ETHNOBOTANICAL SURVEY OF PLANTS USED IN TREATMENT OF VIRAL INFECTIONS IN JOS, AND ANTIVIRAL EVALUATIONS OF *BOSWELLIA DALZIELLI* HUTCH (BURSERACEAE) AND *ENANTIA CHLORANTHA* OLIVER (ANNONACEAE)

BY

TEMITAYO LUCIA OHEMU

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

JUNE, 2014

ETHNOBOTANICAL SURVEY OF PLANTS USED IN TREATMENT OF VIRAL INFECTIONS IN JOS, AND ANTIVIRAL EVALUATIONS OF *BOSWELLIA DALZIELLI* HUTCH (BURSERACEAE) AND *ENANTIA CHLORANTHA* OLIVER (ANNONACEAE)

BY

Temitayo Lucia OHEMU
B.Pharm. (UNIJOS) 2006; FPC Pharm.2013
(MSC/PHARM-SCI/04911/2009-2010)

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTERS OF SCIENCE IN PHARMACOGNOSY

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

JUNE, 2014

DECLARATION

I declare that the work reported in this thesis entitled '**Ethnobotanical survey of plants used in treatment of viral infections in Jos, and antiviral evaluations of *Boswellia dalzielli* Hutch (Burseraceae) and *Enantia chlorantha* Oliver (Annonaceae)'** was carried out by me in the Department of Pharmacognosy And Drug Development. Information derived from literature have been duly acknowledged and referenced. To the best of my knowledge, no part of this thesis has been previously presented for another degree or diploma.

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

Temitayo Lucia OHEMU Date

CERTIFICATION

This thesis entitled "**ETHNOBOTANICAL SURVEY OF PLANTS USED IN TREATMENT OF VIRAL INFECTIONS IN JOS, AND ANTIVIRAL EVALUATIONS OF *BOSWELLIA DALZIELLI* HUTCH (BURSERACEAE) AND *ENANTIA CHLORANTHA* OLIVER (ANNONACEAE)**" by Temitayo Lucia OHEMU meets the regulations governing the award of the Degree of Masters of Science in Pharmacognosy of Ahmadu Bello University, Zaria, Nigeria and is approved for its contribution to knowledge and literacy presentation

_____ _____

Dr A. Agunu, B. Pharm, M.Sc; PhD — Date
Chairman, Supervisory Committee

_____ _____

Prof. M.S. Abubakar, B. Pharm, M.Sc; PhD — Date
Member, Supervisory Committee

_____ _____

Dr G. Ibrahim B.Sc, M.Sc; PhD — Date
Head, Department of Pharmacognosy
and Drug Development

_____ _____

Prof. Adebayo A. Joshua — Date
Dean, School of Postgraduate Studies

DEDICATION

This thesis is dedicated to the Almighty God, the Giver of life for His faithfulness and kindness.

ACKNOWLEDGEMENTS

I sincerely appreciate my esteemed supervisors, Dr A. Agunu and Prof. M.S. Abubakar, for their commitment, encouragement and dedication in ensuring that this work was properly done. It has been a great privilege to work under you; you are my career mentors. May the Almighty God bless you.

A big thank you to the Head of Department, Dr G. Ibrahim and the postgraduate coordinator Dr. Ahmed Abubakar. To all the staff of the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, I really appreciate your support and encouragement.

My dear husband, Ohemu Benson, my children Daniel and Joy, thank you for being a solid support. I will also like to specially appreciate the Bamidele family for housing me, and supporting me both morally and otherwise throughout the programme.

My appreciation also goes to my dear friends who have contributed in one way or the other to make this research a success; Pharm. U. Ajima, Pharm P.N. Olotu, Mr Solomon, Mr Azila, Mr Gbadebo and Pastor I. Ajagbe. My parents, parent in-law, Celina, Titi, Lola, Gbenga and Seun, thank you all for your prayers and moral support.

ABSTRACT

An ethnobotanical survey of plants used in the treatment of viral infection was carried out in Jos North, Jos South and Jos East Local Government Areas of Plateau State, Nigeria by verbal interactions with Traditional Medicine Practitioners, Herbalist, Herb sellers and some indigenes/residents, being guided by a structured questionnaire. The study aimed at collecting and documenting medicinal plants used in the treatment of viral infections like common cold, measles, chickenpox, rabies, birdflu, hepatitis and HIV, within Jos. Pharmacognostic, phytochemical, antiviral and hepatoprotective were carried out on the two most used plants from the survey -*Boswellia dalzielii* stem bark and leaf and *Enantia chlorantha* stem bark. An *invivo* hepatoprotective assay of methanol extracts was analysed in liver injured CCl_4 – treated rats. Biochemical parameters including Aspartate amino transaminase (ASAT), Alanine amino transaminase (ALAT), Alkaline Phosphatase (ALP), total proteins, albumin, total bilirubin and conjugated bilirubin in serum were analysed. The biochemical findings were supported with histopathological examination. The extracts of were also subjected to *in ovo* antiviral activity against Newcastle Disease Virus (NDV). A total of 64 medicinal plants species, represented by 62 genera from 39 families were obtained. Cell wall materials, cell inclusions and other diagnostic characters that can aid in the easy and proper identification of *E. chlorantha* were identified. Preliminary phytochemical screening of the leaves and stem bark of *B. dalzielii* showed the presence of carbohydrate, cardiac glycoside, steroid, triterpene, tannins and flavonoids. *E. chlorantha* stem bark, revealed the presence of alkaloids, carbohydrates, cardiac glycosides, steriods, and triterpenes. The methanol extracts of the two selected plants were found to be safe both in phase one and two of acute toxicity evaluation, with the highest dose of 3000 mg/kg, except for the leaf extract of *B. dalzielii* that showed

toxicity at dose of 3000 mg/kg alone. Biochemical parameters obtained from the hepatoprotective assay showed no significant difference with that of the toxicant control .The activity of NDV was inhibited at lower concentrations for the three methanol extracts tested. These current findings have revealed and documented for the first time, medicinal plants used in treatment of viral infections in Jos, Plateau State, Nigeria. This information will be beneficial in public health, research and in providing lead to plants that can be useful in drug discovery.

TABLE OF CONTENT

Page

Cover page - - - - - - - - i

- -

Title page - - - - - - - - ii

- -

Declaration - - - - - - - - ii

- --

Certification - - - - - - - - iv

- -

Dedication - - - - - - - - v

- -

Acknowledgement - - - - - - - - v

- -

Abstract - - - - - - - - v

-

Table of contents - - - - - - - - ix

- -

List of tables - - - - - - - - x

-

List of figures - - - - - - - - x

- -

List of plates - - - - - - - - x

- -

CHAPTER ONE

1.0 Introduction 1

1.1 Background of the study - - - - - 1

-

1.2 Phytogeography - - - - - - 3

-

1.2.1 Location - - - - - - - 3

- -

1.2.2 Climate - - - - - - - 6

-

1.2.3 Vegetation - - - - - - - 6

-

1.2.4 Agriculture - - - - - - - 6

-

1.2.5 Language and culture - - - - - - 6

-

1.3 Typical traditional Medicine practice in Plateau state - 7

- -

1.4 Research Statement Problem - - - - - - 7

-

1.4 The Need for Ethnobotanical survey of medicinal plants used in the

treatment of viral infection in Jos, Plateau state - - - 7

- -

1.5 Research Question - - - - - - 10

- - -

1.6 Justification - - - - - - - 11

- - -

1.7 Aims - - - - - - - - - 12

- -

1.8 Objectives of the study - - - - - - 12

- -

1.9 Hypothesis - - - - - - - 12

- -

CHAPTER TWO

2.0 Literature Review - - - - - - 13

- -

2.1 Viruses - - - - - - - 13

- -

2.2 Pathogenesis of viral disease - - - - - 14

-

2.3 New Castle Disease Virus (NDV) - - - - 15

- -

2.4 Antiviral potential of medicinal plants - - - 15

- -

2.5 An Ethnobotanical survey of medicinal plants used in treating viral infections- 18

2.6 Future Prospects - - - - - - 20

- -

CHAPTER THREE

3.0 Materials and methods - - - - - 21

- -

3.1 Materials and Instrument used- - - - - 21

- -

3.2 Ethnobotanical survey - - - - - 23

- -

3.2.1 Collection and Documentation of information on medicinal plants - 23

-

3.2.2 Study population - - - - - - - 23

- -

3.2.3 Sampling Technique - - - - - - 23

- -

3.2.4 Method of Data Selection and Collection - - - 23

- -

3.2.5 Data Collection Procedure - - - - - 24

- -

3.2.6 Plant collection, Identification and Authentication - - 25

- -

3.2.7 Herbarium Specimen Preparation - - - - 25

-

3.2.8 Data Analysis - - - - - - - 26

- -

3.3 Pharmacognostic studies of the selected plants - - 26

- -

3.3.1 Preparation of plant material - - - - - 26

- -

3.3.2 Microscopical Examination - - - - - 26

- -

3.3.3 Chemomicroscopy - - - - - - 27

- -

3.4 Determination of Physiochemical Parameters - - 28
- -
3.4.1 Moisture content - - - - - - 28
- -
3.4.2 Total ash value - - - - - - 29
- -
3.4.3 Extractive Values - - - - - - 29
- -
3.5 Preliminary Phytochemical Screening - - - 30
- -
3.5.1 Extraction Procedure - - - - - - 30
- -
3.5.2 Procedures for Phytochemical Screening - - - 30
- -
3.6 Hepatoprotective assay - - - - - 34
- - -
3.6.1 Extraction of plant material - - - - - 34
- -
3.6.2 Acute Toxicity - - - - - - 34
- -
3.7 *Invivo* Hepatoprotective Test - - - - - 35

- -

3.7.1 Experimental Design - - - - - - 35

- -

3.7.2 Experimental procedure - - - - - 36

- -

3.7.3 Data Analysis - - - - - - - 37

- -

3.8 Antiviral assay - - - - - - 37

- -

3.8.1 Source of virus and 9-Day Old Embryonated Chicken Eggs - 37

- -

3.8.2 Determination of Median Embryo Infectious Dose ($EID_{50)}$ of the 37

virus - - - - - -

3.8.2.1 Preparation of viral dilution 38

3.8.2.2 Preparation of embryonated eggs 38

3.8.3 Preparation of inoculum (virus/extract mixture) - - 39

- -

3.8.4 Inoculation of eggs - - - - - - 40

-

3.8.5 Spot haemagglutination test - - - - - 40

- -

3.8.6 Data Analysis - - - - - - - - - - 40

CHAPTER FOUR

4.0 Results - - - - - - - - - - - 41

4.1 Results of ethnobotanical survey - - - - - - - 41

4.2 Response rate - - - - - - - - - - 41

4.3 Distribution of informant's age - - - - - - - 42

4.4 Distribution of respondents based on their practice - - - - - - 43

4.5 Medicinal plants used in the treatment of viral infections in Jos, Plateau State 44

4.6 Recipes - - - - - - - - - - - 62

4.7 Pharmacognostic Evaluation of *Enantia chloratha* Oliv. Stembark and *Boswellia dalzielli* Hutch. Leaves and stembark - - - - - - - 73

4.7.1 Microscopical examination of *Enantia chloratha* Oliv. Stembark 73

- -

4.7.2 Physical constants of the powdered Stem bark of *Enantia chloratha* Oliv.and Leaves and stembark *Boswellia dalzielli* Hutch. - - - - 75

4.8 Results of Preliminary Phytochemical Screening - - - - - 76

4.9 Acute-toxicity study - - - - - - - - 76

4.10 Hepatoprotective assay - - - - - - - 78

4.11 Antiviral assay - - - - - - --- 80

4.11.1 EID_{50} (50 percent Embryo Infectious Dose) - - - - - - 80

4.11.2 Results of antiviral activity of *B. dalzielli* methanol leaf extract against NDV - 84

4.11.3 Results of antiviral activity of *B. dalzielli* methanol stem bark extract against NDV - - - - - - - - - - 86

4.11.4 Results of antiviral activity of *E. chlorantha* methanol stem bark extract against NDV - - - - - - - 88

- - -

CHAPTER FIVE

5.0 Discussion - - - - - - - 90

- -

CHAPTER SIX

6.0 Summary, conclusion and recommendation - - - 98

- -

REFERENCES - - - - - 10

- - -

APPENDIX I- ETHNOBOTANICAL SURVEY SHEET 11

- - -

APPENDIX II- IMAGES OF MICROSCOPICAL EXAMINATION OF *ENANTIA CHLORANTHA* STEM BARK POWDER- - - - - 11

APPENDIX III- IMAGES OF EQUIPMENTS USED FOR ANTIVIRAL ASSAY- - - - - - - - - 12

LIST OF TABLES

Table 4.1	Medicinal plants used in the treatment of viral infections in Jos, Plateau state - - - - - - - -- - -	45
Table 4.2	Recipes and Route of Administration for the different medicinal preparations used in treatment of viral infection - - - - - -	63
Table 4.3	List of Plants frequently mentioned In the Treatment of a Particular Viral Infection by two or more informants - - - - -	71
Table 4.4	Chemomicroscopy of *Enantia chlorantha* Stem bark - - - -	74
Table 4.5	Physical constants of the powdered plant samples - - - -	75
Table 4.6	Summary of Preliminary Phytochemical Screening - - - -	76
Table 4.7	Biochemical parameters of rats after liver function test - - -	78
Table 4.8	EID_{50} - - - - - - - - - -	82

Table 4.9 Results of antiviral activity of *Boswellia dalzielli* methanolic leaf extract against NDV - - - - - - - - - - - 85

Table 4.10 Results of antiviral activity of *Boswellia dalzielli* methanolic bark extract against NDV - - - - - - - - - - 87

Table 4.11 Results of antiviral activity of *Enantia chlorantha* methanolic bark extract against NDV - - - - - - - - - - 89

LIST OF FIGURES

Figure 1.1	Map of Nigeria, showing Plateau State - - - - -	4
Figure 1.2	Map of Plateau, State showing study Areas - - - - -	5
Figure 4.1	Distribution of informant's age - - - - -	42
Figure 4.2	Sources of information - - - - - -	43

LIST OF PLATES

Plate I: *Boswellia dalzielli* Hutch. (BURSERACEAE) - - 50

-

Plate II: *Enantia chlorantha* Oliv. (ANNONACEAE) - - - 51

-

Plate III: *Piliostigma thonningii*(Schum.)(CAESALPINIACEAE) - 52

-

Plate IV: *Parkia biglobosa* (Jacq.) R.Br (MIMOSACEAE) - - 53

-

Plate V: *Annona senegalensis* Pers.(ANNONACEAE) - - - 54

-

Plate VI: *Vernonia amygdalina* Del. (ASTERACEAE) - - - 55

-

Plate VII: *Erythrina senegalensis* DC (FABACEAE) - - - 56

Plate VIII: *Ocimum gratissimum* Linn. (LAMIACEAE) - - - 57

-

Plate IX: *Abrus precatorius* L. (FABACEAE) - - - - 58

-

Plate X: *Carissa edulis* (Forssk.) Vahl. (APOCYNACEAE - - 59

-

Plate XI: *Anogeissus leiocarpus(*DC.)Guill. & Perr. (COMBRETACEAE) 60

-

Plate XII: *Vitellaria paradoxa* C.F. Gaertn. (SAPOTACEAE) - - 61

-

Plate XIII: Liver of a control rat showing normal hepatocytes - - - 79

Plate XIV: Liver section from a CCl4-treated rat - - - - 79

-

Plate XV Liver section from a Silymarin treated rat - - - 80

- -

Plate XVI: Liver section of rat treated with 200mg/kg of methanolic extract of stembark of *Boswellia dalzielii* - -

- - 80

Plate XVII: Liver section of rat treated with 200mg/kg of methanolic extract of leaf of *Boswellia dalzielii* - - -

- - - 81

Plate XVIII: Liver section of rat treated with 200mg/kg of methanolic extract of

stembark of *Enantia chlorantha* - - - - 81

Plate X1X: Bundles of fibres - - - - - 119

- -

Plate XX:	Medullary rays - - - - - - -	119
Plate XXI:	Phloem parenchyma - - - - - - -	120
Plate XXII:	Scleried - - - - - - -	120
Plate XXIII:	starch grain - - - - - - -	121
Plate XXIV:	cork cells - - - - - - -	121
Plate XXV:	prisms of calcium oxalate crystals - - - -	121
Plate XXVI:	Safety Cabinet - - - - - - -	122
Plate XXVII	A crate of embryonated eggs - - - - -	122
Plate XXVIII	An incubator - - - - - - -	122
Plate XXIX	A stand of pipettes - - - - - - -	123
Plate XXX	An autoclave - - - - - - -	123

-

Plate XXXI Laboratory - - - - - - 124

-

Plate XXXII A candler - - - - - - 124

-

Plate XXXIII A centrifuge - - - - - - 124

-

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Since the origin of human civilization on earth, medicinal plants have been used in the treatment of diseases and infection, including viral infections (Mukhtar *et al*, 2008).

In many developing countries, 70% to 80% of the population have some form of alternative or complementary medicine. This population also depends on traditional medicine for primary health care (WHO, 2008). Traditional medicine has increased significantly in industrialised countries, due to the fact that many prescription drugs have originated from the tropical flora (Nelson-Harrison *et al*., 2002).

Nigeria is endowed with many medicinal plants, both domesticated and wild. Like every other developing country, majority of its population depend on these plants to meet their health needs (Oladunmoye and Kehinde 2011). The medicinal uses of the plants may vary from one community to another or even from one culture to another.

Ethnobotany was defined by Kim (2007) "as the study of how people of particular cultures and regions make use of the plants in their local environments. These uses can include as food, medicines, fuel, and shelter and in many cultures, in religious ceremonies."

The aim of ethnobotany is to document, describe and explain complex relationships between culture and uses of plants for food, clothing, currency, ritual, medicine, cosmetics etc across human societies (Acharya and Shrivastava, 2008).

Ethnomedicine is a subfield of Ethnobotany that deals with the study of traditional medicines especially, for those whose practice and knowledge has been orally passed down from generation to generation (Acharya and Shrivastava, 2008).

A great deal of information about traditional use of plants are still intact with the tribal. (Sood, 2001) However, the native healers are often reluctant to accurately share their knowledge with an outsider.

Ethnobotanical survey is very important in the continuous search for natural plant products as medicines (Ogbole *et al*., 2010). It also serves as a major approach for selecting plants for pharmacological screening (Igoli *et al*., 2005). In addition, ethnomedicinal uses of plants remain one of the most successful criteria in finding new therapeutic agents by Pharmaceutical industries (Cox and Balick, 1994). Plants have been found useful in treating many microbial diseases in man and animals as caused by bacteria and viruses.

Viral infection is one of the world's most transmissible diseases. This is because it is almost always followed by a secondary bacterial infection. However available antiviral and vaccines have shown good results (WHO, 1983). The high cost of available antiviral drugs and their toxic side effects, viral resistance coupled with viral latency and conflicting efficacy in recurrent infection in immunocompromised patients have made viral disease remain a major and continuous burden for researchers. Hence, the need for search for new antiviral compounds from plants that is safe, effective, which overcomes resistance and less toxic (Ngono Ngare *et al*., 2011) .

However, recent studies' showing antiviral potential of plant extract against viral strains resistant to conventional antiviral agents, has challenged modern drug discovery practices, and tends towards exploring medicinal plants with antiviral constituents (Mukhtar *et al*., 2008).

1.2 Phytogeography

Phytogeography is the branch of biogeography that is concerned with the geographic distribution of plant species and their influence on the earth's surface. Phytogeography is concerned with every aspects of plant distribution, from the controls on the distribution of individual species ranges (at both large and small scales), to the factors that govern the composition of entire communities and floras. In order to study medicinal plants of Jos Plateau, a brief description of its geography will be appropriate.

