

**ETHNOBOTANICAL SURVEY OF PLANTS
USED FOR THE TREATMENT OF TYPHOID
FEVER IN MINNA, NIGER STATE, AND
EVALUATION OF ANTI-TYPHOID ACTIVITY
OF TERMINALIA AVICENNOIDES GUILL &
PERR. (COMBRETACEAE).**

By

HALIMAT ABDULSALAMI

**DEPARTMENT OF PHARMACOGNOSY AND
DRUG DEVELOPMENT,**

**AHMADU BELLO UNIVERSITY, ZARIA.
NIGERIA.**

AUGUST, 2014

**ETHNOMEDICINAL SURVEY OF PLANTS USED FOR THE
TREATMENT OF TYPHOID FEVER IN MINNA, NIGER STATE
AND EVALUATION OF ANTI-TYPHOID ACTIVITY OF
TERMINALIA AVICENNOIDES GUILL & PERR
(COMBRETACEAE).**

By

Halimat ABDULSALAMI

MSc/PharmSci/1258/2010-2011

A THESIS SUBMITTED TO THE SCHOOL OF POSTGRADUATE
STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
AWARD OF A
MASTER DEGREE IN PHARMACOGNOSY

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

August, 2014

DECLARATION

I hereby declare that the work in this thesis titled “Ethnomedicinal survey of plants used for the treatment of typhoid fever in Minna, Niger state and evaluation of anti-typhoid activity of *Terminalia avicennoides* Guill & Perr (combretaceae)” was performed by me in the Department of Pharmacognosy and Drug Development of the Ahmadu Bello University, Zaria under the supervision of Dr. Abdulkarim Agunu and Prof (Mrs.) N.Ilyas.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

Name of student

Signature

Date

CERTIFICATION

This thesis entitled “ETHNOMEDICINAL SURVEY OF PLANTS USED FOR THE TREATMENT OF TYPHOID FEVER IN MINNA, NIGER STATE AND EVALUATION OF ANTI-TYPHOID ACTIVITY OF TERMINALIA AVICENNOIDES GUILL & PERR (COMBRETACEAE)” by Halimat ABDULSALAMI meets the regulations governing the award of the degree of Masters of Science in Pharmacognosy of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

_____ Dr. Abdulkarim Agunu, B.Pharm, M.Sc, PhD

Date

Chairman, Supervisory committee

_____ Prof (Mrs.) N.Ilyas, B.Sc, M.Sc, PhD

Date

Member, Supervisory committee

Dr .G. Ibrahim, B.Sc, M.Sc, PhD

Date

Head, Department of Pharmacognosy and Drug Development

Prof.A.A.Joshua, B.A, M.A, PhD

Date

Dean, School of Postgraduate studies

DEDICATION

To

Almighty Allah for preserving my soul and seeing me all through the course of my studies.

Also, to my late lovely daughter, Fatima Zahra for being there at some point in my life.

ACKNOWLEDGMENT

All Praise and Glory be to Allah, Lord of the Universe who taught men what they knew not, for his guidance and for making the completion of this research work a reality.

My enormous appreciation goes to my supervisor Dr. Abdulkarim Agunu, for his effective Supervision, constructive criticism and proficient suggestion during this Research. Also, the contribution of my second Supervisor, Prof (Mrs.) N. Ilyas is greatly appreciated for her valuable advice and meticulously reading the entire Manuscript.

My sincere gratitude goes to the Laboratory technologists; Mal. Kabir, Mal. Kamilu, and Mal Mustapha of the Department of Pharmacognosy and Drug Development, also to Mr. Ezekiel of the Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

To the Taxonomists of the Department of Biological Sciences, Faculty of Sciences, Ahmadu Bello University, Zaria. I really appreciate your effort.

I equally wish to thank Dr. I.C.J Omalu, the Head of Department of Biological Sciences, F.U.T Minna and the Management of Federal University of Technology Minna, Niger state.

To my Parents, Alhaji Ibrahim Abdulsalami and Hajia Risikatu Abdulsalami for your encouragement and for always being there for me.

To my Husband, Mal. Abdulwaheed Dauda, words alone cannot express how appreciative I am even if they were as big as the Ocean. Thanks for your encouragement and for being patient and understanding. I will always Love You.

Special thanks to Alhaji Adamu Usman and his Family, my siblings and all my Friends.

ABSTRACT

An ethnobotanical survey was conducted from December 2011 to April 2012 in Minna, Niger State, Nigeria. The survey was aimed at identifying and documenting medicinal plants used for the treatment of typhoid fever in 6 communities in Minna, Niger State and establishing resourceful information on the pharmacognostic, phytochemical screening and anti- salmonella activity on the most frequently used plant. Ethnobotanical data were collected with the aid of a tape recorder and supported with a structured questionnaire during the interview interactive session with the Traditional medical practitioners. The survey revealed a total of 49 plants species belonging to 31 families and the most frequently used plant was *Terminalia avicennioides* Guill and Perr. The plant habit and habitat of collection showed that 63.34% of the medicinal plant species grow up to the height of a tree and are mostly found in the wild (50%). The survey also revealed that the leaves were the major parts used for herbal preparation. The main methods of preparation are decoction and infusion. The microscopic evaluation carried out on the leaves of *T. avicennioides* revealed: anomocytic stomata on both upper and lower surface, covering trichomes, rosette calcium oxalate crystals, phloem fibres, annular and spiral xylem vessels. The chemomicroscopy indicated the presence of cellulose, lignin, starch, calcium oxalate crystals, fat and tannins. The phytochemical screening carried out on the methanol extract of the leaves of the plant using thin layer chromatography (TLC) showed that phenolic compounds and terpenes were present and alkaloid absent. The anti-typhoid activity of *T. avicennioides* extract against *Salmonella typhi* collected from Ahmadu Bello University (ABUTH) Shika, Zaria showed significant inhibitory activity against *Salmonella typhi* with the methanolic extract (Defatted) showing the highest zone of inhibition (31mm). The Minimum Inhibitory Concentration (MIC) value obtained using

the Broth dilution method was 6.25mg/ml while Minimum Bactericidal Concentration (MBC) was 12.5mg/ml. It can be concluded that the leaves of *Terminalia avicennioides* possess anti- typhoid activity.

TABLE OF CONTENTS

Title page -----	i
Declaration-----	ii
Certification -----	iii
Dedication -----	iv
Acknowledgement-----	v
Abstract -----	vi-vii
Table of Contents -----	viii-
xii	
List of Tables -----	xiii-
xvi	
List of Figures -----	xv
List of Plates -----	xvi-
xvii	
List of Abbreviations -----	xviii

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study -----	1
1.2 Ethnobotany of Medicinal Plants used for the treatment of Typhoid fever in Minna, Niger State. -----	3

1.2.1	History and Location -----	3
1.2.2	Ethnic Composition, Languages, Culture and the Arts -----	6
1.2.3	Economy and Occupation -----	6
1.3	Statement of research problem -----	7
1.4	Justification for the study -----	7
1.5	Specific objectives -----	8
1.6	Statement of research hypothesis -----	8

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1	Ethnobotanical survey -----	9
2.2	Integrating modern and traditional medicine -----	11
2.2.1	Biodiversity and sustainability -----	13
2.2.2	New techniques for old treatments -----	15
2.3	Role of Ethnopharmacology in the treatment of Typhoid fever -----	16
2.4	<i>Terminalia avicennoides</i> -----	18

CHAPTER THREE

3.0	MATERIALS AND METHODS	19
3.1	Study Design/Study Population	21
3.2	Sampling Techniques Employed	21
3.3	Method of Data Selection/Collection	21
3.3.1	Data Collection Procedure	22
3.4	Material and Instruments	23
3.5	Plants Collection and Herbarium Preparation	23
3.5.1	Data Analysis	24
3.6	Equipments/Apparatus and Solvents/Reagents	24
3.6.1	Equipments/Apparatus	25
3.6.2	Solvents/Reagent	26
3.7	Microscopic Evaluation of <i>T. avicennioides</i>	27
3.7.1	Preparation of Transverse and Longitudinal Section	27
3.8	Quantitative Microscopy	28
3.8.1	Determination of Stomatal Number and Stomatal Index	28
3.8.2	Determination of vein-islet and vein let termination numbers	29
3.8.3	Determination of Palisade Ratio	30
3.9	Chemomicroscopy of Powdered Drugs	30
3.10	Physicochemical Evaluation of Powdered Drug	31
3.10.1	Determination of Moisture Content	31
3.10.2	Determination of Total ash Values	32
3.10.3	Determination of Acid-Insoluble Ash	32

3.10.4	Determination of water soluble Ash -----	33
3.10.5	Alcohol-soluble Extractive -----	33
3.10.6	Water Soluble Extractive -----	33
3.12	Extraction of the leaves of <i>T. avicennioides</i> -----	34
3.13	Phytochemical screening of the leaves of <i>T. avicennioides</i> -----	34
3.14	Test Organism -----	35
3.14.1	Antimicrobial Susceptibility Tests -----	35
3.14.2	Determination of Minimum Inhibitory Concentration (MIC) -----	36
3.14.3	Determination of Minimum Bactericidal Concentration (MBC) -----	37

CHAPTER FOUR

4.0 RESULTS

4.1	Demography/Personal Information on Respondents -----	38
4.2	Medicinal Plants Used in Treatment of Typhoid Fever in Minna, Niger State ---	39
4.3	Medicinal Plants and their Parts used for the Treatment of Typhoid Fever -----	48
4.4	Medicinal Plants used for the Treatment of Typhoid Fever mentioned by three or more informants -----	52
4.5	Some of the surveyed Plants Images -----	54
4.6	Some Traditional Medicinal Practioners Recipes used for the Treatment of Typhoid Fever in Minna, Niger State -----	54
4.7	Microscopic Evaluation -----	64
4.8	Quantitative Leaf Microscopy of the Leaves of <i>T. avicennioides</i> -----	66

4.9	Chemomicroscopic Evaluation of the Leaves of <i>T. avicennioides</i> -----	68
4.10	Physicochemical Determination of <i>T. avicennioides</i> -----	69
4.11	Chromatographic analysis of the leaves of <i>T. avicennioides</i> -----	70
4.12	Anti-salmonella studies on the leaves of <i>T. avicennioides</i> -----	73
4.13	Minimum Inhibitory Concentration (MIC) -----	76
4.14	Minimum Bactericidal Concentration (MBC) -----	77
CHAPTER FIVE		
5.0	DISCUSSIONS -----	78
CHAPTER SIX		
6.0	SUMMARY, CONCLUSION AND RECOMMENDATION	
6.1	Summary -----	84
6.2	Conclusion -----	85
6.3	Recommendations -----	85
REFERENCES -----		87
APPENDICES -----		98

LIST OF TABLES

Table	Page
2.1: Selected modern drugs that come from traditional medicine	12
2.2: Key differences between Traditional and Modern medicine	14
2.3: How to create modern drugs from traditional compounds.	15
4.2: Medicinal Plants used in the Treatment of Typhoid Fever in Minna, Niger State.	40
4.3 : Medicinal Plants and their Classified habits/habitats	49
4.4: Medicinal Plants used for the treatment of typhoid Fever mentioned By three or more informants	52
4.5: Some Recipes used for the Treatment of Typhoid Fever in Minna, Niger state.	63
4.6: Quantitative Leaf Microscopy of the Leaves of <i>T. avicennioides</i> 70	
4.7: Physicochemical Determination of <i>T. avicennioides</i>	69
4.8: Chromatographic Resolution of Methanolic Extract of <i>T. avicennioides</i>	73
4.9: Zone of Inhibition (mm) at Different Concentration of Plant Extract	74
4.10: Minimum Inhibitory Concentration (MIC) of plant (<i>T. avicennioides</i>) extract	76
4.11: Minimum Bactericidal Concentration (MBC) of plant (<i>T. avicennioides</i>) extract	77

LIST OF FIGURES

Figure

Page

1.1: Map of Niger State showing Minna

5

4.2: Some microscopic diagnostic features from the Powdered Leaves

65

of *T.avicennioides* ; x400

LIST OF PLATES

Plates

Page

I : <i>Alchornea cordifolia</i>	-----	54
II : <i>Anogeissus leiocarpus</i>	-----	54
III : <i>Calotropis procera</i>	-----	55
IV : <i>Daniellia oliveri</i>	-----	55
V : <i>Erythrina senegalensis</i>	-----	56
VI : <i>Ficus platyphylla</i>	-----	56
VII : <i>Grewia mollis</i> Juss.	-----	57
VIII : <i>Khaya senegalensis</i>	-----	57
IX : <i>Nauclea latifolia</i>	-----	58
X : <i>Piliostigma thonningii</i>	-----	58
XI : <i>Plumera rubra</i>	-----	59
XII : <i>Senna occidentalis</i>	-----	59
XIII : <i>Terminalia avicennioides</i>	-----	60
XIV : <i>Vitex doniana</i>	-----	60
XV : <i>Ximenia americana</i>	-----	61
XVI: Developed Chromatogram of Methanolic Extract of <i>T. avicennioides</i>		

Leaves after spraying with 10% sulphuric acid. -----	74
XVII: TLC Profile of Methanolic Extract of <i>T. avicennioides</i> -----	75
XVIII: Agar Plate showing zones of inhibition at different concentration of plant extract (Methanolic fraction) -----	78

LIST OF ABBREVIATIONS

gm - gram

TLC - Thin layer chromatography

Rf - Retention factor

MIC - Minimum inhibitory concentration

MBC - Minimum bactericidal concentration

MHA - Mueller Hinton Agar

SEM - Standard error of mean

WHO - World health organisation

CAM - Complementary and alternative medicine

% - Percentage

CDC - Center for disease control and prevention

LGA - Local government areas

TMPs - Traditional medical practitioners

CHAPTER ONE

1.0

INTRODUCTION

1.1 Background of the Study

Plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well-being (Iwu *et al.*,1999). The knowledge of traditional medicine is a custom which has preoccupied mankind since his creation from generation to generation to maintain health and wellbeing. According to the World Health Organisation (WHO, 2005) the definition of traditional medicine may be summarized as the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used in the maintenance of health and in the prevention, diagnosis, improvement or treatment of physical and mental illness. Traditional medicine covers a wide variety of therapies and practices which vary from

country to country and region to region. It is referred to as alternative or complementary medicine (CAM). In Africa, Up to 80% of the population uses traditional medicine for primary health care.

In China, traditional herbal preparations account for 30-50% of the total medicine consumption whilst in Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children with high fever resulting from malaria is the use of herbal medicine at home (WHO, 2003).

