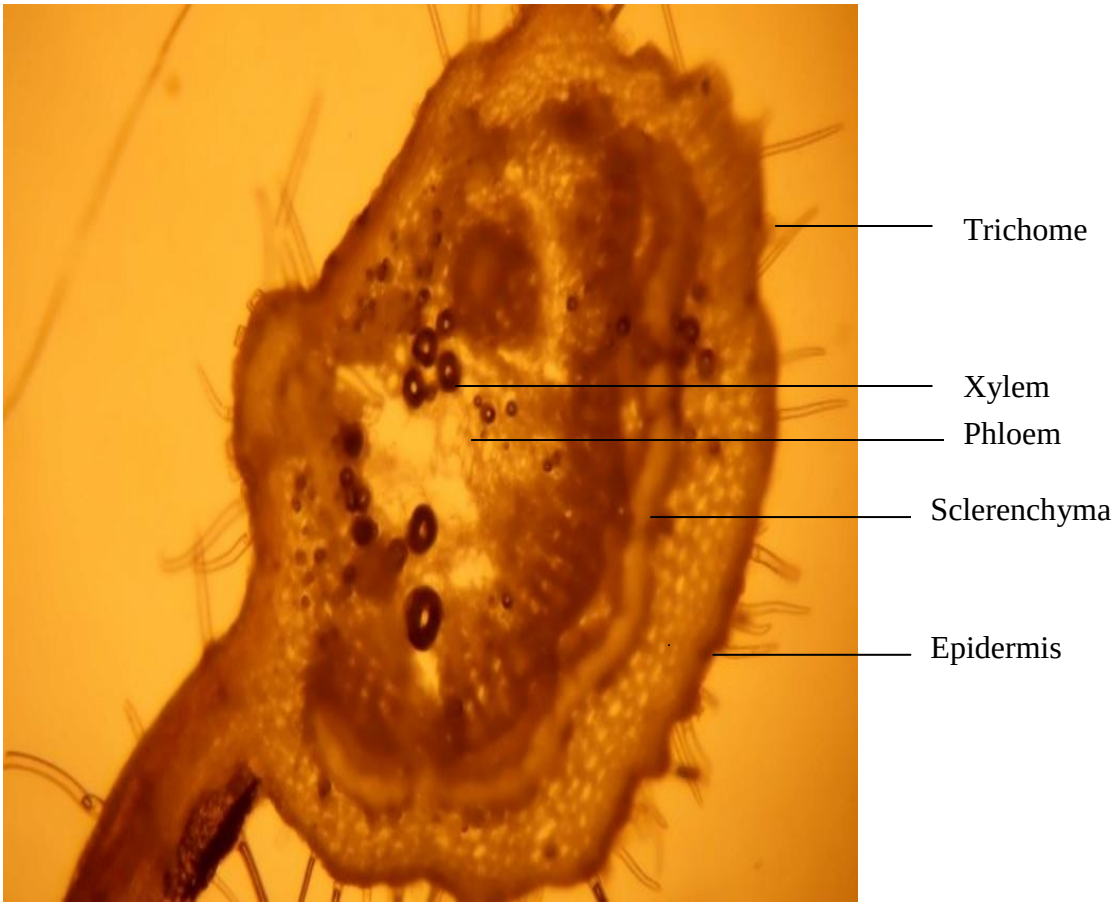


Plate VIII: **Transverse section of *Desmodium velutinum*midLeaf viewed under microscope (X40)**