1.2.1 Location

The Jos Plateau is a plateau that lies between latitudes 8^0 22′ and 10^0 24′ North and longitudes 8^0 32′ and 10^0 38′ East. Thus, Plateau State which derives its name from the Jos Plateau is located right in the centre of Nigeria-North central zone . The plateau has given its name to the State and the state's capital is Jos. It covers 8600 km^2 and bounded by 300-600 meter escarpments around much of its circumference. With an average altitude of 1280 meters and a high point of 1829 meters in the Shere Hills . The State shares common boundaries with the following states: Nasarawa, in the South West, Kaduna in

the North West, Bauchi in the North East and Taraba in the South East (see Figure 1.1).

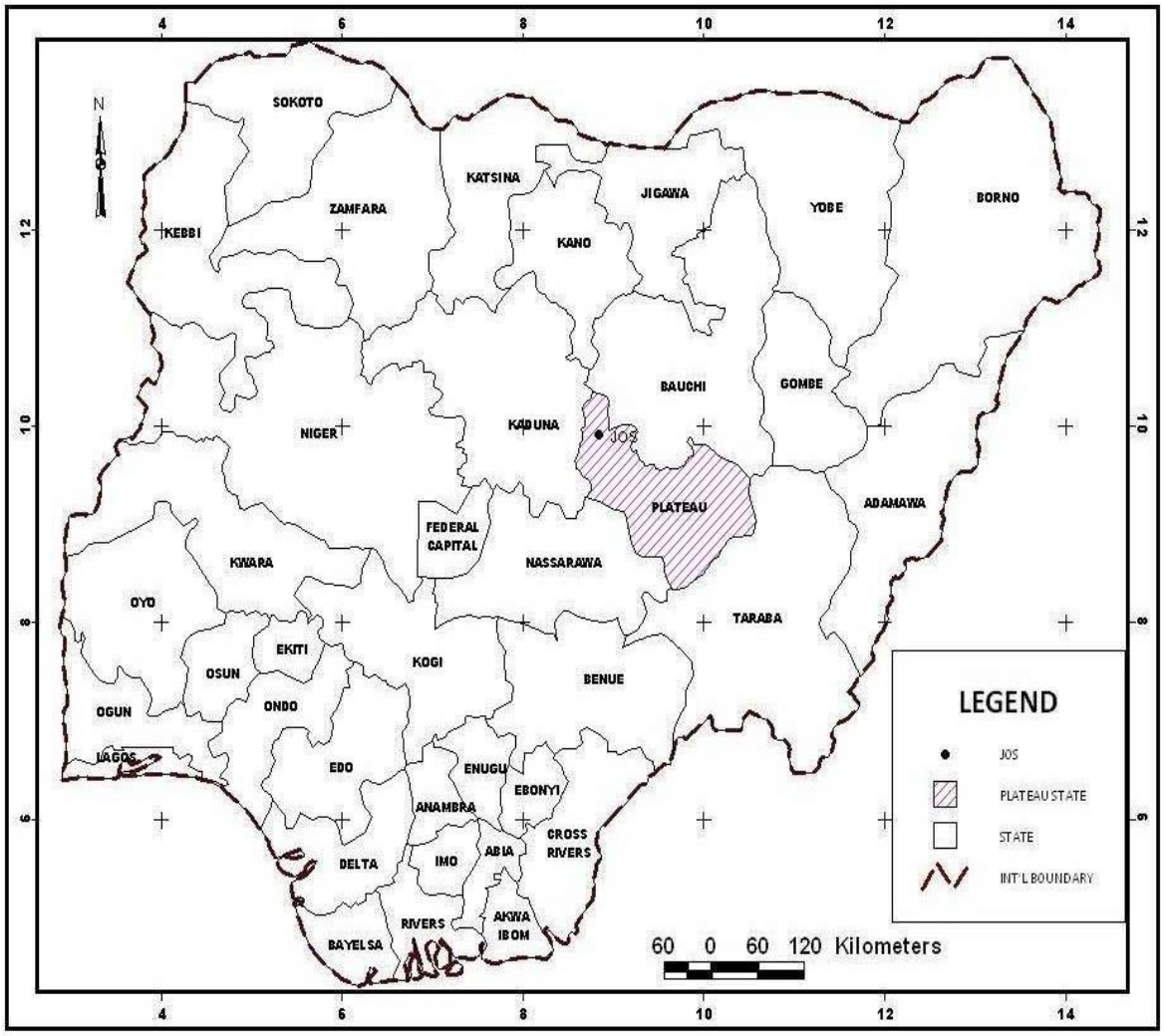


Figure 1.1: Map of Nigeria, showing Plateau State
Source: Geographic Information Systems (GIS) Laboratory, Department of Geography and Planning, University of Jos (2011)

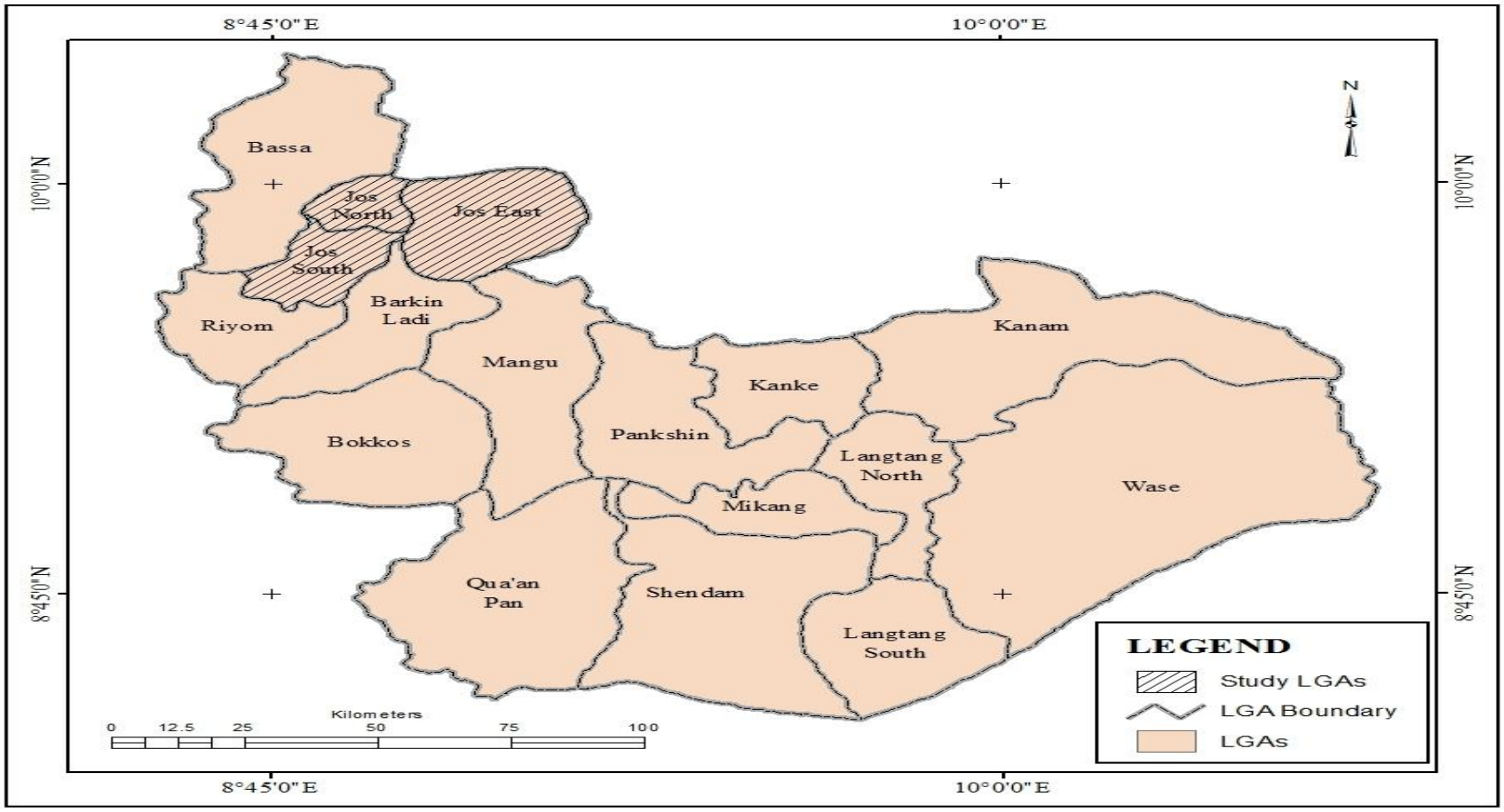


Figure 1.2: Map of Plateau, State showing study Areas
Source: Geographic Information Systems (GIS) Laboratory, Department of Geography and Planning, University of Jos (2011)

1.2.2 Climate

The Jos – Plateau is the Upper part, and the Northern highland area of Plateau State, with a near temperate type. Weather conditions are warm during the rainy season (April-October) and cold during the Harmattan period (December-February). The mean annual temperatures in the state range between 20^0 and 25^0 centigrade, while the mean annual rainfall figures range from 131.75cm in the Northern part to 146cm in the Southern part (Plateau State Government, 2007).

1.2.3 Vegetation

The broad vegetation cover of Plateau State is in the Guinea savannah with short trees and tall grasses. Near some villages are thick hedges of cactus planted around households or farmsteads or compounds. Fringing woodlands or gallery forests can be found along some river systems. Most of the natural vegetation has been tampered with as a result of human activities especially, farming and mining.

1.2.4 Agriculture

Agriculture is the main stay of the economy, with about 80% of the population engaged in farming in almost all the rural areas. Plateau state has 2,714,700ha of land (Plateau State Government, 2007). About 1.5 million hectares is under cultivation and over two third of the land is arable.

1.2.5 Language and culture

The Jos Plateau lies in the Nigerian middle belt, and this region known for cultural diversity, it is unusually diverse. Barbour *et al*. (1982) show over 60 ethno-linguistic

groups on the Plateau. Most of the Plateau's languages are in the Chadic family (Isichei, 1982), which is part of the Afro-Asiatic phylum. Two of the Plateau's largest ethnic groups are the Berom, in the northern Plateau, and the Angas in the southeast. Smaller groups include the Mwaghavul, Pyem, Ron, Eggon, Chokfem, and Kofyar.

1.3 Typical traditional Medicine practice in Plateau state

The Traditional medicine Practitioners in Plateau, practice different form of traditional medicine, the most common is herbal medicine. Some of their practices involve incantation, prayers and consultation of oracle.

1.4 Research Statement Problem

1.4.1 The Need for Ethnobotanical survey of medicinal plants used in the treatment of viral infection in Jos, Plateau state.

Survey and documentation of a country's or community's natural resources is an important prerequisite for proper utilization of its raw materials. Full knowledge of various plants is necessary, so as to enhance proper utilization (Choudhary *et al*., 2008).

These natural resources are being threatened by Environmental and cultural changes, therefore serious measure have to be taken to ensure that medicinal plants in the natural ecosystems and other suitable environments are conserved. This can be achieved through Ethnobotanical studies (**Tesfaye *et al.*, 2009).**

The World Health Organization (WHO) encourages and supports developing countries to identify and provide quality, safe and effective remedies and practices for

use in the public and private health services. Various resolutions passed by the WHO at various levels indicate that:

i. **Most of the world's population depends on traditional medicine for primary healthcare;**
ii. **The workforce represented by the practitioners of traditional medicine is a potentially important resource for the delivery of health care;**
iii. **Medicinal plants are of great importance to the health of individuals and communities (Sofowora, 2008)**

Indigenous people have long history and expertise in the use of medicinal plants, but information on these plants and their uses is mainly passed from one generation to the other orally and even to date is poorly documented (Gurib-Fakim, 2006). The main obstacle to the acceptance of traditional medicine in developed countries is the lack of documentation and stringent quality control (Thomas *et al*., 2008). The lack of an organized documentation for medicinal plant knowledge may also contribute to the loss of medicinal plant knowledge, particularly for plants that are neglected or non-preferred (Musa *et al*., 2011).

Like every communities in the developing nation, the people of Plateau state depend on medicinal plants to meet their domestic and health needs. Majority of rural communities depend on traditional medicine, while most urban settlements depend on orthodox medicine due to civilization and modernization. Most of the plants used by these people are obtained from the wild; hence there is a problem of extinction and dissemination of the flora of this area. This calls for urgent conservation of the biodiversity. There is also the problem of

holding information on use of plants as secrets due to distrust of researchers by traditional medicines practitioners because of previous bad experiences, the desire to pass down information to offspring, family members alone, and avoidance of competition in the practice. Eventually these people get older and die, resulting into lost of vital information (Sofowora, 2008).

Therefore, in order to preserve the medicinal plants knowledge on their uses, there is need for Ethnobotanical survey of the area.

1.5 Research Question

The following research question was raised for the study:

1. Can ethnobotanical findings and documentation of medicinal plant lead to discovery of medicinal plants with interesting pharmacological activities, especially antiviral medicinal plants?

1.6 Justification

Traditional Medicine Practitioners, Herbalist, Herb sellers and even indigenes within the Jos- Plateau have claims that some medicinal plants within their environment have been used effectively over time in the treatment of viral infections. These claims stimulated the interest to gather and document information on these medicinal plants and selection of two plants to test for antiviral activity so as to provide scientific bases, for these practices in traditional treatment of viral infections, and possible development of new and effective antiviral agent(s).

1.7 Aims

The overall aim of this study is to survey the medicinal plants used in the treatment of viral infections and to carry out antiviral evaluation of *Boswellia dalzielii* Hutch. and *Enantia chlorantha* Oliver.

1.8 Objectives of the study

i. To collect and document medicinal plants used within Jos for treatment of viral infections

ii. To carry out pharmacognostic studies and phytochemical screenings of two most commonly used plants during the survey; *Boswellia dalzielii* Hutch. (Stem bark and leaf) and *Enantia chlorantha* Oliv. (stembark)

iii. To study the antiviral and hepatoprotective properties of the crude extract of two most commonly used plants during the survey; Boswellia *dalzielii* Hutch. (Stem bark and leaf) and *Enantia chlorantha* Oliv. (Stem bark)

1.9 Hypothesis

Medicinal plants used in treatment of viral infection within Jos metropolis may have constituents with antiviral properties.

CHAPTER TWO

LITERATURE REVIEW

2.1 Viruses:

Viruses are the smallest infectious agents (ranging from about 20 nm to about 300 nm in diameter) and contain only one type of nucleic acid either RNA or DNA, never both as their genome. The nucleic acid is encased in a protein shell, which may be surrounded by a lipid-containing membrane. The entire infectious unit is termed a virion (Jawetz *et al*, 2007). They possess no functional organelle and are completely dependent on their host for the machinery of energy production and synthesis of macromolecules. Outside the host, the viruses are metabolically inert; this is the transmission phase. Inside the host cell, the viruses become metabolically active; this is the replicative phase in which the viral nucleic acid contains information necessary for programming the infected host cell to synthesize virus-specific macromolecules required for the production of viral progeny. During this replicative cycle, numerous copies of viral nucleic acid and coat proteins are produced. The coat proteins assemble together to form the capsid, which encases and stabilizes the viral nucleic acid against the extracellular environment and facilitates the attachment and penetration by the virus upon contact with new susceptible cells. The virus infection may have little or no effect on the host cell or may result in cell damage or death (Jawetz *et al*, 2007).

Most viruses have defied all antiviral drug development. This is because viruses incorporates itself into the host cell, both functionally and physically during the replicative

cycle, making it difficult to differentiate unique biochemical features suitable for selective attack (Ibezim *et al*, 2009). Therefore, drugs that interfere with viral replication nearly always interfere with the essential cell functions. Unlike bacteria which have a unique metabolic pathway different from those of their host, and antibiotics are directed at these pathways.

2.2 Pathogenesis of viral disease

The fundamental process of viral infection is the viral replicative cycle. The cellular response to that infection may range from no apparent effect to cytopathology with accompanying cell death to hyperplasia or cancer. Most viral infections are subclinical because they do not produce any symptoms in the host and does not result in the production of disease (Jawetz *et al*, 2007).

Viral pathogenesis refers to the process by which viral infection leads to disease production. A virus is pathogenic for a particular host if it can infect and cause signs of disease in that host.

To produce disease, viruses must enter a host, come in contact with susceptible cells, replicate, and produce cell injury. Understanding mechanisms of viral pathogenesis at the molecular level is necessary to design effective and specific antiviral strategies. Much of our knowledge of viral pathogenesis is based on animal models, because such systems can be readily manipulated and studied.

2.3 New Castle Disease Virus (NDV)

The virus used to carry out antiviral assay is the NDV. This virus is of the order, mononegavirales and the family; Paramyxoviridae. Paramyxovirus virions are enveloped, covered with large pepplomer and contain a herringbone- shaped helically symmetrical nucleo- capsid. The genome consists of a single linear molecule of negative – sense, single stranded RNA. The envelop contains two glycoproteins, a hemagglutinin (in most species with neuramindase activity) and a fusion protein (White and Fenner, 1994).

The viruses have only been identified primarily in mammals and birds. Transmission is mainly by aerosol and droplets.A lot of human viruses falls into this family, for example; human parainfluenza virus1, measles virus, mumps virus and human respiratory syncytial virus (White and Fenner, 1994).

2.4 Antiviral potential of medicinal plants

Since medieval times, medicinal plants have served as a source of inspiration for drug development of medicines or phytomedicines used in the treatment of diseases (Iwu, 1993).

It has been shown from studies that a total of at least 35000 plants species are widely used for medicinal purposes. Due to the harmful effect of orthodox drugs, there has been an increase in the demand for traditional herbs (Oladunmoye and Kehinde, 2011). Medicinal plants find wide usage in developing countries particularly in China, India, Japan, Pakistan, Sri Lanka, Thailand and a number of African countries. Usage of plant-based medicinal products is now encouraged in health care systems in developed countries. An example is the Natural Health Product Regulations of Canada, which encourages the use of evidence-based scientific support and modern technology towards promoting medicinal plant and associated products (Mukhtar *et al*., 2008).

A review showed that approximately 25% of modern medications are derived from plants, also between 1981 and 2002, 75% of new drugs against infectious diseases originate from natural products (Bedoya *et al*., 2009).

Among several other ailments, viral infections, particularly infections associated with human immunodeficiency virus type 1 (HIV-1) and 2 (HIV-2), and newly emerging infectious viruses have challenged mankind survival. Many medicinal plants have shown promise to treat a number of viral infections, and some of them possess broad-spectrum antiviral activity. Previously, exploration into the antiviral activity of various promising medicinal plants was limited due to: (a) highly infectious nature of viruses and (b) lack of appropriate separation techniques for the identification of antiviral components from plants. Development of vector-based strategies, in which non-infectious molecular clone of a virus could be used for antiviral screening purposes, and advancement in separation technologies offers promise for medicinal plants usage in modern drug discovery (Mukhtar *et al*., 2008).

The first recognized interest in development of antiviral agents from medicinal plants was shown by Boots drug company England, where 288 plants were screened for anti- influenza activity. There have been several other studies on the antiviral properties of plants, most of these studies utilize either water soluble or alcoholic extracts of medicinal plants (Mukhtar *et al*., 2008).

Studies in the past and recent literature suggest that a variety of natural products isolated from several plants possess antiviral properties. Such studies include; Aqueous extract from the roots of *Carisa edulis* (Forssk.) Vahl (Apocynaceae), a medicinal plant grown in Kenya, showing remarkable anti- Herpes Simplex Virus (HSV) activity *in vitro* and *in vivo*

for both wild type and resistant strains of HSV (Tolo *et al*., 2006). In another study, ethanolic extracts of two Nigerian plants; *Bambusa vulgaris* and *Aframomum melegueta* were analysed for antiviral activities against measles, yellow fever and polio viruses by standard laboratory tests. *Bambusa vulgaris* showed antiviral activity against measles virus while *Aframomum melegueta* inhibited measles and yellow fever viruses respectively. Polio virus was not susceptible to any of the extract (Ojo *et al*., 2009).

In another study, traditional Korean medicinal herbs that exhibit potency against hepatitis B virus (HBV) and hepatitis C virus (HCV) infections were carried out. Antiviral activity of an aqueous extract of herbal formulation KYH-1 in surrogate *in vitro* assays for HBV and HCV was demonstrated by the study and the mechanisms of action of the extract was also identified. KYH-1 exhibited potent antiviral activity against Woodchuck Hepatitis Virus (WHV) and to a lesser extent against Bovine Viral Diarrhoea Virus (BVDV). KYH-1 and its constituent components inhibited HBV polymerase priming *in vitro*. Additionally, KYH-1 suppressed HBV replication in a human hepatoblastoma cell line. The study provided significant results justifying preclinical evaluation of KYH-1 as an antiviral therapy for HBV infections (Jacob *et al*., 2004).

