EVALUATION OF HONEY FROM SOME STATES IN NIGERIA BY THE DETERMINATION OF POLLEN SPECTRA, PHYSICO-CHEMICAL PARAMETERS, ANTI-BACTERIAL ACTIVITY AND CONSUMERS' ACCEPTANCE

BY

JUDITH ADESOLA IBEGBULEM

DEPARTMENT OF BIOLOGICAL SCIENCES,
AHMADU BELLO UNIVERSITY, ZARIA,
NIGERIA

JUNE, 2014

EVALUATION OF HONEY FROM SOME STATES IN NIGERIA BY THE DETERMINATION OF POLLEN SPECTRA, PHYSICO-CHEMICAL PARAMETERS, ANTI-BACTERIAL ACTIVITY AND CONSUMERS' ACCEPTANCE

BY

Judith Adesola IBEGBULEM
B.Sc. (UNILAG, 2002), M.Sc. (A.B.U., 2007)
Ph.D/SCIEN/01956/2006-2007

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

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY (Ph.D) IN BIOLOGY

DEPARTMENT OF BIOLOGICAL SCIENCES,
AHMADU BELLO UNIVERSITY, ZARIA,
NIGERIA

JUNE, 2014

DECLARATION

I declare that the work in the dissertation entitled "EVALUATION OF HONEY FROM SOME STATES IN NIGERIA BY THE DETERMINATION OF POLLEN SPECTRA, PHYSICO-CHEMICAL PARAMETERS, ANTI-BACTERIAL ACTIVITY AND CONSUMERS' ACCEPTANCE" has been performed by me in the Department of Biological Sciences under the supervision of Dr. (MRS) D.N. Iortsuun, Dr. E. Kogi and Prof. P.I. Bolorunduro. The information derived from the literature has been duly acknowledged in the text and the list of reference provided. No part of this dissertation was previously presented for another degree or diploma at any university.

JUDITH ADESOLA IBEGBULEM ....

Signature Date

CERTIFICATION

This dissertation titled "EVALUATION OF HONEY FROM SOME STATES IN NIGERIA BY THE DETERMINATION OF POLLEN SPECTRA, PHYSICO-CHEMICAL PARAMETERS, ANTI-BACTERIAL ACTIVITY AND CONSUMERS' ACCEPTANCE" meets the regulations governing the award of the degree of Doctor of Philosophy (Ph.D) (Biology) of Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

Dr. (MRS) D.N. Iortsuun Chairman, supervisory committee Department of Biological Sciences Ahmadu Bello University, Zaria	…… (Signature)	……... (Date)
Dr. E. Kogi Member, supervisory committee Department of Biological Sciences Ahmadu Bello University, Zaria	…… (Signature)	……... (Date)
Prof. P. I. Bolorunduro Member, supervisory committee N.A.E.R.L.S. Ahmadu Bello University, Zaria	…… (Signature)	……. (Date)
Prof. A.K. Adamu The Head Department of Biological Sciences, Ahmadu Bello University, Zaria	…… (Signature)	.……... (Date)
Prof. A. A. Joshua Dean, School of Postgraduate Studies Ahmadu Bello University, Zaria	…… (Signature)	……... (Date)

DEDICATION

I dedicate this work to God the Father of our Lord Jesus Christ and the Holy Spirit.

ACKNOWLEDGEMENTS

My sincere thanks and appreciation goes to God, the Father of our Lord Jesus Christ who granted me the opportunity to complete this good work.

Great and unquantifiable appreciation goes to Dr. Mrs D.N. Iortsuun, Dr. E. Kogi and Prof. P.I. Bolorunduro my supervisors for their various contributions towards the success of this work. God bless you all and reward you and yours.

My sincere gratitude goes to Alhaji Y.I. Ilu, Executive Director, National Agricultural Extension and Research Liaison Service (NAERLS). I am also grateful to my programme leaders; Prof. C.K. Daudu, Prof. J.O. Adeosun and Dr. Ahmed Ibrahim, my Head of Department, for granting me the permission to finish up the programme. Mr. Micheal Ogunwole and all staff of Crop and Forestry Department are appreciated for their contributions towards this work. Dr. M. Chia and Dr. Iyiola-Tunji for their statistical contributions.

I thank Vaughn M. Bryant, Professor of Anthropology, Director, Palynology Laboratory, Department of Anthropology, Texas A&M University, U.S.A. and Mr. Orijemie Akpo Emuobosa, Palynology Laboratory, Department of Archaeology and Anthropology, University of Ibadan, Nigeria for their assistance in the identification and counting of the pollen grains.

Special thanks goes to my husband and children, Surveyor Olu Oyekeye, Pastor Joseph, my daddy late Pastor S.O. Adeyeye of blessed and cherished memories and uncles for your prayers, songs of praise to God and contributions towards this project. God bless you all real good and reward you here and in heaven, Amen.

I sincerely appreciate the assistance rendered to me by the staff of the Department of Biological Sciences, the Department of Microbiology Ahmadu Bello University Zaria and the entire management and staff of NAERLS. Not forgetting former Miss Blessing Afuwai, Now Mrs Gbenga, thank you.

ABSTRACT

Fresh honey samples from six States of three ecological zones in Nigerian were analyzed for their pollen spectra, physico-chemical parameters, anti-bacterial activity through the zone of inhibition (ZOI), Minimum inhibitory and bactericidal concentrations and consumers' acceptance. The honey sample from Jigawa State recorded 18 pollen types (1,339 pollen grains), Bauchi State 20 pollen types (50,110), while Adamawa State recorded 31 pollen types (35,915). Dominant pollen types from the Sudan savanna were *Parkia biglobosa* (61%), *Mangifera indica* (80%), and *Combretum* sp. (90%) for Jigawa, Bauchi, and Adamawa States honey samples respectively. The sample from Plateau State recorded 22 pollen types (82,762) while Kaduna recorded 31 pollen types (27,415). Dominant pollen types from the guinea zone were Myrtaceae (95%) and *Pavetta* species (77%) for Plateau and Kaduna State honey samples respectively. Ondo State had 21 pollen types with a total of 23, 886 pollen grains while Oyo State had 20 pollen types and 409 pollen grains. Oyo State honey seemed to be very low in pollen grain. There was a significant difference in the moisture and ash content of honey samples from the different States ($p<0.05$) though all samples were within the standard limit. The pure honey and concentrations of honey in sterile distilled water were tested for anti-bacterial activity against *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Salmonella typhi,* and *Pseudomonas aeruginosa.* There was significant difference ($p<0.05$) in the susceptibility of the organisms against different honey concentrations for all states at $p< 0.05$. Honey sample from Adamawa State showed a minimum bactericidal concentration of 12.5% for *E. coli, B. cereus* and *S. aureus*. There was a strong relationship between the quantity of honey purchased and consumers' income level ($r=0.9$).