In Nigeria traditional medicine is well acknowledged and established as a viable profession (Kafaru, 1994), and almost all plants seem to have some kind of application in traditional medicine (Babayi *et al.*, 2004).

Searches for substances with antimicrobial activity in plants are frequent, due to their popular use as remedies for many infectious diseases (Shibata *et al.*, 2005).

Plants are rich in different types of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties (Cowan, 1999; Lewis and Ausubel, 2006).

Typhoid fever is an acute illness associated with fever that is most often caused by the bacteria *Salmonella typhi*. It can also be caused by *Salmonella paratyphi*, a related bacterium that usually leads to a less severe illness. It is known as an enteric fever transmitted through ingestion of food or drink that is contaminated by the faeces or urine of infected persons. Each year the disease affects at least 16 million persons world-wide, most of whom resides in the developing countries of Southeast Asia and Africa (CDC, 2003). Typhoid fever is uncommon in industrialized regions such as the USA, Canada, Europe, Australia and Japan and new cases of the disease in these countries are related to

travel to the developing countries (Papadimitropoulos *et al.*, 2004). Mortality rates associated with typhoid fever vary from region to region, with highest reported from Indonesia, Nigeria and India (Miller *et al.*, 1994).

Some variants of *Salmonella* have developed multidrug-resistance as an integral part of the genetic material of the organism, and are therefore likely to retain their drug-resistant genes even when antimicrobial drugs are no longer used, a situation where other resistant strains would typically lose their resistance (CDC, 2006).

Consequently, the development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants (Erdogrul, 2002). Today, it is estimated that plant materials are present in or have provided the models relatively for 50% Western drugs (Rodders *et al.*, 1996).

Traditional medical practitioners in Nigeria use herbal preparations to treat microbial infections such as typhoid and para-typhoid infections and they claimed that the primary benefit of using plant –derived medicines is that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments.

1.2 Ethnobotany of Medicinal Plants used for the treatment of Typhoid fever in Minna, Niger State.

The phytogeography of a particular region needs to be considered in order to study the ethnobotany (Gilmore, 2005). Phytogeography is the study of wild Plants and the laws governing the distribution of cultivated Plants. In determining the medicinal plants used for the treatment of typhoid fever in Minna, Niger State, it would be more appropriate to

consider their history, which includes : Location, Origin, ecologic, archeological, ethnologic and botanic evidences of their past activities.

1.2.1 History and Location

Niger State is located between Latitude 8°22'N and 11°30'N and Longitude 3°30'N and 7°20'E. The area making up Niger State today comprised of the old Nupe and Kontagora Kingdoms, Abuja (Suleja) with link to the famous kingdom of Zazzau and host of other political entities. Niger State was excised from the defunct North-Western State and made a full-fledged State in the Federation in April 1976. The State is bordered to the North by Zamfara State, West by Kebbi State, South by Kogi State, South West by Kwara State, North-East by Kaduna State and South East by FCT. The State also has an International Boundary with the Republic of Benin along Agwara and Borgu LGAs to the North West. The land mass covers 76, 469.903 square kilometers (about 10% of the total land area of Nigeria) out of which about 85% is arable. This makes the state the largest in the country.

Based on the 2006 Census the population figure of Niger State is 3,950,249. Niger State experiences distinct dry and wet seasons with annual rain fall varying from 1,100mm in the northern parts to 1,600mm in the southern parts. The soil types in Niger State are two: Ku soil and Ya soil. The Ku soil has little erosion hazards, while the Ya soil has better water holding capacity.(Niger state Planning Commission, 2011).



Fig. 1.1: Map of Niger State showing Minna (Niger state Planning Commission, 2011).

1.2.2 Ethnic Composition, Languages, Culture and the Arts

Several ethnic groups are found in the State. However, there are three most prominent ethnic groups: the Nupes, the Gbagis (Gwari) and Hausas. Other ethnic groups include the Kadara, Koro, Baruba, Fulani, Ganagana, Dibo, Kambari, Kamuku, Pangu, Dukkawa, gade and Ingwai. Most of these groups can be said to have evolved and instituted a system of political leadership with either a king or chief surrounded by other title holders. Tribes from othe states like Igbo and Yoruba also settle in the State. Islam and Christianity are the major religions in the state. Niger State is popular for its brass works, particularly at Bida,

and also for pottery, weaving and several cottage industries which can be found throughout the length and breadth of the State. (Niger state Planning Commission, 2011)

1.2.3 Economy and Occupation

Agriculture is the backbone of the economy of Niger State, More than 85% of the population depends either directly or indirectly on it for their livelihood while others constituting (15%) are involved in vocations such as white collar jobs, business, craft and arts. By reason of its location, climate and soil, the state is one of the largest and most fertile agriculture lands in the country and has the capacity to produce most of Nigeria's stable crops. It also has ample opportunities for grazing, fishing and forestry. Unique opportunities exist in the State for the establishment of large scale farms. (Niger state Planning Commission, 2011).

The Gbagis, Koro, Kadara and Kambari are noted for yam and guinea corn production, while the Nupe are the major rice producers. The Hausa and Fulani in Mriga LGA are well-known in the field of animal husbandry. Some natural and mineral resources found in the State include: Talc, Gold, Ball clays, Silica, Sand, Marble, Copper, Iron, Felsper, Lead, Kaolin, Casserole, Columbite, Mica, Quartzite, and Limestone. The Three Hydro Electric Power Stations in the Country (Kainji, Jebba and Shiroro) are situated in Niger State. The State is one of the richest in the country in terms of tourism. Some of the Tourist attractions are Zuma Rock, Gurara falls, Baro Empire Hill, Lord Lugard Colonial ruins at Zungeru, Nagwamatse Well and Kainji Lake National Park (Niger state Planning Commission, 2011).

1.3 Statement of the problem

The people of Minna, Niger state , Nigeria utilize medicinal plants for their primary health care needs .Some of these plants and their parts collected from various habitat are used either singly or in combination for the treatment of typhoid fever. However, there was no scientific evidence to either support or debunk the claims by the traditional herbalist of some of the plants successfully used as remedies.

1.4 Justification for the study

The major challenges to the management of typhoid fever are diverse and formidable. The conventional drugs available for the treatment of typhoid fever in the health care system are expensive. Resistance has since been noticed with virtually all drugs used in the treatment of typhoid fever.

As such, the search for medicinal plants which are accessible and cheap as remedy for the treatment of typhoid fever is worthy of scientific investigation.

1.5 Aim and Objectives

The aim of the study is to conduct ethnobotanical survey on some plants used for the treatment of typhoid fever in Minna, Niger State.

The specific objectives are:

- a) To use Ethnobotanical Survey as a new phytomedicinal research approach to establish a data base of Medicinal Plants used in the treatment of Typhoid Fever in Minna, Niger State.
- b) To study the macroscopical and microscopical features as well as determine the physico-chemical constants of the most commonly used Plant (*Terminalia avicennioides*) in the survey.

- c) To Evaluate the anti Salmonella activity of the methanol, acetone and pet ether extracts of the leaves of *T. avicennioides*.

1.6 Statement of research hypothesis

The most frequently used plant found in the survey contain bioactive compounds which are responsible for its anti-salmonella activity.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Ethnobotanical survey

The revival of interest in the use and importance of African medicinal plants by many developing countries has led to intensified efforts on the documentation of ethno medicinal data of medicinal plants, since most traditional healers keep scanty records and their information is passed on, mainly verbally, from generation to generation (Hatil and Kamali, 2009).

The word “ethno” means the way people see the world. When used as a prefix in an academic discipline such as botany or pharmacology, it implies that the researcher is exploring the ordinary man’s (native of an environment) perception of cultural, natural or scientific knowledge (Martin, 1995). Ethnobotany is the study of useful plants prior to commercial exploitation and eventual domestication. It is based on the knowledge of plants by the local people and their usefulness as understood by the people of a particular ethnic group, since information concerning a particular plant varies from one ethnic group to another. The use of medicinal plants as remedies is common and widespread in Nigeria (Igoli *et al.*, 2005).

Olajide (2003) reported that Nigerian vegetations are naturally endowed with arrays of floristic composition of different plant forms including trees, shrubs, herbs and other non-wood forest resources. Within the natural forest abound several valuable non-timber resources of edible and highly nutritious plants whose fruits, twigs, barks, roots, gum, latex or dyes are of medicinal value (Owonubi and Otegbeye, 2004). Mgeni (1991) opined that with the unique diversity of plant and animal life, tropical rainforest represents biologically renewable resources of food, medicine and fuel if well managed.

Due to unavailability of modern health facilities, most people especially rural people are still forced to practice traditional medicines for their common day ailments, most of these people form the poorest link in the trade of medicinal plants (Khan, 2002). A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance (Diallo *et al.*, 1999). The continuous search for natural plant products for use as medicines is encouraged

by ethnobotanical survey; Igoli *et al.* (2005) recognized ethnobotanical survey as one of the major approaches for selecting plants for pharmacological screening.

Several workers have conducted ethnobotanical surveys among various tribes of the African continent and some other parts of the world (Adjanohoun *et al.*, 1991; Gbolade and Soremekun, 1998; Rashid, 2001; Gbolade 2000; Ajaiyeoba *et al.*, 2002; Osowole *et al.*, 2005; Khan and Rashid, 2006) in search of plants with antibacterial, antiviral and antifungal properties. The medicinal values of these plants lie in some chemical substances they contain that produce a definite physiological action on the human body (Edeoga *et al.*, 2002). Ethnobotanical surveys are important in order to understand the social-cultural and economic factors influencing ideas and actions concerning health and illness and also to get information on type of diseases and health problems prevalent among the people of a particular locality. Such studies, as suggested by Lawal *et al.* (2010), may help to provide the basic health care services needed to improve health challenges of the rural population. The potentials of the plants are far from being tapped. This study is intended to document such valuable information.

2.2 Integrating modern and traditional medicine

Modern medicine is desperately short of new treatments. It takes years for a new drug to get through the research and development pipeline to manufacture and the cost is enormous. The increasing rate of drug resistance, caused by the misuse of medications has rendered several antibiotics and other life-saving drugs useless. These trends mean that scientists and pharmaceutical companies are seriously searching for new drug sources and are increasingly turning their eyes to traditional medicine (Ghalib, *et al.*, 2007).

In the past two decades, there has been a global revival of interest in traditional medicine for the treatment of ailments that defile orthodox medicine principally because many diseases have defiled or developed resistance to conventional drugs . As a result of this, a lot of interest and attention have been drawn to the curative claims and norms (ethics) of herbal plants in different parts of the globe especially Africa and Asia (Awoyemi *et al.*, 2012). Nearly a quarter of all modern medicines are derived from natural products, many of which were first used in traditional remedies (Table 2.1).

Table 2.1: Selected modern drugs that originate from traditional medicine

Drug	What it is for	Derived from	Originally used in
Artemisinin	Anti-malarial	Produced by the Chinese herb <i>Qinghao</i> or sweet wormwood	Traditional Chinese medicine for chills and fevers.
Cromoglycate	Asthma prophylaxis	Synthetic compound based on khellin, active ingredient of the khella plant	Traditional Middle Eastern remedies for asthma. Khellin has also traditionally been used in Egypt to treat kidney stones
Etoposide	Anti-cancer	Synthesized from podophyllotoxin, produced by the mandrake plant	Various remedies in Chinese, Japanese and Eastern folk medicine
Opiates	Analgesic	Unripe poppy seeds	Traditional Arab, Chinese, European, Indian and North African medicines as pain relief

			and to treat range of illnesses including diarrhoea, coughs and asthma
Quinine	Anti-malarial	Bark of the cinchona tree	Traditional remedies to treat fevers and shivers in South America
Vinca alkaloids (vincristine, vinblastine)	Anti-cancer	Rosy periwinkle	Various folk remedies across the world, including use as an anti-diabetic in Jamaica, to treat wasp stings in Indian traditional medicine, as eyewash in Cuba, as love potion in medieval Europe

(Ghalib, *et al.*, 2007)

2.2.1 Biodiversity and sustainability

Patient safety is an important issue but then a growing herbal market needs to be checked because its great commercial benefit might pose a threat to biodiversity through the over harvesting of the raw materials for herbal medicines and other natural health care products. These practices, if not regulated, may lead to the extinction of endangered species and the destruction of natural habitats and resources (Shah *et al.*, 2011).

In order to sustain the sensible utilization of medicinal and aromatic plants, conservation has to be kept as the central focus. In conducting research and developmental activities, plant parts from which extracts are obtained should be done such that it will not destroy the plant. Furthermore, the methods of harvesting the desired plant parts should take

cognizance of the conservation of the plant. Ex situ cultivation of the desired medicinal and aromatic plants would be necessary so as to obtain raw materials grown under the same conditions of climate, ecology etc. (Ernest, 2001).

Beyond the sustainability of natural resources, marrying traditional and modern medicine faces numerous challenges that stem from key differences in how each is practiced, evaluated and managed (Shah *et al.*, 2011).

Table 2.2: Key differences between Traditional and Modern medicine

	Traditional medicine	Modern medicine
Knowledge protection	Open access	Closed, patent-protected
Formulation	Ad hoc during consultation with the patient	Pre-determined, and once tested in clinical trials cannot be changed unless re-tested
Regulation	Virtually none, though some countries are trying to introduce rules and standardization	Extremely tight, to the point that bringing drugs to market now costs billions of dollars
Testing	No formal testing as knowledge of the effectiveness is handed down through generations	Rigorous trials that happen in different phases, first testing for safety, then efficacy

Dosage	Unfixed: the amount of medicine given might be roughly similar, but the active ingredient (which is what dosage really is) can vary widely	Fixed doses that tend to vary only slightly with age or weight, or disease severity
Consultation	Lengthy, and the patient is asked about a wider range of questions than just their symptoms	Consultations in both primary and secondary care tend to be brief and focused, especially as national health systems come under strain
Training	Both systems of medicine require lengthy training over many years but with traditional medicine, knowledge is often passed one-to-one through families, and practitioners are often born into a family of healers	Often vocational: health professionals go through formal training in schools and universities

(Shah *et al.*, 2011).

2.2.2 New techniques for old treatments

New scientific techniques are also being applied to traditional medicine in the search for modern drugs. These innovative approaches are developing at breakneck speed (Patwardhan *et al.*, 2009)

Table 2.3: How to create modern drugs from traditional compounds.