Similarly, in another study, aqueous extracts of *Enantia chlorantha* plant used by traditional medical practitioners or in phytomedical practice in the treatment of diseases such as fevers and influenza were investigated for their inhibitory activities on the yellow fever virus in the tissue cell culture using Vero cells. These extracts were mixed with equal volumes of $100TCID_{50}$ Yellow Fever Virus (YFV) in confluent monolayer of Vero cells. The extract showed antiviral activities against yellow fever virus. The result of the study revealed that the water extract of *E. chlorantha* showed significant antiviral activity. The

extracts were considered effective against YFV as they completely inhibited the infectivity of YFV as evident in complete absence of Cytopathic effects (CPEs) (Fasola *et al*., 2011).

Studies aim at identifying medicinal plants with anti-HCV is ongoing, especially due to its prevalence in poor countries and lack of good vaccine. The methanolic extract of *Acacia nilotica* L. Wild ex Delile, *Boswellia carterii, Embelia schimperi, Quercus infectoria, Trachyspermum ammi* and aqueous extracts of *Piper cubeba* L., *Syzygium aromaticum* L., showed potentials to inhibit HCV (Hussein *et al*., 2000).

2.5 An Ethnobotanical survey of medicinal plants used in treating viral infections

A survey on medicinal plants used in treatment of some common viral diseases such as chickenpox, poliomyelitis, influenza, hepatitis, measles and jaundice was carried out Ekiti, Ondo, Osun, and Oyo states in South Western Nigeria. Information was gathered by using questionnaire and oral discussion (Oladunmoye and Kehinde , 2011).

Two hundred and eight (208) data were collected, with the family Annonaceae(10.3%) being the most frequently used, followed by Leguminosae (9.9%), and Zingiberaceae (7.9%).

The study also revealed that decoction was the mostly the mostly employed method of preparation in the study area for viral infection, while concoction was not a common practice.

Another interesting study is the use of medicinal plants for the treatment of measles in Nigeria. This was an Ethnobotanical survey of three local Government areas of Ogun State

in Southwest Nigeria. Unstructured interview was used in the information gathering (Sonibare *et al*., 2009).

Twenty three (23) plant species belonging to 18 Angiosperm families were collected. *Elytraria marginata* Vahl, *Peperomia pellucida* (L.) Humb., Bonpl. & Kunth, *Vernonia amygdalina* Del., *Momordica charantia* L., *Newbouldia laevis* (P .Beauv.) Seem. exBureau, and *Ocimum gratissimum* L. were ranked highest based on Informant consensus**.**

An Ethnobotanical study of medicinal plants used in treatment of viral infections was carried out in the central and southern region of Cameroon, revealed five (5) species namely: *Enantia chlorantha* Oliv., *Pteleopsis hylodendron* Mildbr., *Spathodea companulata* P. Beauv., *Costus afer* Ker-Gawler and *Mormodica charantia* L. belonging to five families, used for treatment of chicken pox, measles, influenza, shingles and viral hepatitis. The study showed the need for the enlightenment of traditional healers and the public in general on selective use of plants for the treatment of viral diseases

Fourteen (14) medicinal plants were reported from an ethnobotanical study carried out on the treatment of viral hepatitis in Rewa district, Madhya Pradesh, India. *Sphaeranthus indicus* was discovered to the best in treatment of viral hepatitis based on informant consensus (Manisha and Khalid, 2012).

2.6 Future Prospects

Molecular biology and modern technologies are combining to devise novel approaches to vaccine development. Many of these approaches avoid the incorporation of viral nucleic acid in the final product, improving vaccine safety.

There is need for production of vaccines and protein-based therapeutics from plants (Mukhar *et al*., 2008). Scientific reports put forward that plants can offer a good source for the production of pharmaceutical grade peptides/proteins (Glenz and Warzecha, 2006).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Material / Instruments used

Ethnobotanical survey

i. Materials used

A structured questionnaire, Digital camera, tape recorder, note books, newspaper prints, plant press

Pharmacognostic Studies

i. Apparatus

Microscope, Glass slide, cover slips, test tubes, filter paper, funnel, evaporating dish, 25ml beakers, 25 ml conical flasks, 10ml,50ml &100ml measuring cylinder, pipette, wash bottle, water bath, oven, furnace

ii. Solvents/ Reagents

Methanol , distilled water, Benzene, chloral hydrate, glycerol, Sudan IV, sulphuric acid ,hydrochloric acid ,phloroglucinol ,ruthenium red, N/50 iodine, 90% alcohol, Molisch's reagent, Fehling's solution A and B, 10% Ammonium solution, ferric chloride, 2% of 3,5- dinitrobenzioc acid in methanol,sodium hydroxide, Dragendroff's, Mayer's, Wagner reagents.

Biological studies

i. Animals

Colony bred healthy male and female Wistar rats (150- 200 g) from Nigerian Institute for Trypanomiasis and Onchocerciasis Research, Vom, Plateau State were used for the test.

ii. Apparatus

Syringe and needles (1ml, 2ml, 5ml & 10ml), dissecting kit, sample bottles, incubator, egg puncher, Candler, bio safety cabinet, molten wax, centrifuge, autoclave, ice parks, micropipette, tile, wire loop.

iii. Solvents/ Reagents and Drugs

Methanol, 30% carbontetrachloride in olive oil, sodium carbon methyl cellulose, 10% formalin, distilled water, phosphate buffered saline, silymarin

3.2 Ethnomedicinal survey

3.2.1 Collection and Documentation of information on medicinal plants

3.2.2 Study population

The study area was Jos-Plateau, which constitutes three Local Government Areas (LGA); Jos North, Jos South and Jos East respectively as shown on Fig. 1.2. At least 10 individual were the target per LGAs. The sample population constitutes mainly of Traditional Medicine practitioner, Traditional healers and herb sellers, a few individuals with claims of medicinal plants knowledge according to the methods of Sofowora (2008).

3.2.3 Sampling Technique

The study area has not been investigated for plants with antiviral activity. The sampling technique employed for this survey research was snowball sampling or referral sampling. This is a sampling technique used to obtain research and knowledge, from extended associations, through previous acquaintances.

3.2.4 Method of Data Selection and Collection

A list of the common viral diseases was made, and enquiry was made of their treatment. These viral infections include common cold, measles, chickenpox, rabies, bird flu, hepatitis and HIV/ AIDS respectively. The data for this study were obtained by oral discussion with the respondents from September 2011 to March 2012. Informed consent was obtained orally from each of the respondent, before an interview. Since most of the respondents were not educated, oral interview was adopted to obtain the relevant ethno medicinal information. Each respondent was visited two to three times in order to verify the authenticity of the information obtained, and to gather more not mentioned during a previous visit. Any discrepancy between information obtained at different visit on a particular ailment and plant used in its treatment, makes the information unreliable and hence, rejected.

3.2.5 Data Collection Procedure

The data for this study were obtained from direct interviews, using the communicable dialect within the area, although a well structured questionnaire which is a modification of those designed by Sofowora, (2008) was used as a guide. The questionnaire was divided into five sections (see Appendix I).

Section (A) deals with demographic information such as: age, sex, address, duration of practice, tribe, and the specialty.

Section (B) is on plant identity, local name, part used, form of preparation, route of administration and recipes for treatment of common cold 'mura', measles 'bakon dauro', chickenpox, rabies 'chiwon kare', bird flu 'chiwon kaji', hepatitis and HIV/ AIDS 'chiwon kanjanmo'. Other questions centered on whether he or she uses incantations or prayers and possible reasons for using two or more plants or plant parts.

Section (C) is on preparation for medicinal use.

In last section (D) herbarium and herbarium information was considered.

3.2.6 Plant collection, Identification and Authentication:

The plant species mentioned during the interview were collected by the respondent or the person who normally prepares the remedies, so as to avoid collection of the wrong plant

(Sofowora , 2008). Most of the plants were collected fresh, while few were preserved into Herbarium specimen. Photograph of collected plant species were also made so as to enhance their identification. The plant species obtained from the survey were identified using keys and description given in the Flora of West Tropical Africa (Hutchison and Dalziel, 1963) and The Useful plants of West tropical Africa (Burkill, 1985) at College of Forestry Herbarium Jos, by Mr Azila and Dr Jemilat Ibrahim of the herbarium unit at the National Institute of Pharmaceutical Research and Development (NIPRD), Abuja.

The identities of the plants were established at the herbarium unit in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria by the Taxonomists of the unit:- Mallam Gallah and Mallam Musa. Some were also authenticated at the Forest Herbarium of the Forestry Research Institute of Nigeria, Ibadan by Mr Adeniji. Voucher numbers were obtained for each specimen.

3.2.7 Herbarium Specimen Preparation

Herbarium specimens of 23 of the collected plant species were prepared according to standard procedures. From the families of the plants collected from the survey one plant was picked for herbarium specimen preparation.

3.3 Pharmacognostic studies of the selected plants

3.3.1 Preparation of the plant material

The plant material was dried under shade, and then grounded using grinding machine .The powdered plant material (10 g) was used for the study. The remaining was preserved in an air tight container.

3.3.2 Microscopical Examination

Powdered sample

Powdered samples of the stem bark of *E. chlorantha* were used for this study. Few drops of chloral hydrate were placed on a glass slide, and then small quantity of the powdered sample was transferred into the drop of fluid on the slide. This was stirred thoroughly and carefully, and then the cover slip was applied. The slide was then heated over Bunsen burner flame for few seconds. When the powder was thoroughly and sufficiently cleared, few drops of dilute glycerol were added and the slide was observed under the compound microscope at magnification of x10 and x40. The microscopical features observed, were noted, drawn, snapped and labelled (WHO, 1998).

3.3.3 Chemomicroscopy

The chemomicroscopy of the powdered drug was carried out as described in by WHO (1998) for the histochemical detection of the cell wall materials and cell content.

i. Test for lignin

The cleared powdered sample on the slide was mounted on the microscope, then moistened with a drop of phloroglucinol, followed by a drop of conc. HCl. It was observed under a microscope.

ii. Test for mucilage

Cleared powdered sample was placed on a slide and mounted; a drop of ruthenium red was then added. It was observed under a microscope.

iii. Tests for oils

Cleared powdered sample was placed on a slide, it was then mounted and a drop of glycerol and Sudan IV was added to it. It was observed under a microscope.

iv. Test for cellulose

Cleared powdered sample was placed on a slide and mounted, then, a drop of glycerol was added, followed with a drop of N/50 iodine and 66 % Sulphuric acid. It was observed under a microscope.

v. Test for calcium oxalate crystals and calcium carbonaten

Small quantity of powdered plant sample was cleared with chloral hydrate. It is then mounted on a slide and with a drop of glycerol. One drop of Hydrochloric acid was added to it . It was observed under a microscope.

3.4 Determination of Physicochemical Parameters

3.4.1 Moisture content: The moisture content of the plant samples was determined by loss on drying method as follows;

i. An evaporating dish was heated to a constant weight which was noted
ii. Powdered sample (3.0 g) was accurately weighed into an evaporating dish of known weight above (i).
iii. The evaporating dish with its content was then placed in an oven at temperature of 105 ^{0}C. After 30 minutes, the weight of drug and the dish was determined and returned to the oven. The weight was then taken subsequently after every 30 minutes until a constant weight was obtained.

iv. The total loss in weight (weight of the moisture) was determined by subtracting the constant weight of the dish and powdered drug after heating from the weight of the dish and its content before heating. The percentage of the moisture contents with reference to the initial weight of the powered drug was then calculated. This was achieved by dividing the weight of the moisture by the original weight of the sample and multiplied by a hundred, as shown in the formulae below. Three different determinations were carried out and the average of the three taken as the moisture content of the drug (Evans, 2009).

The percentage moisture content was then calculated following the formulae:

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

3.4.2 Total ash value

To measure the total amount of material remaining after ignition, the following procedure was observed.

i. A crucible was heated at 105 ^{o}C to a constant weight and the weight was noted.

ii. Powdered sample (2 g) was accurately weighed, into the crucible of known weight.

iii. The sample and the crucible were then ignited in a furnace by gradually increasing the heat to 600 ^{o}C until it turned white.

iv. The ash and the crucible were then weighed after heating,

The total ash value was then computed in percentage as follows

$$\% \text{ total ash value} = \frac{\text{weight of residual ash}}{\text{weight of original sample}} \times 100$$

3.4. 3 Extractive Values

a. Alcohol soluble extractive value

It is the percentage soluble material obtained after dissolving the powder in alcohol. The procedure observed was as followed;

i. Powdered sample (4.0 g) was accurately weighed into a 250 ml conical flask with a stopper.
ii. Ethanol 90 % (100 ml) was added to the flask
iii. The stopper was replaced, and then the flask was shaken on a mechanical shaker for 6 hr and was then left to stand for 18 hr.
iv. The mixture was filtered and 20 ml of the filtrate was put into a clean pre weighed evaporating dish and then evaporated to dryness on water bath.
v. The residue was dried to a constant weight in an oven at 105 ^{o}C
vi. After that, the weight was taken and the value computed in percentage as follows

$$\% \text{ Alcohol soluble ext value} = \frac{\text{wt of residue in 20 ml ext}}{20} \times 100$$

Water Soluble Extractive value:

This was achieved using same procedure as in alcohol soluble extractive value. Chloroform water was used in place of the 90 % ethanol.

3.5 Preliminary Phytochemical Screening

The different morphological parts of the two plants selected from the survey (based on frequency of usage) were screened for the presence or absence of secondary metabolites such as carbohydrates, anthraquinone glycosides, saponins, cardiac glycosides, flavonoids, steroids and terpenoids, tannins and alkaloids using standard methods.

3.5.1 Extraction Procedure

The morphological parts of the plants were air dried and the pulverized using pestle and mortar. The powdered plant material (300g) was extracted by maceration for 48 hr using

methanol. It was filtered, and the filtrate was concentrated to dryness using a rotary evaporator, dried and then stored in a suitable container.

3.5.2 Procedures for Phytochemical Screening

The procedures for the detection of the various Phytochemical investigated were:

a. Test for carbohydrate

Molisch's test: The plant extract (0.5 g) was dissolved in distilled water. 4 drops of Molisch's reagent and 4 drops of Sulphuric acid were added to it without mixing. The above was allowed to form a lower layer, after which a purple to violet colour interface was taken as a positive result (Evans, 2009).

b. Anthracene derivatives

i. Borntrager's test: The powdered sample (5 g) was shaken with 10ml of benzene, filtered and 5 ml of 10 % ammonium solution was added to the filtrate. The mixture was shaken and the presence of a pink, red or violet colour in the ammonical (lower layer) phase is an indication of the presence of free hydroxyl anthraquinone (Sofowora, 2008).

ii. Combined anthraquinones: The plant extract(5 g) was boiled with 10 ml aqueous sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of benzene, the benzene layer separated and half its own volume of 10 % ammonium solution added. A pink, red or violet coloration in the ammonical phase (lower layer) indicates the presence of combined anthraquinone derivatives in the extract (Sofowora, 2008).

c. Cardiac glycosides test

i. Keller-Killiani test: The plant extract (0.5 g) was dissolved in 2 ml of glacial acetic acid containing a drop of ferric chloride solution. This was then underlayed with 1 ml of concentrated sulphuric acid. A brownish ring in the interface indicates the presence of deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer; a greenish ring may form just above the brown ring and gradually spread throughout this layer (Sofowora, 2008).

ii. Kedde test: The plant extract in methanol(1 ml of an 8 % solution) was mixed with 1ml of 2 % solution of 3,5- dinitrobenzoic acid in methanol and 1 ml of 5.7 % aqueous sodium hydroxide. An immediate violet colour indicated the presence of cardenolides in the extract, the colour fading gradually through reddish-brown to brownish-yellow with the precipitation of whitish crystalline solid. This test indicates the presence of a lactone ring in the cardenolide (Sofowora, 2008).

d. Test for Steriods and Triterpenes

i. Lieberman- Burchard's test: The extract (1 ml) was dissolved in 0.5 ml of acetic anhydride and this was followed by the addition of 1 ml of concentrated sulphuric acid to form a layer underneath. A blue to blue-green colour in the upper layer and reddish ring indicates the presence of steroids and red, pink or purple colour indicates the presence of triterpenoids (Sofowora, 2008).

e. Test for Tannins

i. Ferric chloride test: The powdered plant (0.2 g) was extracted with 1 ml of water and filtered. To the filtrate in test tube, 2 drops of 5 % ferric chloride was added. A greenish black

precipitate indicates the presence of condensed tannins, while a blue black precipitate showed the presence of hydrolysable tannins (Evans, 2009).

ii. Lead Sub acetate test: To 1 ml of aqueous extract in test tubes, 3 drops of lead sub-acetate solution was added. A coloured precipitate indicates the presence of tannins (Harborne, 1991).

f. Tests for Flavonoids

i. Shinoda test: The powdered plant (0.5 g) was boiled in ethanol on water bath for 3minutes. It was filtered and cooled, and the filtrate in the test tube was diluted with ethanol. Few pieces of magnesium turnings were added followed by the addition of 2 to 3 drops of concentrated HCl. A pink or red colour indicates the presence of flavonoids (Harborne, 1991).

ii. Sodium hydroxide test: The powdered plant (0.2 g)was detanned with acetone and the acetone discarded. The residual acetone was evaporated on a water bath after which the residue was extracted with warm water and filtered while hot. The filtrate was allowed to cool in test tube before the addition of an equal volume of 1 % aqueous NaOH solution. A resultant yellow solution which on addition of dilute HCl becomes colourless indicates the presence of flavonoids (Evans, 2009).

g. Test for Alkaloids

The plant material (2.0 g) in 5 ml of 1.25 % Sulphuric acid in ethanol (12.5 %) was heated on water bath for 20minutes. It was cooled and filtered

i. Drangendorff's test: The filtrate (1 ml) was tested with few drops of Drangedorff's reagent. An orange-yellow/reddish brown precipitate observed was an indication for the presence of alkaloids in the extract (Evans, 2009).

ii. Mayer's test: The filtrate (1 ml) was tested with few drops of Mayer's reagent. A white/cream precipitate observed was an indication for the presence of alkaloids in the extract (Evans, 2009).

iii. Wagner's test: The filtrate (1 ml) was tested with few drops of Wagner's reagent. A reddish brown precipitate observed was an indication for the presence of alkaloids in the extract (Evans, 2009).

3.6 Biological studies

The following biological studies were carried out on the crude extract and fractions of *Boswellia dalzielii* Hutch. (stem bark and leaf) and *Enantia chlorantha* Oliv. (stem bark)

3.6.1 Acute Toxicity

The acute toxicity of the extracts in rat was conducted according to the method of Lorke (1983).

The method was divided into two phases.

In the first phase, 3 groups of three rats each were treated with the methanol extracts of the plants at doses of 10, 100 and 1000 mg/kg body weight (i.p.) and observed for signs of toxicity and death within 24 hours.

In the second phase, 3 groups each containing one rat was injected with 1000, 2000 and 3000 mg/kg doses of the extract based on the result of obtained from first phase.

3.6.2 *Invivo* Hepatoprotective Tests

These were done to determine the hepatoprotective activity of methanol extracts of the plants against hepatoxicity induced into wistar rats, by administration of carbon tetrachloride (CCl_4).

The procedure was a modification of Raj *et al*, 2010. Thirty six (36) rats were divided into six groups of six (6) rats each. . Liver damage was induced by administration of 30% CCl_4 suspended in olive oil (1 ml/kg body weight, i.p). With the result of the acute toxicity studies performed, the dose was fixed at 200 mg/kg b.w. and 250 mg/kg body weight for crude extract and standard Silymarin, respectively

Group I: received the vehicle (Sodium Carboxyl Methyl Cellulose 0.3 %) and served as control and was not treated with the toxicant.