TABLE OF CONTENTS

Title Page..... i

Declaration..... ii

Certification..... Iii

Dedication..... Iv

Acknowledgement..... v

Abstract..... vi

Table of Contents..... Vii

List of Tables..... xiii

List of Figures..... xv

List of Plates..... Xvi

List of Appendices..... xvii

Abbreviations and Symbols..... xviii

CHAPTER ONE..... 1

1.0 INTRODUCTION..... 1

1.1 Background information..... 1

1.2 Statement of Research Problem..... 5

1.3 Justification of Research..... 7

1.4 Objectives of Study..... 9

1.5 Hypotheses..... 9

CHAPTER TWO..... 10

2.0 LITERATURE REVIEW..... 10

2.1 Definition of Honey..... 10

2.2 Honeybees and Bee Keeping..... 11

2.3 Types of Hives..... 12

2.3.1 Fixed Comb Hive..... 13

2.3.2 Improved Comb Hive..... 14

2.3.3 Removable Frame Hive..... 14

2.4 Bee Forage and Vegetation Influence on Honey..... 14

2.5 Nigerian Vegetation..... 20

2.6 Production of Honey..... 25

2.7 Honey Constituents..... 26

2.8 Variation in Honey Sugars and Sugar Properties..... 28

2.9 Types of Honey..... 30

2.10 Deterioration of Quality..... 31

2.11 Moisture/Water Content..... 32

2.12 Physico-Chemical Parameters of Honey..... 34

2.12.1 Mineral and Ash Content..... 36

2.12.2 Ph and Acidity..... 39

2.13 Uses of Honey..... 40

2.14 Anti-Microbial Activity of Honey..... 41

CHAPTER THREE..... 49

3.0 MATERIALS AND METHODS..... 49

3.1 Honey samples Collection Sites..... 49

3.2 Collection and Preparation Of Honey Samples..... 49

3.3 Melissopalynological Analysis of Honey..... 51

3.3.1Pollen Characterization..... 51

3.4 Determination of Physico-Chemical Parameters..... 52

3.4.1 Determination of Water Content..... 52

3.4.2 Determination of Ash Content..... 52

3.4.3 Determination of Mineral and heavy metal Content..... 53

3.4.4 Determination of Ph and Acidity..... 53

3.4.5 Determination of Glucose..... 54

3.4.6 Determination of Reducing Sugar..... 54

3.4.7 Determination of Sucrose..... 55

3.4.8 Determination of Maltose..... 55

3.4.9 Determination of Protein..... 56

3.4.10 Determination of Fat..... 57

3.4.11 Determination of Hydroxymethylfurfural..... 58

3.5 Anti-Bacterial Assay..... 59

3.5.1 Agar Well Diffusion..... 59

3.5.2 Minimum Inhibitory Concentration..... 60

3.5.3 Minimum Bactericidal Concentration..... 60

3.6 Consumers'acceptance of Honey..... 60

3.7 Statistical Analysis..... 61

CHAPTER FOUR..... 62

4.0 RESULTS..... 62

4.1 Pollen Spectra of Honey Samples..... 62

4.1.1 Pollen Spectra of Honey Samples from Sudan Savanna Zone..... 62

4.1.2 Pollen Spectra of Honey Samples from Guinea Savanna Zone..... 67

4.1.3 Pollen Spectra of Honey Samples from the Rain Forest Zone..... 72

4.1.4 Abundance of Plant Families Visited by Bees..... 72

4.1.5 Pollen Spectrum of Honey from Bauchi State..... 72

4.2 Physico-Chemical Analysis..... 79

4.2.1 Moisture, Ash, pH and Acidity of Honey Samples..... 79

4.2.2 Carbohydrate Content of Honey Samples..... 81

4.2.3 Protein, Fat, Dextrin and Hydroxymethylfurfural Content of Honey Samples..... 83

4.2.4 Heavy Metal Composition of Honey Samples..... 85

4.2.5 Mineral Composition of Honey Samples..... 85

4.3 Anti-bacterial Activity of Honey Samples..... 85

4.3.1 Anti- bacterial Activity of Honey Sample from Adamawa State..... 88

4.3.2 Anti- bacterial Activity of Honey Sample from Jigawa State..... 88

4.3.3 Anti- bacterial Activity of Honey Sample from Kaduna State..... 88

4.3.4 Anti- bacterial Activity of Honey Sample from Plateau State..... 92

4.3.5 Anti- bacterial Activity of Honey Sample from Ondo State..... 92

4.3.6 Anti- bacterial Activity of Honey Sample from Oyo State..... 92

4.3.7 Anti- bacterial Activity of Honey Sample from Bauchi State..... 96

4.3.8 Organisms Reactions to Honey from Different States..... 96

4.3.9 Effect of Concentration on Microorganisms..... 103

4.3.10 Effect of Concentration and Source of Honey on Zone of Inhibition..... 103

4.4 Minimum Inhibitory Concentration..... 106

4.5 Minimum Bactericidal Concentration..... 108

4.6 Demographic Features of Respondents..... 110

4.7 Reasons for Honey Purchase..... 110

4.8 Factors Affecting Purchase..... 110

4.9 Relationships between Demographic Features and Honey Purchase..... 114

4.9.1 Level of Education and Criteria for Honey Purchase..... 114

4.9.2 Level of Education and Quantity of Honey Purchased..... 114

4.9.3 Effect of Income and Quantity of Honey Purchased..... 114

4.9.4 Income and Preference for Imported Honey..... 114

4.9.5 Income and Opinion about Honey Price..... 119

CHAPTER FIVE..... 121

5.0 DISCUSSION..... 121

5.1 Pollen Spectra of Honey Samples..... 121

5.2 Physico-Chemical Parameters of Honey Samples..... 125

5.3 Anti-Bacterial Assay..... 132

5.3.1 Agar Well Diffusion..... 132

5.3.1.1 Anti- bacterial Assay for Adamawa State Honey Sample..... 133

5.3.1.2 Anti- bacterial Assay for Jigawa State Honey Sample..... 135

5.3.1.3 Anti- bacterial Assay for Kaduna State Honey Sample..... 136

5.3.1.4 Anti- bacterial Assay for Plateau State Honey Sample..... 136

5.3.1.5 Anti- bacterial Assay for Ondo State Honey Sample..... 137

5.3.1.6 Anti- bacterial Assay for Oyo State Honey Sample..... 138

5.3.1.7 Microorganisms Response to Honey Samples..... 138

5.3.1.8 Response of Microorganisms to Different Concentrations of Honey..... 139

5.3.1.9 Comparison of the Anti- bacterial Activity of Honey Samples from different States..... 140

5.3.1.10 Anti- bacterial Assay for Bauchi State Honey Sample..... 141

5.3.2 Minimum Inhibitory Concentration..... 142

5.3.3 Minimum Bactericidal Concentration..... 144

5.4 Criteria for Honey Acceptance..... 145

5.4.1 Motivators for Honey Purchase..... 145

5.4.2 Factors Affecting Honey Purchase..... 145

5.4.3 Relationships between Demographic Features and Honey Purchase..... 145

CHAPTER SIX...... 147

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS..... 147

6.1 Summary..... 147

6.2 Conclusions..... 148

6.3 Recommendations..... 148

REFERENCES..... 150

APPENDICES..... 159

LIST OF TABLES

Table 4.1 Plant Taxa Identified and Their Pollen Percentage Occurrence in Nigerian Sudan Savanna zone Honey 65

Table 4.2 Plant Taxa Identified and Their Pollen Percentage Occurrence in Nigerian Guinea Savanna zone Honey 70

Table 4.3 Plant Taxa Identified and Their Pollen Percentage Occurrence in Nigerian Rain Forest Zone Honey..... 75

Table 4.4 Plant Taxa Identified and Their Pollen Percentage Occurrence in Bauchi State Honey 78

Table 4.5 Moisture, Ash, pH and Acidity of Honey Samples from States within Three Vegetation Zones in Nigeria 80

Table 4.6 Carbohydrate Content of Honey Samples..... 82

Table 4.7 Protein, Fat, Dextrin and Hydroxymethylfurfural Content of honey Samples..... 84

Table 4.8 Heavy Metal Composition of Honey Samples from

parts of Nigeria..... 86

Table.4.9 Mineral Composition of Honey Samples from Parts of Nigeria... 87

Table 4.10 Anti-bacterial Activity of Honey from Adamawa State..... 89

Table 4.11 Anti- bacterial Activity of Honey from Jigawa State 90

Table 4.12 Anti- bacterial Activity of Honey from Kaduna State 91

Table 4.13 Anti- bacterial Activity of Honey from Plateau State..... 93

Table 4.14 Anti- bacterial Activity of Honey from Ondo State..... 94

Table 4.15 Anti- bacterial Activity of Honey from Oyo State..... 95

Table 4.16 Anti- bacterial Activity of Honey from Bauchi State..... 97

Table 4.17a Mean Inhibitory Zones of Honey against Test Organisms at 100%..... 99

Table 4.17b Mean Inhibitory Zones of Honey against Test Organisms at 50%..... 100

Table 4.17c Mean Inhibitory Zones of Honey against Test Organisms at 25%..... 101

Table 4.17d Mean Inhibitory Zones of Honey against Test Organisms at 12.50%..... 102

Table 4.18 Organisms Response to Different Honey Concentrations..... 104

Table 4.19 Anti-bacterial Activity of Honey from Different States..... 105

Table 4.20 Minimum Bactericidal Concentration of the honey samples..... 109

Table 4.21 Demographic Features of Respondents..... 111

Table 4.22 Highest Level of Education and Honey Purchase 115

Table 4.23 Highest Level of Education and Quantity of Honey Purchased. 116

Table 4.24 Quantity of Honey Purchased Based on Monthly Income..... 117

Table 4.25 Honey Preference Based on Monthly Income 118

Table 4.26 Respondents Opinion about Price of Honey Based on their Monthly Income..... 120

LISTS OF FIGURES

Figure 3.1. Vegetation map of Nigeria..... 50

Figure 4.1. Percentage distribution of major pollen plant family from Adamawa State..... 63

Figure 4.2. Percentage distribution of major pollen plant family from Jigawa State... 64

Figure 4.3. Percentage distribution of major pollen plant family from Kaduna State 68

Figure 4.4. Percentage distribution of major pollen plant family from Plateau State 69

Figure 4.5. Percentage distribution of major pollen plant family from Ondo State 73

Figure 4.6. Percentage distribution of major pollen plant family from Oyo State 74

Figure 4.7. Abundance of each plant family from six honey samples.... 76

Figure 4.8. Percentage distribution of major pollen plant family from Bauchi State..... 77

Figure 4.9. MIC of honey samples from the different States to bacteria species..... 107

Figure 4.10. Motivators of honey purchase..... 112

Figure 4.11. Criteria for honey purchase..... 113

LIST OF PLATES

Plate I. Zones of inhibition of different bacteria species against honey from Bauchi State..... 98

Appendix III-Plate 2 Pollen Micrographs Identified from Samples..... 167

Appendix IV–Plate 3 Zone of Inhibition of Ciprofloxacin on Test Organisms..... ` 169

APPENDICES

Appendix I	Questionnaire.....	159
Appendix II	Performance of Microscopical Examination of Honey Samples.....	163