Technique	Process
Reverse pharmacology	Researchers start with the end product, a clinically useful compound for example, and work backwards to find out what it

	contains and how it functions. This can offer clues about how particular medicines work, and where they act in the body.
High-throughput screening	This advanced screening relies on high-speed data processing and sensitive detectors to conduct millions of biochemical, genetic or pharmacological tests in a few minutes. The process can quickly identify active compounds that affect particular biological pathways.
Ethnopharmacology	The systematic study of how specific ethnic groups use medicinal plants.
Systems biology	This holistic approach aims to understanding the way different chemicals and metabolic processes interact within the body. Since traditional medicines often have numerous active ingredients, it could be used to measure the whole body's response to the mixture of compounds.

(Patwardhan *et al.*, 2009)

2.3 Role of Ethnopharmacology in the treatment of Typhoid fever

A number of workers have evaluated the anti typhoidal activities of medicinal plants and some of them proved to be promising. For example: Aliero and Wara (2009) evaluated the efficacy of *Leptadenia hastata* (Pers.) Decne extracts against five selected bacterial species and two fungal species. Aqueous extract markedly inhibited the growth of *Salmonella paratyphi* and *Escherichia coli* and *Pseudomonas aeruginosa*. The result provided a scientific support for the claimed ethnomedical uses of aqueous extracts of *L. hastata* in

the treatment of bacterial diseases and suggest the potential of methanol extract as a source of antifungal agent.

Evans *et al.* (2002) evaluated the efficacy of *Euphorbia hirta*; *Citrus aurantifolia*, *Cassia occidentalis*, and *Cassia eucalyptus* claimed by the Nupes tribe of Nigeria to be effective in the treatment of typhoid fever. The result of *invitro* antimicrobial analysis showed that, only *Cassia eucalyptus* showed inhibition of *Salmonella typhi* growth and concluded that the plant is efficacious and contains natural compounds that could be used in the treatment of typhoid fever.

Similarly, In Cameroon, Nkuo-Akenji *et al.*, (2001) evaluated the effects of herbal extracts derived from plants commonly prescribed by traditional practitioners for the treatment of typhoid fever (caused by *Salmonella typhi*, *S. paratyphi* and *S. typhimurium*) using formulations often prescribed by the traditional healers which includes:

- 1) Formulation A: comprising *Cymbogogon citratus* leaves, *Carica papaya* leaves, and *Zea mays* silk.
- 2) Formulation B comprising *C. papaya* roots, *Mangifera indica* leaves, *Citrus limon* fruit and *C. citratus* leaves.
- 3) *C. papaya* leaves.
- 4) *Emilia coccinea* whole plant.
- 5) *Comelina bengalensis* leaves.
- 6) *Telfaria occidentalis* leaves.
- 7) *Gossypium arboretum* whole plant.

The result obtained in this study, showed that Formulation A elicited inhibitory activity at a lower range of 0.02 to 0.06 mg/ml. Similarly, Formulation B elicited bacterial activity at the lowest range of 0.06 to 0.25mg/ml. *C. bengalensis* leaves on the other hand, showed

the lowest activity with a concentration range of 0.132 to 2.0 mg/ml and 1 to 4 mg/ml in MIC and MBC assays respectively. The result demonstrated that *S. paratyphi* was most sensitive to the formulations (concentration range of 0.02 to 1 mg/ml in both MIC and MBC assays) while *S. typhimurium* was the least sensitive and concentrations of up to 4 mg/ml were required to be bactericidal.

Iroha *et al.* (2010) evaluated the anti *Salmonella typhi* activity of ethanol, hot and cold crude water extracts of *Vitex doniana* (root), *Cassia tora* (Leaf), *Alstonia boonei* (bark), *Stachytarpheta jamaicensis* (leaf), and *Carica papaya* (leaf) used as typhoid medicine in Ebonyi state, Nigeria. The results of these findings showed that ethanol and hot water extracts of *V. doniana* and *A. boonei* had the best anti typhoid activity.

Oluduro and Omoboye (2010) investigated the antibacterial potency and synergistic effect of crude aqueous and methanolic extracts of nine plant parts against multidrug resistant *S. typhi*. The nine plant parts includes: unripe *Carica papaya* fruit, *Citrus aurantifolia*, *Anana sativus*, *Citrus paradisi*, *Cymbopogon citratus*, *Cocos nucifera* leaves of *Carica papaya*, leaves of *Euphorbia heterophylla* and *Gossypium* spp. Both the aqueous and methanol extracts of each plant material and mixture showed appreciable antimicrobial activities on *S. typhi*.

2.4 *Terminalia avicennoides*

Terminalia avicennoides Guill. & Perr. (Combretaceae) is a tropical herb common in North central vegetation of Nigeria. It is locally called *baushe* (Hausa), *Kpace* (Nupe), *Idin* (Yoruba) and *Edo* (Igbo). It is a tree with yellowish brown hard and durable wood (Burkill, 1985).

Macroscopically, the leaves are evergreen with alternate arrangement and petiole present. The leaves have simple and pinnatifid incision, lanceolate in shape ranging from 12.4-24.3cm long and 4.1- 9.3cm wide. The lamina is thick with pinnate-reticulate venation, entire margin, acute apex and asymmetric base. Organoleptically, the leaves are greenish in color, characteristic taste and odourless.

In ethnomedicine, the plant is known to be active against trypanosomes (Bulus *et al.*, 2008). Extracts of the stem bark and roots of *T. avicennioides* are used for the treatment of bacterial infections such as diarrhea and gastrointestinal disorders (Abdullahi *et al.*, 2001), bloody sputum (tuberculosis) and cough in humans (Mann *et al.*, 2007). The stem bark of *T. avicennioides* have been found to be fungicidal against the dermatophytic *Epidermophyton floccosum*, *Microsporum gypseum* and *Trichophyton mentagrophytes* and fungistatic against *Candida albicans* (Baba-Moussa *et al.*, 1999).

Extracts of the stem bark of *T. avicennioides* contain large quantities of saponins and tannins (Baba-Moussa *et al.*, 1999), which might be responsible for the good antifungal effects.

CHAPTER THREE

3.0

MATERIALS AND METHODS

This Research is divided into four parts. The first part is the survey, which involves the collection of information from the informants and the collection of the plants used in the treatment of typhoid Fever in Minna, Niger State. Followed by their identification,

preparation of herbarium sample and classification of data. The second, third and fourth part constitute the pharmacognostic evaluation, phytochemical screening and the anti salmonella activity on the most frequently used plant from the survey.

The parts and contents are divided as follows:

I Ethnobotanical survey

- i. Study design/ study population
- ii. Sampling technique
- iii. Method of data selection and collection procedure
- iv. Materials/Instruments
- v. Method of data analysis

II Pharmacognostic evaluation of the leaves of *Terminalia avicennioides*.

- i. Macroscopic Evaluation
- ii. Microscopic Evaluation
- iii. Physicochemical constants
- iv. Extractive values

III Preliminary phytochemical study using Thin layer chromatography (TLC)

IV Anti-Salmonella studies

- i. Maintenance of Test Organism

- ii. Anti-salmonella Susceptibility tests
- iii. Determination of Minimum Inhibitory Concentration (MIC)
- iv. Determination of Minimum Bactericidal Concentration (MBC)

I Ethnobotanical survey

3.1 Study Design/Study Population

Ethnobotanical study mainly involves a field research work, hence a survey research approach becomes the adopted strategy. The research therefore provides information on facts, opinions and attributes of person towards the use of plants in local communities.

To ensure an effective coverage of the study area and a large data of plants used in the treatment of typhoid fever in Minna Niger state, six major locations in Minna were targeted. These are Ketteren gwari, Kpakungu, New Market, Bosso, Mobile fish market and Tunga. A selected sample frame of (at least) 10 interviews was administered in each settlement. The sample population is principally of traditional medicine practitioners (TMPs), Herbalist (also known as traditional healers), Elders with claims of medicinal plant knowledge, Herb sellers, House wives and Mothers according to the method of Sofowora (1993).

3.2 Sampling Techniques Employed

The study area which is a part of the Guinea-savannah vegetative zone of North Central Nigeria has not been investigated for its medicinal plant potentials. Therefore, the sampling technique used to obtain Data for the study is based on the facts and information on ethnomedicinal plants provided by the group of persons (mentioned in section 3.1 above) interviewed. This therefore makes the entire population a target.

3.3 Method of Data Selection/Collection

The method described by Betti (2004) was used in this study to serve as a guide (and as an affirmation of information). It consists of gathering data on the commonly used medicinal plants in a given area rather than asking which plant was used to treat which ailment. The originality and the strength of this method is derived from its qualitative and quantitative approach.

3.3.1 Data Collection Procedure

The ethnobotanical Data obtained for this study were from interviews with the local people, conducted from December 2011 to April 2012. The ethnobotanical survey which

was aimed at identifying and documenting the useful medicinal plants used in the treatment of typhoid fever in Minna, Niger state involved mainly informants between the ages of 30 and 80 years. Data acquisition was successively collected using the communicable dialect within the area in line with standard enquiry procedure and with the aid of the research instruments.

On the bases of opinion put forward by Cordell (1995), data collection procedure for the expedition of this study was conducted taking into consideration the following measures:

- a. On the usage of medicinal Plant within the community for the treatment of typhoid fever.
- b. Traditional medicine practice of the people in line with the ailment above was inquired on: Medicinal plants, method of preparation, mode of application, dosage and duration of treatment, years of practice, sources of knowledge, extent of patronage, gender, economic and educational status of the patients and the level of success with the used prescription/ recipes.
- c. Information on culture such as beliefs with regard to modern health care system, socio-cultural values, societal norms etc.
- d. Ecological/ Enviromental finding on activity of people and its effects on medicinal wild flora conservation, the rate of genetic depletion of medicinal plant resources of the community, extinction and destruction of medicinal plant flora and questions such as how readily are the informants ready to part with the information they possess as well as their experiences with other people with knowledge on medicinal plants were asked.

3.4 Materials and instruments

The information acquisition on an ethnobotany survey is mostly obtained from unlearned people and societies. Consequently the method where a researcher fill in a questionnaire for his informant during an interview session is not definitely binding on the informant as this could also lead to bias answers. To this end, a tape recorder was employed during the interview session to record information by the informants. A questionnaire guided tool structured to furnish data on the itemized considerations earlier mentioned under 3.3.1(a-d) was also used to serve as supplementary tool. The structured questionnaire was an integration of those designed by Sofowora (1993) and the information collection data formed by the Ministry of Health (Government Chemistry Laboratory) in Dar-es-salaam Tanzania (Adjanohoun *et al.* 1991).

3.5 Plant Collection and Herbarium Preparation

For each selected plant species collected, a record was made of its habitat in the area which were classified under wild and field under cultivation . The plant species were collected by the person who normally prepares the herbal remedy/informant, so as to avoid the collection of the wrong specimens through variation in local names (Sofowora, 1993). The collected plant species were then prepared according to standard procedures of herbarium specimen preparations and preservation . Photograph images of collected plant species were also made to facilitate their identification process. Final identification was made at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria with the help of the Taxonomists in the unit; Mr Musa Muhammed and Mr U.S.Gallah.

3.5.1 Data Analysis

Analyses of data according to and Akuezilo (1993) refers to those techniques whereby the investigator extracts from the data information that was not apparently there before and which would enable a summary description of the subject studied to be made. Hence, descriptive statistical tool; tables and frequency distribution tables for grouped and ungrouped data were then utilized to analyze the findings.

3.6 Equipments/Apparatus and Solvents/Reagents Used

All the equipments, solvents and reagents used were of analytical grade as available in the Department of Pharmacognosy and Drug Development and the Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria.

3.6.1 Equipments/Apparatus

Compound Microscope

Slides and Cover slips

Razor blades

Cotton wool

Beakers

Test tubes

Micropipette

Measuring cylinder

Electronic weighing balance

Mechanical shaker

Conical Flask of various sizes

Funnels

Photographic camera

Filter papers/ Ashless filter papers

Water bath

Dessicators

Petri dishes

Nickel crucibles

Oven

Evaporating dish

Wire loop

Cork borer

Burnsen burner

3.6.2 Solvents/ Reagents

Ethyl acetate

Distilled water

Pet ether

Acetone

Methanol

Chloral hydrate

Concentrated HCl

Chlor-zinc iodine

Phloroglucinol

N/50 iodine

Sudan red

Ferric chloride

Normal saline

II Pharmacognostic Evaluation of the leaves of *Terminalia avicennioides*

3.7 Microscopic Evaluation of *T. avicennioides*

The microscopic evaluation of the powdered sample and anatomical sections were carried out using standard laboratory methods (Evans, 2008). The powdered drug, transverse and longitudinal sections of the leaves were studied.

3.7.1 Preparation of Transverse and Longitudinal Sections

For transverse section, a thin section was cut horizontally across the mid rib of the leaf while for the longitudinal sections a thin section was cut vertically across the veins and veinlets between the midrib and margin of the leaf using a sharp razor blade. The sections were viewed under the Microscope (Kokate, 2001).

3.8 Quantitative microscopy

Fragments of freshly prepared leaves were used for quantitative microscopy using standard laboratory methods (Kokate, 2001) to determine the following parameters: Palisade ratio, Stomatal number, Stomatal index, Vein-islet number and Veinlet termination number.

3.8.1 Determination of stomatal number and stomatal index

Stomatal number is defined as the average number of stomata per sq mm of epidermis of the leaf while stomata index is the percentage which the number of stomata form relative to the total number of epidermal cells, each stoma being counted as one cell.

A fragment of leaf from the middle of lamina was cleared by boiling in chloral hydrate solution. The upper and lower epidermis of the leaf were peeled using forceps and mount separately in glycerine water. A square measuring 2×2 mm was drawn using stage micrometer and camera Lucida on a drawing paper. The stage micrometer was replaced by the leaf preparation and focused under the same magnification. The epidermal cells and stomata were traced by looking through the microscope when the superimposed image of the leaf was seen at the same time. The number of the stomata and epidermal cells were

counted (two guard cells and ostiole being counted as one) within the square. The stomata were calculated as the number of stomata per square millimeter of leaf preparation while the stomatal index was calculated using the formula:

$$S.I = \frac{S}{E + S} \times 100$$

Where,

S.I = Stomatal Index

S = Number of stomata per unit area

E = Number of epidermal cells in the same unit area

3.8.2 Determination of vein-islet and vein let termination numbers

Vein-islet number is defined as the number of vein islets per sq mm of the leaf surface midway between the midrib and the margin. A vein let termination is the ultimate free termination of veinlet.

Few leaves were boiled in chloral hydrate solution in a test tube placed in boiling water bath until it was cleared. It was then mounted in glycerine water. A camera Lucida was set up to divide the paper into 2 square millimeter by means of the stage micrometer. The

stage micrometer was then replaced by the cleared leaf preparation, and the veins were traced in a square of $2\text{mm} \times 2\text{mm}$.