Group II: served as CCl_4 -treated control

Group III: received methanol extract of leaves of *B. dalzielii* (200 mg/kg body weight)

Group IV: received methanol extract of stem bark of *B. dalzielii* (200 mg/kg body weight)

Group V: received methanol extract of stem bark of *E. chlorantha* (200 mg/kg body weight)

Group VI: received the standard Silymarin (250 mg/kg body weight). This was done for a period of 7 days.

On the seventh day except group I, all other groups received 30 % CCl_4 suspended in olive oil (1 ml/kg b.w.) i.p.

After 24 h of intoxication, on the 8th day, blood was collected through heart puncture into sterile centrifuge tubes and allowed to clot. Serum was separated and used for the estimation of Aspartate amino transaminase (ASAT), Alanine amino transaminase (ALAT), Alkaline Phosphatase (ALP), total proteins, albumin, total bilirubin and conjugated bilirubin.

The rats were sacrificed later and the liver were perfused and excised. Part of the liver was stored in 10% formalin saline for histopathological examination.

3.6.3 Antiviral assay

This was done to determine the antiviral potential of methanol extracts of the bark and leaf of *B. dalzielli* and bark of *E. chlorantha* against Newcastle Disease Virus (NDV) using embryonated egg

3.6.3.1 Sources of virus and 9-Day Old Embryonated Chicken Eggs

A velogenic strain of Newcastle Disease Virus (NDV) was obtained from Viral Research Department while embryonated chickens eggs were obtained from Poultry Division both of National Veterinary Research Institute, Vom, Nigeria.

3.6.4 Determination of Median Embryo Infectious Dose (EID_{50}) of the virus

The EID_{50} of the virus was determined using method of Young *et al*. (2002).

3.6.4.1 Preparation of the viral dilutions

Using an automatic pipette, 4.5ml each of phosphate buffered saline (PBS) was dispensed into ten (10) sterile McCartney bottles, labeled 10^{-1} to 10^{-10}. 0.5ml of Newcastle disease virus viral stock was added to bottle1 labeled 10^{-1} and mixed thoroughly. 0,5ml of dilution

in bottle 1 was transferred into bottle 2 and mixed thoroughly. The same dilution was done across the bottles until bottle 10 to make a 10-fold serial dilution (10^{-1} to 10^{-10}).

3.6.4.2 Preparation of embryonated eggs

Nine –day-old embryonated chicken eggs were candled, labeled and swabbed with 70 % ethanol. The eggs were divided into 12 groups of fives each, and labeled 10^{-1} to $10^{-10.}$ Group 11 and 12 served as control. Eggs in each group were inoculated with 0.2 ml of appropriate dilutions (e.g 10^{-1} dilution into 10^{-1} labeled eggs). They were sealed and incubated at 37 ^{0}C for 48 hr. Eggs were candled after 24 hr to confirm viability and also after 48 hr. Results were recorded appropriately. EID_{50} was calculated according to the method of Reed and Muench (1938)

Dilution=

$$\frac{\text{\% Mortality at dilution just above 50\% - 50\%}}{\text{\% Mortality at dilution just above 50\% - \% Mortality at dilution just below 50\%}}$$

The above formula will give $1EID_{50}$/ 0.2ml

From this, $100EID_{50}$/ 0.2ml of the virus stock was made for the experiment.

3.6.5 Preparation of inoculum (virus/extract mixture)

A 1:1 v/v dilution of the 100 EID_{50}/0.2 ml of virus with predetermined extract concentrations was made to put extract final concentration in the virus/extract mixture at 250, 200, 150, 100 and 50 mg/ml. The virus/extract mixtures were kept at 4 °C for 1 h to react before inoculation.

3.6.6 Inoculation of eggs

The procedure is according to the method of Chollom *et al*. (2012). Nine-day-old embryonated chicken eggs were divided into eight groups of five each. The embryonated chicken eggs were labelled according to the extract concentrations used. A set of plastic egg trays were thoroughly cleaned with Virkon ® (a disinfectant), the eggs were swabbed with 70 % ethanol in cotton wool and transferred into the cleaned trays.

The swabbed eggs were placed in the micro-safety cabinet where they were punched and immediately inoculated with the extract/virus mixture via the allantoic route. Groups 1 to 5 were inoculated with 0.2 ml of virus/extract mixtures at final concentrations of 250, 200 ,150, 100 and 50 mg/ml.

Group 6 was inoculated with 0.2 ml of 100 EID_{50}/0.2 ml standard NDV (virus control), Group 7 was inoculated with 0.2 ml phosphate buffered saline (diluent control) while group 8 was not inoculated (uninoculated control). The eggs were sealed with molten wax and incubated at 37 °C and embryo survival was observed at 24 hr daily. Allantoic fluids from treated eggs were collected for spot test and haemagglutination test to detect NDV in the eggs.

3.6.7 Spot haemagglutination test

Dead embryos that had been chilled were brought out of the refrigerator and kept at room temperature for about 30 min. The eggs were swabbed and placed in the biosafety cabinet. The shell of each egg was opened to reveal the air space and a pipette was used to dispense a drop of 1% washed chicken red blood cells on a white tile. A wire loop was thoroughly flamed and used to pick a drop of the allantoic fluid which was mixed with the drop of blood. The tile was gently rocked and observed for visible agglutination, indicating viral

activity (Murakawa *et al*., 2003). This was done for every egg and the observations were recorded.

3.6.8 Data Analysis

The data gathered during the ethnobotanical survey were analysed by extracting information from data available, so as to give a summary description of the subject. Descriptive statistical tools such as tables and multiple bar charts were used.

The data obtained from the Pharmacognostic studies were expressed using descriptive statistical tools; table and mean ± standard error of the mean (Mean ± SEM) were used.

The data obtained from the biological studies were expressed as the mean±SEM using the SPSS Statistical package. One-way analysis of variance (ANOVA) was used to detect further differences between groups. $P < 0.05$ was considered significant.

CHAPTER FOUR

RESULTS

4.1 Results of ethnobotanical survey

4.2 Response rate

The target for the interview sessions was at least 30 interviews for the three (3) Local Government Area in Plateau State (Fig.1.2). Only 24 interview sessions were possible, resulting in an overall response rate of 80%. The respondents were made up of 2 males and 7 females for Jos North, 7 males and 4 females for Jos South and 4 males only for Jos East **.** The respondents comprise mainly of the following tribes; Berom, Anaguta, Jarawa, Hausa and Yoruba.

4.3: Distribution of informant's ages

The age distribution of informants showed that most of the informants encountered during the survey are within the age range 40-49 and 50-59. This is shown on Fig 4.1

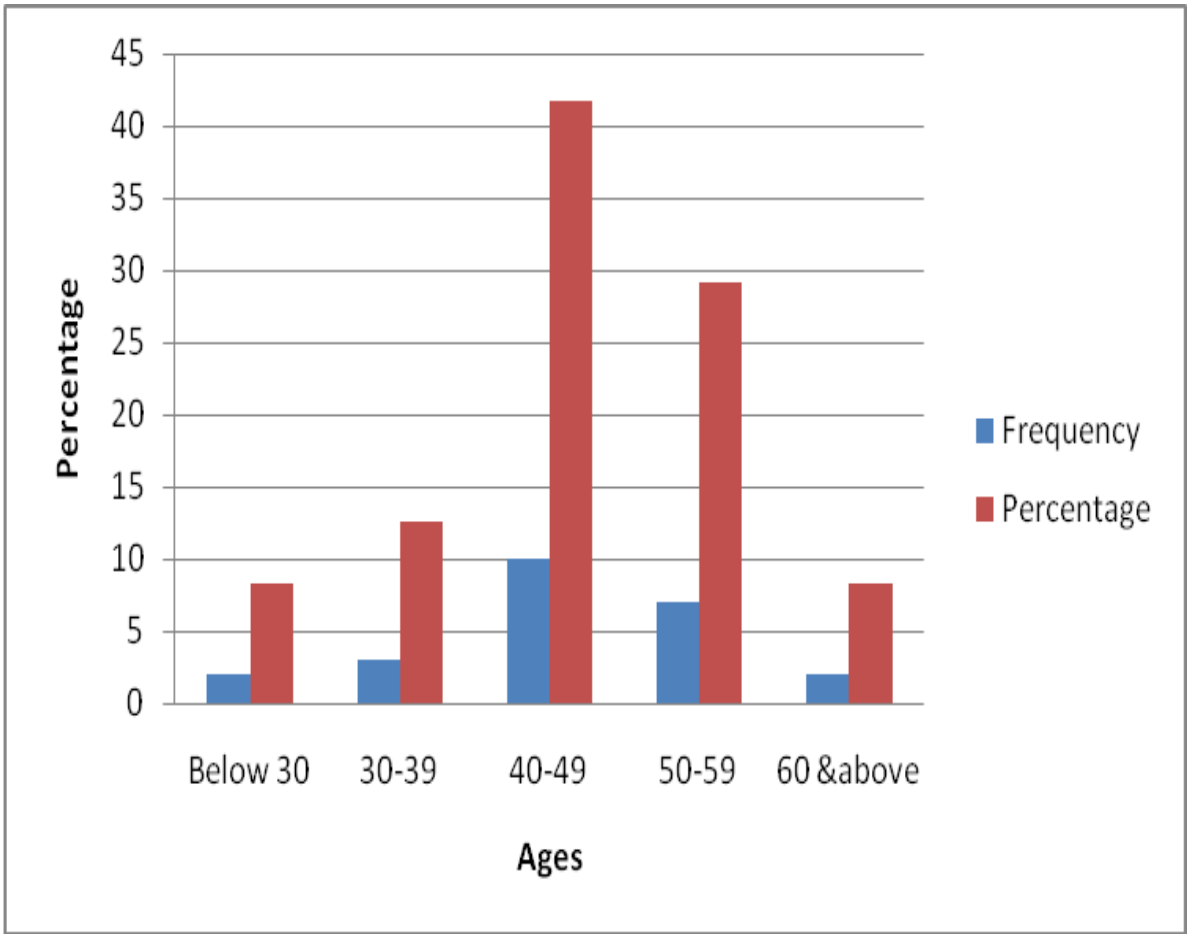


Fig 4.1: Distribution of Informant's Age in the Ethnobotanical Survey of Plants Used in Treatment of Viral Infections in Jos.

4.4 Distribution of respondents based on their practice

The sources of information in the study are Traditional Medicine Practitioners, Herbalist, Herb sellers, indigenes and residents. Herbalist and Traditional Medicine Practitioners were the major source of information, while the herb sellers, indigenes and residents gave less information as presented by Fig 4.2

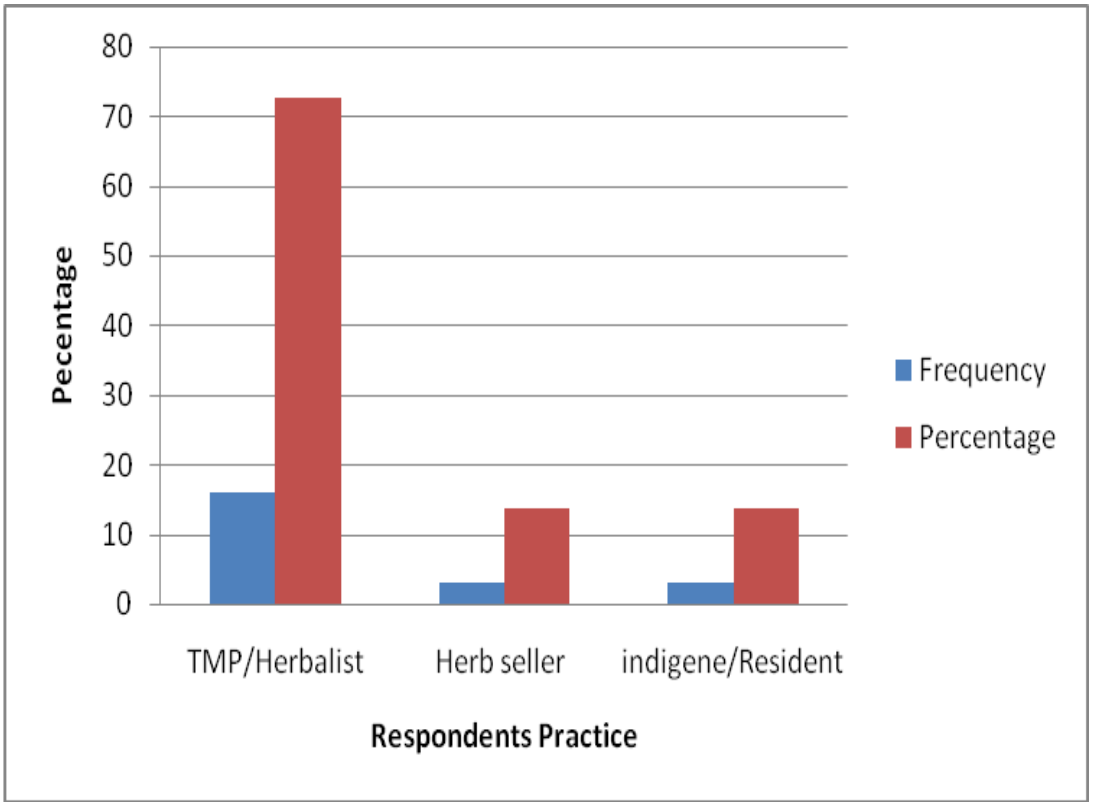


Fig 4.2 Distribution of Respondents Based on their Practice in the Ethnobotanical Survey of Plants Used in Treatment of Viral Infections in Jos.

4.5 Medicinal plants used in the treatment of viral infections in Jos, Plateau State

A total of 64 medicinal plants species, represented by 62 genera were obtained from 39 families. Tables 4.1 gives a concise information on the medicinal plant species, their families, plant parts used, medicinal uses and their vernacular names in Hausa, Yoruba , Berom, Jarawa and Anaguta.

The Rubiaceae family have the highest number of plant species (5 plants), followed by Fabaceae (4 plant species), and Annonaceae, Combretaceae, Moraceae and Solanaceae all with 3 plant species each.

The infection with the highest number of medicinal plant for its treatment is Hepatitis, followed by measles, then chickenpox, HIV and common cold.

Table 4.1: Medicinal plants used in the treatment of viral infections in Jos, Plateau state

Family	Scientific Name	Local Name	Plant Part Used	Voucher Number	Medicinal Use
AGAVACEAE	*Sansevieria liberica* Geromes Labroy	Mooda (H)	Stem Bark	ABU 1821	Hepatitis
AMARANTHACEAE	*Aerva lanata* (L.) Juss. ex Schult.	Efun (Y)	Leaves	ABU 70736	Common cold
AMARYLLIDACEAE	*Crinum giganteum* Andr.	Gadaali (H)	Whole plant	ABU 1408	Hepatitis
ANACARDIACEAE	*Magnifera indica* Linn.	Mangoro (H)	Stem Bark	ABU 1944	Hepatitis
ANONNACEAE	*Annona senegalensis* Pers.	Gwanda daji (H)	Leaves	ABU 90012	Measles
	Xylopia aethiopica (Dun.)A. Rich.	Eruje (Y)	Stem Bark	FHI 108075	Hepatitis, HIV
	Enantia chloranta Oliv.	Awopa(Y)	Stem Bark	FHI 101821	Hepatitis
APOCYANACEAE	*Landophia owarienses* P.Beauv.	Ciwoo (H)Ree (B)	Stem Bark	ABU 1225	Hepatitis
	Alstonia boonei de Wild	Awun (Ahun) (Y)	Stem Bark	FHI 103096	Hepatitis
	Carissa edulis (Forssk.) Vahl.	Lemun tsuntsu(H)	Root	ABU 900086	HIV
ASTERACEAE	*Vernonia amygdalina* Del.	Shuwaka(H)	Leaves	ABU 595	Chicken pox
					Hepatitis
					Rabies

					Measles
BORAGINACEAE	*Heliotropium ovalifolium* Forssk.	Shaanikasani(H)	Root	ABU 2037	HIV
BURSERACEAE	*Boswellia dalzielli* Hutch.	Ararabi(H)	Leaves and Stem bark	ABU 1314	Rabies Chicken pox Hepatitis HIV
CAESALPINIACEAE	*Piliostigma thonningii* (Schum.)	Kargo (H)	Leaves and fruits	ABU 1132	Measles
	Cassia singuena (Del.) Lock.	Runfu(H)	Flowery tops	ABU 6855	Measles Hepatiti

Family	**Scientific Name**	**Local Name**	**Plant Part Used**	**Voucher Number**	**Medicinal Use**
	Deuterium microcarpum(Guill and Sperr.)	Tawra(H)	Stem Bark	ABU 551	Hepatitis
CARICACEAE	*Carica papaya* Linn.	gwanda (H)	Leaves	ABU 005	Measles
CHENOPODIACEAE	*Chenopodium ambrosiodes* Linn.	kwalinsan (B)	Whole plant	ABU 1921	Measles
COCHLOSPERMACEAE	*Cochlospermum planchoni* Hook.f.ex.Planch	Rawaya (H)	Root	ABU 900011	Hepatitis HIV
COMBRETACEAE	*Anogeissus leiocarpus (*DC.) Guill. & Perr. *Terminalia avicennoides* Guill. & Perr. Fl.	Marke (H)	Stem Bark and leaves	ABU 900389	Common cold

	Seneg. Tent.		Stem Bark		
		Baushe(H)		ABU 900309	Common cold
	Guiera senegalensis J.F.Gmel	Sabara(H)		ABU 900165	Chicken pox
CURCUBITACEAE	*Cucumis metuliferus* E. Mey	Buurar-zaakii(H)	Fruits	ABU 3232	Bird flu
	Adenopus breviflorus Benth.	Tagiri (Y)	Fruit	FHI 107945	Measles
EBENACEAE	*Diospyros mespiliformis* Hochst .ex.A. DC.	Kanya (H)	Stem bark	ABU 901431	HIV
EUPHORBIACEAE	*Jatropha curcas* Linn.	Biydazogu (H)	Leaves and root/	ABU 1911	Hepatitis
					HIV
	Manihot esculenta Linn.	Rogo (H)	Leaves	ABU 2347	Measles
FABACEAE	*Dialium guinese* Willd.	Tsamiyar biri (H)	Leaves	ABU 3792	Measles
					Chicken pox
	Erythrina senegalensis DC	Minjirya (H)	Stem bark	ABU 7721	HIV
					Hepatitis
	Abrus precatorius L.	Idon zakara (H) Chakala (J)	Whole plant/	ABU 1496	Common cold
	Tamarindus indica L.	Tsamiya (H)	Leaves	ABU 900265	Measles

Family	Scientific Name	Local Name	Plant Part Used	Voucher Number	Medicinal Use
GUTTIFERAE	*Garcinia kola* Heckel	Orogbo (Y)	Fruit/nut	ABU 1614	Common cold
LAMIACEAE	*Ocimum gratissimum* Linn.	Efirin (Y)	Leaves	ABU 661	Measles
LILIACEAE	*Allium sativum* Linn.	Tafarnuwa (H)	Bulb	ABU 423	Common cold
LORATHACEAE	*Tapinanthus dodoneifolius* (DC.) Danser.	Kauchi(H)	Stem bark	ABU 6517	Hepatitis
MALVACEAE	*Hibiscus rostellatus* Guill. & Perr.	Dakwan (B) maratum (A)	Whole plant	ABU 1774	Rabies Hepatitis HIV
MELIACEAE	*Khaya grandifolia*	Oganwo (Y)	Stem bark	ABU 900181	Chicken pox Rabies
MIMOSACEAE	*Parkia biglobosa* (Jacq.) R.Br	Dorawa (H)	Stem bark, leaves	ABU 2846	Chicken pox Measles

					Hepatitis
MORACEAE	*Ficus thonningii* Blume	Chediya(H)	Stem bark	ABU 651	HIV
	Ficus vallis chodae Delile	Ogunro (Y)	Stem bark	ABU 547	HIV,chicken pox
	Ficus sycomorus L.	Baore (H)	Root	ABU 1942	Hepatitis
MORINGACEAE	*Moringa oleifera* Lam.	Zogallagandi(H)	Root	ABU 571	Hepatitis
MYRTACEAE	*Syzygium guineense* Wall.	Malmo (H)	Root	ABU 900295	Hepatitis
	Psidium guajava L.	Guaaba (H)	Leaves	ABU 2846	Measles