ABBREVIATIONS AND SYMBOLS

ABU Ahmadu Bello University

AD	Number of years since Jesus Christ was born
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BC	Before Christ
Cipro®	Ciprofloxacin
Cm	Centimetre
c	Circa
cf	Looks like
$CuSO_4$	Copper Sulphate
DMRT	Duncans Multiple Range Test
e.g.	For Example
***et al*.**	And Others
FCT	Federal Capital Territory
F/G	Fructose over Glucose
F+G	Fructose plus Glucose
HCl	Hydro Chloric acid
HDE	Honey Dew Elements
HMF	Hydroxy Methyl Furfural
H_2O	Water
H_2O_2	Hydrogen Peroxide
H_2SO_4	Hydrogen Sulphate
hr	Hour
g	Gram
gm/cc	Gram per Cubic Centimetre

IBRA	International Bee Research Association
ICPBR	International Commission for Plant Bee Relationships
IHC	International Honey Commission
Kcal	Kilocalorie
Kg	Kilogram
K_2SO_4	Pottasium Sulphate
L	Litre
M	Molar
MBC	Minimum Bactericidal Concentration
Meq or mEq	Melli Equivalents
MG	Methyl Glyoxal
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSSA	Methicillin Sensitive *Staphylococcus aureus*
mg	Milligram
mg/kg	Milligram per Kilogram
ml	Milli-Litre
mm	Millimetre
N	Normal
₦	Naira sign
NaOH	Sodium Hydroxide
Na_2CO_3	Sodium trioxo-carbonate VI

NEST	Nigerian Environmental Study Team
NHB	National Honey Board
NPGN	Number of Pollen Grains from Nectariferous species
nm	Nanometre
O_2	Oxygen
pH	Hydrogen ion concentration
ppm	Parts Per Million
RI	Refractive Index
S/m	Siemens per metre
sp.	Species
USA	United States of America
v/v	Volume per Volume
X	Magnification Sign
ZOI	Zone of inhibition
/	Per
^{0}C	Degree Centigrade
^{o}N	Degree North
µ	Micro
µg/l	Microgram per Litre
µg/day	Microgram per day
%	Percentage
>	Greater Than
≥	Greater than or Equal to
<	Less Than

≤	Less than or Equal to
=	Equal
±	Plus or Minus
*	Multiplication sign

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND INFORMATION

Honey is produced by honeybees. Honeybees depend wholly on plants for their food and both climate and soil determine what plants are able to grow and flower within the foraging range of bees from the colonies in a region. Honeybees collect nectar or honeydew and transform it by combining it with specific substances of their own, deposit, hydrate, store and leave in honeycombs to ripen and mature (Crane, 1990; Downey *et al*., 2005). Bees use a variety of plants to create honey, consequently compositional differences that can influence the value of a specific honey for medicinal or health promoting purposes arise (Flodhazi, 2004).

Bees that produce enough honey worth harvesting belong to two subfamilies of the family Apidae: Apinae (honeybees) and Meliponinae (stingless bees). Apinae has only one genus *Apis* of which the species *Apis mellifera* is of much greater economic importance than any other. The bases of the world's beekeeping industry are races and strains of the honeybee *Apis mellifera* (Crane, 1990). The common African honeybee (*Apis mellifera adansonii*) lives in colonies throughout the year. A colony consists of a queen bee, which is a fertile, egg-laying female, and from 10,000 to 20,000 worker bees, which are infertile females. The male bees, called drones, are present in the reproductive season, but may be absent in times of food scarcity or dearth period (Crane, 1990).

Although the origin of beekeeping in Nigeria is not certain, beekeeping is fast becoming an occupation. Constructed hives aid the colonization and increased production of honey. The type of hives used varies from area to area (Chinaka, 1995). Basically we have the traditional, semi modern and modern types of hives which fall into fixed comb hive, movable comb hive and movable frame hive technology respectively. Modern practice of beekeeping makes use of simple single bars called Top bar hive and movable frames in box hives (IBRA, 1997).

Melissopalynology also known as Melitopalynology is the branch of palynology which deals with the study of the botanical and geographical distribution of honey by subjecting honey sediments to microscopic analysis. It includes the study of pollen in the honey as well as the source of the pollen (Brooks and Shaw, 1968). It is possible to gain evidence of the geographical location by observing the honey samples for the presence of or a combination of pollen that is typical only to that particular location. It is also possible to identify taxonomically the genera of the plants the honey bees visited (Louveaux *et al*., 1978). No honey produced by bees flying free

is entirely unifloral, unless the bees were reared in apiaries and exposed to a particular species of bee pollinated plant (Terrab *et al*., 2003).

Honey as a natural product has various constituents (Bertoncelj *et al.*, 2007). Honey is a supersaturated sugar solution of two sugars, glucose and fructose, with small amounts of other more complex sugars. Many other substances also occur in honey such as flavonoids, phenolic acids, water, vitamins, organic acids, proteins, phytochemicals and minerals which are largely responsible for the differences among individual honey types (White and Doner, 1980; Bertoncelj *et al*., 2007). The concentration of the sugar is frequently characteristic of the plant species but varies with environmental conditions (Flodhazi, 2004). Fructose (levulose) is the dominant sugar with about 38.5g in honey, glucose (dextrose) 31g, with at least twenty two other more complex sugars such as sucrose 1.5g, maltose, isomaltose, erlose, kijibiose, melezitose and all others with 4g per 100g (White and Doner, 1980; Bertoncelj *et al*., 2007). The mineral content of honey usually varies and is recognized as an environmental indicator at least since 1984. Honey contains all of the trace minerals that are essential to health; iron, copper, manganese, silicon, chlorine, calcium, potassium, sodium, phosphorus, aluminium and magnesium. The mineral content of honey is closely related to the floral type, mineral resources in the soil and environmental factors. Per 100g of honey 6mg calcium, 4mg phosphorus, 4mg sodium, 52mg potassium, 0.42mg iron, 0.22mg zinc, 2mg magnesium, 0.80mg selenium, 0.04mg copper and 0.08mg manganese is found (White and Doner, 1980; Terrab *et al*., 2003; Bertoncelj *et al*., 2007). Honey contains vitamin C (0.50mg), vitamin B (0.02mg), folate (2mg), pantothenic acid (0.07mg), niacin (0.12mg), riboflavin (0.04mg) per 100g (White and Doner, 1980). Organic- acids add to honeys antibacterial properties. Gluconic acid is the major organic acid, it

is the product of the enzymatic glucose oxidase reaction. It increases calcium absorption. Other organic acids in honey are butyric, acetic, formic, succinic, malic, citric, maleic, oxalic and pyroglutamic acids. As with other components in honey, the organic acid content varies according to the floral source (Crane, 1975). Honey also contains protein, enzymes, dietary fibre (0.20g per 100g), no fat and cholesterol, ash (0.20g per 100g) (NHB, 2003).

From ancient times honey has been used as a natural sweetener (NHB, 2003). The traditional significance of honey as marriage requirement cannot be overemphasized as it signifies sweetness, the marriage is expected to be sweet from the beginning to the end the way honey is sweet from beginning to the end. It also signifies unity, it is expected that no one will be able to separate the couple. Traditionally, honey is used to ease labour pains in women, half a cup of pure honey is taken at the onset of labour, in the treatment of pimples, honey is mixed with the albumen of egg and a few drops of lemon or lime. Honey is also used in the treatment of infertility, malaria, gastric ulcer, burns, earache, dressing of wounds, hangover, chapped hands, boils, witlows and for hair growth (Adebiyi Personal Communication, 2010).

In Ancient Egypt, honey was used to sweeten cakes and biscuits, and was used in many other dishes. Ancient Egyptian and Middle-Eastern people also used honey for embalming the dead. In the Roman Empire, honey was possibly used instead of gold to pay taxes (NHB, 2003). In Nigeria modern use of honey is for the treatment of ear ache, at Ghana, for infected leg ulcer, in India for cough, sore throat and eye diseases, in China for scars, cancer, small pox and worms, in Turkey ‘mad honey’ was used to treat diabetes and the Greeks use honey to treat measles in the eyes, gastric ulcer and constipation (Molan, 2001; Oztasan *et al*., 2005).

For at least 2,700 years, honey has been used by humans to treat a variety of ailments through topical application, but only recently have the antiseptic and antibacterial properties of honey been chemically explained (Abdulla and Abdul-Aziz, 1998; Wahdan, 1998). Wound gels that contain antibacterial raw honey and have regulatory approval for wound care are now available to help conventional medicine in the battle against drug resistant strains of bacteria methicillin sensitive *Staphylococcus aureus* (MRSA) (Molan, 2001).