The vein-islet and veinlet termination were traced by looking through the microscope when a superimposed image of the leaf portion and paper were seen at the same time. The number of vein-islet and veinlet termination present within the square were counted and the total numbers of each were divided by four to get the value for one square mm. A total of ten sets of counts were made.

3.8.3 Determination of Palisade Ratio

Palisade ratio is defined as the average number of palisade cells beneath each epidermal cell. Pieces of leaves were cleared by boiling with chloral hydrate solution and mounted in glycerin water. It was then focused under high power ($\times 40$) magnification. A camera Lucida was attached and the outlines of four continuous epidermal cells were traced and marked using pencil. The palisade cells within each group were counted including those

cells in the count which were more than half covered by the epidermal cells. The values obtained from the counts were divided by four to obtain the palisade ratio.

3.9 Chemomicroscopy of Powdered Drugs

The powdered drug were treated with reagents and the microscopic evaluation were carried out using standard methods to detect the presence or absence of cell contents such as calcium carbonate, calcium oxalate crystals, fats, lignin, mucilage, starch and cellulose (WHO, 1999).

a) Cellulose : The powdered drug was treated with iodinated zinc chloride and allowed to stand for a few minute. A blue to blue violet coloration indicates the presence of cellulose.

b) Lignin : The powdered drug was mounted in phloroglucinol and was allowed to stand for two minutes, a drop of concentrated hydrochloric acid was added to it. A red coloration indicates lignification.

c) Starch : The powdered drug was mounted in N/50 iodine. Bluish coloration indicates the presence of starch.

d) Calcium Oxalate Crystals : The powdered drug was treated with hydrochloric acid. The presence of calcium oxalate crystals which were observed as bright structures disappears.

e) Tannins : The powdered drug was mounted in ferric chloride solution and observed under microscope. Bluish or greenish black coloration indicates the presence of tannins.

f) Fats: The powdered drug was mounted in sudan red and allowed to stand for a few minutes. Orange red to red coloration indicates the presence of fats.

3.10 Physicochemical Evaluation of Powdered Drug

Physicochemical parameters such as moisture content, total ash value, acid insoluble ash, water soluble ash, alcohol soluble extractive and water soluble extractive were carried out using standard laboratory methods (Kokate, 2001; WHO, 1999).

3.10.1 Determination of Moisture Content

The moisture content was determined by loss on drying method. 3g of powdered leaves were accurately measured and placed into a clean nickel crucible of a known weight. It was then heated in an oven at 105⁰C. After an hour the crucible was cooled in a dessicator and weighed. The weighing was repeated at 30 minute intervals until constant weight was obtained. The moisture content was calculated with reference to the original weight of the powder used as shown below:

$$\% \text{ Moisture Content} = \frac{\text{Weight of moisture}}{\text{Original weight of Sample}} \times 100$$

3.10.2 Determination of Total ash values

The powdered drug (3g) was accurately weighed into a nickel crucible and heated at a temperature not exceeding 450⁰C until it was white, indicating the absence of carbon. The content was cooled and weighed, until a constant weight was obtained for three successive readings. Percentage of ash was calculated with reference to the original weight of the powder used as follows:

$$\text{Total Ash} = \frac{\text{Wt. of residual ash}}{\text{Original weight of sample}} \times 100$$

3.10.3 Determination of Acid-Insoluble Ash

The total ash obtained above was used for the determination of the acid insoluble ash. Dilute hydrochloric acid (25ml) was added to the crucible containing the total ash. The mixture was boiled gently for 5 minutes and filtered through an ash less filter paper. The washing of the filter paper was repeated many times until the filtrate was neutral .

The filter paper and contents were dried in an oven at 105°C. The weight of clean crucible was accurately determined. The filter paper with its contents were folded into a small cone and transferred into the crucible. The crucible with its contents were gently heated over a Bunsen burner flame until the filter paper was completely burnt to ashes. The crucible and contents were cooled and the final weight noted. The weight of the residue was calculated thus:

$$\% \text{ Acid insoluble ash} = \frac{\text{Wt of residual ash}}{\text{Wt of original sample}} \times 100$$

3.10.4 Determination of water soluble Ash

The procedure above was repeated but water was used instead of dilute hydrochloric acid.

3.10.5 Alcohol-soluble extractive

Accurately weighed powdered leaves (5 gm) were transferred into 250ml stoppered conical flask. 100 ml of 90% alcohol was added to it and shaken for 6hr in an electrical shaker and then allowed to stand for 18 hours. The extract was filtered immediately. The filtrate (25ml) was transferred into a clean and accurately weighed tarred evaporating dish.

It was evaporated to dryness. The residue was dried to constant weight at 105°C in an oven and the final weight determined. The % w/w alcohol soluble extractive value was calculated with reference to the powdered drug.

3.10.6 Water Soluble extractive

The procedure above was repeated but chloroform water (1:4) was used instead of 90% ethanol.

3.11 Extraction of the Leaves of *Terminalia avicennioides*

The leaves were air dried for five days and pounded in a mortar. The powdered leaves (50g) were macerated with 300ml of petroleum ether and drained after 24 hrs to remove the non-polar fraction. The marc was further macerated with 70% methanol for 24 hours to get the polar fraction. Another 30 grams of the powdered leaves were macerated with 200ml of acetone. The filtrate of the acetone, pet-ether and methanol were concentrated to dryness on a water bath which were used for the anti - salmonella test.

III Preliminary phytochemical study using Thin layer chromatography (TLC)

3.12 Phytochemical screening of the Leaves of *T. avicennioides*

Thin Layer Chromatography (TLC) technique was used to determine the type of phytochemicals present in the leaves of the plant.

The methanolic extract of the leaves was analyzed using thin layer chromatography (WHO,1999). Already prepared pre-coated TLC plates were used. Using a micropipette spots of the plant extract were placed onto the starting line which was parallel to and also 1cm above the lower edge. The spots were allowed to dry, the plates were placed vertically into the chromatographic chamber containing ethylacetate/Methanol/water (8:1:1) ensuring that the point of application were above the surface of the mobile phase, after which the chamber was closed. The chromatograms were developed at room temperature and visualized under daylight and U.V light. The location of the separated compounds were ascertained and marked. The components were analysed by calculating the retention factor (Rf) values of the spots.

The different chromatograms developed were sprayed with sulphuric acid (a general spraying reagent), dragendorff spray, p-anisaldehyde and ferric chloride spray.

IV Anti-Salmonella Studies

3.13 Test Organisms

The organism *Salmonella typhi*, was obtained from the Medical Microbiology Laboratory of Ahmadu Bello University Teaching Hospital, Zaria for the susceptibility tests. The

organism was used after the identity was confirmed in the Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria. The stock culture was maintained on agar slants at 4°C in the refrigerator. Active culture for experiments were prepared by transferring a loopful of cells from the stock culture to sterile bottles of Nutrient broth and then incubated for 24 hours at 37°C.

3.13.1 Antimicrobial Susceptibility tests

The Susceptibility of the test organism to the extract was evaluated using the agar well diffusion method. Suspension of organisms were adjusted to 0.5 McFarland turbidity standard (Coyle, 2005). The inoculum was swabbed on the surface of sterile Mueller-Hinton agar plate, it was allowed for 10 to 15 min to pre-diffuse. Wells were made in the agar using 8mm sterile cork borer and sealed with one drop of melted Mueller-Hinton agar. Different concentrations of the extract from 12.5mg/ml to 100mg/ml were used. Using a sterile syringe, 0.1ml of the extract were introduced into the wells. The plates were then allowed to stay for one hour at room temperature for effective diffusion to take place and then incubated at 37°C for 24 hours. The diameters of the zones of inhibition were measured in millimeter with a transparent ruler and recorded.

This was repeated three times and average diameters were recorded. A positive control (Ciprofloxacin disc 5µ, oxoid) was used while 0.1ml distilled water dispensed into one of the wells served as the negative control.

3.13.2 Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the extract was determined using the Broth dilution method. Nutrient broth was prepared according to the manufacturer's

instructions. It was dispensed into ten test tubes followed by the dilution of the extract in the nutrient broth to obtain concentration of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.12mg/ml, 1.56mg/ml, 0.78mg/ml, 0.39mg/ml, 0.19mg/ml. using a syringe, 1ml of the test organism adjusted to 0.5 McFarland standard was inoculated into each test tube and mixed thoroughly on a vortex mixer and then incubated at 37⁰C for 24hours . The test tubes were observed for turbidity. The tube with the lowest concentration of the extract showing a clear solution was considered as the MIC (Coyle, 2005).

3.13.3 Determination of Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration was carried out after the result of the Minimum Inhibitory Concentration (MIC) was obtained. Mueller Hinton agar (MHA) was prepared according to the manufacturer 's instructions and aseptically poured into plates. The plates were labeled accordingly to correspond to the MIC test tubes.

The content of the test tubes were sub cultured into the plates by dipping a sterile wire loop and streaking the surface of the agar in the plates and then incubated at 37⁰C for 24hours after which they were checked for growth. The plate with the lowest concentration of the extract without growth was regarded as the MBC. (NCCLS, 2000).

CHAPTER FOUR

4.0 RESULTS

4.1 Demography/personal information on respondents

A total of 70 respondents were interviewed. The respondents were mainly Herbalists/Traditional medical practioners (TMPs) (50%), Herb sellers (19%), Elders (17%) and others (House wives and Mothers) (14%). The demographic survey of respondents is presented in Table 4.1.

Table 4.1: Demographic survey of respondent on the Medicinal Plants used in the treatment of typhoid fever.

Parameter	Specification	N (%)
Practice specification	Herbalists/Traditional medical practioners (TMPs)	35 (50)
	Herb sellers	13 (19)
	Elders	12 (17)
	Others	10 (14)
Sex	Male	30 (43)
	Female	40 (57)
Age (years)	1 - 20	0
	21 - 40	15 (21)
	41 - 60	31 (44)
	>60	24 (34)
Religion	Islam	61 (87)
	Christianity	9 (13)

N = number of respondents; % = percentage of respondent

4.2 Medicinal plants used in the Treatment of Typhoid Fever in Minna, Niger State

A total of 49 medicinal plants species were collected from 31 families. Table 4.2 (a-h) gives a concise analysis on medicinal plant species, their families, common Names and their corresponding Vernacular Names in Hausa, Igbo, Yoruba and Nupe.

Table 4.2a: Medicinal plants used in the Treatment of Typhoid Fever in Minna, Niger State

Family	Botanical names/Authority	Common Names	Vernacular names	Voucher number
Amaranthaceae	<i>Alternanthera sessilis</i> (L.) DC	Sessile joy weed	Maikai dubu ^h , Masantogi ⁿ , Dagonro ^y .	970
Anacardiaceae	<i>Anacardium occidentale</i> L.	Cashew	Kashew ^h , Okpo kpo ⁱ , Eko nasara ⁿ , Kaju ^y .	184
“	<i>Mangifera indica</i> L.	Mango	Mangworo ^h , Mungoro ⁿ , Mangoro ^y , Mangolo ⁱ .	1944
“	<i>Spondias mombin</i> L.	Hog plum	Tsadar masar ^h , jinjerechi ⁿ , Akika ^y , Jinkara ⁱ .	2384
Annonaceae	<i>Annona senegalensis</i> Pers.	African custard apple.	Gwandar daji ^h , Nungberechi ⁿ , Abo ^y , Uburu-ocha ⁱ	382
“	<i>Enantia chlorantha</i> Oliv.	African yellow wood	Awopa ^y , Dokita -igbo ^y .	

Key : h – Hausa ; n – Nupe ; y – Yoruba ; i – Igbo; NA- Not available

Table 4.2b: Medicinal plants used in the Treatment of Typhoid Fever in Minna, Niger State

Family	Botanical names/Authority	Common Names	Vernacular names	Voucher number
Apocynaceae	<i>Alstonia boonei</i> De Wild.	Pattern wood	Ahun ^y , Egbu ⁱ	
“	<i>Plumera rubra</i> L.	Temple tree, Frangipani.	Karya ^h .	131
Asclepiadaceae	<i>Calotropis procera</i> (Ait.)Ait.F	Sodom Apple	Tumfafiya ^h ,Epuko ⁿ ,Bomubomu ^y ,Otokwuru ⁱ	900219
Asteraceae	<i>Chromolaena odorata</i> (L.) R.M. King & Robinson	Siam weed	Akintola ^y , Obiarakara ⁱ , Ekpe gbakun ⁿ .	1128
“	<i>Vernonia amygdalina</i> Del.	Bitter leaf	Shìwáákáá ^h , ,Tsula ⁿ , Ewuro ^y , Olubo ⁱ	675

Key : h – Hausa ; n – Nupe ; y – Yoruba ; i – Igbo; NA- Not available

Table 4.2c: Medicinal plants used in the Treatment of Typhoid Fever in Minna, Niger State

Family	Botanical names/Authority	Common Names	Vernacular names	Voucher number
Balanitaceae	<i>Balanites aegyptica</i> (L.) Del	Soap berry tree, Desert date.	Aduwaa ^h , Aduwa ⁿ , Budare ^y , Enyi-ndi- mmuo ⁱ .	2064
Bromeliaceae	<i>Ananas Comosus</i> (L) Merr.	Pineapple	Abarbaa ^h , Akwu olu ⁱ , ope- oyinbo ^y ,	2446
Burseraceae	<i>Commiphora kerstingii</i> Engl.	NA	Hánà gobara ^h , Enagunbochi ⁿ .	006
Caricaceae	<i>Carica papaya</i> Linn.	Pawpaw	Gwanda ^h , Okworo- beke ⁱ , Ibepe ^y , Konkeni ⁿ .	185
Combretaceae	<i>Anogeissus leiocarpus</i> (DC) Guill. & perr.	Axle wood tree	Marike ^h , kukunchi ⁿ , Ayin ^y , Atara ⁱ	167
“	<i>Terminalia avicennioides</i> Guill. & perr.	NA	Báushè ^h , kpace ⁿ , Edo ⁱ , Idin ^y	901452
Cucurbitaceae	<i>Citrullus colocynthis</i> (Linn) Schrad	Bitter gourd, Wild gourd.	Kwartowa ^h , Egwusi ⁱ , Baara ^y .	1266
Euphorbiaceae	<i>Alchornea cordifolia</i> (Schum. & Thonn.) Muell.Arg.	Christmas bush	Bambami ^h , Dzudzanci ⁿ , Ipa ^y , Ububo ⁱ .	4324