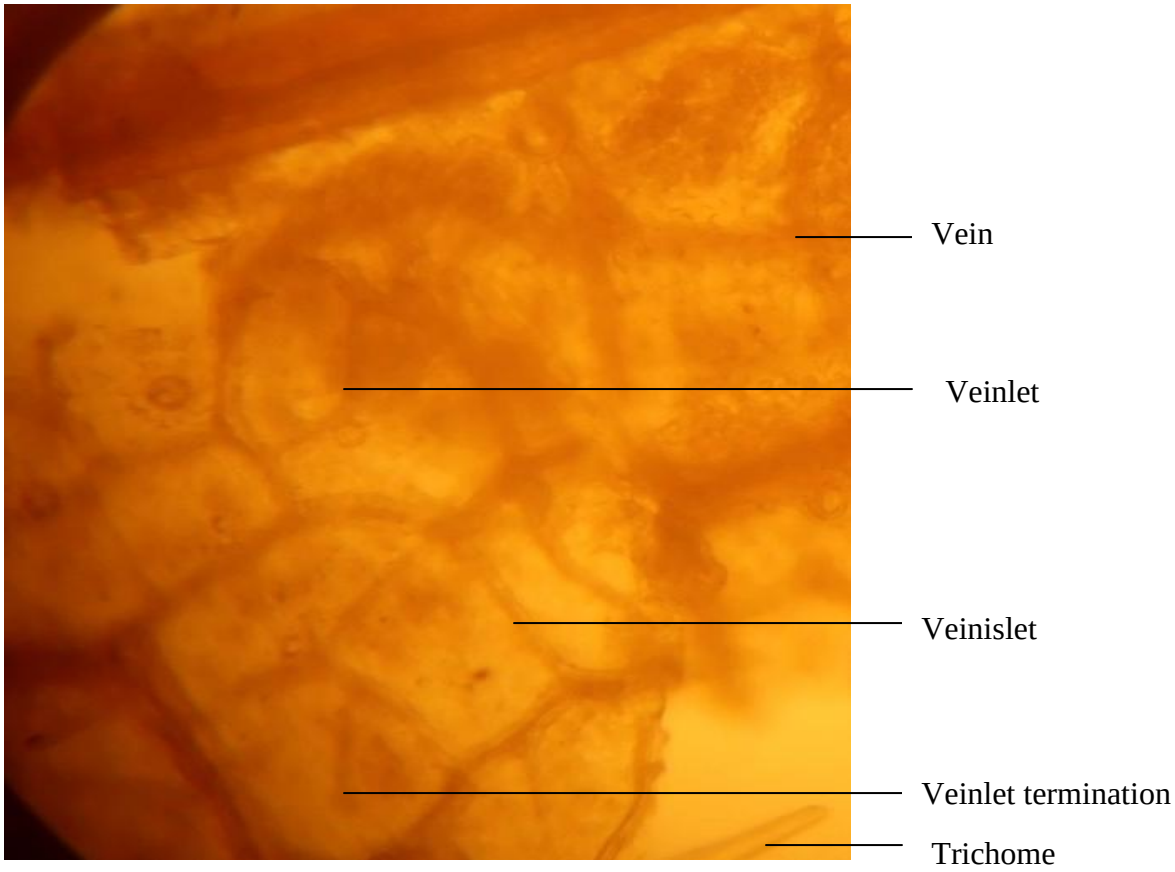


Plate IX: **Epidermis of*D. velutinum*with veins, veinlet, veinislet and vein as viewedunder microscope (X40)**

4.11.2.2 *Quantitative leaf microscopy*

The result of the average stomatal number, veinlet number, veinlet termination number and veinislet number for upper and lower epidermis were determinedunder microscope (plate IX). The results are as shown in table 4.9.

Table 4.9: The results of quantitative leaf microscopy

S/N	Parameter	Value (Mean ± SEM)
1.	Stomatal number	4.3 ± 0.23
2.	Stomatal index	9.09 ± 0.31
3.	Veinlet number	4.7± 0.06
4.	Veinlet termination number	2.40 ± 0.26
5.	Vein islet number	3.20 ± 0.60

n = 4

1. Trichomes

The trichomes were seen as unicellular , non glandular structures, covering innature. They are numerous and are found on both upper and lower epidermis.

2. Calcium oxalate crystal

These were seen as acicula structures and are few in numbers and scattered along the veins of the leaf.The transverse section of the leaf through the midrib revealed a network venation as well as open and markedly differentiated vascular bundles which are characterisctic

features of dicotyledonous plants (plate VIII). Spongy parenchyma cells are conspicuous with much air space in between. The xylem vessels are located just above the phloem without cambium (Plate VIII). The petiole had a single layer of epidermis and parenchyma cells with a set of vascular bundle in the middle. Trichomes are also observed along the tip.

4.12Summary of the Chemomicroscopical Examination of the Leaf of *D. velutinum*

Table 4.10: Summary of the Chemomicroscopical Examination of the Leaf of *D. velutinum*

S/N	**Constituents/Reagents**	**Observation**	**Inference**
1	Cellulose + N/50 iodine + 66% H_2SO_4	bluish colouration	present
2	Starch + N/50 iodine	blue-black colouration	present
3	Tannin + $FeCl_3$	greenish colouration	present
4	Oils + Sudan (IV) reagent	no pink colouration	absent
5	Calcium oxalate crystal + Chlorahydrate solution + Conc. HCl	Disappeared on addition of HCl	present

n = 4

4.13Summary of Results of Quantitative Evaluation of Physical constants

The summary of the results of the quantitative evaluation of the plant, *D. velutinum* are shown in table 4.8. Each was obtained as mean and standard error of mean from three samples.

Table 4.11 Summary of Results of Quantitative Evaluation of Physical Constants

S/N	**Parameter**	**Value (Mean ± SEM) % w/w**
1	Moisture (% w/w)	6.11 ±0.09
2	Ash value (%w/w)	8.50 ±0.24
3	Acid insoluble ashvalue (% w/w)	0.50 ±0.21
4	Alcohol solubleextractive value (%w/w)	20.80 ±0.09
5	Water soluble extractive value (%w/w)	31.83±0.07

n = 3

4.14.1Plates of thin layer chromatograms developed from methanolic extract of*D. velutinum*Leaf.