1.2 STATEMENT OF RESEARCH PROBLEM

Pollen is an essential tool in the analysis of honey. Taxa of pollen are used to indicate the floral nectar sources utilized by bees to produce honey (Louveaux *et al*., 1970; Sawyer, 1988). Thus, the relative pollen frequency is often used to verify and label a honey sample as to the major or minor nectar source (s). This information has important commercial value because honey such as sourwood, tupelo, buckwheat, or citrus honey command a premium price. Identifying and quantifying the pollen in honey samples is one of the best ways to determine the range of nectar types used to produce honey, and therefore label it correctly based on actual foraging resources. Another reason that pollen analysis of honey is often required is to identify the geographical source or origin (Jones *et al.*, 2001). The first melissopalynological study in Africa was reported by Smith in Sowunmi (1976) but the study of pollen in honey is becoming more popular in Nigeria only recently (Sowunmi, 2001). In spite of this, very few mellisopalynological studies

have been carried out in Nigeria (Ebenezer and Olugbenga, 2010) and few attempting to give botanical names to honey samples. With the rapidly increasing interest and research into natural health remedies and supplements, there is a resurgence of interest in the therapeutic use of honey (Weston, 2000).

Scientific evidence for the effectiveness of honey in several experimental and clinical conditions is beginning to emerge (Al-mamary *et al*., 2002). All honeys are not equal in its effectiveness and care must be taken to ensure that the type used has adequate anti-microbial activity. Observed effect attributed to using honey for wound dressing include: rapid healing, stimulation of tissue regeneration, clearance of infection, cleansing action on wounds, reduction of inflammation and comfort of dressings due to lack of adhesion to the tissues (Efem, 1988; Molan, 2001; Molan, 2002). The antibacterial activity of honey is well established *invivo* and *invitro* but the mechanism of its action is still a matter of debate (Aljadi and Yusoff, 2003; Nagia *et al*., 2006). Medical professionals and consumers need to be aware of the variation in the composition and properties of honey from different floral sources which is invariably dependent on the plants available in the region (Molan, 2001; Aljadi and Yusoff, 2003).

Escherichia coli is an enteric pathogen from the family Enterobacteriaceae. It is an aetilogical agent of diarrhoea. Diarrhoea ranks second as a major cause of morbidity among noticeable diseases in Nigeria (Ogunsanya *et al*., 1994). Gram negative bacilli such as *E. coli* and *Pseudomonas aeruginosa* have replaced *Staphylococcus aureus* as the most frequent causes of nosocomial infections although the staphylococci have remained a problem (Todar, 2005). *Staphylococcus aureus* causes infections at many sites in the body; these include skin, eyes,

gastrointestinal tract and vagina (Shinefield, 1976; Musher and McKenzie, 1977; White and Brooks, 1977; Dillon and Ware, 1981).

Honey collecting is a traditional activity in almost all parts of Africa. Traditional beekeepers use simple hives often made from locally available materials for example, logs, barks and straws. The empty hives are placed in trees and become occupied by passing swarms and in due course are plundered. This method results in the destruction of the colony and a yield of low quality honey (IBRA, 1997).

1.3 JUSTIFICATION OF RESEARCH

In the wake of reappearance of agriculture in Nigeria, farmers and beekeepers need information to know the plant source(s) from where bees collect pollen with a view of encouraging bee farmers to maximize the flowering season for increased honey production and to ensure conservation and increased planting of these plants.

In recent years, mellisopalynology has attained a topic of global status. This is borne out of the fact that not only is honey useful as a food supplement; it is now increasingly being used in the treatment of various ailments (Molan, 2001). Honey has been shown to be bactericidal rather than bacteriostatic (Wahdan, 1998). Therapeutic uses of pharmaceutical anti-inflammatory substances have serious limitations; corticosteroids suppress tissue growth and the immune response while the non-steroidal anti-inflammatory drugs are harmful to cell especially in the

stomach. But honey has an anti-inflammatory action free from adverse side effect. Honey has no side effects other than a stinging sensation by some people when applied to open wounds. Antibiotics are sometimes associated with adverse effects on host with hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immune-suppression and allergic reactions. Although allergy to antibiotics is fairly common, allergy to honey is rare and it may be a reaction to either the pollen or the bee protein in honey (Al- Jabri, 2005). Antibacterial properties of honey are the result of the low water activity causing osmosis, hydrogen peroxide effect, high acidity, and the antibacterial activity of methylglyoxal. Honey appears to be effective in killing drug-resistant biofilms which are implicated in chronic rhinosinusitis (Molan, 2001).

Honey is a good alternative to drugs; it gives quick recovery rates and healing time, when honey was used for wound closure in women who had wound disruption following cesarean sections they were healed within two weeks (Phuapradit and Saropala, 1992). Honey offers low cost for wound dressing (Molan, 2001). Honey is farmed and used all over Nigeria. Initially local farmers harvested the honey from the wild but today apiculture is a growing industry in many parts of the country making honey more available (Adebiyi *et al*., 2004).

As food stuff used for healing purposes, honey must be free of objectionable contents. It should contain only small amounts of pollutants such as heavy metals (Przybylowski and Wilczynska, 2001). Since metals are non-biodegradable, they tend to accumulate in various organs therefore, exposure to trace concentration of various metal ions can lead to long term toxic effects (Adraino, 1995). Heavy metal content is variable in honey and is a sign of environmental pollution (Przybylowski and Wilczynska, 2001). Determination of heavy and transitional metals

in honey is of interest for quality control when considering it as food. High levels of metals are undesirable because of their known or supposed toxicity (Buldini *et al*., 2001). Each honey is unique on the basis of the number and combinations of the various components that give it a specific individualistic note. To ensure the authencity of honey, it is important to perform extensive honey compositional analyses (Serrano *et al*., 2004). There is need to determine the therapeutic potential of honey from different vegetation zones in Nigeria and classify the honey according to the floral source.

1.4 OBJECTIVES OF STUDY

The general aim of the study was to evaluate the anti-microbial activity, physico-chemical parameters, pollen spectra, and consumers' acceptance of some honey samples from some States in Nigeria. The specific objectives were to:

i. Determine the pollen spectra composition of honey from the three vegetation zones of Nigeria.
ii. Evaluate the physico-chemical properties of the honey samples from the three vegetation zones of Nigeria.
iii. Determine the percentage of sugars in the honey samples.
iv. Determine the efficacy of different concentrations of honey samples as anti-microbial agents.
v. Assess the criteria of honey acceptability by consumers.

1.5 HYPOTHESES

i. H_o: The pollen spectra composition of honey from the three vegetation zones of Nigeria is from the same source.

ii. H_o: The physico-chemical properties of the honey samples from the three vegetation zones do not differ.

iii. H_o: The sugar composition of honey samples from the three vegetation zones is similar.

iv. H_o: The efficacy of honey samples as anti-microbial agents do not differ with concentrations.

v. H_o: There is no relationship in the acceptability of honey by consumers.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DEFINITION OF HONEY

Honey is the natural sweet substance produced by honeybees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants. Blossom honey or nectar honey is the honey which comes from nectars of plants. Honeydew honey is the honey which comes mainly from excretions of plant sucking insects (Hemiptera) on the living parts of plants or secretions of living parts of plants (Codex Alimentarius, 2001). Honeydew is a sugar-containing substance excreted by some plant-sucking insects. The bees gather the honeydew, transforming and mixing it with glucose oxidase (the enzyme responsible for hydrogen peroxide production in honey) and they leave it to mature in the honeycomb. The honeydew can have a plant or insect origin. Vegetal honeydew is produced as a consequence of the digestive processes of the insects, which suck the sap. The main honeydew-producing plants are conifers, such as fir (*Abies* species, *Picea* sp. (Linneaus) H. Karst, *Pinus* sp. L.) or larch (*Larix decidua* Miller), different oak species (*Quercus* sp. L.), chestnut (*Castanea sativa* Miller), beech (*Fagus sylvatica* L.), hazel (*Corylus avellana* L.), elm (*Ulmus* sp.) and ash tree (*Fraxinus* sp.). The main honeydew indicators in honey are fungal spores and mycelium and algae cells belonging to Chlorophyceae and Cyanophyceae. They are found in the sediment along with pollen grains and microscopic impurities (plant tissues, plant hairs, soot, oxalate crystals, bee hairs and yeast cells). Limits defining the floral, honeydew or mixed honeys are not published by the different legislations of the European Community, but the International Commission for Plant Bee relationships (ICPBR) establishes that a honey is floral

when the honeydew elements over number of pollen grains from nectariferous species (HDE/NPGN) ratio is <1; if this ratio is three or more, the honey is considered as a honeydew honey, while those honeys with a ratio between one and three are considered as mixed honeys. The honeydew honeys are characterized by their very dark colour and strong flavour, and are heavily bodied honeys; they are also slow to granulate. In general, the honeydew honeys are characterized by their high values of pH, ash and mineral content and electrical conductivity (Diez *et al*., 2004).

2.2 HONEYBEES AND BEE KEEPING

Since the late 1700s four species of honeybees have been recognized which occupy various regions of the world. *Apis cerana* occupies tropical Asia and eastern Asia as far north as part of the Soviet Union (USSR), Europe and Asia near the Pacific coast. *A. mellifera* occupies the continents of Africa except for the desert regions and Europe. It extends as far north as Southern Scandinavia and the forests of Russia, and as Far East as the Urals and further South Iran. *A. dorsata* and *A. florea* are confined to tropical Asia. Tropical forms of *A. mellifera* are smaller than temperate zone forms, and they have a more slender abdomen and are called *A. m. adansonii*. Their colonies produce many more swarms. *A. m. adansonii* has a natural distribution extending along the coastal lowlands of West Africa (Crane, 1990).