Family	**Scientific Name**	**Local Name**	**Plant Part Used**	**Voucher Number**	**Medicinal Use**
OLACACEAE	*Ximenia americana* L.	Tsaada (H)	Root/	ABU 1612	Hepatitis
OCHNANCEAE	*Lophira lanceolata* Tiegh. ex Keay	Jan magani (H)	Roots	ABU 900121	Measles
POACEAE	*Sorghum guineense* Staph.	Doro (H) oka baba(Y)	Seeds, stem	ABU 8501	Measles Hepatitis

POLYGALACEAE	*Securidaca longipenduculata* Fers.	Sanya(H)	Leaves	ABU 900141	Common cold
			Whole plant		Measles
			Root		HIV
RUBIACEAE	*Pavetta crissipe* K. Schum.	Rubatari (H)	Leaves	ABU 904	Common cold
	Mitracarpus scaber	Googamassu (H)	Leaves	ABU 70701	Measles
	Nauclea latifolia Sm.	Egbesi (Y)	Stem bark	ABU 005	Chicken pox ,Hepatitis
	Spermococe verticellata	Karyangarma(H)	Whole plant	ABU 672	Chicken pox
	Oldenlandia gorensis DC	Raatsa-hanji(H)	Whole plant	ABU 9558	Chicken pox
RUTACEAE	*Citrus aurantifolia*	Lemun tsami(H)	Leaves	ABU 1440	Measles

Family	Scientific Name	Local Name	Plant Part Used	Voucher Number	Medicinal Use
SAPOTACEAE	*Vitellaria paradoxa* C.F. Gaertn.	Ori (Y), Kadanya (H)	Nuts	FHI90709	Common cold
			Stem bark		Chicken pox
SCROPHULARIACEAE	*Striga hermontheca* (Del.) Benth.	Kujiji (H)	Seeds and leaves	ABU 1058	Common cold
SOLANACEAE	*Solanum nodiflorum* Jacq.	Guota kaji (H)	Fruits	ABU 1664	Birdflu
	Capsicum frutescens L.	Barkoonoo (H)	Fruits/		Birdflu
	Nicotiana tabacum L.	Taba (H)	Leaves	ABU 1611	Chicken pox
VERBENACEAE	*Vitex chrysocarpa* Planch. ex Benth.	Magani kaji(H)	Leaves		Birdflu
ZINGIBERACEAE	*Zingiber officinale* Roscoe.	Chitta(H)	Rhizomes	ABU 2261	Common cold
		Atale (Y)			
	Aframomum melegueta K. Schum.	Atare (Y)	Leaves and seeds	FHI 108004	Common cold

Key: H: Hausa; Y: Yoruba; B: Berom; A: Anaguta



Plate I: *Boswellia dalzielli* Hutch. (Burseraceae)



Plate II: *Enantia chlorantha* Oliv. (Annonaceae)



Plate III: *Piliostigma thonningii*(Schum.)(Caesalpiniaceae)



Plate IV: *Parkia biglobosa* (Jacq.) R.Br (Mimosaceae)



Plate V: *Annona senegalensis* Pers.(Annonaceae)



Plate VI: *Vernonia amygdalina* Del. (Asteraceae)



Plate VII: *Erythrina senegalensis* DC (Fabaceae)



Plate VIII: *Ocimum gratissimum* Linn. (Lamiaceae)



Plate IX: *Abrus precatorius* L. (Fabaceae)



Plate X: *Carissa edulis* (Forssk.) Vahl. (Apocynaceae)



Plate XI: *Anogeissus leiocarpus(*DC.)Guill. & Perr. **(Combretaceae)**



Plate XII: *Vitellaria paradoxa* C.F. Gaertn. **(Sapotaceae)**

74.6: Recipes

The mode of preparation and route of administration of the medicinal preparation used in treatment of the viral infections are presented in Table 4.2 below.

Table 4.2: Recipes and Route of Administration for the different medicinal preparations used in treatment of viral infection

S/N	Ailment/ Species	Recipes/ dosage regimen	Route of Adminstration
A	**COMMON COLD**		
1	*Garcinia cola*	Dried fruits are cut into small sizes, and chewed with salt. This is done until the cold subsides.	Oral
2	*Anogeissus leiocarpus* *Pavetta crissipe*	Aqueous decoction is made from fresh leaves. Preparation is taken for a week. OR Aqueous decoction of the dried leaves of *Pavetta crissipe* and dried bark of *Anogeissus leiocarpus* is made with red potash. The decoction is filtered and taken twice daily.	Oral Oral
3	*Aerva lanata* *Zingiber officinale*	Dried leaves of *Aerva lanata* and rhizomes of *Zingiber officinale* are decocted with alum. The preparation is taken for two days.	Oral
4	*Vitellaria paradoxa*	Fats from nuts of the plant are melted to be taken and rubbed on the nose.	Topical
5	*Terminalia avicenniodes* *Pavetta crissipe* *Striga hermontheca*	Aqueous decoction of bark of *Terminalia avicenniodes,* leaves of *Pavetta crissipe*, leaves of *Striga hermontheca*, sheabutter and potash is made. The decoction is taken warm.	Oral

S/N	Ailment/ Species	Recipes/ dosage regimen	Route of Adminstration
6	*Anogeissus leiocarpus* *Pavetta crissipe* *Striga hermontheca* *Allium sativum*	Dried bark of *Anogeissus leiocarpus,* dried leaves of *Pavetta crissipe*, dried seeds of *Striga hermontheca*, and bulb of *Allium sativum* are decocted with red potash. , The decoction is filtered, a teacup of the preparation is taken twice daily for adult, and a teaspoon trice daily for children.	Oral
7	*Abrus precatorius*	Aqueous decoction of the Fresh or dried leaves is made with alum, and drunk. OR Aqueous decoction of leaves is made and taken hot twice daily for one week	Oral
8	*Securidaca longependunculata*	Dried leaves is powdered and then sniffed	Nasal
B	**MEASLES**		
1	*Chenopodium ambrosiodes*	Whole plant is soaked in cold water for a day. The extract is taken morning and evening and used to bath also in the morning and evening.	Oral and Topical
2	*Ocimum gratissimum*	Fresh leaves are squeezed to obtain the juice with small amount of water; the juice is then mixed with palm wine. One glass cup is taken twice daily for five days and also rubbed on the body.	Oral and Topical
3	*Cassia singuena*	Flowery tops or roots is powdered and soaked in cold water. The extract is taken twice daily OR The powdered drug is mixed with pap and taken twice daily.	Oral

S/N	Ailment/ Species	Recipes/ dosage regimen	Route of Adminstration
4	*Vernonia amygdalina*	Fresh leaves are squeeze in small amount of cold water to obtain the plant juice, it then filtered. A small tumbler is taken three times daily and also used to bath thrice a day. OR Fresh leaves are squeeze in small amount of cold water to obtain the plant juice, it then filtered. The extract is mixed with gin, one tablespoon is taken thrice daily, and also rubbed on the body.	Oral and Topical
5	*Aframomum melegueta* *Boswellia dalzielii*	Aqueous decoction of Seeds and leaves of *Aframomum melegueta* and leaves of *Boswellia dalzielii* are made. The decoction is drunk and also sprinkled on the body.	Oral and Topical
6	*Psidium guajava* *Carica papaya* *Citrus aurantifolia* *Parkia biglobosa*	Aqueous decoctions of the leaves are made. The decoction is taken orally and also used to bath.	Oral and Topical
7	*Adenopus breviflorus*	Fruit is sliced and boiled in water. The decoction is taken twice daily	Oral
C	**CHICKENPOX**		
1	*Oldenlandia gorensis DC*	Aqueous decoction of the dried or fresh leaves, seeds and stem is made. The decoction is taken warm and used in bathing morning and evening.	Oral and Topical
2	*Ocimum gratissimum*	Fresh leaves are rinsed and the juice is squeezed out by rubbing it between the palms. The juice is mixed with palm wine. One glass cup is taken twice daily for five days and also rubbed on the body.	Oral and Topical

S/N	Ailment/ Species	Recipes/ dosage regimen	Route of Adminstration
3	*Boswellia dalzielli*	Dried stem bark is pounded into powder. Cold water maceration of the powder is made. The extract is filtered and taken daily, while the marc is rubbed on the body.	Oral and Topical
4	*Veronia amygdalina*	Fresh leaves are rinsed and the juice is squeezed out by rubbing it between the palms in small amount of cold water. It is then filtered; a small tumbler of the extract is taken thrice daily, and also used to bath.	Oral and Topical
5	*Spermococe verticellata*	The whole plant is boiled with red potash, and the decoction is taken	Oral
6	*Boswellia dalzielli* *Guiera senegalensis* *Dialium guineese*	Aqueous decoction of leaves of *Boswellia dalzielli, Guiera senegalensis* and stem bark of *Dialium guineese* is made. The decoction is taken and also used in bathing.	Oral and Topical
D	**RABIES**		
1	*Ocimum gratissimum* *Vernonia amygdalina*	Fresh leaves are rinsed and the juice is squeezed out by rubbing it between the palms in small amount of cold water. It is then filtered; the extract is taken while the marc is used as a poultice at the site of bite.	Oral and Topical

S/N	Ailment/ Species	Recipes/ dosage regimen	Route of Adminstration
2	*Hibiscus rostellatus*	Aqueous decoction of the fresh whole plant is made. The decoction is to be taken. OR The dried whole plant is powdered, and then cold water maceration of the powder is made. The extract is taken twice daily.	Oral
3	*Boswellia dalzielli*	Pound fresh bark of plant, squeeze the juice out to drink and apply the marc as poultice at the site of bite	Oral and Topical
E	**BIRD FLU**		
1	*Solanum nodiflorium*	Dried fruit is crushed into cold water and given to the chicken to drink	Oral
2	*Solanum nodiflorium* *Vitex chrysocarpa*	Dried fruit of crushed *Solanum nodiflorium* and dried leaves of *Vitex chrysocarpa*, soaked in cold water and given to the chicken to drink	Oral
4	*Cucumis metuliferus*	Fresh fruit is broken into cold water and given to chicken to drink OR Fresh fruit may be eaten by patient	Oral

S/N	Ailment/ Species	Recipes/ dosage regimen	Route of Adminstration
F	**HEPATITIS**		
1	*Alstonia boonei* *Xylopia aethiopica*	Fresh barks are boiled in pap water, allowed to cool and taken.	Oral
2	*Jatropha curcas*	Dried leaves are powdered and mixed with every meal	Oral
3	*Moringa oleifera*	Aqueous decoction of fresh roots is made. The decoction is allowed to cool and then taken	Oral
5	*Vernonia amygdalina* *Citrus aurantifolia*	Fresh leaves of *Vernonia amygdalina* squeezed in small amount of cold water to extract its juice. The extract is filtered. One and half coke bottle of the filtrate is mixed with half bottles of *Citrus aurantifolia* and half bottle of gin. Half a glass cup is taken twice daily.	Oral
6	*Crinum gigantenum*	Aqueous infusion of the fresh whole plant is made. the infusion is taken thrice daily	Oral
7	*Ximenia Americana* *Syzygium guineense*	Aqueous decoction of roots and red potash are made, the decoction is filtered and then taken daily	Oral
8	*Sansevieria liberica* *Cassia singuena* *Enantia chlorata* *Manifera indica* *Khaya grandifolia*	Aqueous decoction of bark of *Sansevieria liberica* and roots of *Cassia singuena* is made. The decoction is taken three times daily. It may also be used to bath. Aqueous decoction of stem barks is made and taken twice daily	Oral and Topical

S/N	Ailment/ Species	Recipes/ dosage regimen	Route of Adminstration
9	*Enantia chlorata* *Citrus aurantifolia*	Dried barks are pounded with small quantity of potash. The powdered drug is taken with hot pap	Oral
10	*Enantia chlorata* *Azadirachta indica* *Nauclea latifolia* *Alstonia boonei* *Citrus aurantifolia*	Aqueous decoction of the stem barks of *Enantia chlorata, Azadirachta indica, Nauclea latifolia, Alstonia boonei,* and fruit of *Citrus aurantifolia* is made. The warm decoction is taken orally; the hot decoction is used for inhalation and afterwards used to bath.	Oral and Nasal
11	*Jatropha curcas* *Allium sativum*	Aqueous decoction of the leaves of *Jatropha curcas* and bulbs of *Allium sativum* is made,. The decoction is taken three times daily.	Oral
12	*Boswellia dalzielli* *Parkia biglobosa* *Nauclea latifolia*	Aqueous decoction of the stem barks of *Boswellia dalzielli, Parkia biglobosa* and the roots of *Nauclea latifolia* is made. The decoction is taken orally	Oral
13	*Hibiscus rostellatus*	Dried leaves are powdered by pounding. The powdered drug is taken with ‘kunu’ four times daily.	Oral

S/N	Ailment/ Species	Recipes/ dosage regimen	Route of Adminstration
G	**HIV**		
1	*Boswellia dalzelli*	Fresh leaves are dried under shed, when dried it is powdered by pounding. The powder drug is taken by mixing with pap, food, tea or 'kunu'	Oral
2	*Heliotropium ovalifolium*	Dried roots are powdered by pounding. The powder is taking with kunu, early in the morning.	Oral
3	*Hibiscus rostellatus*	Aqueous decoction of whole plant is made. The decoction is taken orally	Oral
4	*Jatropha curcas* *Boswellia dalzeilli* *Securidaca longepedunculata* *Cochlospermum planchoni* *Diospyros mespilformis*	Aqueous decoction of the roots of *Jatropha curcas*, stembark of *Boswellia dalzeilli*, whole plant of *Securidaca longepedunculata*, roots of *Cochlospermum planchoni*, and stem bark of *Diospyros mespilformis* is made. One teacups of decoction is taken thrice daily.	Oral
5	*Ficus thonningii*	Aqueous decoction of stem bark with little quantity of red potash is made. The decoction is taken orally.	Oral

Table 4.3: List of Plants frequently mentioned In the Treatment of a Particular Viral Infection by two or more informants

Viral Infection	Scientific Name Of Plant	No. of Informants
Common cold	*Anogeisus leioparus*	8(50%)
	Pavetta crissipes	7(44%)
	Striga hermontheca	5(31%)
	Allium sativum	4(25%)
	Abrus precatorus	4(25%)
Measles	*Vernonia amygdalina*	2(13%)
	Chennepodium ambrosiodes	2(13%)
	Manihot esculenta	2(13%)
Chickenpox	*Vernonia amygdalina*	2(13%)
Birdflu	*Cucumis metuliferus*	3(19%)
	Solanum nigrum	7(44%)
Rabies	*Boswellia dalzielii*	3(19%)
Hepatitis	*Jatropha curcas*	2(13%)
	Boswellia dalzielii	2(13%)
	Enantia chlorantha	3(19%)
HIV	*Moringa oleifera*	4(25%)

The number of informant is a function of 16 plant species

Formula: Number of informants divided by total number of species multiply by 100

Table 4.3 above shows that some of the medicinal plants discovered during the survey are more popular in viral infection therapy than others. This confirms the authenticity of information gathered and also the importance of such plants. Hence, informant's consensus

revealed that, *Anogeisus leioparus* used in the treatment of common cold is the most popular plant, cited by 8 informants (50 %) , followed by *Pavetta crissipes* and *Solanum nigrum* used in the treatment of common cold and bird flu respectively, mentioned by 7 informants (44 %) . The next plant is *Striga hermontheca* cited by 5 informants (31 %). *Allium sativum* and *Abrus precatorus* used in the treatment of common cold and *Moringa oleifera* used to treat HIV are the next, mentioned by 4 informants (25 %)

4.7 Pharmacognostic Evaluation of *Enantia chlorantha* Stem bark and *Boswellia dalzielli* leaves and stem bark

4.7.1 Microscopical examination of *Enantia chloratha* stem bark

Chemomicroscopy

The result of chemomicroscopy as shown on Table 4.4 indicating the presence of cell inclusions such as calcium oxalate and tannins. Cell wall materials such as lignin, suberin were also found to be present.

Table 4.4 Chemomicroscopy of *Enantia chlorantha* Stem bark

Test	**Observation**	**Inference**
1 drop of Phloroglucinol + 1 drop of Hydrochloric acid	Red colouration was observed on the fibres	Lignified cell walls present
1 drop of Sudan Red	Distinct red colouration was observed on a fibre	Suberized or cuticular cell walls presents
1 drop of Ruthenium Red	Brownish colouration was observed	Mucilage absent
1 drop of Ferric chloride	Bluish black colouration was observed	Tannins present
1 drop of Hydrochloric acid	Prism shaped crystals which dissolved in hydrochloric acid was observed	Calcium oxalate crystals present

Microscopy

The microscopical examinations of the powdered stem bark of *E. chloratha* revealed the following characteristic features: polygonal sclereids, cork cells, fibres with narrow lumen and bundles of fibre and polygonal parenchyma. Numerous prism shaped calcium oxalate crystals, and several phloem parenchyma (see Appendix II)

4.7.2 Physical constants of the powdered Stem bark of *E. chloratha* and leaves and stem bark of *B. dalzielli*

Physical constants such as moisture content, total ash value, extractive value (alcohol soluble and water soluble extractives) are presented in table 4.5

Table 4.5 Physical constants of the powdered plant samples

Parameters	***Boswellia dalzielli leaf***	***Boswellia dalzielli stem bark***	***Enantia chloratha stem bark***
Moisture content	11.0±0.100	10.0±0.340	8.0±0.070
Ash value	8.3±1.400	5.0 ±0.000	6.7±1.400
Alchohol extractive	0.5±0.000	0.9±0.003	0.3±0.007
Water extractive	0.9±0.003	0.9±0.030	0.2±0.060

(Values are mean of three determinants±SEM) %w/w

4.8 Results of Preliminary Phytochemical Screening

The results of the Preliminary Phytochemical Screening carried out for *E. chlorantha* and *B. dalzielli* are presented by in table 4.6.

Table 4.6: Summary of Preliminary Phytochemical Screening

Constituents	***B. dalzielli*** **leaves**	***B. dalzielli*** **bark**	***E. chlorantha*** **bark**
1. Carbohydrates	+	+	+
2. Unsaturated steroid & triterpene	+	+	+
3. Cardiac glycoside	+	+	+
4. Tannins	+	+	-
5. Flavonoids	+	+	-
6. Alkaliods	-	-	+

Key = + present
- Absent

4.9 Acute-toxicity study

Stem bark of *B. dalzielii:* In both the first phase and second phase of treatment with the methanol extract, no death and no sign of toxicity was observed after 48hr of observation.

Leaf of *B. dalzielii: i*n the first phase of treatment with methanol extract no death was recorded. The second phase no death was also recorded at doses 1000 mg/kg and 2000 mg/kg, while death was recorded at 3000 mg/kg

Stem bark of *E. chlorantha:* In both the first phase and second phase of treatment with the methanol extract, no death was observed, and no sign of toxicity was observed after 48hr of observation.

4.10 Hepatoprotective assay

Table 4.7: Biochemical parameters of rats after liver function test

	Total protein	Albumin	Total bilirubin	Conjugate bilirubin	Alkaline phosphatase	ALAT	ASAT
Control	76.60±1.08	44.80±1.59	15.00±0.00	10.00±0.00	266.80±26.00	107.20±11.50	478.00±133.00
CCl_4	77.20±0.49	44.80±0.08	15.00±0.00	10.00±0.00	225.00±20.10	125.40±14.50	499.00±51.10
Silymarin	75.80±1.62	43.20±1.20	15.00±0.00	10.00±0.00	269.80±22.00	101.20±9.06	458.80±61.40
E.chlorantha (bark)	79.20±0.37	43.60±0.93	14.00±1.00	9.00±1.00	213.80±22.60	95.60±9.36	379.00±81.20
B. dalzielli (leaf)	76.80±1.74	44.00±1.26	15.00±0.00	10.00±0.00	264.60±36.00	125.80±14.10	567.00±103.00
B. dalzielli (bark)	77.20±0.80	43.20±0.80	15.00±0.00	10.0±0.00	260.60±27.10	120.60±22.70	450.00±89.70

(Values are mean of five determinants±SEM)

Key
$CCl_{4=}$ Carbon tetrachloride
ALAT= Alanine amino transaminase
ASAT= Aspartate amino transaminase

Level of significance (α) = 0.05

The ANOVA calculation gave a P-value of 0.999. This P-value is greater than the level of significance and therefore we accept the null hypothesis and conclude that there is no significant difference between the CCl_4 group and the other groups.