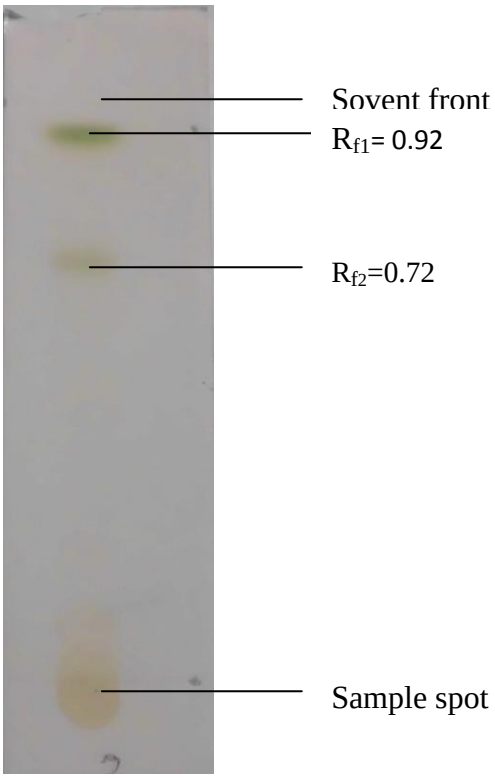


Plate X: Chromatogram developedin chloroform - ethylacetate - formic acid (5:4:1), sprayed with 1% Ethanolic solution of aluminum chloride and viewed under ultra violet light (360nm), yellow fluorescence. R_{f2} indicates the presence of flavonoids.

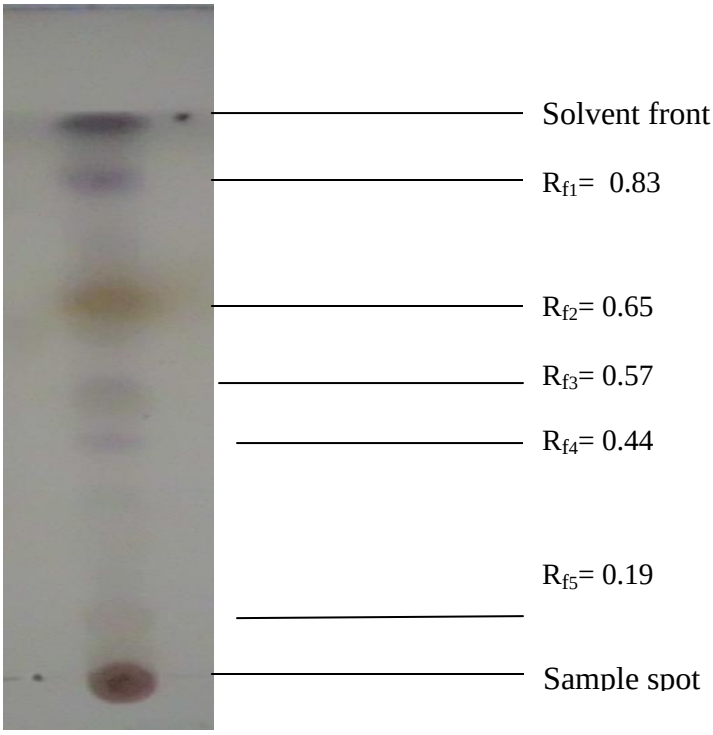


Plate XI: Chromatogram developedin chloroform - ethylacetate - formic acid (5:4:1) and sprayed with vanillin ethanolic sulphuric acid (purple-R_{f3} colour forsteroidal presence)



Plate XII: Chromatogram developedin chloroform - ethylacetate - formic acid (5:4:1) and sprayed with p-anisaldehyde in ethanloic sulphuric acid (green/grey-R_{f1} colour for terpenepresence) respectively.



Plate XIII: Chromatogram developedin chloroform - ethylacetate - formic acid (5:4:1) and sprayed with ferric chloride solution (dark green colour at R_{f2} shows the presence of phenolic compounds). Further treatment with bromine water shows a dull yellow colour at R_{f1} which indicates the presence of tannins.

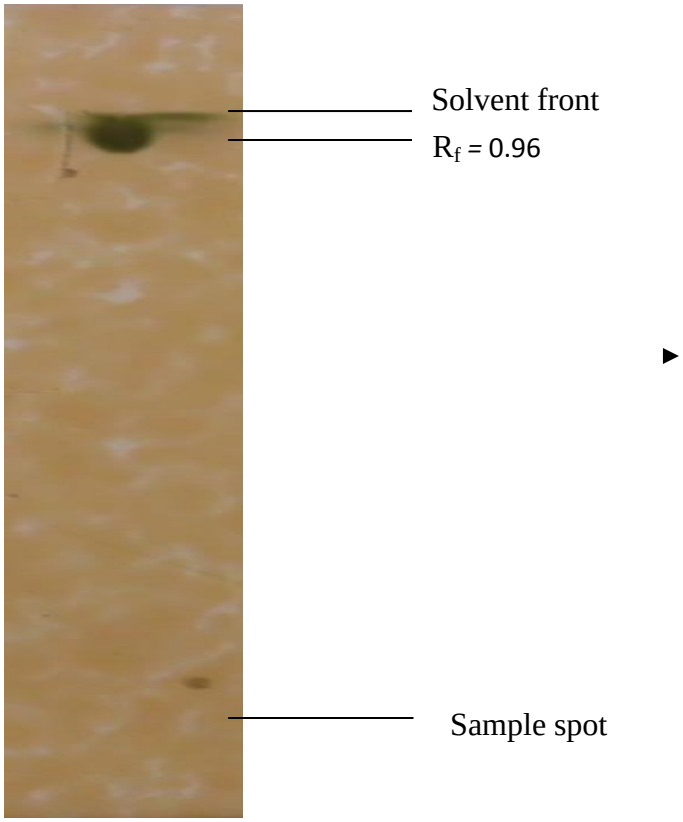


Plate XIV: Chromatogram developedin chloroform – ethylacetate - formic acid (5:4:1) and sprayed with Dragendorff's reagent (no orange or red colour indicating alkaloids are absent).