The honeybee is able to store energy foods by converting nectar and honeydew into honey which has high sugar content. When nectar or honeydew is being brought into the colony, the foraging bee adds secretions from the hypopharyngeal glands which contain enzymes invertase, glucose oxidase and diastase, no water is evaporated at this point. In the hive the forager passes the

nectar or honeydew to one or more house bees proboscis which elaborates (ripens) the honey from the nectar in an uncrowded part of the brood nest where the temperature is a few degrees below 35^0C. They manipulate the liquid in their mouth parts reducing the water content of the liquid and adding to it more salivary secretions containg enzymes. When little honey is produced, it is much manipulated and the finished honey contains more of the enzymes compared to the honey produced during a strong flow (Crane, 1990).

Bee keeping is defined as an applied science for the rearing of honey bees for man's economic benefits. Pharmaceutical demand for pure honey is increasing and most of them have resulted to importation. According to the bee keeping expert from Europe, the only gold Nigeria is yet to tap into is honey (Nwali, 1996). It is likely that early man hunted for wild nests of bees and took their honey. Beekeepers have used hives and harvested honey from them for at least 4500 years. Egypt in North Africa may have been the first place where honey was harvested from bees kept in hives (Crane, 1990).

2.3 TYPES OF HIVES

There are various types of hives ranging from the local primitive types to the improved types. A beehive is a suitable home for a colony of bees designed to keep the bees from adverse weather, pests and will allow the honey to be harvested with minimal disturbance to the bees. Beehives can be classified into the following types; fixed comb hives, moveable–comb hives and moveable frame hives which can be single or multichambered. Single chambered hives consist of only one unit in which the bees maintain their brood nest and their honey stores while multi-chambered hives consist of more than one unit intended for brood or honey. Choice of hive

design is to make management easy as much as it is possible while the choice of hive technology is based on the cost, ease of hive production, availability of materials and resources with the ultimate aim to maximize the amount of honey produced and cash returns (Paterson, 2006).

2.3.1 Fixed Comb Hive: comprises of traditional hives such as; the gourd hive. Holes are drilled in the gourd to form a suitable entrance for the bees. A branch is cut from a shrub, the stalk of the gourd is forced into the stump remaining on the main stem and suitable bee bait is put into the hive to attract bees into it. In Nigeria it is used in Kwara, Kogi and Ogun State. The gourd is broken to harvest honey and new empty ones put on the stump. This leads to reduction of bee colony; Pot hive, earthen cooking pots are used. The pot is made from earth or mud that is moulded into the shape of a cooking pot. The mouth is closed with a smaller size of pot or other suitable material. Holes are made in the closure to give the bees entrance. It is used in Adamawa, Bauchi, Benue, Niger and Plateau States and at Abuja FCT. Though the pot is not broken when harvesting the honey, the colony of bees has little chances of survival after harvesting because everything is harvested; Basket hive, is made of woven reeds like a round basket tapering to a point at the top . The basket is made water proof with a thick layer of dung and mud. Grass is put on top to protect the basket hive from rain. A large hole is left in the side of the basket so that the owner is able to put his/her hand inside and take out combs. Used in Kafachan and Kagoro Southern parts of Kaduna State. Beekeepers harvest too frequently with this kind of hive thus leading to production of low quality/unripe honey; Straw hive, is made from woven grass. It can be covered with clay or a mixture of clay and cow dung. It is usually found in Jigawa, Katsina, Kano, Bauchi, Borno, Gombe and Sokoto States and Zaria in Kaduna State; Bark hive, the bark is peeled from a tree trunk and is sewn together to form a cyclinder

and sealed with more bark or with grass disc at both ends. Removing all the bark from a tree kills the tree, therefore bark hive is not encouraged to be used. However, it is used in Jema'a, Kaduna State, parts of Adamawa State up to Cameroon and some other tropical African countries (Chinaka, 1995).

2.3.2 Improved Comb Hives: comprises the intermediate or semi modern technology or low technology (removable comb hive) and the modern technology (removable frame hive) of bee hives. It allows individual combs to be removed, inspected and returned into the hive. Removable comb hive allows hives to be managed e.g. Top bar hive. It is normally made of timber and designed to take 28 top bars each; 480mm long, 32mm wide and 10mm thick. The ends are vertical with the long sides sloping inwards at an angle of about 60 degrees. The entrance can be at the end or by the side (Paterson, 2006).

2.3.3 Removable Frame Hive: have various types of removable comb hives with frames for example; Langstroth, WBC, Dadant or Simplex hives. The langstroth hive is mainly used in North and South America, Africa and Australia (Paterson, 2006).

2.4 BEE FORAGE AND VEGETATION INFLUENCE ON HONEY

Bees require a variety of nectar and non-nectar yielding plants to make honey. All natural honey comes from floral sources except for some honeydew honey (Codex Alimentarius, 2001). There are varieties of honey depending on flora which the bees foraged. Honey from one particular flower is known as unifloral honey. Honey can be edible or non edible depending on the floral source. Different geographical areas present particular floral associations therefore some plant

species may do well as a bee plant in one region but not in another (Ghosh and Ghosh, 1999). Bee forage plants in Tanzania include *Acacia* species (Mill.), *Adansonia digitata* (Linn.), *Albizia* sp. (Durazz), *Azadirachta indica* (A. Juss.), *Brachystegia* sp. (Benth), *Citrus* sp., *Combretum* sp. (Loefl.), *Cordia* sp. (Linn.), *Croton* sp. (Linn.), *Dalbergia melanoxylon* (Guill. and Perr.), *D. sissoo* (Roxb.), *Delonix regia* (Hook.) Raf., *Dichrostachys cinerea* (Linn.) Wight and Arn. (Mbuya *et al*., 1994). Generally the families with great number of bee forage species are Anacardaceae (*Anacardium occidentale* Linn., *Lannea* sp. A. Rich and *Mangifera indica* Linn.), Compositae *Chromolaena odorata* (L.), *Guizotia abyssinica* (L.f.) Cass and *Vernonia* sp. Schreb), Euphorbiaceae (*Croton* sp. Linn., *Hevea brasiliensis* (A. Juss.) Muell and *Ricinus communis* Linn.), Lamiaceae (*Ocimum* sp. Linn.), Fabaceae/Mimosoidae (*Acacia* sp. Mill., *Albizia* Durazz., *Brachystegia* sp. Benth., *Glycine max* (Linn.) Merr., *Phaseolus* sp. Linn. and *Tamarindus indica* Linn.), Melastomataceae, Moraceae,, Myrtaceae (*Eucalyptus* sp. L Herit. and *Syzygium* sp. Gaertn.), Arecaceae (*Cocos nucifera* Linn.), Rubiaceae (*Coffea arabica* Linn.) and Rutaceae (*Citrus* sp. Linn.) (Crane, 1990). At Ibadan, south –west Nigeria, the main sources of nectar for bee plants are *Albizia glaberrima* (Schum. and Thonn.) Benth, *Berlinia grandifolia* (Vahl) Hutch. and Dalz, *Bombax buonopozense* (P. Beauv), *Ceiba pentandra* (Linn.), *Chromolaena odorata* (Linn.) King and H. Robison, *Combretum* sp. (Loefl.), *Daniellia oliveri* (Rolfe) Hutch. and Dalz., *Elaeis guineensis* (Jacq.), *Hildergardia barteri* (Mast.) Kosterm, *M. indica*, maize (*Zea mays* Linn.), *Parkia biglobossa* (Jacq.) R.Br. ex G. Don, *Vitellaria paradoxa* (Gaertn.f.) and *Syzygium* sp. (Mutsaers, 1991). Some of the most important plants for honey samples from Imo State, Nigeria a lowland forest were; *Elaeis guineensis, Pentaclethra macrophylla* Benth., *Irvingia gabonensis* Baill., *Azadirachta indica*, *Lycopersicon esculentum* Mill., *Solanum* sp., *Citrus* sp., *Milicia excelsa* (Wilw.) C.C. Berg, *Treculia africana* Decne.,

Pterocarpus sp., *Alchornea cordifolia* Schum and Thon., and *Lannea* sp. (Agwu and Njokuocha, 2004).

The honeys of the world show great variety, especially in colour, flavour and aroma because different plants contribute their own characteristic constituents. Honey is produced under many different climatic conditions but the main constituents in all honey are the same (Crane, 1990). The largest constituents in honey are sugar (81%) and water (17%) (White *et al.*, 1962). The remaining 1-2% contains various enzymes and compounds such as minerals, vitamins whose composition plays a significant role in the bactericidal activity of honey (Molan, 1999). Taking into account that honey is a complex natural food obtained under conditions which are difficult to control, an unequivocal characterization of honey samples requires the use of most parameters. Several honey types were produced, due to the wide variety of botanical sources associated with the markedly different climatological and topographic conditions which were observed in Madrid rural areas. While mountain zones (>900 m altitude) were mainly covered by shrubs and trees such as *Pinus* L. and *Quercus* L. species, nectar-producing plants (e.g. *Rosmarinus officinalis* (L.), *Thymus* sp. (L.), *Echium* sp. (L.), Rosaceae shrubs) were more abundant in the plain zones. Honey characterization was based on the determination of its chemical, physical or biological properties (Soria *et al*., 2004).