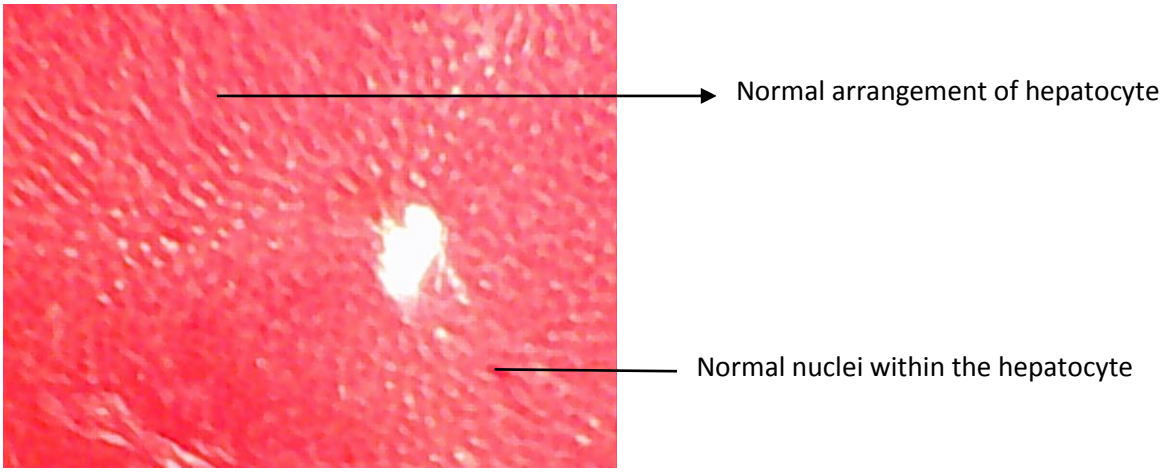


Plate XIII: Liver of a control rat showing normal hepatocytes and normal architecture (X100)

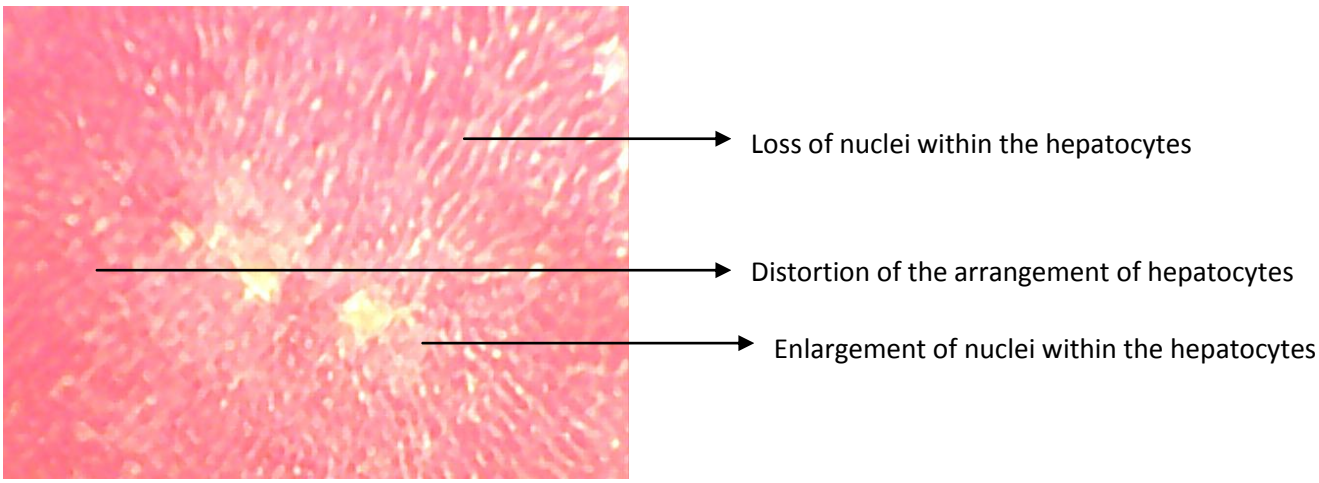


Plate XIV: Liver section showing hepatotoxic effect of CCl_4-treated (X100)

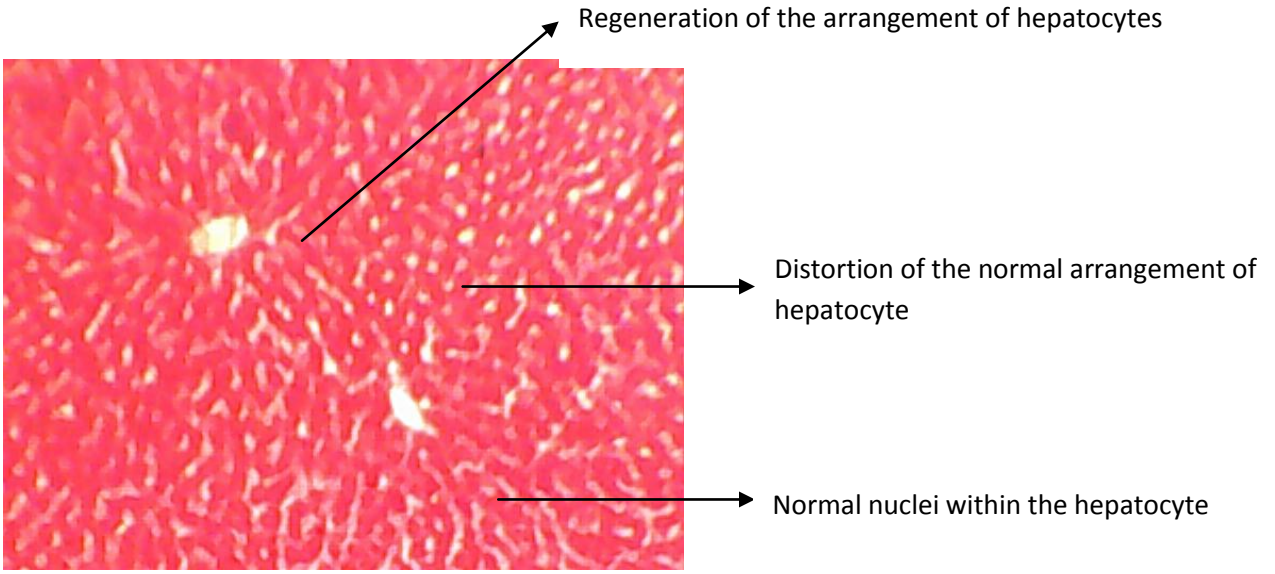


Plate XV: Liver section from a Silymarin treated rat (X100)

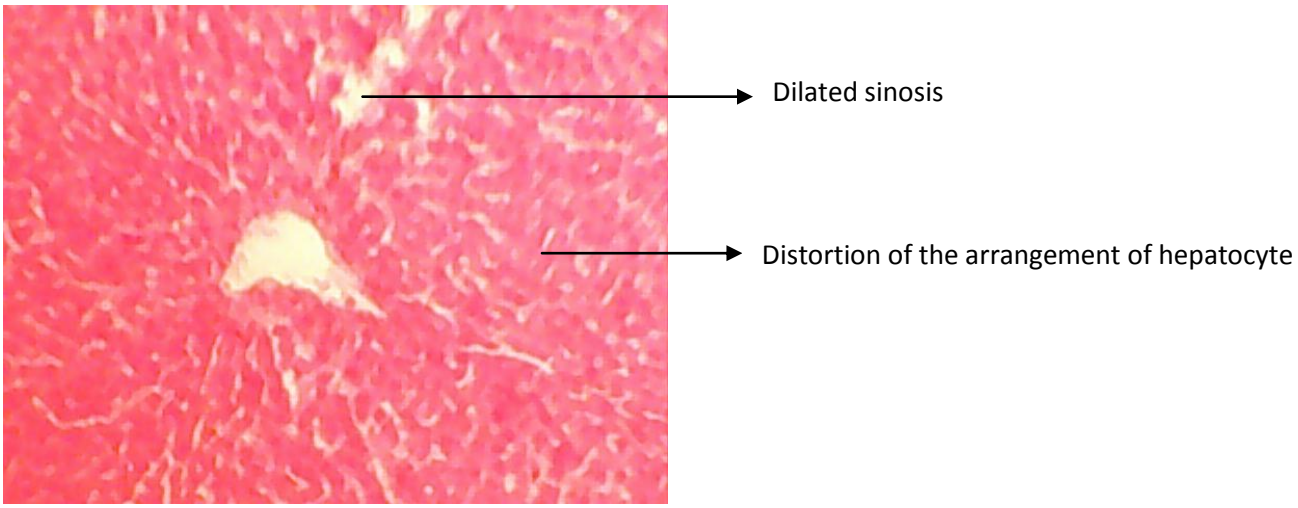


Plate XVI: Liver section of rat treated with 200 mg/kg of methanol extract of stem bark of *Boswellia dalzielii* (X100)

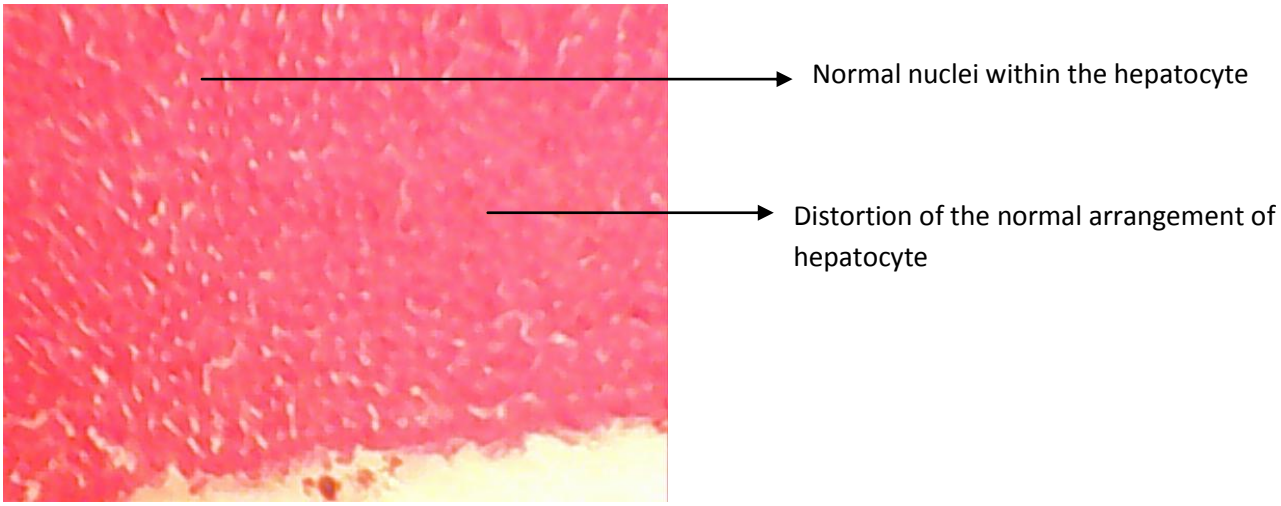


Plate XVII: Liver section of rat treated with 200 mg/kg of methanol extract of leaf of *Boswellia dalzielii* (X100)

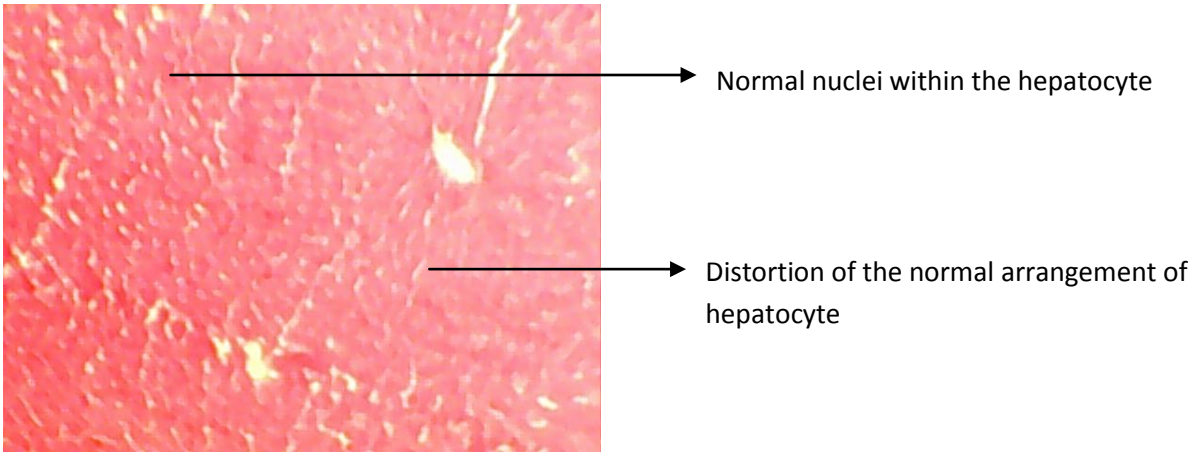


Plate XVIII: Liver section of rat treated with 200 mg/kg of methanol extract of stem bark of *Enantia chlorantha* (X100)

4.11 Antiviral assay

4.11.1 Median percent Embryo Infectious Dose (EID_{50})

One EID_{50} unit is the amount of virus that will infect 50 percent of inoculated eggs. The concentration of Newcastle disease virus in a suspension is expressed as an Infectivity Titre. The Infectivity Titre is established by carrying out a titration.

Table 4.8: **Median percent Embryo Infectious Dose**

Dilutions	No. of eggs	No. of eggs alive	No. of eggs dead	Percentage Mortality
10^{-1}	5	0	5	100%
10^{-2}	5	2	3	60%
10^{-3}	5	2	3	60%
10^{-4}	5	3	2	40%
10^{-5}	5	5	0	0%
10^{-6}	5	5	0	0%
10^{-7}	5	5	0	0%
10^{-8}	5	5	0	0%
10^{-9}	5	5	0	0%
10^{-10}	5	5	0	0%
Virus control	5	0	5	100%
PBS control	5	5	0	0%

From this data, the percentage figures entered in the Reed and Muench formula are as follows:

Dilution of 10^{-3} is % mortality at dilution just above 50%= 60 percent

Dilution of 10^{-4} is % mortality at dilution just below 50%= 40 percent

Dilution=
% Mortality at dilution just above 50% - 50%
% Mortality at dilution just above 50% - % Mortality at dilution just below 50%

$$= \frac{60\%-50\%}{60\%-40\%}$$

$$= \frac{10\%}{20\%} = 0.5$$

Apply the index calculated using this formula to the dilution that produced the infection rate immediately above 50 percent=$10^{-3.5}$

This dilution of the virus suspension contained one EID_{50} unit of virus in 0.2ml.

1ml of the virus suspension will contain ten times the reciprocal of the calculated dilution.

Therefore infectivity Titre of virus suspension in EID_{50}/ml= 10 x $10^{3.5}$= $10^{4.5}$ EID_{50}/ml

4.11.2 Results of antiviral activity of *B. dalzielli* methanol leaf extract against NDV

B. dalzielli methanol leaf extract at 250 mg/ml concentration did not inhibit virus growth, but rather was toxic to the embryo as revealed by the 80% death of the embryo 24hr after

inoculation. The spot HA test of allantoic fluids of eggs inoculated at this concentration, showed agglutination.

Bacterial infection was not seen in the allantoic fluid, ruling out bacteria as the cause of embryo death. Embryos of inoculated eggs with extract at concentration 200 mg partially inhibited virus growth probably due to toxicity of the extract at this concentration or mechanical injury during inoculation. Concentrations of 150 mg, 100 mg and 50 mg completely inhibited virus growth in embryonated eggs as revealed by the survival of 80 %, 100 % and 100 % respectively, of the embryo of the inoculated eggs. The diluent and uninnoculated controls had live embryo throughout the duration of the experiment, while the entire embryo in the virus control died 48hours post inoculation. This result was further confirmed by results of spot agglutination test (Table 4.9).

Table 4.9: Results of antiviral activity of *B. dalzielli* methanol leaf extract against NDV

Mortality (Pi)					HA			
Extract Dilutions(mg/ml)	No of Eggs	24hr	48hr	72hr	%Mortality	Agglutination (+ve)	No Agglutination (-ve)	% Agglutination due to virus
250	5	4/5	0/1	1/1	100	5	0	100
200	5	0/5	1/5	¼	40	2	3	40
150	5	1/5	0/4	0/4	20	1	4	20
100	5	0/5	0/5	0/5	0	0	5	0
50	5	0/5	0/5	0/5	0	0	5	0
Vc	5	0/5	5/5	-	100	5	0	100
Dc	5	0/5	0/5	0/5	0	0	5	0
Uc	5	0/5	0/5	0/5	0	0	5	0

KEY
Vc:Virus control
Dc: Diluent control
Uc: Uninoculated control
Pi: Post inoculation
HA: Heamagglutination

4.11.3: Results of antiviral activity of *B. dalzielli* methanol stem bark extract against NDV

250 mg/ml concentration of *B. dalzielli* methanol stem bark extract did not inhibit virus growth , but killed all the embryo in the inoculated egg after 48hr of post inoculation. The spot HA test of allantoic fluids of eggs inoculated at this concentration, showed agglutination.

The bark extract partially inhibited the virus growth at 200 mg concentration. The bark extract at 150 mg, 100 mg and 50 mg completely inhibited virus growth in embryonated eggs as revealed by survival of embryos of the inoculated eggs. The diluent and uninnoculated controls had live embryo throughout the duration of the experiment, while the entire embryo in the virus control died 48hr post inoculation. This result was further confirmed by the results of spot agglutination test (Table 4.10)

Table 4.10: Results of antiviral activity of *B. dalzielli* methanol stem bark extract against NDV

		Mortality (Pi)				HA		
Extract Dilutions(mg/ml)	No of Eggs	24hr	48hr	72hr	%Mortality	Agglutination +ve	No Agglutination -ve	% Agglutination due to virus
250	5	3/5	2/2	-	100	5	0	100
200	5	1/5	2/4	2/2	100	2	3	40
150	5	0/5	0/5	0/5	0	0	5	0
100	5	0/5	0/5	0/5	0	0	5	0
50	5	0/5	0/5	0/5	0	0	5	0
Vc	5	0/5	5/5	-	100	5	0	100
Dc	5	0/5	0/5	0/5	0	0	5	0
Uc	5	0/5	0/5	0/5	0	0	5	0

KEY
Vc:Virus control
Dc: Diluent control
Uc: Uninoculated control
Pi: Post inoculation
HA: Heamagglutination

4.11.4: Results of antiviral activity of *E. chlorantha* methanol stem bark extract against NDV

The methanol stem bark extract of *E. chlorantha* at 250 mg/ml concentration also did not inhibit virus growth, but killed all embryos in the inoculated eggs 72 hr post inoculation. The spot HA test of allantoic fluids of eggs inoculated at this concentration, also gave a positive result. Embryos of inoculated eggs with extract at concentrations of 200 mg and 150 mg partially inhibited virus growth as various degrees of mortality in the embryos of the eggs were observed.