4.14.2 Results ofthin layer chromatography of *D. velutinum* leaf

Table 4.12: Thin layer chromatographic analysis of phytochemical constituents of the leaf of *Desmodium velutinum*

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S/N	**CONSTITUENTS**	**SOLVENTS**	**REAGENTS**	**OBSERVATION**	**R_f**	**INFERENCE**
1	Alkaloids	Chloroform, Ethylacetate, Formic acid(5:4:1)	Dragendorff's reagent	No red or orange spot	-	Absent
2	Terpenes	,,	P-anisaldehyde	Green spot	0.65	Present
3	Flavonoids	,,	Aluminum Chloride and viewed at UV (360nm)	Yellow fluorescence	0.72	Present
4	Steroids	,,	Vanillin sulphuric acid	Purple spot	0.57	Present
5	Tannins	,,	(a)Ferric chloride (b)Bromine water	Dark green spot Dull yellow	0.87	Present

CHAPTER FIVE

5.0 DISCUSSION

Medicinal plants are used for the management/treatment of many diseases including pain. The present study describes the identification and documentation of the medicinal plants of Lokoja, Nigeria. The survey covered 52 informants consisting of 14 informants from Igala, 13 informants from Ebira and 25 informants from Okun. Male is 35, female is 17, traditional medicine practitioners is 40, herb sellers is 9 and elders is 3. From the results of the survey, traditional medicine has a long history of acceptability in Lokoja, Nigeria.

Traditional plant based medicines, which are used to treat and manage various health related problems particularly pain are still patronized and sought for by the majority of the rural populace. Its easy accessibility, acceptability to body systems and low cost makes it first choice of healthcare system in spite of the influence of modern medicine. These observations are in agreement with those earlier observed by the World Health Organization (WHO). It has also been noted that traditional medicine remains popular among rural communities of the world since it is readily accessible, affordable and more importantly, is an integral part of the traditional and cultural beliefs and practices of the people (WHO, 2002). The use of plants to manage health and well–being is an age long practice. Many conventional and high efficacious drugs are produced from plant sources.

The plants cited for the management of pain include *Desmodium velutinum, Morinda lucida, Spilanthes filicaulis, Ananas comosus, Azadirachta indica*. The respondents revealed that pain management with these plants was mostly done using their recipes with relatively high success. They further indicated that although in most cases this form of treatment was

effective in managing pain and related conditions, in a situation where the sickness persisted, the patient could resort to orthodox medicine. Osemene, *et al*., (2011) in an earlier study comfirmed the foregoing by documenting findings on the application of both orthodox and alternative sources of medicine by rural communities.

Plant habit/habitat from the collection showed that many of the medicinal plants are ubiquitously wild, herbs and shrubs. Trees are less represented as medicinal plants from Lokoja. The forest plants made up only 11% of the medicinal plants collected. Plant conservation was poor; as most of the informants admitted they just collect these plants from the wild, some are burnt by annual bush fire and during cultivation of farmlands. These plants have become endangered since no consideration for cultivation or preservation was observed. These findings are in agreement with those of Obute (2005).

Observation of the few medicinal plants documented during the survey show that Lokoja area has a fair distribution of medicinal plants along the huge hills and mountain reliefs surrounding the region as shown on the map (plateI). Most of the respondents lamented their ordeal in climbing the mountain to obtain medicinal plants. Because of this , such trips are usually planned before execution. Some however claimed to have a huge reserve of medicinal plants with higher efficacy, including those for use as therapy against spiritual ailments.

The survey results as shown in table 4.3 showed recipes of the surveyed medicinal plants. Generally, most of the remedies involved a single plant with water and sometimes local drinks such as “kunu”, “burukutu”, palm wine etc as the common extraction solvents, either by maceration, decoction, concoction or infusion. The survey results also show that, oral

method of administering traditional medicine is the most popular practice in Lokoja.The mode of administration and dosage-regimen were poorly designed and regulated. This poor precision in prescription of traditional medicine has earlier been observed as its major disadvantage (WHO, 2002).

Plants which the informants claimed to have analgesic potential were screened and selected for laboratory investigation on the basis of the number of occurrence (frequency of usage) and the number of scientific information from the literature studies. Plants like *Morinda lucida, Spilanthes filicaulis, Ipomea asarifolia, Euphorbia hirta, Uvaria chamae, Momordica charantia, Azadirachta indica, Mangifera indica* and *Carica papaya* etcshow high occurrence from the survey and have a lot of scientific information on analgesic properties. For this reason, they were not selected for scientific investigation in this research. Plants like *Ficus capensis, Chlomolaena odorata, Cochlospermum planchonii*, show few occurrence and considerable scientific information. For this reason, they were not selected for laboratory investigation. However, plants such as *Aspilia Africana,Mucuna pruriens, Hyptis suaveolens, Crossopteryx febrifuga* and *Desmodium velutinum* show considerable occurrence from the survey result and little scientific information. For this reason, they are deemed fit for the purpose of this study and therefore were selected for scientific investigation in the area of analgesic activities.