Indeed, honey is a natural substance made when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honey bees. Consequently, its composition is defined by numerous factors and subject to variation (Devillers *et al*., 2004). The wide range in parameters observed for most of the honeys agree with the variability of honey sources for honey

samples produced in Madrid province. The minimum and maximum values obtained for the physicochemical variables are; pH 3.63 and 5.01, free acidity 13.1 and 51.2, lactonic acidity 0.00 and 13.9, total acidity 14.5 and 59.6, water content 13.0 and 18.7%, ash content 0.003 and 0.990%, electrical conductivity 0.117 and 1.116, glucose 19.3 and 31.2% and fructose 23.2 and 39.9% respectively. Physicochemical parameters measured could be related to the different botanical and climatological characteristics of the honey collection places (Soria *et al*., 2004).

Honey samples that are available commercially, differ in quality on account of various factors like geographical, seasonal and processing conditions, floral source, packaging and storage period. Commercial honey in India is generally of assorted type as it is collected from regions having different climatic conditions and very often from forests or wild flower sources. Hence, they have varied sensory and physico–chemical properties (Anupama *et al*., 2003). Where comparison was made of the composition of the same types of honey from two crop years, relatively small or no differences was found (White and Doner, 1980). This is true of Manuka honey (Molan, 2001).

Manuka honey from New Zealand from *Leptospermum* plant has been confirmed to have a higher anti-microbial activity compared to honey from most other plants (Molan, 1999; Molan, 2002; Aljadi and Yusoff, 2003; Weston *et al*., 1999). Weston *et al.* (1999) stated that while most of the phenolic products possess antibiotic activity, they do not individually or collectively account for the antibacterial activity of 'active' manuka honey. Essentially, all of these activities are associated with the carbohydrate fraction of honey. While Weston *et al.* (2000) stated that phenolics do not account for the non-peroxide activity but that it is the carbohydrate fraction that

is responsible for the activity and that the phenolics might contribute to the activity but does not account for the observed non-peroxide antibacterial activity of manuka honey. They, therefore, hypothetically concluded that the non-peroxide activity could be interpreted as residual hydrogen peroxide. Molan (2002) disagreed with Weston's observation and stated that the peroxide system does not account for all the observed activity in manuka honey rather he proposed that a unique plant derived product was responsible for the non-peroxide antibacterial activity. Aljadi and Yusoff (2003) further stated that hydrogen peroxide may be destroyed by tissue catalase, therefore there is a non-peroxide activity of honey which is important to know what it is.

Honey has several properties that contribute to its antimicrobial activity. In most honeys, low pH and high osmolarity are combined with the enzymatic production of hydrogen peroxide that exerts an antimicrobial effect. Phytochemical components derived from the floral source of the honey can confer additional activity that is stable in the presence of catalase, an enzyme that destroys hydrogen peroxide. This non-peroxide activity was first identified in manuka (*Leptospermum scoparium*) honey from New Zealand where it is often marketed as the Unique Manuka Factor (UMF®). Variations in the type and level of antimicrobial activity in honey are associated with their floral source. However, while some floral sources appear to be associated with particular levels of hydrogen peroxide activity, variation in this activity among honeys from within the same floral species has also been observed. This may be due to the geographical location of the floral source and the prevailing environmental conditions, which affect the physiology of the floral species or to bee-related factors such as age or colony health, which may affect the production or activity of glucose oxidase (the enzyme responsible for hydrogen peroxide production in honey). Honeys with non-peroxide antimicrobial activity are more closely

associated with floral source being generally derived from *Leptospermum* species although this type of activity has also been found in a small number of non-*Leptospermum* honeys (Molan and Russell, 1988; Brady *et al*., 2004).

Non-peroxide activity was strongly associated with *Leptospermum* honeys collected from the Northern Rivers region of New South Wales and the adjacent Southeast Coast region of Queensland, indicating that these regions are a potentially valuable source of therapeutically beneficial honey. Among the *Leptospermum* species, *L. polygalifolium* (jellybush) produced honey that was particularly high in activity. Although *L. scoparium* (manuka) is the primary source of honey with non-peroxide activity in New Zealand, none of the 11 samples of *L. scoparium* honey from Australia had detectable non-peroxide activity. These findings suggest that environmental conditions in different regions play a role in the relationship between floral source and non-peroxide antibacterial activity, or alternatively that different regions contain as yet uncharacterised subspecies of *Leptospermum* that are responsible for providing honeys with non-peroxide activity. The antibacterial activity of the Australian honey samples suggests that the antibacterial activity in fresh honey is largely all-or-nothing, although what governs this is not known as there was substantial variation in activity both among and within floral sources. Plant-derived factors that contribute to the antimicrobial activity in honey may be influenced by local environmental conditions such as climate, water and nutrient availability (Irish *et al*., 2011).

2.5 NIGERIAN VEGETATION

Natural vegetation provides us with an array of products and services that are vital for our survival and balanced development. The vegetation cover of the country forms habitats for a great variety of wildlife which are of economic value. Some scientists believe that before human beings came with their growing numbers, economies and culture to dominate the environment, Nigeria was covered by three major types of vegetation; tropical rain forest, covering the southernmost 39% of the country; tropical deciduous forest, immediately to the North and also covering about 39% of the country and tropical xerophytic woodland, covering the northernmost 22% part of the country. Human activities such as bush burning, farming, grazing and urbanization have, over several millennia, changed this original vegetation cover (Nigerian Environmental Study Team (NEST), 1991).

Forest vegetation is dominated by woody species, majority of which are trees. The forest zone contains the following major types of vegetation; coastal vegetation, mangrove forest, fresh water swamp communities, Riparian forest and lowland forest. Logging, farming, particularly bush-fallowing/land rotation associated with bush burning, urban and infrastructural development and conversion to plantations have reduced the true forest area to patches found in forest reserves. Outside the reserves, the vegetation in the forest zone is a mosaic of farms, fallow vegetation of various ages and degraded or converted land. Relics of forest maintained as sacred grooves are to be found scattered among farmlands but these are usually small, often less than one hectare (NEST, 1991). Coastal vegetation occurs as strand vegetation which consists mainly of shrubs such as *Conocarpus erectus* and *Hibiscus tiliaceus* and herbs such as *Ipomoea pes-capeae*. The most common plant species are *Ipomoea aquatica* and *Paspalum vaginatum*.

The mangrove region occurs mostly in the Niger –Delta. Mangrove in Nigeria is dominated by red mangroves (Rhizophoraceae) in association with white mangroves (*Avicennia*) and *Laguncularia racemosa*. The red mangrove *Rhizophora racemosa* is the commonest species followed by *R. harrisonii* and *R. mangle*. *Avicennia,* a smaller tree than *Rhizophora* grows singly on firm land while *Laguncularia* are less abundant. Freshwater swamp communities is made up of grasslands, freshwater swamp forests, deltaic swamps and undifferenciated swamps which succeed mangrove and continue in low lying places along waterways inland in the forest zone. Nigerian freshwater swamp communities are largely non-peaty and are classified as follows; aquatic grassland and herbaceous swamps; they are commonly observed at the outer edge of swamp forests and frequently consist of floating vegetation of the grass *Vossia cuspidata*, large communities of *Cyperus* sp., or of grass vegetation consisting of *Acroceras amplectens*, *Paspalum* sp., *Echinochloa pyramidalis*, the aroid *Cyrtosperma senegalense*, the fern *Nephrolepis biserrata* or dicotyledonous herbs such as *Jussiaea*, *Polygonum* and *Ipomoea* sp. Floating or submerged plants include *Nymphaea lotus*, *N. micranthus*, *Pistia stratiotes*, *Impatiens*, *Eleocharis*, *Salvinia*, *Lemna* and *Utricularia* species. This habitat is being invaded by water hyacinth, *Eichhornia crassipes*; Terrestrial swamp forest, these formations have an outer fringe, close to the water edge which is dominated by species of *Raphia*, *Pandanus*, *Calamus* and *Alchornea*. The best known species of this formation is *Mitragyna ciliata*. Other conspicuous and well known species are *Symphonia globulifera*, *Lophira alata*, *Alstonia boonei* and *Nauclea gilletti*. Less well known species include *Spondianthus preussii*, *Carapa procera*, *Uapaca* sp., *Garcinia* sp., *Cleistopholis patens* and *Oxystigma mannii*; Undifferenciated inland swamps, freshwater swamp forest farther inland in the Niger Delta is seasonally flooded. The main canopy of these forests is more or less open and in gaps, dense tangles of shrubs and lianas