Key : h – Hausa ; n – Nupe ; y – Yoruba ; i – Igbo; NA- Not available

Table 4.2d: Medicinal plants used in the Treatment of Typhoid Fever in Minna, Niger State

Family	Botanical names/Authority	Common Names	Vernacular names	Voucher number
Fabaceae				
Caesalpinoideae	<i>Daniellia oliveri</i> (Rolfe) Hutch. & Dalz.	Ilorin balsam	Maje ^h , Kadaura ^h , Ozabwa ⁱ , Danchi ⁿ , Iya ^y .	90021
“	<i>Detarium microcarpum</i> Guill.& perr.	Tallow tree	Taura ^h ,Gungorochi ⁿ , Ogbobo ^y ,Ofo ⁱ .	900676
“	<i>Piliostigma thonningii</i> (Schum.) Milne –Redh.	Wild bauhinia	Kalgo ^h ,Okpoatu ⁱ , Bafin ⁿ , Abafe ^y	1911
“	<i>Senna occidentalis</i> L.	Negro Coffee	Râi d' oòré ^h , Gaya ⁿ , Rere ^y ,Okamo ⁱ	1611
Papilionoideae	<i>Eriosema psoraleoides</i> (Lam) G. Don.	NA	Taba jiki ^h , Egwagutagi ⁿ , otili ^y	887
“	<i>Erythrina senegalensis</i> DC.	Senegal coral tree	Minjirya ^h , Eshcibgan ⁿ , Ologun sheshe ^y , Echiechi ⁱ ,	7095

Key : h – Hausa ; n – Nupe ; y – Yoruba ; i – Igbo; NA- Not available

Table 4.2e: Medicinal plants used in the Treatment of Typhoid Fever in Minna, Niger State

Family	Botanical names/Authority	Common Names	Vernacular names	Voucher number
Lamiaceae	<i>Ocimum gratissimum</i> L	Tea bush	Daidoya ta gida ^h , Tanmotswangi-wawagi ⁿ , Efinrin ^y , Nehonwu ⁱ	1285
Lythraceae	<i>Lawsonia inermis</i> (L)Keay	Henna Plant	Lalle ^h , Lali ⁿ , Lali ^y	0342
Malvaceae	<i>Gossypium hirsutum</i> L.	Cotton Plant	Auduga ^h , Lulu ⁿ , Ela-owu ^y	453
Meliaceae	<i>Azadirachta indica</i> A. Juss	Neem Tree	Dogonyaro ^h , Nimu ⁿ , Oke oyinbo ^y .	900151
“	<i>Khaya senegalensis</i> (Desr.) A.Juss	Mahogany	Madaci ^h , Wuchi ⁿ , Oganwo ^y , Ono ⁱ	1886
“	<i>Pseudocedrela kotschy</i> (Schweinf.) Harms.	NA	Tónáá ^h , Emi-gbegi ^y	900243
Moraceae	<i>Ficus platyphylla</i> Del.	Flake Rubber Tree	Gamji ^h , Gbagun ⁿ , Afomo ^y .	7230

Key : h – Hausa ; n – Nupe ; y – Yoruba ; i – Igbo; NA- Not available

Table 4.2f: Medicinal plants used in the Treatment of Typhoid Fever in Minna, Niger State

Family	Botanical names/Authority	Common Names	Vernacular names	Voucher number
MYRTACEAE	<i>Eucalyptus camadulensis</i>	River Red Gum	Turaren turawa ^h ,	2510
“	Dehnh <i>Psidium guajava</i> Linn.	Guava	Goiba ^h , Goyiba ⁿ , Guafa ^y , Ugwoba ⁱ .	3253
Olacaceae	<i>Ximenia americana</i> L.	Wild Olive	Tsaada ^h , Igo ^y	1973
Palmae	<i>Cocos nucifera</i> Linn.	Coconut Palm	Kwakwa ^h , Yikunu Kpauta ⁿ , Agbon ^y , Akibeka ⁱ .	1708
POACEAE	<i>Cymbopogon citratus</i> (DC.) Stapf.	Lemon Grass	Isauri ^h , Eto Lemu ⁿ , Ewe tea ^y , Iriru- Oyibo ⁱ .	1882
“	<i>Sorghum bicolor</i> (L.) Moench	Sorghum	Dawa ^h , Eyi Kpan ⁿ , Oka baba ^y , Oka maajali ⁱ .	2319
Polygalaceae	<i>Securidaca</i> <i>longependunculata</i> Fres.	Violet Tree	Uwar magunguna ^h , Sanya ^h , Jechi ⁿ , Kyiritoo ^y , Ofodo ^y .	1880

Key : h – Hausa ; n – Nupe ; y – Yoruba ; i – Igbo; NA- Not available

Table 4.2g: Medicinal plants used in the Treatment of Typhoid Fever in Minna, Niger State

Family	Botanical names/Authority	Common Names	Vernacular names	Voucher number
Rhamnaceae	<i>Ziziphus abyssinica</i> Hochst.	NA	Magariya kura ^h , Dangodi ⁿ .	1283
Rubiaceae	<i>Gardenia aqualla</i> Stapf and Hutch	NA	Gáúf è ^h ,	1114
“	<i>Morinda lucida</i> Benth.	Brimstone Tree	Oruwo ⁱ , Eze ogu ⁱ .	8560
“	<i>Nauclea latifolia</i> J. E. Smith.	African Peach	Tafashiya ^h , Gbashi ⁿ , Egbesi ^y , Adi ⁱ	
Rutaceae	<i>Citrus aurantifolia</i> (Christm) Swingle	Lime	Lemun tsami ^h , Lemu bakagi ⁿ , Osan wewe ^y , Afofanta ⁱ .	1690
“	<i>Citrus paradisi</i> Macf	Grape fruit	Furuntu ^h , Furuntu ⁿ , osan gerepu ^y , Oroma orji ⁱ .	1440
Sapindaceae	<i>Blighia sapida</i> (konig)	Akee apple	Gwanja kusa ^h , Yilanchi ⁿ , Isin ^y , Okpu ⁱ	1180
Tiliaceae	<i>Grewia mollis</i> Juss	Raisin	Dargaji ^h , Rwarwanchi ⁿ , Oraigbo ^y .	1899

Key : h – Hausa ; n – Nupe ; y – Yoruba ; i – Igbo; NA- Not available

Table 4.2h: Medicinal plants used in the Treatment of Typhoid Fever in Minna, Niger State

Family	Botanical names/Authority	Common Names	Vernacular names	Voucher number
Verbanaceae	<i>Vitex doniana</i> Sweet	Black plum	Dinya ^h , Dinchi ⁿ , Oriri ^y	1162
Zingiberaceae	<i>Zingiber officinale</i> Roscoe.	Ginger	Tsita mai yatsu ^h , Tsutafu ⁿ , Atale ^y	3021

Key : h – Hausa ; n – Nupe ; y – Yoruba ; i – Igbo; NA- Not available

4.3 Medicinal Plants and their parts used for the treatment of typhoid Fever

The recorded plant species in relation to their parts used and their habits/habitats are shown in table 4.3 (a-c). The result showed that medicinal plants which grow up to the size of a tree are represented by 63.34% while 22.45% of the plant species are shrub in nature. Herbs, grass and underground stem are represented by 8.16%, 4.08%, 2.04% respectively. Analysis of the table also showed that the plants obtained from the wild represented 53.57% while those cultivated made up 46.43%.

Table 4.3a : Medicinal Plants and their Classified habits/habitats

Species	Plant Part	Habit / Habitat
<i>Alchornea cordifolia</i>	Leaves	Tree / Wild
<i>Alstonia boonei</i>	Stem bark	Tree / Wild
<i>Alternanthera sessilis</i>	Whole Plant	Herb / Cultivated
<i>Anacardium occidentale</i>	Stem bark and leaves	Tree / Cultivated
<i>Ananas comosus</i>	Unripe Fruit	Herb / Cultivated
<i>Annona senegalensis</i>	Stem bark and leaves	Shrub/ Wild
<i>Anogeissus leiocarpus</i>	Stem bark	Tree / Wild
<i>Azadirachta indica</i>	Stem bark and leaves	Tree / Cultivated
<i>Balanites aegyptica</i>	Leaves	Tree / Wild
<i>Blighia sapinda</i>	Leaves	Tree / Cultivated
<i>Calotropis procera</i>	Leaves	Shrub / Cultivated
<i>Carica papaya</i>	Leaves	Tree / Cultivated
<i>Chromolaena odorata</i>	Leaves	Shrub / Wild,Cultivated
<i>Citrullus colocynthis</i>	Fruits	Herb / Wild,Cultivated.
<i>Citrus aurantifolia</i>	Fruits	Tree / Cultivated
<i>Citrus paradisica</i>	Fruits	Tree / Cultivated
<i>Commiphora kerstingii</i>	Stem bark	Tree / Wild ,Cultivated.
<i>Cymbopogon citratus</i>	Leaves	Grass / Cultivated
<i>Daniellia oliveri</i>	Stem bark	Tree / Wild
<i>Detarium microcarpum</i>	Leaves	Tree / Wild

Table 4.3b : Medicinal Plants and their Classified habits/habitats

Species	Plant Part	Habit / Habitat
<i>Enantia chlorantha</i>	Stem bark	Tree/ Wild
<i>Eriosema psoraleoides</i>	Leaves	Shrub / Wild
<i>Erythrina senegalensis</i>	Stem bark and leaves	Tree / Wild
<i>Eucalyptus camadulensis</i>	Leaves	Tree / Wild ,Cultivated.
<i>Ficus platyphylla</i>	Leaves	Tree / Wild
<i>Gardenia aqualla</i>	Roots and Leaves	Shrub / Wild
<i>Gossypium hirsutum</i>	Leaves	Herb / Cultivated
<i>Grewia mollis</i>	Stem bark	Shrub / Wild
<i>Khaya senegalensis</i>	Stem bark and Leaves	Tree / Wild ,Cultivated.
<i>Lawsonia inermis</i>	Leaves	Shrub/ Cultivated
<i>Mangifera indica</i>	Stem bark	Tree / Cultivated
<i>Morinda lucida</i>	Stem bark and Leaves	Tree / Wild
<i>Nauclea latifolia</i>	Stem bark and Leaves	Tree / Wild
<i>Ocimum gratissimum</i>	Leaves	Shrub/ Cultivated
<i>Piliostigma thonningii</i>	Leaves	Shrub / Wild
<i>Plumera rubra</i>	Leaves	Tree / Cultivated
<i>Pseudocedrela kotschy</i>	Root	Tree / Wild
<i>Psidium guajava</i>	Stem bark and leaves	Tree / Cultivated
<i>Securidaca longipendunculata</i>	Leaves ,Stem bark and Roots	Tree / Wild

Table 4.3c : Medicinal Plants and their Classified habits/habitats

Species	Plant Part	Habit / Habitat
<i>Senna occidentalis</i>	Leaves	shrub / Wild ,Cultivated
<i>Sorghum bicolor</i>	Leaves	Grass / Cultivated
<i>Spondias mombin</i>	Stem bark and leaves	Tree / Wild
<i>Terminalia avicennioides</i>	Leaves	Tree / Wild
<i>Vernonia amygdalina</i>	Leaves	Shrub/ Cultivated
<i>Vitex doniana</i>	Stem bark and Leaves	Tree / Wild
<i>Ximenia americana</i>	Stem bark and Leaves	Tree / Wild
<i>Zingiber officinale</i>	Rhizome	Underground stem / Cultivated
<i>Ziziphus abyssinica</i>	Root and Stem bark	Tree / Wild

4.4 Medicinal Plants used for the treatment of Typhoid Fever mentioned by three or more informants.

The ethnobotanical survey of medicinal plants showed that some of the medicinal plants are more utilized than the others, thereby confirming their importance. The informants (15.71%) revealed that *Terminalia avicennioides* is most popular, followed by *Lawsonia inermis* cited by 7 informants (10%). *Alstonia boonei* , *Enantia chlorantha*, *Khaya senegalensis* and *Nauclea latifolia* cited by 6 informants (8.57%) came in third position. *Annona senegalensis*, *Cymbopogon citratus*, *Erythrina senegalensis* and *Mangifera indica* were reported by 5 informants (7.14%) while *Azadirachta indica* and *Ficus platyphylla* were mentioned by 4 informants (5.71%). Finally the others reported were mentioned by one, two or three informants. (Table 4.4).

Table 4.4: Medicinal Plants used for the treatment of typhoid Fever mentioned by three or more informants.

Scientific Name	Family	Number of Informants (%)
<i>Alstonia boonei</i> De Wild.	Apocynaceae	6 (8.57)
<i>Annona senegalensis</i> Pers.	Annonaceae	5 (7.14)
<i>Azadirachta indica</i> A. Juss	Meliaceae	4 (5.71)
<i>Cymbopogon citratus</i> (DC.) <i>stapf</i>	Poaceae	5 (7.14)
<i>Enantia chlorantha</i> Olive.	Annonaceae	6 (8.57)
<i>Erythrina senegalensis</i> DC.	Fabaceae	5 (7.14)
<i>Ficus platyphylla</i> Del.	Moraceae	4 (5.71)
<i>Khaya senegalensis</i> (Desr) A. Juss	Meliaceae	6 (8.57)
<i>Lawsonia inermis</i> (L) keay	Lythraceae	7 (10)
<i>Mangifera indica</i> L.	Anacardiaceae	5 (7.14)
<i>Nauclea latifolia</i> J.E. Smith	Rubiaceae	6 (8.57)
<i>Terminalia avicennioides</i> Guill. & perr.	Combretaceae	11 (15.71)

4.5 Some of the Surveyed Plants Images

The following plants (Plates I to XV) were some of the plants surveyed for this research in their Natural Habitats.



Plate I : *Alchornea cordifolia*



Plate II : *Anogeissus leiocarpus*



Plate III : *Calotropis procera*



Plate IV : *Daniellia oliveri*



Plate V : *Erythrina senegalensis*



Plate VI : *Ficus platyphylla*



Plate VII : *Grewia mollis*



Plate VIII : *Khaya senegalensis*



Plate IX : *Nauclea latifolia*



Plate X : *Piliostigma thonningii*



Plate XI : *Plumera rubra*



Plate XII : *Senna occidentalis*



Plate XIII : *Terminalia avicennioides* Guill. & perr.



Plate XIV : *Vitex doniana*



Plate XV : *Ximenia americana* L.

4.6 Some Traditional Recipes used for the Treatment of Typhoid Fever in Minna, Niger state.

Most of the recipes used in the treatment of typhoid fever contain a combination of medicinal plants while some contain a single plant as shown below.