The LD_{50} (>3000mg/kg) obtained from the leaf methanolic extract of *D. velutinum* showed no observed side effect which probably suggests that the plant extract is relatively safe for mice. This as well indicates that the leaf extract of the plant may be relatively safe for human consumption. It is a great advantage for any plant extract used for herbal treatment to have a

very low toxicity with high $LD_{50.}$ According to Clarke and Clarke, (1979), any substance whose LD_{50} is higher than 1000mg/kg is considered relatively safe.

The number of writhing in mice at 300mg/kg of the extract (27.20±3.34) was significantly lower ($P \leq 0.05$) when compared with that obtained with the normal saline (42.80±3.96) while at 100mg/kg of the extract (23.40±2.15) was even significantly much more lower when compared to the normal saline showing a dose response relationship. However, there is statistically significant difference at ($P \leq 0.05$) in the number of writhing in the mice administered with standard drug (21.80±1.27) compared with normal saline (table 4.6). Similarly, the percentage inhibition of pain in the mice treated with the extract is higher than that treated with normal saline. The standard drug gave the highest inhibition among the experimental groups. The acetic acid-induced writhing method showed that at lowest dose, it gave the highest analgesic properties which were comparable with those of pentazocine, a peripherally acting analgesic.

The acetic acid-induced writhing is a visceral pain model that has been associated with release of arachidonic acid, cyclooxygenase (COX), bradykinins and substance P which induce pain. Prostaglandin biosynthesis plays a role in nociceptive mechanisms (Satyanarayana *et al.*, 2004). Therefore this model of pain might have been inhibited by peripheral analgesics through the inhibition of COX activity. The results show that lowest dose of 100mg/kg has peripheral analgesic properties similar to pentazocine by inhibiting the release of endogenous pain mediators.

The number of seconds expended by mice before jumping off the plate at a dose of 300mg/kg was significantly higher ($P \leq 0.05$) when compared with that obtained in the normal

saline. So also, the number of seconds expended by mice before jumping off the hot plate at a dose of 100mg/kg was significantly higher than normal saline. However, there is statistically significant difference at ($P \leq 0.05$) in the number of seconds expended by mice administered with standard drug (morphine) when compared with normal saline (table 4.7).

The hot-plate test was utilized to assess the central anti-nociceptive properties of *D. velutinum* leaf extract. This test is sensitive to drugs which exert their analgesic effects through the central nervous system (CNS). At doses of (100, 200 and 300mg/kg), the extract significantly increased reaction time to thermal stimulation from 30 to 120 minutes of post treatment. The crude extract and morphine behaved the same way by showing high activity at 30 and 60 minutes of administration and later decrease at 90 and 120 minutes. It was also observed that the lowest dose exhibited the highest activity indicating that could be the appropriate dose for medication. This is in line with what is called "carrier mechanism" a phenomenon that explains the zero increase in the activity of some drugs as dose is increased (Sayyah *et al*., 2004). Morphine, a central acting analgesic increased the reaction time from 30 minutes post treatment. Since the extract is able to increase the latency to thermal stimulation, it could therefore have some central analgesic properties. Although the exact chemical compound or compounds responsible for the analgesic effectof the leaf extract of *D. velutinum* are not known, the observed pharmacological effects may have been due to a single constituent or by the complex interaction among the various chemical constituents in the leaves of the plant.

The leaf extract showed anti-nociceptive and analgesic properties. The results of the analgesic activity tests were expressed as mean ± standard error of mean (SEM). Analysis of

Variance (ANOVA) test was used to analyze the data. Comparison between control and drug treated groups were considered to be significant ($P<0.05$). The results of the extract on pain (tables 4.6 and 4.7) were compared based on the effect on the number of writhing produced by acetic acid in acetic acid induced writhing test and the time in seconds expended before jumping off the hot plate in the hot plate induced pain method along with negative control (normal saline) and positive control (standard drug) in mice.

From the preliminary analgesic evaluation of the five plants, *D. velutinum*, family-PAPILIONACEAE gave one of the highest activities and has the fewest scientific information from the literature survey (no scientific information on analgesic activity). For this reason, it was selected for further laboratory work.

The macroscopical examination of the leaves of *D. velutinum leaf* showed that it has entire margin, symmetrical base, blunt apex and hairy epidermis which are in line with those described earlier by Anowi *et al*.,(2012). These features can be employed in identifying and classifying the plants on the field. The unicellular non glandular trichomes observed from the microscopy of the powder and thin sections of the leaf could also serve as a diagnostic feature since the presence of a particular type of trichomes are normally employed in the taxonomy of some families, genera and species (Evans, 2009). Trichomes maintain a layer of still air on the leaf surface which prevents excess water loss by transpiration (Evans, 2009). The absence of stomata on the upper epidermis of the leaf and their few numbers on the lower epidermis could be a mechanism by which *D. velutinum* reduces the amount of water loss through transpiration as it is a tropical plant.