practically form an impenetrable growth. The undergrowth is mostly of climbing palms, *Alchornea cordifolia* and species of *Urera, Cissus, Dalbergia, Combretum, Afromomum* and *Megaphrynium*. Common weed species include *Mimosa pudica, Costus afer, Aspilia africana, Asystasia* sp., and increasingly *Chromolaena odorata*. In some places *Raphia* palms, *Carthormion, Uapaca, Symphonia globulifera* and *Pterocarpus santaniloides* can be found in abundance; Riparian forest, is commonly found by stream banks. It is less complex than the lowland moist forests of forest zone but denser than similar woodlands in the savanna area. Characteristic tree species include *Brachystegia eurycoma, Cola laurifolia, Cleistopholis patens, Cynometra vogelii, Irvingia grandifolia, I. smithii, Khaya grandifolia, K. senegalensis, Myrianthus serratus, Pterocarpus santaniloides, Trichilia heudelotii* and *Uapaca* sp.; Lowland rain forest, is the main block of Nigerian forest formation at low and medium altitudes. Lowland rain forest is the county's main source of timber. Extensive traditional bush fallowing system of cropping has meant that over 60 percent of lowland rain forest has been converted to farmlands at different stages of cultivation or regrowth to forest. Extensive groves of oil palm (*Elaeis guineensis*) marks areas that have been long under cultivation. Other more or less permanent conversions are the cocoa and kola farms, the rubber plantations, oil palm plantations and plantations of forest trees such as *Gmelina arborea* and *Tectona grandis*. Lowland rain forest can be found in Ondo, east of river Niger, Anambra forest reserve at Ogurugu, and forest of Obudu. Lowland rain forest consists of trees such as mahogany (*Khaya*, *Entandrophragma*), obeche (*Triplochiton*), afara (*Terminalia*), iroko (*Chlorophora* now *Milicia*), African walnut (*Lovoa*), ekki (*Lophira*) and *Mansonia* which are some of the main sources of timber. Some spice yielding species *Xylopia*, *Piper*, *Dennettia* and *Tetrapleura* leaves, barks, roots and fruits play vital roles in ethnomedicine and nutrition (NEST, 1991).

Forest outside the forest belt, are forest outliers which include riparian forests and forest islands in savanna zone. Forest outliers exist in Gongola State in Amboine, Baissa, Bissaula, Gangumi, Wurkam, in Benue Okura-Iyale, west Okura, Acharane and Dekina forest reserves. In Kwara Eba river, Olle, Gbo escarpment and Gbedege forest reserves. Other islands occur in Niger State, southern extensions of Kaduna and Plateau States and in Gongola. These forests mostly occupy moist lowlands or hollows and river valleys but may, as in part of Kainji lake National park be found as relics on stony hill tops or other relatively inaccessible sites unsuitable for farming (NEST, 1991).

Savanna occupies nearly 80 percent of the land surface of Nigeria, extending from about 6^{o}N to the northern borders of the country. There is seasonal vegetation in which there is closed or nearly closed cover of grasses at least 80cm high with flat, usually cauline leaves. Savanna is usually burnt annually and has various densities of trees and shrubs. On the basis of the density and proportion of woody species, savanna is usually distinguished into; savanna woodland, with fairly closed canopy of trees and shrubs; tree savanna, where the woody plants are scattered; shrub savanna, where the trees are absent and grass savanna, where woody plants are absent. The various forms may exist side by side but tend to occur in varying proportions in the different zones into which savanna is subdivided; Derived savanna; Guinea savanna; Sudan savanna and Sahel savanna (NEST, 1991).

Derived savanna is the transitional forest-savanna mosaic, occurring immediately north of the lowland rain forest belt. This zone covers parts of Ogun, Oyo, Kwara, Bendel (now Benin/Edo), Anambra, Imo, Benue, Cross River and Gongola (now Adamawa/Yola) States, reaching $8^{o}30'$ N in the west and $6^{o}40'$ N in the east. The dry season in this zone is about 3 months. The vegetation is believed to have been derived from the forest. The oil palm *Elaeis guineensis* is still abundant in this zone. The typical savanna species are *Daniellia oliveri*, *Vitex doniana*, *Ficus capensis*, *Lophira lanceolata* and *Hymenocardia acida*. Relict forest species include *Albizia* species, *Cola* sp., *Combretum* sp., and *Dialium guineense*. The dominant grasses in this belt are *Andropogon tectorum* (Southern gamba grass) and *Loudetia arundinacea* (NEST, 1991).

Guinea savanna is the most extensive vegetation zone in the country, covering 50 percent of the land area. It occurs immediately north of the Derived savanna belt in an area where the dry season lasts 4 to 6 months. The guinea savanna encompasses much of the thinly populated middle belt region. In the southern parts, *Daniellia oliveri* is a common tree while *Andropogun gayanus* replaces *A. tectorum* as the major grass. Northwards, *Afzelia africana* and *Isoberlinia* sp., replace *Daniellia* as dominant trees while *Hyparrhenia* sp., *Andropogun* sp., and *Schizachyrium* become co-dominant grasses. Therefore guinea savanna is thus divided into southern and northern zones. On the basis of structure and species composition guinea savanna can be grouped into; mixed deciduous woodland, covering most of the Niger and Benue troughs; *Afzelia africana* and semi- deciduous forests around Abuja; *Isoberlinia* savanna, around Kaduna and extending southwestwards; a patch of *Burkea africana* savanna, north of the Kainji lake and wooded savanna to the northeast of the zone (NEST, 1991).

Sudan savanna is the northernmost savanna belt in the country; the dry season lasts 5 to 7 months. The vegetation is typically mixed combretaceous woodland with *Vitellaria paradoxa* formerly *Butyrospermum paradoxum* (shea butter), *Acacia senegal*, *Acacia albida*, *Zizyphus*, *Adansonia digitata* and *Piliostigma reticulatum* being the common trees. The common grasses in this region are *Aristida*, *Brachiaria*, *Panicum*, *Chloris*, *Digitaria*, and *Eragrostis*. Cultivation is intense together with heavy grazing (NEST, 1991). Sahel savanna's dry season lasts 7 to 8 months. The main vegetation layer consists of low growing shrubs, mostly *Acacia* sp., *Anogeisus leiocarpus*, *Balanites aegyptiaca*, *commiphora quadricincta*, *cordial rothii* and *Salvodora persica*. *Sorghum* grass is dominant (NEST, 1991).

Combretum species inhabits the savanna and flowers between February and May. It is widespread in tropical Savanna (Keay, 1989). It is a tree that flowers in dry season. Medically the plant has choleretic properties, treats intestinal disorders, headaches, colics, aches and pains, wound (Arbonnier, 2004). *Mangifera indica* (Linn.) flowers between February and April. *Parinari kerstingii* (Engl.) habits the fringing forest in Sudan Savanna and Guinea low land regions. This plant is a tree that flowers almost all the year round (Keay *et al.*, 1964). This plant is found in Northern Nigeria, it is much localized and locally very common (Arbonnier, 2004).

2.6 PRODUCTION OF HONEY

The bee's food resources which are; nectar, pollen and honeydew have little or no economic use unless honeybees collect them. The ecomomic yield of honey produced per hive varies greatly in different parts of the world. It depends on: the duration of the season when temperatures are high enough for the bees to fly and for plants to grow and flower, the amount of nectar, honeydew and

pollen within flight range of bees, the genetic honey producing capability of the colonies, and the expertise and equipment of the beekeepers. Migratory beekeeping can greatly increase honey yeilds and some beekeepers move their hives up to eight times a year to take advantage of honey flows from different plants. Among the species of *Apis* and their races, those native to regions with cold winters (North Temperate Zone) in general store more honey than those native to subtropical and tropical regions where flight and foraging are not interrupted by a long cold period (Crane, 1990). Honey production by wild bees (*Apis melifera*) in Nigeria is relatively moderate and non-seasonal, because of the varied vegetation pattern resulting in species abundance and diversity with different blooming seasons typical of the tropics (Agwu and Njokuocha, 2004).

The total world production of honey is estimated at 1,170,000 tons and China, the largest producer, exports 70,000 tons of honey compared to 1,000 tons exported by India. In India, the consumption of honey as food is very low, (8.4 g/year/person) compared to 120–1,800 g in other countries, due to food habits, high cost and utilization of a major quantity for medical purposes (Nanda *et al*., 2003).

2.7 HONEY CONSTITUENTS

There is a great variability for all honey constituents. This variation is due to differences in the nectar produced by different species of plant and to variations in the nature of soils on which plants grow. Honey, as it is found in the hive, is a truly remarkable material, elaborated by bees with floral nectar, and less often with honeydew. Nectar is a thin, easily spoiled sweet liquid that is changed ("ripened") by the honey bee to a stable, high-density, high-energy food. United

States Food and Drug Act defined honey as "the nectar and saccharine exudation of plants, gathered, modified, and stored in the comb by honey bees (*Apis mellifera* and *A. dorsata*) levoratory; contains not more than 25% water, not more than 0.25% ash, and not more than 8% sucrose." The limits established in this definition were largely based on a survey published in 1908. Today, this definition has an advisory status only, but is not totally correct, as it allows too high a content of water and sucrose, is too low in ash, and makes no mention of honeydew (White and Doner, 1980).