Table 4.5: Some Recipes used for the Treatment of Typhoid Fever in Minna, Niger state.

Plant Species	Preparation/ Dosage
<i>Mangifera indica</i>	Grind the dried bark of Mango tree, sieve and macerate with water. 1 cup twice a day
<i>Enantia chloranta, Alstonia boonei</i>	The stem barks are boiled together with red ginger. Half cup to be taken thrice a day.
<i>Plumera rubra</i>	Fresh leaves are boiled together with potash. One cupful twice a day.
<i>Annona senegalensis, Senna occidentalis, Terminalia avicennioides</i>	The leaves are boiled together with potash and taken twice a day.
<i>Lawsonia inermis</i>	Dried leaves are pounded smoothly and a spoonful is taken with pap or 7 up (beverage) for seven days.
<i>Enantia chloranta , Khaya senegalensis, Daniellia oliveri</i>	The stem barks are broken into pieces and macerated with cold water over night. A cup twice daily.
<i>Terminalia avicennioides</i>	Dried leaves are pounded smoothly and a Tablespoonful is taken with pap thrice a day.
<i>Ananas comosus, Citrus aurantifolia Citrus paradisiaca</i>	The fruits are boiled together with Lemon grass. Half cup to be taken thrice a day.
<i>Ocimum gratissimum</i>	Fresh leaves are pounded and infused in cold water. One cupful twice a day. Aqueous decoction of whole plant. Half cup to be taken thrice a day
<i>Anacardium occidentale, Carica papaya , Psidium guajava , Azadirachta indica</i>	The leaves are boiled together and taken twice a day. The patient also baths with it.
<i>Morinda lucida</i>	Aqueous decoction of the leaves. Half cup to be taken thrice a day

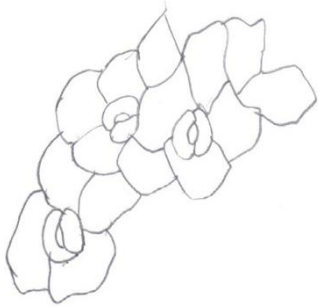
4.7 Microscopic Evaluation of the Leaves of *Terminalia avicennioides*

The transverse section of the leaf through the midrib showed the precise position and arrangement of the various tissues present.

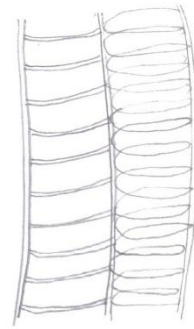
The epidermal cells are tabular and straight walled. They are narrowly placed with no intercellular spaces. Covering trichomes are more abundant on the adaxial surface than on the abaxial surface. Anomocytic type of stomata is present on both surfaces but more frequent on the abaxial surface.

The mesophyll is dorsiventral, the palisade layer is compact, single layered with elongated narrow cells. The spongy layer is made up of loosely arranged cells and vascular bundles which are annular, spiral xylem and phloem fibres. A few rosette type of calcium oxalate crystals are present in the mesophyll.

The midrib is biconvex in shape with the lower part more curved than the upper part. The epidermal layers of the lamina are continuous in the midrib region, underneath it lies rows of parenchymatous layer, the remaining ground tissues being collenchymatous. A collateral vascular bundle made up the stele.



a: Anomocytic stomata



b: Annular and spiral vessel



c: Trichomes



d: Calcium oxalate crystals (Rosette)

Figure 4.2 (a-d) : Some microscopic diagnostic features from the powdered leaves of *T. avicennioides* ; x400

4.8 Quantitative leaf microscopy of the leaves of *T. avicennioides*

The Quantitative leaf microscopy of the leaves of *T. avicennioides* shows the number of stomata (50.5 – **59.4** – 68.3 and 86.0 – **101.2**– 116.4) and stomatal index on both the upper and lower epidermis (5.6 – **6.7** – 7.9 and 9.2 – **10.8** – 12.4), Palisade ratio (9.7 – **11.4** – 13.1), Vein-islet number and Veinlet termination number (5.2 – **6.2** – 7.1 and 3.7 – **4.4** – 5.1) present in the leaves (Table 4.6).

Table 4.6: Quantitative leaf microscopy of the leaves of *T. avicennioides*

Parameter	Range
Palisade ratio	9.7 – 11.4 – 13.1
Stomatal Number	
Upper epidermis	50.5 – 59.4 – 68.3
Lower epidermis	86.0 – 101.2 – 116.4
Stomatal index	
Upper epidermis	5.6 – 6.7 – 7.9
Lower epidermis	9.2 – 10.8 – 12.4
Vein islet number	5.2 – 6.2 – 7.1
Veinlet termination number	3.7 – 4.4 – 5.1

*Mean values of five counts

*Range = Mean $\pm$ 15%

4.9 Chemomicroscopic Evaluation of the leaves of *T. avicennioides*

The chemomicroscopic evaluation shows the presence of the following Cell wall Materials:

Cellulose

A blue black color was observed on the walls of some epidermal cells and other parenchymatous cell.

Lignin

Bright-red colorations were observed on the phloem fibres & xylem vessels.

Starch

Blue-black coloration was observed on starch grains of some parenchymatous cells.

Calcium oxalate

The bright crystals observed dissolves with the addition of HCl.

Fat

Orange red coloration was observed in some epidermal and parenchymatous cells

Tannin

Greenish-black color was observed in some epidermal cells, parenchymatous cells and collenchymatous cells

4.10 Physicochemical Determinations of the Leaves of *T. avicennioides*

The physical constants determined from the powdered leaves of the plant were total ash value, acid insoluble ash, water soluble ash, moisture content, water soluble extractives and alcohol soluble extractives. (Table 4.10)

4.7 Physicochemical Determinations of the Leaves of *T. avicennioides*

S/N	Parameters	Value (% w/w) $\pm$ SEM
1	Moisture content	6.10 $\pm$ 0.24
2	Total ash value	6.33 $\pm$ 0.47
3	Acid insoluble ash	1.11 $\pm$ 0.25
4	Water soluble ash	3.33 $\pm$ 0.20
5	Water soluble extractive	19.67 $\pm$ 0.44
6	Alcohol soluble extractive	19.33 $\pm$ 0.88

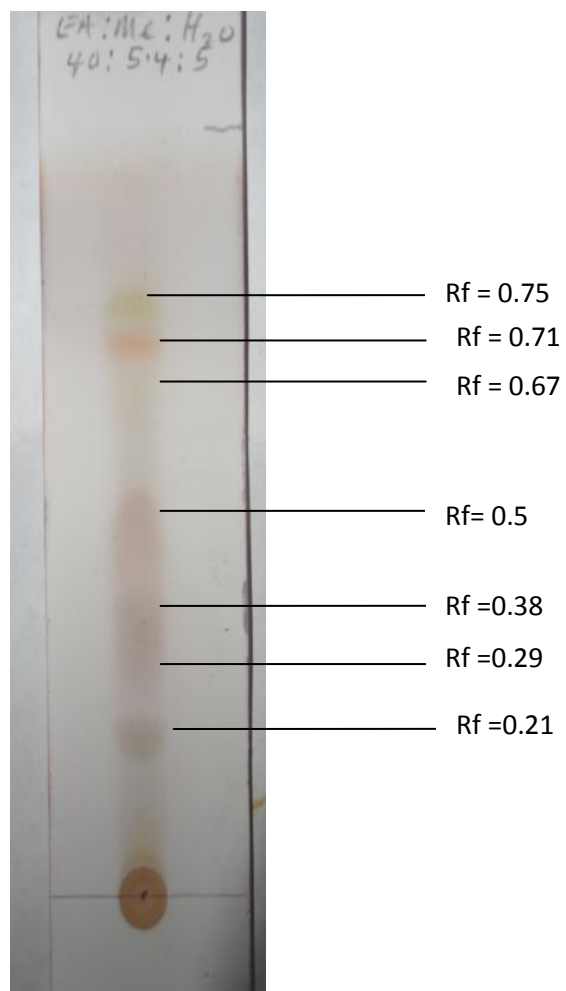
* Mean value of 3 counts.

4.11: Chromatographic Analysis of the Leaves of *T. avicennioides*

The developed chromatogram revealed seven spots after spraying with sulphuric acid (A general spray), greenish black spots and a red colored spot after spraying with ferric chloride spray and p-anisaldehyde spray respectively and one spot when viewed under U.V light (254nm)

Table 4.8: Chromatographic resolution of Methanolic Extract of the Leaves of *T. avicennioides*

DETECTING AGENT	OBSERVATION	INFERENCE
Day Light	No visible spot	-
U.V Light	One red spot	-
Spraying reagent		
Sulphuric spray	Seven colored spots	-
Ferric chloride	Greenish black colored spots	Phenolic compounds present
p-anisaldehyde	Red colored spot	Terpenoids present
Dragendorff	No change in color	Alkaloid absent



PlateXVI: Developed Chromatogram of Methanol Extract of *T. avicennioides* Leaves after spraying with 10% sulphuric acid.

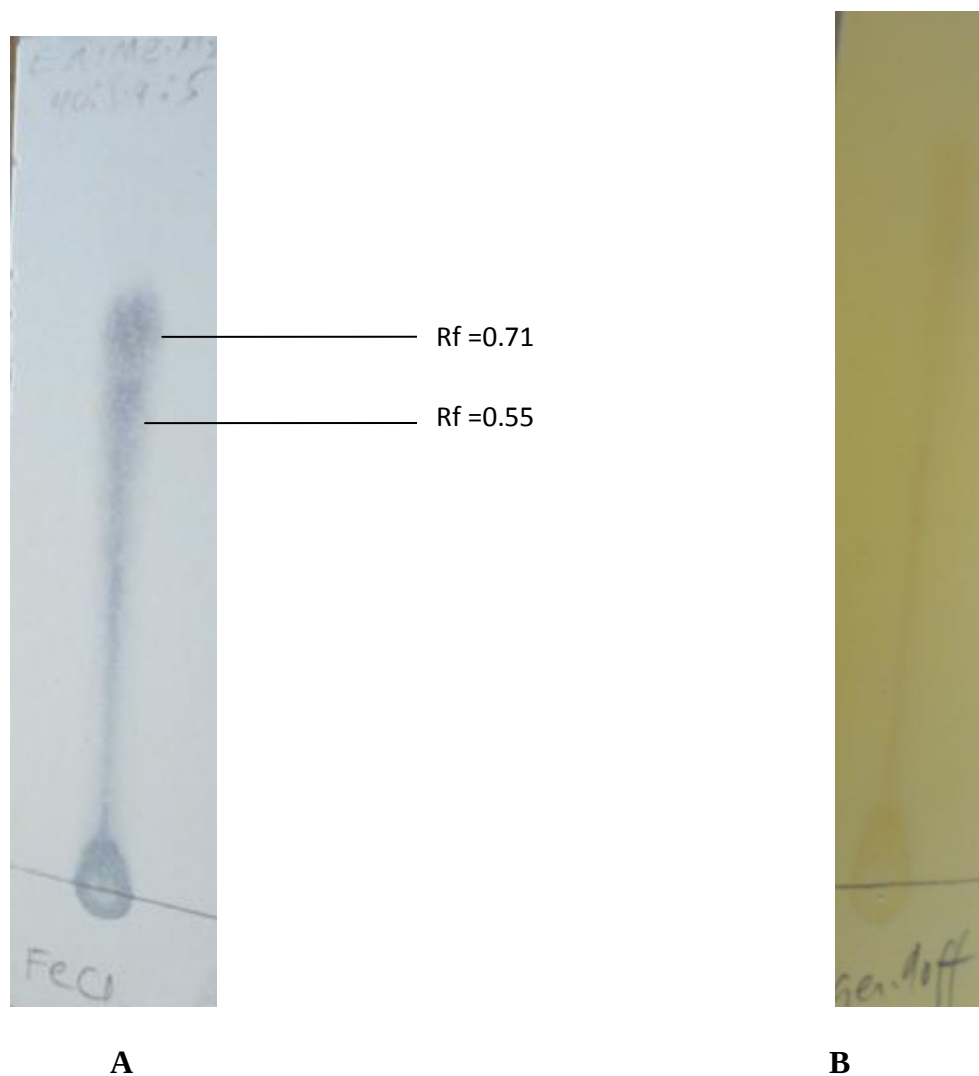


Plate XVII: TLC profile of methanol extract of *T. avicennioides* leaves

Developed chromatogram after spraying with ferric chloride (A) and Dragendorff's reagent (B)

4.12 Anti-salmonella studies on the leaves of *T. avicennioides*

The evaluation of the anti- salmonella activity shows that the methanol extract(defatted) showed the highest zone of inhibition ($31\text{mm} \pm 0.62\text{mm}$) followed by the Methanol extract (crude) and the acetone extract ($18\text{mm} \pm 0.88\text{mm}$) (Table 4.12).

The pet ether fraction only showed the zone of inhibition $17\text{mm} \pm 0.58\text{mm}$ at a concentration of 100mg/ml. The organism showed resistance to the other levels of concentration of Pet ether fraction at 50mg/ml, 25mg/ml and 12.5mg/ml (Table 4.12).

Table 4.9: Zone of inhibition (mm) at different concentration of plant (*T. avicennioides*) extract .