The chemomicroscopic examination of the leaf powder and thin sections showed calcium oxalate crystals (acicula) type which are found along the veins of the leaves and this could serve as a diagnostic feature of this plant species.

The moisture content (6.11% w/w) is low and unlikely to encourage microbial or fungal thrive and enzymatic or chemical reaction which can spoil the potency of the crude drug. This is comparable to that of digitalis leaves (6.0% w/w). Hence the leaves can be stored for a considerable period of time under standard conditions.

The total ash value (8.50% w/w) obtained for the leaves of *D. velutinum* was considerably higher than the acid insoluble ash value (0.50% w/w) and this indicates the physiological state of the plants. *D. velutinum* has low ash value and so has low adulteration. Ash value is used for identification and standardization of powdered drug. It is a critical tool for detecting adulteration in drugs or resulting from poorly prepared drug. The higher the value, the more likely is the adulteration for poor preparation or woody drug. Ash represents inorganic salts naturally occurring in drug or adhering to it or deliberately added to it as a form of adulteration sources. The low ash value shows that the amount of extraneous materials in the drug is very low. The acid insoluble ash is 0.5% w/w. It is used to measure the amount of silica especially sand and siliceous earth present in a drug. The acid insoluble ash is the "physiological ash" that does not normally dissolve in the dilute acid.

The value obtained for the alcohol extractive value (20.53% w/w) was significantly less than that of the water extractive value (31.50% w/w) and this indicates that more of the constituents of the leaf are more soluble in water than in alcohol.

Generally, information from phytochemical test of *D. velutinum* (table 4.12) as well as those of other plants obtained from literature survey including the data on their therapeutic uses (table 4.4), can be used to study a possible pattern of the chemosystematics of these surveyed plant species, judging from fact that these phytochemical constituents reported or detected in different plants can be compared across species for similarities and differences. In this way, species sharing same compounds can be presumed to be more closely related by descent. This information would play a leading role towards the bioassays of these plants for the discovery and development of novel drugs. The data on table 4.4 can offer information of chemotaxonomic importance, which can be applied to eliminate species which are likely to yield known active secondary metabolites that are beneficial to humanity. Moreover, it can serve as lead in the type of chemical, pharmacognostic and pharmacological evaluation to be carried out in these plants.

The results of phytochemical screening of *D. velutinum* showed the presence of tannins, flavonoids, terpenes and steroids. This is in line with the investigation made by Anowi *et al.*,(2012). Similarly, Frank and Buckingham, (2009) and Bojaxa *et al*., (2010) studies on the phytochemical screening of species belonging to the Fabaceae showed that they possess tannins, flavonoids, phenols and steroids in common.

Flavonoids and terpenes are known to possess antimicrobial, anti-inflammatory and analgesic activities (Anilkumar, 2010). Similarly, this therapeutic property exhibitedprobably due the presence of tannin, flavonoid or steroid is in agreement with the work of Zahida *et al*., (2007) and Sankaradoss *et al*., (2012). These chemical components detected in the plants therefore may serve as the active ingredients responsible for the claimed therapeutic indication.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

Ethnomedical survey and evaluation of the traditional uses of plants are important in development of traditional medicine.

The physical constants areuseful in determination of level of adulteration and improper handling of drugs. The moisture content of the drug will not encourage the growth and action of micro-organism overtime and chemical or enzymatic action. The total ash value is also vital in the evaluation of the purity of the drug, i.e. the presence or absence of foreign inorganic matter such as metallic salts and /or silica.

The macroscopic, microscopic and chemomicroscopic results in this research could serve as vital tools in the preparation of the monograph of plant. The vein islet number, vein termination number and other parameters evaluated in the quantitative microscopy are useful and can help to differentiate closely related species of the plants.

The LD_{50}(>3000mg/kg) of *D. velutinum* shows that the plant is relatively safe for human consumption.

The leaf extracts showed potent analgesic properties in a non dose dependent manner in mice models. Therefore, findings of the present study provide pharmacological support for the folkloric use of the plant in the management of pain.

6.2 Conclusion

Medicinal plants have contributed greatly to traditional and orthodox medicine through the provision of ingredients for drug development.

In Lokoja, there is poor practice in regenerating medicinal plants especially the wild type. Their exploitation poses danger to some of these species as it can result in their extinction in the nearest future.

This survey is the first of its kind on medicinal plants used for pain management/treatment in Lokoja. Some of the plants from the findings have already been scientifically evaluated. The selected *D. velutinum* has been evaluated for its macroscopy, microscopy, physical constant, chromatography and biological activities.

The findings in this research have provided useful information for a monograph of the plant and have validated the traditional use of the plant for pain management/treatment.

6.3 Recommendation

The survey explored the plants used by the people of Lokoja in managingpain. *D. velutinum* proved to be relatively safe for human consumption and showed considerable analgesic property. Thin layer chromatography revealed the presence of tannin, terpene, phenol, flavonoid and steroid.