The growth of the virus in the embryonated eggs was however; completely inhibited by the stem bark extract at concentration of 100 mg and 50 mg. The survival of the entire embryo in the eggs inoculated with concentrations 100 mg and 50 mg respectively, and the negative results of the spot heamagglutination test proves this complete inhibition. The diluents control had live embryo throughout the duration of the experiment, while the entire embryo in the virus control died 48 hr post inoculation. This result was further confirmed by the results of spot agglutination test. 20 % mortality was observed in the uninoculated control. (Table 4.11)

Table 4.11: Results of antiviral activity of *E. chlorantha* methanol stem bark extract against NDV

		Mortality (Pi)				HA		
Extract Dilutions(mg/ml)	No of Eggs	24hr	48hr	72hr	%Mortality	Agglutination (+ve)	No Agglutination (-ve)	% Agglutination due to virus
250	5	0/5	4/5	1/1	100	5	0	100
200	5	0/5	2/5	0/3	40	2	3	40
150	5	2/5	0/3	1/3	60	0	5	0
100	5	0/5	0/5	0/5	0	0	5	0
50	5	0/5	0/5	0/5	0	0	5	0
Vc	5	0/5	5/5	-	100	5	0	100
Dc	5	0/5	0/5	0/5	0	0	5	0
Uc	5	0/5	1/5	0/4	20	0	5	0

KEY
Vc:Virus control
Dc: Diluent control
Uc: Uninoculated control
Pi: Post inoculation
HA: Heamagglutination

CHAPTER FIVE

DISCUSSION

5.1 Ethnobotanical survey

Plants used in the treatment of the seven (7) viral infections were surveyed. These viral infections are common cold or flu, measles, chickenpox, rabies, bird flu, hepatitis and HIV/AIDS. In all the areas visited, the respondents mentioned the effectiveness of medicinal plants in the treatment of viral infections. This makes one to assume that plant materials are effective and safe (Igoli *et al*., 2005).

The study revealed that most knowledge on herbal remedies is handled by members of the community between the age range of 40-49 and 50-59, as shown on Fig. 4.1. This indicates that there is a wide gap of ethnomedicinal knowledge between the elderly and the younger generation. The majorities of the informants are elders and said that they had learned about medicinal plants during their childhoods and the knowledge had been orally passed down from family members, particularly grandparents and parents. Most of the adults reported that they learned about medicinal plants when trailing with their parents or grandparents to gather remedies in the forest. This situation seems to be the same in many parts of the world (Musa *et al*., 2011, Bussmann and Sharon, 2006).

Cultural changes as a result of westernization and modernization (Voeks and Leony, 2004) has contributed in making the younger generation undermine our traditional values (Giday *et al*., 2003). Since traditional medicine remains the most popular medicine in solving health problems in the developing world. It is important to publicize medicinal plant knowledge within the young generation to raise awareness of and appreciation for their

traditional values and for the conservation and sustainable use of the plants as well as to keep the traditional medical knowledge left in their community alive.

Fig. 4.2 shows that Traditional Medicine Practitioners (TMP) and Herbalist gave the highest response, while herb sellers and indigenes/residents showed little response. This was due to availability and willingness of the TMPs and Herbalists to share their knowledge. Some of the herb sellers were resistant, while the residents and indigenes had little knowledge on plants used in the treatment of these diseases and due to easy access to modern health they see no need for traditional medicine.

In this ethno botanical survey, a number of 64 medicinal plants from 39 families and their uses in treating some common viral infections are reported as shown by table 4.1. This demonstrates the depth of the knowledge of the people of Jos- Plateau on medicinal plants and their uses. This study like various studies before, it has shown that different areas in different part of the world have considerable amount of indigenous ethno medicinal knowledge (Tesfaye *et al.*, 2009).

Table 4.2 showed the recipe of the surveyed medicinal plants. Most of the remedies involved the use of a single plant, and water is the most commonly used solvent. Other solvents include gin, gruel and pap. Decoction and maceration are the most common mode of preparation. Oral route is the major route of administration, followed by a combination of both oral and external route of administration, depending on the type of viral infection been treated. This finding is in agreement with previous studies by Hunde *et al*, (2004) and Musa *et al*, (2011) which also revealed that oral ingestion is the most frequently used route of administration in traditional medicine.

Like most studies in ethno botany, it was observed during this study that the TMP s usually have no knowledge of the strength of their remedies, dosing depends on each practitioner (Tesfaye *et al.,* 2009). This lack of standardization and precision in dosage is seen as one of the main disadvantage of traditional medicine (Sofowora, 2008).

The frequently mentioned plants in the treatment of a particular viral infection are shown on Table 4.3. Sixteen (16) plants were identified and grouped according to their use in different viral infections. The fact that some of the plants are having similar uses in different LGAs surveyed indicates their pharmacological effectiveness (Oladumoye and Kehinde, 2011). It also confirms the authenticity of the information gathered during the study. In addition, it implies a probable exchange of ideas among the Practitioners.

5.2 Pharmacognostic Evaluation

The organoleptic, microscopic and phytochemical evaluation of *B. dalzielli* leaves and stembark and *E. chlorantha* stem bark were determined. Pharmacognostic studies helps in identification, authentication and standardization of plant material (Thomas *et al*., 2008).
The observations made will be helpful in the botanical identification and in achieving the goal of standardizations of crude drugs, as well as distinguishing morphologically related plant by differentiating between the desired plant species from its other species (Priyanka *et al*., 2010).

The determination of physiochemical parameter is important in the determination of adulterants and improper handling of crude drugs. Ash values are useful in the determination of the quality and purity of crude drug, particularly in powder form. The

residue left behind after incineration is the ash content of the drug which simply represents inorganic salts, naturally occurring in drugs or adhering to it or deliberately added to it as a form of the adulteration. Extractive values are useful indicators of the approximate amount of the chemical constituents in a crude drug. This was achieved by using ethanol and water, *B. dalzielli* stembark and leaves gave more yield with water as the solvent than with ethanol. On the other hand, *E. chlorantha* gave more yield from ethanol extraction as shown on table 4.5. These solvents are polar in nature, hence can extract polar constituents present in the plants.

The moisture contents for *B. dalzielli* stem bark, *B. dalzielli* leaves and *E. chlorantha* stem bark were 10, 11 and 8 percent respectively as shown on table 4.5. These values are high, hence care must be taken during storage of the crude drugs to maintain stability and avoid microbial contamination and deterioration (Uraih and Izuagbe, 1990).

5.3 Preliminary Phytochemical screening

The results of the preliminary phytochemical screening are shown on table 4.6. carbohydrates, steroids, triterpenes, cardiac glycosides, tannins and flavonoids were found to the present in the leaves and stem bark of *B. dalzielli,* while alkaloid was absent. These findings are in agreement with those of Hassan *et al*., 2009 and Odeghe *et al*., 2012.

The stem bark of *E. chlorantha* was found to contain carbohydrates, alkaloids, cardiac glycosides, steriods, and triterpenes, while flavonoids and tannins were absent . Gill (1992) implicated the alkaloid –berberine as the active ingredient of *E. chlorantha,* saponin and tannins were also detected. A similar study on the phytochemical studies of the stembark of

E. chlorantha resulted in the isolation of berberine and protoberberine alkaloids (Tan *et al*.,2007) . Studies by Odoh *et al*, 2010 and Adesokan *et al*., 2008 on the *E. chlorantha* roots and stembark respectively also revealed the presence of same phytochemicals.

5.4 Acute Toxicity

The LD_{50} of methanol extracts of *B. dalzielli* stem bark showed that at high dose of 3000 mg/kg body weight the extract was safe without any physically observed side effect, while *B. dalzielli* leaf extract showed toxicity at 3000 mg/kg body weight. A previous toxicological study on aqueous stem bark extract of *B. dalzielli* by Etuk *et al* (2006) showed that no sign of toxicity was seen in the tested animals at oral dose of 3000 mg/kg body weight.

E. chlorantha was also found to be safe, because no toxicity or physical side effect was observed after oral dose of 3000 mg/kg body weight. This finding is similar to that of Adebiyi and Abatan (2013) where four extracts of *E. chlorantha* stem bark at a dose of 3000 mg/kg., did not produce any mortality in the rats during the pilot acute toxicity study. These plants can be said to be generally safe, since studies by Clarke and Clarke (1979), have shown that any substance whose LD_{50} is above 1000 mg/kg body weight is regarded as relatively safe.

5.5 Hepatoprotective assay

Liver is a major organ responsible for metabolism, detoxification and excretion of various xenobiotics from the body (Rajesh and Manoj, 2011). Liver disease is any condition that causes liver inflammation or tissue damage that affects liver function. The common causes

of liver disease include infection, injury, therapeutic drugs (e.g., antibiotics, antitubercular drugs, etc), toxic compounds or chemicals (e.g.,carbontetrachloride (CCl_4), aflatoxin, etc.), alcohol and microbial agents (e.g,. hepatitis virus) genetic defects that lead to the deposition and build-up of damaging substances such as iron and copper (Bera *et al*., 2012)

Liver damage caused by hepatotoxic drugs and chemicals are dose related. The clinical picture of the liver damage often resembles that of acute viral hepatitis (Martin, 2012).The hepatotoxicity induced by carbon tetrachloride (CCl_4) is due to its metabolite trichloromethyl free radical (CCl_3) , a free radical that binds to lipoproteins and lead to peroxidation of lipids of endoplasmic recticulum (Brattin *et al*., 1985; Pradeep *et al*., 2009). CCL_4 has been found to induce extensive liver damage within a period of 24 hr following intraperitoneal administration. It is also known to cause marked elevation in serum enzymes (Raj *et al*., 2010).

This serum enzymes include alanine and aspartate aminotransferases (ALT and AST) and alkaline phosphatise (ALP). These serum enzymes are not direct measure of hepatic injuries, but they help show the status of the liver. Raised serum enzymes concentrations are indicative of liver damage. ALT is the most specific for hepatic diseases, because it is only present in the liver, while the other enzymes may be present in other organs and tissues (Martin, 2012).

Silymarin is used as standard hepatoprotective compound because it has been reported to have a protective effect on the plasma membrane of hepatocytes (Raj *et al*., 2010). In CCl_4-induced hepatitis, administration of methanol extract of leaf and stem bark of *B. dalzielli*

and stem bark of *E. chlorantha* showed no significant reductions in ALP, ASAT and ALAT and no significant increase in Total protein, total bilirubin, conjugated bilirubin and albumin, as shown in table 4.8. .This impiles that methanolic extract of leaf and stem bark of *B. dalzielli* and stem bark of *E. chlorantha* showed no hepatoprotective property. The biochemical observations were confirmed by histological examinations of the liver sections. However, the histological result of the standard drug, Silymarin showed signs of regeneration of the damaged hepatocyte, indicating its hepatoprotective property.

This observation is contrary to that of Odeghe *et al*. (2012) in which the methanolic extract of *B. dalzielii* leaves was found to alleviate the damaging action of carbon tetrachloride in the liver of rats. Another study revealed that hexane, chloroform, ethyl acetate and methanol extracts of *E. chlorantha* stem bark exert significant hepatoprotection against acetaminophen-induced toxicity. Although, the hexane extract of *E. chlorantha* showed better hepatoprotective activity in acetaminophen induced liver damage compared to chloroform, ethyl acetate and methanol extracts (Adebiyi and Abatan, 2013).

5.6 Antiviral assay

The results of the antiviral assay of the leaf and stem bark of *B. dalzielli* confirm that the plant has antiviral potential against NDV. This was revealed by the complete inhibition of the virus growth *in ovo* at 150mg/ml, 100mg/ml and 50mg/ml. No mortality (0%) was observed in the embryo of all the inoculated eggs at these concentrations. Higher concentration of 250mg/ml was toxic, as all the embryo in the inoculated eggs died 24hrs post inoculation. The stem bark of *B. dalzielli* has been found to contain phenolic compounds such as protocatechuic acid, gallic acid and ethylgallate as well as a

diterpeniod- incensole and triterpeniods- boswellic acid derivatives (Olukemi *et al.*, 2005). These compounds may be responsible for the antiviral activity seen.

The stem bark of *E. chlorantha* also had antiviral potential against NDV. Complete inhibition of virus growth was seen at 100mg/ml and 50mg/ml. Toxicity was also observed at 250mg/ml. In a similar study, the water extract of *E. chlorantha* showed significant antiviral activity against yellow fever virus (YFV) as it completely inhibited the infectivity of YFV as evident in complete absence of Cytopathic effects (CPEs) (Fasola *et al*., 2011).

Gill, 1992 implicated the alkaloid –berberine as the active ingredient of *E. chlorantha* .The alkaloid has been reported to possess a broad spectrum antiviral activity (Jaioyang *et al*. 2013). A study by Cecil *et al*, (2011), revealed that berberine may be useful for the treatment of infections with influenza A. The antiviral effect observed may be attributed to the alkaloid present in the plant.

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 Summary

A total of 64 medicinal plants used in the treatment of eight viral infections in Jos, Plateau State were obtained from the ethno botanical survey, and the information gathered was documented. These viral infections include common cold, measles, chickenpox, rabies, birdflu, hepatitis and HIV.

The macroscopy, microscopy and chemomicroscopy of *E. chlorantha* revealed cell wall materials, cell inclusions and other diagnostic characters that can aid in the easy and proper identification of the plant were identified. The physiochemical evaluation of *B. dalzielii and E. chlorantha* were done, in order to ascertain quality and purity.

Preliminary phytochemical screening of the leaves and stem bark of *B. dalzielii* showed the presence of carbohydrates, steroids, triterpenes, tannins and flavonoids while alkaloid was absent. The stem bark of *E. chlorantha* was found to contain; alkaloids, carbohydrates, steroids, and triterpenes, while flavonoids and tannins are absent.

An *invivo* hepatoprotective assay of methanolic extracts of *B. dalzielii* (stembark and leaf) and *E. chlorantha* (stem bark) were evaluated on their effects on biochemical parameters such as Aspartate amino transaminase (ASAT), Alanine amino transaminase (ALAT),Alkaline Phosphatase (ALP), total proteins, albumin, total bilirubin and conjugated bilirubin in serum.

For the antiviral assay, methanolic extracts of *B. dalzielii* (Stem bark and leaf) and *E. chlorantha* (Stem bark) at final concentrations of 250, 200, 150, 100 and 50mg/ml in that order were evaluated for their antiviral activity against New Castle Disease virus (NDV) in

nine day old embryonated chicken eggs . Allantoic fluids from treated eggs were tested for spot haemagglutination test to detect NDV in the eggs.

6.2 Conclusion

The result of the ethnobotanical study revealed that there is high knowledge on the use of medicinal plants in Jos, Plateau state. This study has revealed for the first time medicinal plant with the potential to treat or prevent viral infections. The information from this study can serve as criteria in finding new antiviral agents from plants.

The Pharmacognostic evaluation *B. dalzielii* and *E. chlorantha* showed characteristic features that can help in proper identification and standardization.

The phytochemical screening *B. dalzielii and E. chlorantha* revealed the presence of secondary metabolites that may be responsible for the pharmacological actions of the plants

The biochemical studies of the hepatoprotective assay of methanol extract of *B. dalzielii* and *E. chlorantha* show that the plants do not possess hepatoprotective property.

The antiviral assay of the methanolic extract of *B. dalzielii* and *E. chlorantha* on nine day old embryonated chicken egg showed that both plants possess antiviral activity, particularly at lower concentrations , as higher concentration were found to be toxic to the embryo .

6.3 Recommendation

There is need for ethnobotanical survey in every state of the nation on medicinal plants used in treatment of viral infection. In order to preserve knowledge on medicinal plants and to update existing information.

Most of the medicinal plants in used in plateau state are from the wild, there is need to encourage and enforce cultivation of medicinal plants, so as reduce exploitation of plants growing in the wild, otherwise, extinction of useful medicinal plants.

Traditional medicine is relatively cheap, its raw materials are readily available, it is a potential source of new drugs and of course, a source of cheap starting products for the synthesis of known drugs. Hence, the sale and use of medicinal preparations should be encouraged and supported by the government

Since viral infection is one of the world most transmissible diseases , there is need for both private and public organizations to invest in researches that will lead to discovery of new antiviral compounds that are safe, effective and less toxic, particularly from plants. The information on medicinal plants gathered during this study are based on claims by the TMP. Hence, researchers need to carry out investigations on these plants, so as to ascertain the claims. In addition, more research should be carried out in structural elucidation of the secondary metabolites present in the plants.

REFERENCES

Acharya, D. and Shrivastava, A. **(2008).** *Indigenous Herbal Medicines: Tribal Formulations and Traditional Herbal Practices,* Aavishkar Publishers Distributors, Jaipur –India. ISBN 978-81-7910-252-7. Pp 440.

Adebiyi, O. E and Abatan, M. O**. (2013).**Protective Effects of *Enantia chlorantha* Stem Bark Extracts on Acetaminophen Induced Liver Damage in Rats. *Jordan Journal of Biological Sciences*. 6(4), 284 - 290

Adesokan, A. A., Yakubu, M. T., Owoyele, B. V., Akanji, M. A., Soladoye, A. O. and Lawal,O. K. **(2008).** Effect of administration of aqueous and ethanolic extracts of Enantia chlorantha stem bark on brewer's yeast-induced pyresis in rats. *African Journal of Biochemistry Research* 2 (7),165-169.

Barbour, K. M., Oyintoyinbo, J. S., Onyemelukwe, J. O. and Nwafor, J. C. **(1982).** *Nigeria in maps*. Hodder and Stoughton, London. pp 143- 148.

Bedoya, L. M., Bermejo, P. and Abad, M. J.**(2009).** Anti-infectious activity in the Cistaceae family in the Iberian Peninsula. *Mini Reviews in Medicinal Chemistry*, 9(5), 519-525.

Bera,T. K., Chatterjee, K., Jana, K., Ali, K. M., Maiti, S. and Ghosh, D**.(2012).** Antihepatotoxic effect of "Livshis," a polyherbal formulation against carbon tetrachloride- induced hepatotoxicity in male albino rat. *Journal of Natural Pharmaceuticals.* 3(1),17-24.

Brattin, W. J., Glende, E. A. Jr. and Recknagel, R. O. **(1985)**. Pathological mechanism in carbon tetrachloride hepatotoxicity. *Journal of Free Radicals in Biology and Medicine* 1, 27-38.

Burkill, H. M. **(1985)**. *The Useful Plants of West Tropical Africa.* Royal Botanical Garden, Kew.

Bussmann, R. W. and Sharon, D. **(2006).** Traditional medicinal plant use in Loja province, southern Ecuador. *Journal of Ethnobiology and Ethnomedicine* 2,doi:10.1186/1746-4269-2-44.

Cecil, C. E., Davis, J. M., Cech, N. B. and Laster S. M. **(2011).** Inhibition of H1N1 influenza A virus growth and induction of inflammatory mediators by the isoquinoline alkaloid berberine and extracts of goldenseal (Hydrastis Canadensis). *International Journal of Immunopharmacology*. Pp1706-1714.

Chollom, S. C., Agada, G. O. A., Bot, D. Y., Okolo , M. O., Dantong, D. D., Choji, T. P., Echeonwu. B. C., Bigwan, E. I., Lokason, S. and Banwat, E. **(2012)**. Phytochemical Analysis and Antiviral potential of aqueous leaf extract of Psidium guajava against Newcastle disease virus in ovo. *Journal of Applied Pharmaceutical Sciences* ; 2(10), 045-049.

Choudhary, K., Singh, M. and Pillai, U. **(2008)**. Ethnobotanical survey of Rajasthan- An Update. *American. –Eurasian Journal Botany*, 1(2), 38-45.

Clarke, E. G. C. and Clarke, M.L. **(1979)**. Veterinary Toxicology. 1st Edition. Bailiere Tindall Ltd. London.p10.

Cox P.A.and Balick M. **(1994)**. "The ethnobotanical approach to drug discovery" Scientific American, 270, 82-87.

Etuk, E. U., Agaie, B. M., Onyeyilli, P. A. and Ottah, C. U. **(2006)**. Toxicological studies of aqueous stem bark extract of *Boswellia dalzielli* in albino rats. *Indian Journal of Pharmacology* 38, 359-360.

Evans, W. C. **(2009)**. '*Trease and Evans' Pharmacognosy*. Fourteenth Edition. W. B. Saunders Company Ltd. London.pp119-120; 194-227&343.

Fasola, T. R., Adeyemo, F. A., Adeniji, J. A.and Okonko, I.O. **(2011)**. Antiviral Potentials of *Enantia chlorantha* Extracts on Yellow Fever Virus .*Nature & Science*, 9(9), 99.