EXTRACT	Zone of Inhibition			
	100(mg/ml)	50(mg/ml)	25 (mg/ml)	12.5 (mg/ml)
Methanol Extract (Crude)	21 $\pm$ 0.44	16 $\pm$ 0.62	16 $\pm$ 0.44	15 $\pm$ 0.44
Acetone Extract	18 $\pm$ 0.88	17 $\pm$ 0.71	17 $\pm$ 0.82	15 $\pm$ 0.62
Methanol Extract (defatted)	31 $\pm$ 0.62	24 $\pm$ 0.94	22 $\pm$ 0.62	18 $\pm$ 0.71
Pet ether extract	17 $\pm$ 0.58	-	-	-
Ciprofloxacin disc (5 μ)	32 $\pm$ 0.58			

$\pm$ Standard error of mean

- = No zone of inhibition

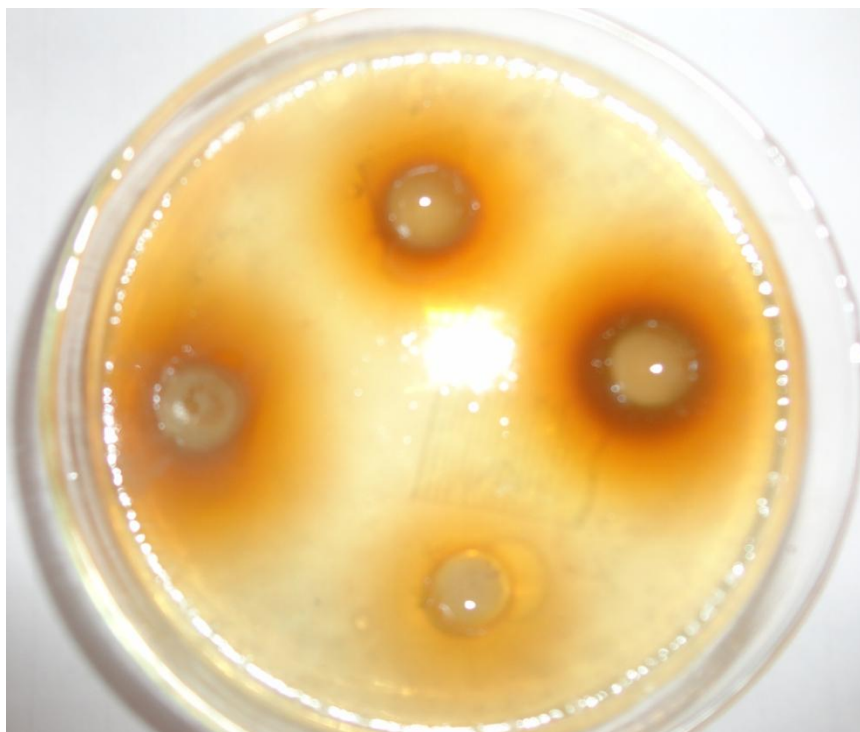


Plate XVII: Agar Plate showing zones of inhibition at different concentrations of plant extract (Methanol fraction)

4.13 Minimum inhibitory concentration of plant (*T. avicennioides*) extract

The Minimum inhibitory concentration of methanol extract of *T. avicennioides*.

was 6.25mg/ml (Table 4.11).

Table 4.10 Minimum Inhibitory Concentration (MIC) of plant (*T. avicennioides*) extract

Test organism	MIC (mg/ml)									
	100	50	25	12.5	6.25	3.125	1.56	0.78	0.39	0.19
<i>S.typhi</i>	-	-	-	-	-*	+	++	++	++	++

Key: (-) clear , (+) light growth, (++) dense growth , (*) MIC

4.14 Minimum Bactericidal Concentration of plant (*T. avicennioides*) extract

The Minimum Bactericidal Concentration of methanol extract of *T. avicennioides*.

was recorded as 12.5mg/ml. (Table 4.12).

Table 4.11 Minimum Bactericidal Concentration (MBC)

Test organism	MBC(mg/ml)					
	100	50	25	12.5	6.25	3.125
<i>S.typhi</i>	-	-	-	-*	+	++

Key: (-) clear , (+) light growth, (++) dense growth , (*) MBC

CHAPTER FIVE

5.0 DISCUSSIONS

Although traditional medicines are highly recognized and commonly used both in the rural and urban communities in Nigeria, the accurate knowledge of these plants and their medicinal properties are known only by few individuals in the community. Those with this knowledge are

elders as reported in table 4.1. It can be presumed that the the younger generations are not eager to acquire this knowledge owing to the wrong belief that the practice is only for illiterate and old people. The continuation of this trend might lead to the loss of traditional knowledge on medicinal plants.

Plants are more easily recognized by their local names in every part of the world. These local names play a vital role in ethnobotanical study of a specific tribe or region (Singh, 2008). Respondents interviewed gave local names of plants in recipes for treating typhoid fever. The local names mentioned were authenticated with their respective botanical names using standard texts. Although local names are not recommended directly for scientific accounts of plants as they lack uniformity and consistency (Singh, 2008), yet they may certainly be considered as a useful tool for obtaining useful information on plants. Local names provide means of reference by local people in a particular area. Information gathered showed that increasing number of people are turning to herbal remedies for prevention and cure of various diseases. The 49 medicinal plant species mentioned were represented by all plant forms. Trees were found to be the most used plants followed by, shrubs, herbs, grass and underground stem.

The plant leaves are important ingredient in traditional treatment of typhoid fever as it is the component that featured most in many herbal preparations which were in agreement with Adekunle (2008).

Some of the plants revealed in the survey have been cited in the ethnobotanical survey of some African countries (Adekunle, 2008; Ogbole and Ajayioba, 2010; Soladoye *et al.*, 2010; Idowu *et al.*, 2010; Oni, 2010). The most commonly used plant in the survey was *Terminalia avicennioides* mentioned by eleven (11) informants.

In recent years there has been an emphasis in standardization of medicinal plants with therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken (WHO, 1999) .

Histologically, the powdered leaves of *T. avicennioides* compose of the rosette type of calcium oxalate crystals, sclereids, heavily lignified spiral and annular xylem vessels, covering trichomes and fibre elements. The occurrence of anomocytic stomata both abaxially and adaxially is of diagnostic importance likewise results obtained on the quantitative leaf microscopy. The presence of numerous unicellular covering trichomes on both sides of the epidermal cells is also of diagnostic importance. Trichomes are hair-like appendages that develop from cells of the aerial epidermis and are produced by most plant species (Werker, 2000). Leaf trichomes may also increase resistance to abiotic stress.

They may increase tolerance to drought by reducing absorbance of solar radiation and increasing the leaf surface boundary layer (Choinski and Wise 1999; Benz and Martin, 2006). Trichomes may further protect living cells from damages caused by solar UV-radiation (Skaltsa *et al.*, 1994) and low temperatures (Agrawal *et al.*, 2004).

Trichomes may thus have multiple functions and trichome density may evolve in response to variation in several environmental factors. In general, plants with high density of leaf trichomes can be expected in environments that are dry or cold, where UV-radiation is intense, and in areas where the risk of being damaged by herbivorous insects are high (Loe *et al.*, 2007).

Drugs which contain excess water is uneconomical and in conjunction with a suitable temperature, moisture will lead to the activation of enzymes and giving suitable conditions to the proliferation of Micro-organisms. As most vegetable drugs contain all the essential food requirements for moulds, insects and mites. Thus during storage deterioration can be very rapid once infestation has taken place (Evans, 2008). The general requirement of moisture content in crude drug is that, it should not be more than 14% (British pharmacopoeia, 1980) and the value obtained (6.1% w/w) is within an accepted range.

The total ash figure is of importance and indicate to some extent the amount of care taken in the preparation of the drug. Ash is the residue remaining after incineration of plant material , which simply represents inorganic salts, naturally occurring in crude drug or adhering to it or deliberately added to it, as a form of adulteration. The ash value was determined by three different methods, which measured total ash, acid-insoluble ash and water soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition. This includes both 'physiological ash' which is derived from the plant tissue itself, and 'non-physiological ash', which is the residue of the extraneous matter adhering to the plant surface. Acid-insoluble ash is a part of total ash and measures the amount of silica present, especially as sand and siliceous earth. Water soluble ash determines that part of total ash which is soluble in water, and it is a good indicator of either previous extraction of the water soluble salts in the drug or incorrect preparation. These ash values are important quantitative standards. The total ash value of 6.33% w/w obtained is moderate. The acid insoluble ash of 1.11% w/w is an indication that the powdered leaves contains some amount of dirt and the water soluble ash of 3.33% is moderate. The water soluble and alcohol soluble extractive values were 19.67% w/w and 19.33% w/w respectively. This result showed that more constituents are soluble in water than alcohol.

The alcohol and water soluble extractive values are meant to detect exhausted and already utilized drug which could be fraudulently used as substitutes and adulterants (Elujoba, 1999).

Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and detection of plant constituents. The results showed seven spots after spraying with sulphuric acid and one spot when viewed under U.V light. No color change was observed after spraying with Dragendorff, this indicates the absence of alkaloid in the leaves. Greenish-black spots were observed after spraying with ferric chloride which indicates that phenolic compounds are present. Red spots were observed after spraying with p- anisaldehyde which confirms the presence of terpenoids. Terpenoids have antiseptic, anti-inflammatory and antimicrobial properties. They constitute the active constituents of a number of medicinal plants and are of current interest for their potential as future drugs (Evans, 2008). Phenolic compounds such as tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Sodipo *et al.*, 1991). The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins (Chung *et al.*, 1998). Flavonoids are naturally occurring phenols which possess numerous biological activities including anti-inflammatory, anti-allergic, anti-thrombotic and vasoprotective effects (Ogunleye and Ibitoye, 2003).

The highest zone of inhibition recorded for *S. typhi*, was found in methanol extract (defatted) at a concentration of 100mg/ml (31mm) indicating the highest degree of activity against the organism. The crude extract also showed a significant activity at a concentration of 100mg/ml (21mm) followed by acetone extract. The pet ether fraction exhibited a zone of inhibition only at a concentration of 100mg/ml (17mm). The zones of inhibition decrease as the concentration of the plant extracts were reduced as shown in table 4.12. It can be deduced that the bioactive

constituents in the Methanol fraction were responsible for the anti salmonella activity which could be comparable to that of ciprofloxacin which showed a zone of inhibition of 32mm.

Although the latter exhibited a wider zone of inhibition, increasing the concentration of the plant extract might be more effective against *S. typhi*.

The MIC result obtained was 6.25mg/ml which suggests that at this concentration the growth of the organism was inhibited. The MBC was 12.5mg/ml which implies that the organism was killed at this concentration.

Minimum inhibitory concentrations (MICs) are considered the ‘gold standard’ for determining the susceptibility of organism to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing. MICs are defined as the lowest concentration of antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation and the minimum bacteria concentrations (MBCs) as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic - free media (Jennifer, 2001).

Typhoid fever caused by *S. typhi* is a major health problem in developing countries, particularly in Sub-Saharan African countries and hence the considerable anti-salmonella activity shown by the test extracts shows the great potential of the plant for treatment of typhoid fever.

The results of the macroscopic, microscopic, physical constants and chemomicroscopic evaluations provide a useful information which can be included in a monograph of the plant .

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATION

6.1 SUMMARY

An ethnobotanical survey was conducted in six communities in Minna Niger State, Nigeria. The survey revealed a total of 49 plants species belonging to 31 families and the most frequently used plant was *Terminalia avicennioides* Guill. and Perr.

The microscopic evaluation carried out on the leaves of *T. avicennioides* revealed: anomocytic stomata on both upper and lower surface, covering trichomes, rosette calcium oxalate crystals, phloem fibres, annular and spiral xylem vessels. The chemomicroscopy indicated the presence of cellulose, lignin, starch, calcium oxalate crystals, fat and tannins.

The phytochemical screening carried out on the methanol extract of the leaves of *T. avicennioides* using Thin layer chromatography (TLC) showed that phenolic compounds and terpenes were present and alkaloid absent.

The anti-typhoid activity of *T. avicennioides* extract against *Salmonella typhi* showed significant inhibitory activity. The Minimum Inhibitory Concentration (MIC) value obtained using the Broth dilution method was 6.25mg/ml while Minimum Bactericidal Concentration (MBC) was 12.5mg/ml.

6.2 CONCLUSION

The present study has established a data bank for some medicinal plants that are used in the management of typhoid fever in Minna Niger State, Nigeria.

The pharmacognostic studies on the leaves of *Terminalia avicennioides* can serve in the correct identification and preparation of a monograph on the plant.

Scientific evidence on the rationale use of the leaves of *Terminalia avicennioides* in the treatment of typhoid fever in Minna, Niger State has been established.

6.3 RECOMMENDATION

This study has provided additional information on the relevance of plants in the treatment of various diseases in our society. It is a step forward towards investigating the medicinal plants diversity in Nigerian flora.

The development of an integrated traditional and scientific knowledge base will serve as a mechanism for accessing, benefit-sharing and documenting traditional knowledge for sustainable socio economic development and poverty alleviation in the country.

Further research on the screening of the secondary metabolites of these medicinal plants for biological and pharmacological studies will be necessary as well as the isolation of active compounds and their structural elucidation for the maximal use of the medicinal plants.

In order to prevent overexploitation that could lead to extinction, efforts should be made to conserve natural resources and to domesticate selected plant species which are commonly used among the herbal practitioners. A high level of pharmacological content should be ensured, using

modern cultivation and preparation methods. The sustainable cultivation of medicinal herbs could facilitate industrial scale processing.

REFERENCES

Abdullahi, A.L., Agho, M.O., Amos, S., Gamaniel, K.S. and Wambebe, C(2001).

Antidiarrhoeal activity of the aqueous extract of *Terminalia avicennioides* roots.

Journal of Phytotherapy Research 15, 431-434.