The four other plants evaluated only for analgesic activity need phytochemical and other pharmacognostic investigation. Since the analgesic activity could be due to a single compound or synergy of two or more compounds, the particular compound(s) responsible require isolation.

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Appendix I

ETHNOMEDICAL SURVEY SHEET (QUESTIONNAIRE)

FOR THE RESEARCH PROGRAM

ETHNOBOTANICAL SURVEY OF THE PLANTS USED FOR PAIN MANAGEMENT IN LOKOJA, NIGERIA

Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University - Zaria.

Sheet number…… Collection number……Group number…… Date ……..

A. Locality: Area …….. Ward……

What is pain?…….

B. Traditional healer/Herb Seller/Informant:

Name…….. Duration of practice……..

Sex……..Age……..
Address…….....

C. Plant Identity/Recipes/Ingredients:

Family name…….Genus……Specie…….…….

Taxonomist ……. Herbarium…….…… Voucher number…….

(For more plants, please write on the back of the sheet)

Common name (English)……..……. Local name(s) (specify dialect) ..……

D. Habit/Habitat/Morphology:

Tree ☐ Shrub ☐ Herb ☐ Mistletoe or other parasite

Aquatic ☐ Savanna ☐ Forest ☐ Flower colour ……
Smell…… Fruit description…….…….……Bark description……..……..

E. Plant Therapeutic Use (Based on Epidemiology):

a. Disease(s) for which the plant is used……
….

b. Level of success in the plant use...……..

c. Which disease is most often treated with the same or other plants? ……...
……..

F. Plant Part Used in Medicine:

Root ☐ Stem ☐ Root bark ☐ Stem bark ☐ Leaf ☐

Flower ☐ Fruit ☐ Seed ☐

G. How plant part is used? Fresh ☐ Dried ☐

Mode of preparation for medicinal use: Powdered ☐ Extracted with cold water ☐ Extracted with hot water ☐ ☐

Others……
……

(Provide detail about the amount or part used; ratio of plant to menstrum etc)
……

H.Mode of Administration (Dosage and regimen)…….

a. Are there any incantation needed? Yes ☐ No ☐
   (If yes, please write the incantation at the back of the sheet)
b. Other plants or ingredients with which the plant is used for preparation:
   1. ……...
      ……
   2. ……
      ……
   3. ……
      ……
c. Any possible reason(s) why two or more plants (multiple plant parts) or plants in combination with other ingredients have to be concocted to effect cure? .....
   ……..
   ……
d. Can the preparation be made in any other way e.g. using another menstrum (e.g. local gin instead of water)?
   .....……..
   a. Plant to be collected at which specific time of the day…….....
   b. Season of collection: Dry ☐ Rainy ☐ Month most found ☐

I. Any other information (please indicate)

……

Appendix II

PHARMACOLOGICAL TESTS OF OTHER FOUR PLANTS

(1) Acute Toxicity Studies of *Aspilia africana*

Mice treated with different doses of 10,100 and 1000 mg/kg for the phase 1 of the experiment did not show any sign of toxicity or death after 24 hours. Out of the mice in the phase 2 (administered with 1600, 2900 and 5,000mg/kg i.p) in the experiment, two which were treated with 2900 and 5000mg/kg died after 24 hours. This shows that the crude drug is to certain extent toxic.

The LD_{50} was the taken as the geometric mean of the lowest lethal dose + the highest non lethal dose or

$$LD_{50} = \sqrt{\text{minimum toxic dose} + \text{max tolerated dose}}$$

$$= \sqrt{2900 \times 1600} = 2154$$

Taking 30% of it, 30/100×2154 = 646.

Table 1: Effect of methanolic extract (ME) of the leaf of*Aspilia africana* on acetic acid induced writhing in mice.

S/N	**Treatment (Mg/Kg)**	**Mean number of Writhing**	**% Inhibition**
1	Normal saline (10)	38.6	0.00
2	Pentazocine (20)	22.2	42.49
3	ME (100)	19.6	49.22
4	ME (200)	16.6	56.99
5	ME (300)	11.8	69.43

n = 5

Table 2: Effect of methanolic extract (ME)of the leaf of *Aspilia africana* on hot plate test induced pain in mice.

S/N	**Treatment in mg/kg**	**0Min**	**30Min**	**60Min**	**90Min**	**120Min**
1	Normal saline (10)	2.0	2.0	1.8	1.6	1.6
2	Morphine (20)	2.4	5.4	4.2	3.0	3.0
3	ME (100)	1.4	2.0	2.4	1.6	1.4
4	ME (200)	2.2	3.8	2.4	2.2	1.6
5	ME (300)	1.4	3.4	3.8	3.0	2.0

n = 5

(2). Acute Toxicity Studies of *Mucuna pruriens*

Out of the mice treated with different doses of 10,100 and 1000 mg/kg for the phase 1 of the experiment, all the mice in 1000mg/kg died after 24 hours. Out of the mice (administered with 140, 225, 370 and 600mg/kg i.p) in the experiment, one which was treated with 600 mg/kg died after 24 hours. This shows that the crude is to certain extent toxic.