Giday, M., Asfaw, Z., Elmqvist, T. and Woldu, Z. **(2003)**. An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia. *Journal of Ethnopharmacology*, 85, 43-52.

Gill, L.S. **(1992)**. *Ethnomedical methods in Nigeria*. Uniben Press, Benin, Nigeria. p 143

Glenz, K. and Warzecha, H. **(2006)**. New medicinal plants for the production of vaccines. *Journal of Consumer Protection and Food Safety*. 1,126-130.

Gurib-Fakim A **(2006)**. Medicinal plants: Traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*., 27, 1-93.

Harborne, J. B. **(1991)**. *Phytochemical Method: A guide to modern techniques of plants analysis* (2nd edition). Chapam and Hall press, London.

Hassan, H.S., Musa, M., Usman, M.A. and Abdulaziz, M. **(2009)**. Preliminary Phytochemical and Antispasmodic studies of the stem bark of *Boswellia dalzielli. Nigerian Journal of Pharmaceutical Sciences.*8(1), 1-6.

Hunde, D., Asfaw, Z., and Kelbessa E. **(2004)**. Use and management of ethnoveterinary medicinal plants by indigenous people in 'Boosat', Wolenchiti area . *Ethiopian Journal of Biological Sciences,* 3(2), 113-132.

Hutchinson, J. and Dalziel, J. M. **(1963)**. *Flora of West Tropical Africa*. Vol. II. Crown Agents for Oversea Governments and Administrations. Milbank, London S. W.

Hussein, G., Miyashiro, H., Nakamura, N., Hattori, M., Kakiuchi, N. and Shimotohno, K. **(2000)**. Inhibitory effects of Sudanese medicinal plant extracts on hepatitis C virus (HCV) protease. *Phytotheraphy Research.*13(1), 31-36.

Ibezim, E. C., Abonyi, D. O., Adikwu, M. U. and Esimone, C. O.**(2009)**. Plants as sources of antiviral agents..*African Journal of Biotechnology*. 8(17), 3989-3994.

Igoli, J. O., Ogaji O. G., Tor-Anyiin, T. A. and Igoli, N. P. **(2005)**. Traditional medicine practice amongst the Igede people of Nigeria. Part II. *African Journal of Traditional , Complementary and Alternative Medicine.* 2, 134-152.

Isichei, E. **(1982)**. *Introduction. In Studies in the History of Plateau State, Nigeria*, ed. by Elizabeth Isichei, Macmillan, London. pp 1–57.

Iwu, M. **(1993)**. *Handbook of African medicinal plants*. CRC Press, Boca. Raton, FL.

Jacob, J.R., Korba, B.E., You, J.E., Tennant, B.C. and Kim.Y. **(2004)**. Korean Medicinal Plants extracts exhibit Antiviral potency against Viral Hepatitis. *Journal of Alternative and Complementary Medicine*, 10(6), 1019- 1026.

Jawetz, E., Melnick, J.L. and Adelberg, E. A. **(2007)** *Medical Microbiology*, 24th Edition, The McGraw-Hill Companies, Inc. ISBN-13: 978-0-07147666-9.

Jiaoyang, L. Dan, Y., Meihua, Y., Xiaoping, D. and Xiaohe, X. **(2013)**. Multicomponent Therapuetics of Berberine Alkaloids. *Evidence- Based Complementary and Alternative medicine.* pp

Kim J.Y. **(2007)**. *Ethno botany. The green world.* Chelsea House, Infobase Publishing, Newyork.pp 1-2.

Lorke, D **(1983)**. A New Approacrch to Practical Acute Toxicity Testing. *Archives of Toxicology*, 54 (4), 275-287.

Manisha, Y. And Khalid, K.K. **(2012)**. Study of Ethnomedicinal Plants Used in The Treatment of Viral Hepatitis, Rewa District, Madhya Pradesh. *Indian Journal of Scientific Research.* 3 (1), 149-151.

Martin, A. C. **(2012)**. *Clinical Biochemistry and Metabolic Medicine.* Hodder and Stoughton Ltd, London.18th edition. Pp 253-260.

Mukhtar, M., Arshad, M., Ahmad, M., Pomerantz, R.J., Wigdahl, B. and Parveen Z. **(2008)**. Review of antiviral potentials of medicinal plants. *Virus Research* 131, 111-120.

Murakawa, Y., Sakaguchi, K., Soejima, K., Eriguchi, S., Takase, K., Sueyoshi, M., Nagatomo, H., Ito, T. and Otuski, K. **(2003)**. Heamgglutinating activity of the lentogenic New Castle disease virus strain MET95. *Avian Pathology*. 32 (1), 39-45.

Musa, M. S., Abdelrasool, F. E., Elsheikh, E. A., Ahmed, L. A. M. N., Mahmoud, A. L. E. and Yagi, S. M. **(2011**). Ethnobotanical study of medicinal plants in the Blue Nile State, South-eastern Sudan. *Journal of Medicinal Plants Research* 5 (17), 4287-4297.

Nelson-Harrison, S.T., King S. R., Limbach, C., Jackson, C., Galiwango, A.,Kato, S. K. and Kanyerezi, B. R. **(2002**). Ethnobotanical research into the 21st century. In: Iwu

M.M., Wootton JC (Eds.), *Ethnomedicinal Drug Discovery Elsevier*, Amsterdam.pp. 283-307.

Ngono Ngare, R. A., Koanga Mogtomo, M. L., Tchinda Tiabou A., Magnifouet Nana, H., Motso Chieffo, P. R., Mballa Bounou Z., Ebelle Etame, R. M., Ndifor, F., Biyiti, L. And Amvan Zollo, P. H. **(2011)**. Ethnobotanical survey of some Cameroonian plants used for the treatment of viral disease. *African Journal of Plant Science.* 5 (1), 15- 21.

Odeghe, O. B., Onoriose, D. A. , Uwakwe, A. A., and Monago C. C. **(2012)**. Hepatoprotective effect of methanolic leaf extract of *Boswellia dalzielii* hutch on carbon tetrachloride induced hepatotoxicity in wistar rats. *Indian Journal of Medicine and Healthcare*.1 (3), 54-63.

Odoh,U. E., Okwor, I. V. and Ezejiofor, M. **(2010)**. Phytochemical, trypanocidal and anti-microbial studies of *Enantia chlorantha* (Annonaceae) root. *Journal of Pharmaceutical and Allied Sciences.* 7 (4).pp

Ogbole O. O., Gbolade A. and Ajaiyeoba E. O. **(2010)**. Ethnobotanical Survey Of Plants Used In The Treatment Of Inflammatory Diseases In Ogun State Of Nigeria. *European Journal of Scientific Research.* 43 (2), 183-191.

Ojo, O. O., Oluyege, J. O.and Famurewa, O. **(2009)**. Antiviral properties of two Nigerian Plants. *African Journal of plant Science.* 3(7), 157-159.

Oladunmoye, M. K. and Kehinde, F. Y. **(2011)**. Ethnobotanical survey of medicinal plants used in treating viral infections among Yoruba tribe of South Western Nigeria. *African Journal of Microbiology Research* . 5(19), 2991-3004.

Olukemi, M. A., Kandakai-Olukemi, Y. T. and Mawak, J. D. **(2005)**. Antibacterial activity of the stem bark of *Boswellia dalzielli*. *Journal of Pharmacy and Bioresources*, 2(2), 131 – 136.

Plateau State Government **(1991)**. A Unique Economic Centre, 17. 38-39.

Plateau State Government **(2007)**. Background Information-History and Development http://www. plateaustategov.org/historical/geo-info: htm retrieved on August 7, 2009.

Pradeep, H. A., Khan, S., Ravikumar, K., Ahmed, M. F., Roa, M. S., Kiranmai, M., Reddy, D. S., Ahamed, S. R. and Ibrahim, M. **(2009)**. Hepatoprotective evaluation of *Anogeissus latifolia*: In vitro and invivo studies. *World Journal of Gastroenterology.*15 (38), 4816-4822

Priyanka, P. S., Patel, M. M. and Bhavsar, C. J. **(2010)**. Comparative Antidiabetic Activity of some Herbal Plants Extracts. *International Journal of Pharmaceutical Sciences.*1 (1), 12-19.

Raj, V. P., Chandrasekhar,R. H., Vijayan, P., Dhanaraj, S. A., Rao, M. C., Rao,V. J. and Nitesh, K. **(2010).** *Invitro* and *invivo* hepatoprptective effects of the total alkaloid fraction of *Hygrophila auriculata* leaves. *Indian Journal of Pharmacology.* 42, 99-104.

Rajesh, K. P. and Manoj, J. **(2011).** Hepatoprotective activity of Alcoholic and Aqeous extracts of fruits of *Luffa cylindrica* Linn. in rats. *annals of Biological Research* . 2 (1), 132

Reed, L. J. and Muench, H. (1938). A Simple Method of Estimating Fifty Percent End Points. American Journal of Hygiene (27), 403

Sonibare, M. A., Moody, J. O and Adesanya, E. O. **(2009**). Use of medicinal plants for the treatment of measles in Nigeria. *Journal of Ethnopharmacology* . 122(2), 268-272.

Sofowora, A. **(2008).** *Medicinal Plants and Traditional Medicine in Africa,* Spectrum Books Limited, Ibadan, Nigeria. 3rd edition. Pp 70-74; 175-180; 201-202.

Sood ,S. K., Nath,R. and Kalia, D. C. **(2001).** *Ethnobotany of cold desert tribes of Lahoul-Spiti (North West Himalaya*).Deep Publications, New Delhi.

Tan, P. V., Boda, M., Enow-Orock, G. E., Francis-Xavier, E. T. O. A. and Bitolog, P**.(2007).** Acute and Sub-acute Toxicity profile of the aqueous stembark extract of Enantia chlorantha Oliv. (Annonaceae) in Laboratory animals. *Pharmacology online* 1,304-313

Tesfaye, H. B., Sebsebe, D. W. and Zemede, A. W. **(2009).** 'An Ethnobotanical study of medicinal plants used by local people in the lowlands of Konta Special Woreda, southern nations, nationalities and peoples regional state, Ethiopia'. Addia Ababa University, *Journal of Ethnobiology and Ethnomedicine*, 5, 26.

Thomas, S., Patil, D. A., Patil, A. G. and Naresh, C. **(2008).** Pharmacognostic Evaluation And Physicochemical Analysis Of *Averrhoa Carambola* L. Fruit. *Journal of Herbal Medicine and Toxicology* 2 (2), 51-54.

Tolo, F. M., Rukunga, G. M., Muli, F. W., Njagi, E. N., Njue, W., Kumon, K.,Mun gai, G. M., Muthaura, C. N., Muli, J. M., Keter, L. K., Oishi, E. and Kofi- Tsekpo, M. W. **(2006**). Antiviral activity of the extracts of a Kenyan medicinal plant Carrisa edulis against herpes simplex virus. *Journal of Ethnopharmacology*. 104(1/2), 92-99.

Uraih, N. and Izuagbe, Y. **(1990).** *Public Health, Food and Industrial Microbiology.*Uniben Press, Nigeria. ISBN: 978-2027-00-6.

White, D. O. and Fenner, F. J**. (1994).** *Medical Viriology* .Academic Press Limited. ISBN 0- 12-746642-8, pg 24-25

WHO **(1983).** Viral vaccines and antiviral drugs : Report of a scientific group of WHO, Geneva, 693, 5-38

WHO **(1998).** Quality Control Methods for Medicinal Plants Materials. ISBN 92 4 154510 0 . Pp 6-16, 28-30

WHO **(2008).** "Fact Sheet No 134.Traditonal medicine" . World Health Organization. 2008-12-01. Retrieved 2012-11-20.

Voeks R. A. and Leony, A. **(2004).** Forgetting the forest: assessing medicinal plant erosion in eastern Brazil. *Economic Botany*, 58, 294-306.

Young, M., Alders, R., Grimes, S., Spradbrow, P., Dias, P., da Silva, A. and Lobo, Q. **(2002).** Controlling Newcastle disease in village village chickens: A laboratory manual ACIAR monograph 87. 142pp

APPENDIX I

ETHNOBOTANICAL SURVEY SHEET (Questionnaire)

FOR THE RESEARCH PROGRAMME ON

SCREENING OF MEDICINAL PLANTS USED IN TRADITIONAL TREATMENT OF VIRAL INFECTIONS IN JOS, PLATEAU STATE, NIGERIA.

Department of Pharmacognosy and Drug Development
Faculty of Pharmaceutical Sciences, ABU – Zaria.

Sheet Number----------

Date----------

LGA----------

Demographic information

Traditional healer/Herb seller/ Informant:
Age ----------
Sex ----------
Address ----------
Duration of practice ----------
Tribe ----------

B)Plant identity/Preparation/Route of Administration and Recipes

	COMMON COLD	MEASLES	CHICKENPOX	RABIES	BIRDFLU	HEPATITIS	HIV
PLANT, ANIMAL OR MINERAL							
VERNACULAR NAMES							
PART USED							
ROUTE OF ADMINSTRATION							

RECIPES

S/NO	DISEASES	RECIPES
1	CARRTARH	
2	MEASLES	
3	CHICKENPOX	
4	RABIES	

5	BIRDFLU	
6	HEPATITIS	
7	HIV	

Are there incantations or prayers needed? (Yes or No). If Yes, write down the incantation or prayers at the back of the sheet.

Any possible reason(s) why two or more plants or plant parts are used in combination with other ingredients, to effect cure?----------
---

C) Preparation for medicinal use. (Please tick the appropriate box)

Disease	Powdered	Cold Water Extraction	Hot Water Extraction	Boiled	Others
Common Cold					
Measles					
Chickenpox					
Rabies					
Birdflu					
Hepatitis					
Hiv					

Any other remark (please indicate below special information indicated by donor of information---------- ----------
---

D) Herbarium

a) In which herbarium has a specimen of the plants been deposited? (Give address below) ----------
---
---

b) Number given to specimen in herbarium

DISEASE	PLANTS	VOUCHER NUMBER
COMMON COLD		
MEASLES		
CHICKENPOX		
RABIES		
BIRDFLU		
HEPATITIS		
HIV		

c) Name and address given of botanist who identified the plant----------
---
-----

d) Name and address of taxonomist who confirmed plant identity in an international herbarium----------
---

APPENDIX II

IMAGES OF MICROSCOPICAL EXAMINATION OF *ENANTIA CHLORANTHA*

STEM BARK POWDER

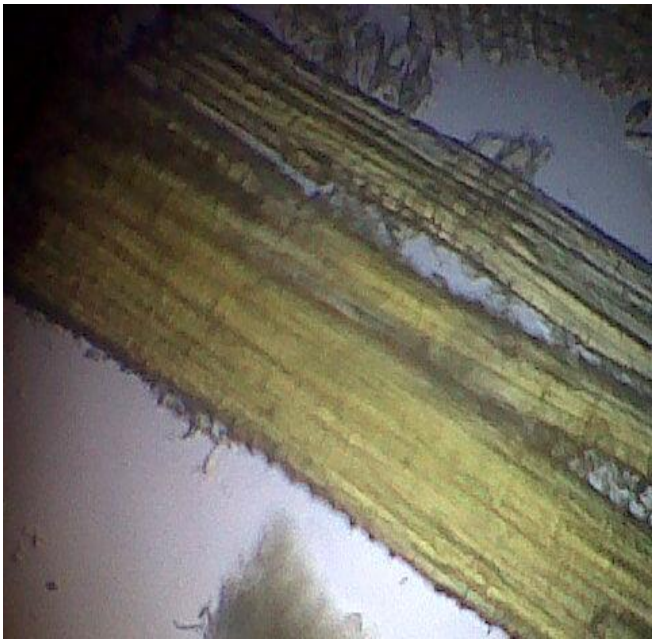


Plate XIX: Bundles of fibres

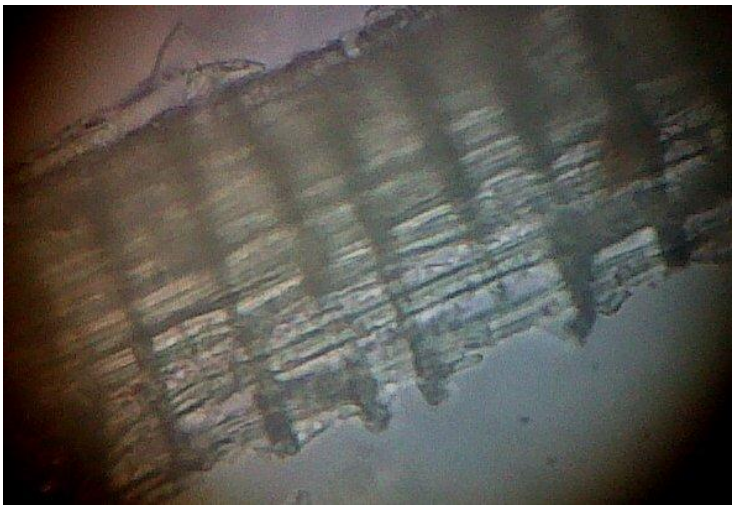


Plate XX: Medullary rays crossing phloem fibre



Plate XXI: Phloem parenchyma



Plate XXII: Scleried



Plate XXIII: starch grain

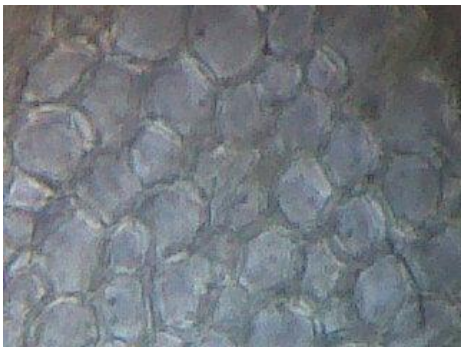


Plate XXIV:cork cells

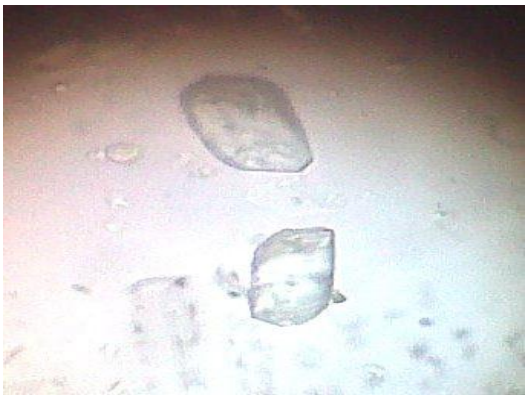


Plate XXV: prisms of calcium oxalate crystal

APPENDIX III

IMAGES OF EQUIPMENTS USED FOR ANTIVIRAL ASSAY



Plate XXVI: Safety Cabinet

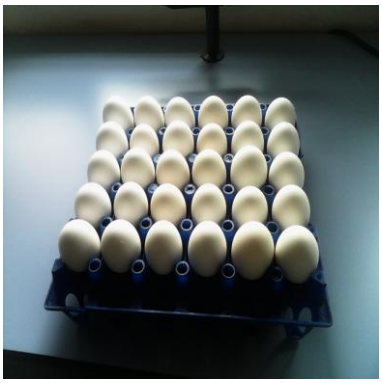


Plate XXVII A crate of embryonated eggs





Plate XXVIII An incubator

Plate XXIX A stand of pipettes

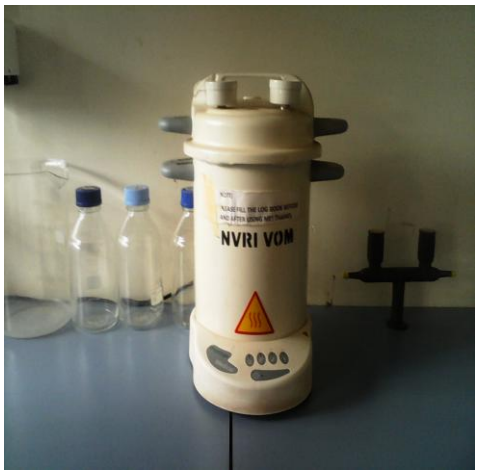


Plate XXX An autoclave



Plate XXXI Laboratory



Plate XXXII A candler



Plate XXXIII A centrifuge