- Adekunle, M.F (2008). Indigenous uses of plants leaves to treat malaria fever at Omo Forest Reserve (OFR), Ogun State, Nigeria. *Ethiopia Journal of Environmental Studies* 1(1): 31-35
- Adjanohoun, E, Ahiyi, M.R.A. Ake-Assi, L. Dramane .K, Eewude J.A, Fadoju S .O, Gbile Z.O, Goudote, .E, Johnson, C.L.A, Keita, .A, Morakinyo .O, Ojewole, J.A.O, Olatunji, A.O and Sofowora, E.A (1991). *Traditional medicine and pharmacopoeia: Contribution to ethnobotanical and floristic studies in Western Nigeria*. Organization of African Unity Scientific, Technical and Research Commission Lagos, Nigeria. pp.420
- Agrawal, A.A., Conner, J.K and Stinchcombe, J.R. (2004). Evolution of plant resistance and tolerance to frost damage. *Ecology Letter* 7:1199–1208
- Ajaiyeoba, E. (2002). Phytochemical and antibacterial properties of *Parkia biglobosa* and *Parkia bicolor* leaf extracts. *African Journal of Biomedical Resources* 5(3): 125-129
- Akuezilo, E. O. (1993). Research method and statistics. Nuel Centi Publishers, Akwa Nigeria. Pp. 11-15
- Aliero, A. A. and Wara, S. H. (2009). Validating the medicinal potential of *Leptadenia hastata*. *African Journal of Pharmacy and Pharmacology*. 3(6): 335-338.
- Awoyemi, O. K. , Ewa, E. E., Abdulkarim, I. A. and Aduloju, A. R. (2012) Ethnobotanical assessment of herbal plants in southwestern Nigeria. *Academic Research International* 2(3) 50-57

- Baba-Moussa, F., Akpagana, K and Bouchet, P (1999). Antifungal activities of seven West African Combretaceae used in traditional medicine. *Journal of Ethnopharmacology* 66, 335-338.
- Babayi, H., Kolo, I., Okogun, J. I and Ijah, U. J. (2004). The antimicrobial activities of methanolic extracts of *Eucalyptus camaldunensis* and *Terminalia catappa* against some pathogenic microorganisms. *Biokemistri* 16 (2): 106-111.
- Benz, B.W and Martin, C.E. (2006) Foliar trichomes, boundary layers, and gas exchange in the species of epiphytic *Tillandsia* (Bromeliaceae). *Journal of Plant Physiology* 163:648–656
- Betti, J. L., (2004). An ethnobotanical study of medicinal plants among the Baka pygmies in the Dja Biosphere Reserve, Cameroon. *African Study Monographs*, 25(1): 1-27.
- British Pharmacopoeia (1980). *Ash value, Acid Insoluble Water Soluble, Extractive and Alcohol Extractive*, Vol. II, Appendix XII, Majesty's Stationery Office, London, pp. 1276 – 1277.
- Bulus, T., Atawodi, S. E. & Mamman, M. (2008)). *In Vitro* antitrypanosomal activity and phytochemical screening of aqueous and methanolic extracts of *Terminalia avicennioides*. *Nigerian Journal of Biochemistry and Molecular Biology*. 23(1):8-14.
- Centers for Disease Control and Prevention (CDC) (2003). *Travelers health. Preventing typhoid fever: a guide for travelers. Atlanta*
(<http://www.cdc.gov/travel/disease/typhoid.htm>).
- Centers for Disease Control and Prevention (CDC). (2006). Coordinating Centre for Infectious Diseases / Division of Bacteria and Mycotic Diseases. *Bulletin*; Nov 4, 2006. pp 1

- Choinski, J.S. and Wise, R.R. (1999) Leaf growth and development in relation to gas exchange in *Quercus marilandica* Muenchh. *Journal of Plant Physiology* 154: 302–309
- Chung, K.T., Wong, T.Y., Wei, C.I., Huang, Y.W and Lin, Y. (1998). Tannins and human health. *Critical Review Food Science Nutrition* 38(6): 421- 464.
- Cordell, G.A., (1995). *Changing strategies in natural products chemistry*. Review article No.109. *Phytochemistry*, Vol.40 (6):1585-1612.
- Cowan, M.M. (1999). Plants products as antimicrobial agents. *Clinical Microbiology Review*. 12: 564-582.
- Coyle, B.M. (2005). *Manual of antimicrobial susceptibility testing*. American Society for Microbiology. Pp 236
- Diallo, D, Hveem B, Mahmoud M.A, Berge G, Paulsen B.S, and Maiga A,(1999): An ethnobotanical survey of herbal drugs of Gourma district, Mali. *Pharmaceutical Biology*, 37:80-91.
- Edeoga, H.O., Okwu E.O. and Mbaebie, M.O (2002). Phytochemical constituents of some Nigerian Medicinal plants. *African Journal of Biotechnology*. 4(7): 685-688.
- Elujoba, A.A. (1999). Pharmacognosy for health and culture. *The PHC jungle connection, inaugural lecture series 134*. OAU Press Ltd. Ile-Ife, Nigeria. pp 9-10
- Erdogrul OT (2002). Antibacterial activity of some plant extracts used in folk medicine. *Pharmaceutical biology*., 40: 269-273.

Ernest .R. (2001). *The African Herbal Industry: Constraints and Challenges*.

Evans, C.E., Banso, A. and Samuel, O. A. (2002). Efficacy of some Nupe medicinal plants against *Salmonella typhi*: an in vitro study. *Journal of Ethnopharmacology*. 80 (1): 21-4.

Evans, W.C. (2008). *Trease and Evans Pharmacognosy* , W.B. Saunders, 15th edition. Pp. 516-525

Gbolade, A.A. and Soremekun, R.O, (1998). A survey of aromatic plants of economic importance in Nigeria. *The Nigerian Journal of Pharmacy*. 29, 50-62.

Gbolade , A.A (2000). Medicinal and pesticidal potential of Nigeria plants. *Proceedings of International Symposium on Natural Products in Developing Countries. Trends and Needs*, pp. 18-24.

Ghalib, H. (2007) The hunt for the next Artemisinin *Tropical Disease Research News*

Gilmore, M.R. (2005). Uses of Plants by the Indians of the Missouri River Region. In: *Thirty third annual report of the bureau of America ethnology*. Pp 6-14

Hatil, H.E. and Kamali, .L (2009). Medicinal Plants in East and Central Africa

Idowu, .O.A., Soniran, .O.T., Ajana, .O, and Aworinde, .D.O (2010). Ethnobotanical survey of anti-malarial plants in Ogun State, South Western Nigeria. *African. Journal of Pharmaceutical Pharmacology*. 4(2): 055-060.

Igoli, J.O., Oyali, O.G, Tor-Ayin, T.A. and Igoli, N.P (2005). Traditional medicine practice amongst the Igede people of Nigeria. Part II. *African Journal of Traditional and Complimentary/ Alternative Medicine*, 2: 37-47.

- Iroha, I. R.I., Lang, D. C., Ayogu, T. E., Oji, A. E. and Ugbo, E. C. (2010). Screening for anti typhoid activity of some medicinal plants used in traditional medicine in Ebonyi state, Nigeria. *African Journal of Pharmacy and Pharmacology* 4(12): 860 – 864.
- Iwu, M.M., Duncan, A.R and Okunji, C. O (1999). New antimicrobials of plant origin. Janick, J (ed.). *Perspectives on new crops and new uses*, pp 457-462.
- Jennifer, M.A., (2001). Determination of minimum inhibitory concentration. *Journal of Antimicrobial chemotherapy* 48 Suppl. SI, 5-16
- Kafaru, E.(1994). *Immense Help From Nature`s Workshop*. Elika Health Services Ltd., Academic Press Plc. Lagos, Nigeria. pp. 1-27.
- Khan, A.U,(2002). History of decline and present status of natural tropical thorn forest in Punjab. *Pakistan Biological Conservation*, 63: 210-250.
- Khan, N.A, Rashid, A.Z.M (2006). A study on the indigenous medicinal plants and healing practices in Chittagony Hill Tracks (Bangladesh). *African Journal of Traditional and Complimentary/ Alternative Medicine*, 2: 37- 47.
- Kokate, C. K.(2001). *Practical Pharmacognosy*. M.K. Jain and Vallabh prakashan Delhi, 4th edition. pp 115-121
- Lawal, I.O, Uzokwe, N.E, Igboanuyo, A.B, Adio, A.F, Awosan, E.A, Nwogwugwu J.O, Faloye, B, Olatunji BP and Adesoya, A.A (2010). Ethnomedicinal information on collation and identification of some medical plants in Research Institutes of Nigeria. *Journal of Pharmacy and Pharmacology*. 4(1): 001-007.

- Lewis, K. and Ausubel, F.M. (2006). Prospects for plant-derived antibacterials. *Natural Biotechnology*. 24 (12): 1504-1507.
- Loe, G., Torang, P., Gaudeul M., A., gren, J. (2007). Trichome production and spatiotemporal variation in herbivory in the perennial herb *Arabidopsis lyrata*. *Oikos* 116:134–142
- Mann, A., Amupitan, J.O., Oyewale, A.O., Okogun, J.I and Ibrahim, K(2007). An Ethnobotanical survey of indigenous flora for treating tuberculosis and other respiratory diseases in Niger State, *Nigeria. Journal of Phytomedicine & Therapy*. 12, 1-12.
- Martin, G.J (1995). *Ethnobotany. A people and plants conservation manual*. Chapman and Hall London, Pp. 288
- Mgeni, A.S (1991). *A more efficient estate: natural forest or plantation*. In: Pyatt N, Williams T (eds). *Tropical Forestry: Third world priorities Versus Western Concern*, pp. 43-58.
- Miller, S.I., Hohmann, E.L., Pegues, D.A, (1994). *Salmonella (including Salmonella typhi)*. In *principles and practice of infectious diseases* Vol. 2 4th ed. Edited by: Mandell GL, Bennet JR, Dolin R. New York: Livingstone, pp. 2013-2033.
- National Committee for Clinical Laboratory Standards (NCCLS) (2000). *Methods for dilution and antimicrobial susceptibility tests for bacteria that grow aerobically*—fifth edition. Approved standard. M7-A5., NCCLS: Wayne, PA.
- Niger state Planning Commission (NPC) (2011) *Facts and figures on Niger State*, State bureau of statistics pp 1-6.

- Nkuo-Akanji, T., Ndip, R., Mcthomas, A. and Fru, E. C. (2001). Anti-salmonella activity of medicinal plants from Cameroon. *Central Africa Journal of Medicine.*, 47(6): 155-158.
- Ogbole, O.O and Ajaiyeoba, E.O (2010). Traditional management of tuberculosis in Ogun State of Nigeria: the practice and ethnobotanical survey. *African Journal of Traditional and Complimentary/ Alternative Medicine*, 7(1): 79-84.
- Ogunleye, D.S. and Ibitoye S.F. (2003) Studies of antimicrobial activity and chemical constituents of *Ximenia americana*. *Tropical Journal of Pharmaceutical Research*, 2 (2), 239-241
- Olajide, O (2003). Steps toward sustainable natural forest management for non-timber forest products in Nigeria. *Proceedings of the 29th Annual conference of the Forestry Association of Nigeria*, Cross River State.
- Oluduro, A. and Omoboye, O. (2010). In Vitro Antibacterial Potentials and Synergistic Effect of South-Western Nigerian Plant Parts Used in Folklore Remedy for *Salmonella typhi* infection. *Nature and Science*; 8 (9): 52-59.
- Oni, P.I (2010). Ethnobotanical survey of a fallow plot for medicinal plants diversity in Idena village, Ijebu-Ode, South-Western Nigeria. *Journal of Medicine and Plant Research.*, 4(7): 509-516.
- Osohole, O., Ajaiyeoba, E., Bolaji, O., Akinboye, D., Fawole, O., Gbotosho, G.,... Oduola, A. (2005). A survey of treatment practices for febrile illnesses among Traditional Healers in the Nigerian Middle Belt Zone. *African Journal of Traditional and Complimentary/ Alternative Medicine*. 2, 337 – 34412.

- Owonubi, J.J and Otegbeye, G.O (2004). Disappearing Forests. A review of the challenges for conservation of genetic resources and environmental management. *Journal of Forest and Reserve Management*, 1: 1-11.
- Papadimitropoulous, V., Vergidis, P.I., Bliziotis, I. and Falagas, M.E (2004). Vaccination against fever in travelers: a cost-effectiveness approach. *Clinical Microbiology Infections*. 10: 681-683.
- Patwardhan, B. (2009) Drug discovery and development: Traditional medicine and ethnopharmacology perspectives. *Science Topics*
- Rodders, J., Speedie, M. and Tyler, V., (1996). *Pharmacognosy and Pharmaco-biotechnology*. Baltimore: Williams and Wilkins pp., 7-12.
- Rashid, M.H (2001). Medicinal plants and their uses in the treatment of various diseases. In: Bose S.K, Roshetko J.M (eds). *Propagation and cultivation of medicinal plants in Bangladesh. Agricultural Research Management Project (ARMP)*. Bangladesh Forest Research Institute, pp. 4-6.
- Shah, A.S.M., Akram, M., Asif, H. M., Rehman, R., Khalil, A., Asadullah, M., Ghazala, S. Shamim, T and Saleem, Q (2011). Harmonization of traditional medicine with modern medicine. *International journal of applied biology and pharmaceutical technology*. 253-260
- Shibata, H., Kondo, K., Katsuyama, R., Kawazoe, K., Sato, Y., Murakami, K., Takaishi, Y., Arakaki, N. and Higuti, T. (2005). Alkyl Gallates, Intensifiers of β -Lactam

- Susceptibility in Methicillin-Resistant *Staphylococcus aureus* Antimicrobial Agents *Chemotherapy*. 49(2): 549-555.
- Singh, H (2008). Importance of local names of some useful plants in ethnobotanical study. *Indian Journal of Traditional Knowledge*. 7(2): 365-370.
- Sofowora, S.A. (1993). *Medicinal Plants and Traditional Medicine in Africa*. Spectrum books Ltd. Nigeria. Pp 142-145.
- Sodipo, O.A, Akanji, M.A., Kolawole, F.B and Odotuga, A.A (1991). Saponin is the active antifungal principle in *Garcinia kola* heckles seed. *Bioscience Resource Community*. 3: 171.
- Soladoye, M.O, Amusa, N.A, Raji-Esan, S.O, Chukwuma, E.C, Taiwo, A.A (2010). Ethnobotanical survey of anti-cancer plants in Ogun State, Nigeria. *Annals of Biological Research*. 1(4): 261-273.
- Werker, .E. (2000) Trichome diversity and development. *Advanced Botany Resources* 31:1–35
- World Health Organisation (1999). *Quality control methods for medicinal plant materials*, Geneva. 8-34
- World Health Organisation (2002). WHO Traditional Medicine Strategy 2002-2005. World Health Organization, Geneva.
- World Health Organisation (2003). Fact Sheet No. 134. Revised May 2003.

World Health Organisation (2005).National Policies on Traditional Medicine and Regulation of Herbal Medicines. Report of a WHO global survey. World Health Organization, Geneva.
Pp. 122

APPENDICES

APPENDIX I

ETHNOBOTANICAL SURVEY SHEET (Questionnaire)

FOR THE RESEARCH PROGRAMME ON

TRADITIONAL TREATMENT OF TYPHOID FEVER IN MINNA, NIGER STATE, NIGERIA.

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

Sheet Number-----

District/Village -----

Date -----

LGA-----

Traditional healer/Herb seller/ Informant:

Age -----

Sex -----

Address -----

Duration of practice -----

A. Epidemiology Survey:

- a) What is your level of success in the use of this particular Plant(s) to cure typhoid fever?
- b) How does your community view traditional medicine ?
.....
- c) Which of the gender patronize you most? Female , Male, Both.
- d) What is the educational status of your patients ? Educated, Not educated.
- e) What is the economic status of your patients ?
Poor, Averagely rich , Rich.

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

PLANT	
VERNACULAR NAMES	

PART USED	
FORM OF PREPARATION	
ROUTE OF ADMINISTRATION	

APPENDIX II

1.0 Preparation of Media

1.1 Nutrient broth

Prepare by dissolving 13g of media in 1litre of distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 121⁰C ,15lbs pressure for 15 mins.

1.2 mueller hinton agar (MHA)

Dissolve 38g in 1litre of distilled water, soak for 10 mins bring to boil and sterilize by autoclaving for 15 mins at 121⁰C.Cool to 47⁰C and mix before pouring into petri dishes.

1.3 Normal saline

It is used for standardization of culture. Dissolve 9g NaCl in 1litre of distilled water.