The LD_{50} was the taken as the geometric mean of the lowest lethal dose + the highest non lethal dose or

$$LD_{50} = \sqrt{\text{minimum toxic dose} + \text{max tolerated dose}}$$

$$= \sqrt{600x370} = 471$$

Taking 30% of it, 30/100×471 = <u>141</u>

Table 3: Effect of mathnolic extract (ME) of the leaf of *Mucuna Pruriens* on acetic acid induced writhing in mice.

.

S/N	**Treatment (Mg/Kg)**	**Mean number of Writhing**	**% Inhibition**
1	Normal saline (10)	43.0	0.00
2	Pentazocine (20)	21.6	50.69
3	ME (100)	43.0	0.00
4	ME (200)	43.0	0.00
5	ME (300)	43.2	-4.64

n = 5

Table 4: Effect of methanolic extract (ME) of the leaf of *Mucuna pruriens* in hot plate induced pain in mice.

.

S/N	**Treatment in mg/kg**	**0Min**	**30Min**	**60Min**	**90Min**	**120Min**
1	Normal saline (10)	1.6	1.2	1.4	1.4	1.4
2	Morphine (20)	2.2	5.4	3.6	2.8	3.0
3	ME (100)	1.4	1.4	1.2	1.3	1.6
4	ME (200)	1.2	1.2	1.2	1.0	1.2
5	ME (300)	1.4	1.2	1.0	1.2	1.6

n = 5

(3) Acute Toxicity Studies of *Crossopteryx febrifuga*

Mice treated with different doses of 10,100 and 1000 mg/kg for the phase 1 of the experiment did not show any sign of toxicity or death after 24 hours. Out of the mice in the phase 2 (administered with 1600, 2900 and 5,000mg/kg i.p) in the experiment, two which were treated with 2900 and 5000mg/kg died after 24 hours. This shows that the crude drug is to certain extent toxic.

The LD_{50} was the taken as the geometric mean of the lowest lethal dose + the highest non lethal dose

$$LD_{50} = \sqrt{\text{minimum toxic dose} + \text{max tolerated dose}}$$

$$= \sqrt{2900 \times 1600} = 2154$$

Taking 30% of it, 30/100×2154

$$= \underline{646}.$$

Table 5: Effect of methanolic extract (ME) of the leaf of *Crossopteryx febrifuga* on acetic acid induced writhing in mice

P

S/N	**Treatment (Mg/Kg)**	**Mean number of Writhing**	**% Inhibition**
1	Normal saline (10)	43.4	0.00
2	Pentazocine (20)	22.2	49.31
3	ME (100)	23.3	47.00
4	ME (200)	24.0	44.70
5	ME (300)	20.0	53.92

n = 5

Table 6: Effect of methanolic extract (ME) of the leaf of *Crossopteryx febrifuga* in hot plate induced pain in mice

.

S/N	**Treatment in mg/kg**	**0Min**	**30Min**	**60Min**	**90Min**	**120Min**
1	Normal saline (10)	1.6	1.2	1.6	1.2	1.2
2	Morphine (20)	2.8	5.0	3.2	2.4	2.4
3	ME (100)	1.4	2.4	2.6	2.2	1.4
4	ME (200)	1.8	3.4	2.4	2.4	1.8
5	ME (300)	1.4	2.4	2.2	142	1.2

n = 5

(4) **Acute Toxicity Studies of *Hyptis suaveolens***

Mice treated with different doses of 10,100 and 1000 mg/kg for the phase 1 of the experiment did not show any sign of toxicity or death after 24 hours. Out of the mice in the phase 2 (administered with 1600, 2900 and 5,000mg/kg i.p) in the experiment, one which was treated with 5000mg/kg died after 24 hours. This shows that the crude drug is to certain extent toxic.

The LD_{50} was the taken as the geometric mean of the lowest lethal dose + the highest non lethal dose or

$$LD_{50} = \sqrt{\text{minimum toxic dose} + \text{maximum tolerated dose}}$$

$$= \sqrt{2900 \times 5000} = 3{,}807$$

Taking 30% of it, 30/100×3808

$$= \underline{1142}.$$

Table 7: Effect of methanolic extract (ME) of the leaf of *Hyptis suaveolens* on acetic acid induced writhing in mice

S/N	**Treatment (Mg/Kg)**	**Mean number of Writhing**	**% Inhibition**
1	Normal saline (10)	41.4	0.00
2	Pentazocine (20)	19.2	54.07
3	ME (100)	25.0	40.19
4	ME (200)	25.6	36.84
5	ME (300)	18.8	55.02

n = 5

Table 8: Effect of methanolic extract (ME)of the leaf of *Hyptis suaveolens* in hot plate induced pain in mice

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S/N	**Treatment in mg/kg**	**0Min**	**30Min**	**60Min**	**90Min**	**120Min**
1	Normal saline (10)	1.6	1.4	1.6	1.2	1.4
2	Morphine (20)	2.2	4.6	3.6	3.4	1.4
3	ME (100)	2.0	3.8	3.2	2.4	1.4
4	ME (200)	1.4	2.8	2.2	1.6	1.4
5	ME (300)	1.4	3.0	2.2	1.4	1.2

n = 5