The consistency of honey can be fluid, viscous or partly to entirely crystallized. The flavour and aroma vary, but are derived from the plant origin (Codex Alimentarius, 2001).The nitrogen is a measure of the protein material, including the enzymes, and diastase is a specific starch-digesting enzyme. Most of these constituents are expressed in percent, that is, parts per hundred of honey (White and Doner, 1980). Honey contains vitamins such as vitamin B_1, B_2, C and nicotinic acid. About 18 organic acids have been detected in honey (Nanda *et al.*, 2003). Honey has a strong hygroscopic character (Hak-Gil *et al*., 1988). Many other substances also occur in honey, but the sugars are by far the major components. The principal physical characteristics and behavior of honey are due to its sugars, but the minor constituents such as flavoring materials, pigments, acids, and minerals are largely responsible for the differences among individual honey types (White and Doner, 1980).

2.8 VARIATION IN HONEY SUGARS AND SUGAR PROPERTIES

Honey is above all a carbohydrate material, with about 95% of the solids being sugars, and the identity of these sugars has been studied for many years. Sugars are classified according to their size or the complexity of the molecules of which they are made. Dextrose (glucose) and levulose (fructose) are simple sugars, or monosaccharides, and are the building blocks for the more complex honey sugars. Glucose and fructose account for about 85% of the solids in honey. Until the middle of this century, the sugars of honey were thought to be a simple mixture of glucose, fructose, sucrose (table sugar), and an ill-defined carbohydrate material called "honey dextrin." With the advent of new methods for separating and analyzing sugars, workers in Europe, the United States, and Japan have identified many sugars in honey after separating them from the complex honey mixture (White and Doner, 1980).

In addition to the common sugars; fructose and glucose in honey, sucrose is also present in honey, and is one of the main sugars in nectar, along with fructose and glucose. Maltose is actually a mixture of several complex sugars (White and Doner, 1980). Nevertheless, glucose and fructose are still by far the major sugars in honey, but 22 others have been found. All of these sugars are more complex than the monosaccharides; ten disaccharides have been identified: sucrose, maltose, isomaltose, maltulose, nigerose, turanose, kojibiose, laminaribiose, B-trehalose, and gentiobiose. Ten trisaccharides are present: melezitose, 3-a-isomaltosylglucose, maltotriose, l-kestose, panose, isomaltotriose, erlose, theanderose, centose, and isopanose; two more complex sugars, isomaltotetraose and isomaltopentaose, have been identified. Most of these sugars are present in quite small quantities (White and Doner, 1980).

Honey contains about 4.5 times as much sugar as water. The very high sugar concentration gives honey its keeping quality, its energy value (304kcal/kg) and its high viscosity. Glucose is a relatively insoluble sugar; fructose is a very sweet sugar. Fructose is very hygroscopic and at room temperature its rate of absorption of water rises rapidly when the relative humidity reaches about 60%. Honey is likely to contain about 2% or 3.9% sucrose. Honeys from many plants have sucrose content above this value. If honey contains substantially more than this, it may have been removed from the hive before the bees finished making it. During heavy nectar flow from plant bees convert the nectar into honey very quickly and thus with less of the enzyme action that inverts sucrose. The honey thus produced is likely to contain more sucrose than normal. Direct adulteration with sucrose may give a sucrose content of 30% or more (Crane, 1990). Anupama *et al.* (2003) reported sucrose content in the range of 1.7–3.0% for honey samples from Molise region of Italy; they also reported sucrose content in the range of 0.062–4.24% from other studies. The average concentration of sucrose varied in individual groups of honeys, however, these differences were not statistically significant (Čelechovská and Vorlova, 2001).

Glucose, a major sugar in honey, can spontaneously crystallize from any honey in the form of its monohydrate. This sometimes occurs when the moisture level in honey is allowed to drop below a certain level. A large part of the honey sold to consumers in the United States is in the liquid form, much less in a finely granulated form known as "honey spread" or finely granulated honey, and even less as comb honey. The consumer appears to be conditioned to buying liquid honey. At least sales of the more convenient spread form have never approached those of liquid honey. Since the granulated state is natural for most of the honey produced in the United States, processing is required to keep it liquid. Careful application of heat to dissolve “seed” crystals and

avoidance of subsequent “seeding” will usually suffices to keep a honey liquid for six months. Honey that has granulated can be returned to liquid by careful heating. Heat should be applied indirectly by hot water or air, not by direct flame or high-temperature electrical heat. Stirring accelerates the dissolution of crystals. For small containers, temperatures of 60º C for 30 minutes usually will suffice (White and Doner, 1980).

2.9 TYPES OF HONEY

Differences between floral honey and honeydew honey have been observed. Floral honey is higher in simple sugars (fructose and glucose), lower in disaccharides and higher sugars (dextrins), and contained much less acid. The higher amount of mineral salts (ash) in honeydew gave it a less active acidity (higher pH). The nitrogen content reflecting the amino acids and protein content was also higher in honeydew. Even though honey contains an active sucrose-splitting enzyme, the sucrose level in honey never reaches zero. Honey varies tremendously in color and flavor, depending largely on its floral source (White and Doner, 1980). Honeys harvested from various parts of the worlds contain a small amount of honeydew honey. Such honeys exhibit the characteristics of honeydew honey to a degree dependent on the proportion of the honeydew contribution. Honeydew honey differs from nectar honey principally because it is derived from the internal phloem sap of the plant instead of from actively secreted nectar and most especially because enzymes from the honeydew producing insects are involved in its production. But the main constituents of both honeys are mostly similar (Crane, 1990).

Honeydew can be produced by a wide variety of sucking insects on a number of different Coniferae, as *Abies alba* L. (Central and Northern Europe), *A. cephalonica* Loudon (Greece), *Picea excelsa* (Lam.) Link. (Central and Northern Europe), *Pinus halepensis* Miller and *P. brutia* Ten. (Greece) (Oddo and Piro, 2004). When compared to nectar honeys, honeydew honeys were generally distinguished on the basis of their higher values of pH, acidity, ash, electrical conductivity and darker colour, as well as by a lower monosaccharide and a higher di- and trisaccharide content (Diez *et al*., 2004; Soria *et al*., 2004).

2.10 DETERIORATION OF QUALITY

Honey sold as such shall not have added to it any food ingredient, including food additives, nor shall any other additions be made other than honey. Honey shall not have any objectionable matter, flavour, aroma, or taint absorbed from foreign matter during its processing and storage. The honey shall not have begun to ferment or effervesce. No pollen or constituent particular to honey may be removed except where this is unavoidable in the removal of foreign inorganic or organic matter. Honey shall not be heated or processed to such an extent that its essential composition is changed and/ or its quality is impaired. Chemical or biochemical treatments shall not be used to influence honey crystallization (Codex Alimentarius, 2001).

Fermentation of honey is caused by the action of sugar-tolerant yeasts upon the sugars glucose and fructose, resulting in the formation of ethyl alcohol and carbon dioxide. The alcohol in the presence of oxygen then may be broken down into acetic acid and water. As a result, honey that has fermented may taste sour. The yeasts responsible for fermentation occur naturally in honey, in that they can germinate and grow at much higher sugar concentrations than other yeasts, and,

therefore, are called "osmophilic." Even so there are upper limits of sugar concentration beyond which these yeasts will not grow. Thus, the water content of a honey is one of the factors concerned in spoilage by fermentation. The others are extent of contamination by yeast spores (yeast count) and temperature of storage. Honey with less than 17.1% water will not ferment in a year, irrespective of the yeast count. Between 17.1% and 18% moisture, honey with 1,000 yeast spores or less per gram will be safe for a year. When moisture is between 18.1 and 19%, not more than 10 yeast spores per gram can be present for safe storage. Above 19% water, honey can be expected to ferment even with only one spore per gram of honey, a level so low as to be very rare (White and Doner, 1980).

2.11 MOISTURE/WATER CONTENT

The natural moisture of honey in the comb is that remaining from the nectar after ripening. The amount of moisture is a function of the factors involved in ripening, including weather conditions and original moisture of the nectar. After extraction of the honey, its moisture content may change, depending on conditions of storage. It is one of the most important characteristics of honey influencing keeping quality, granulation, and body. Beekeepers as well as honey buyers know that the water content of honey varies greatly. It may range between 13 and 25 percent. According to the United States Standards for Grades of Extracted Honey, honey may not contain more than 18.6 percent moisture to qualify for U.S. grade A (U.S. Fancy) and U.S. grade B (U.S. Choice). Grade C (U.S. Standard) honey may contain up to 20 percent water; any higher amount places a honey in U.S. grade D (Substandard). These values represent limits and do not indicate the preferred or proper moisture content for honey. If honey has more than 17 percent moisture and contains a sufficient number of yeast spores, it will ferment. Such honey should be

pasteurized, that is, heated sufficiently to kill such organisms. This is particularly important if the honey is to be "creamed" or granulated, since this process results in a slightly higher moisture level in the liquid part. On the other hand, it is possible for honey to be too low in moisture from some points of view. In the West, honey may have moisture content as low as 13 to 14 percent. Such honey is somewhat difficult to handle, though it is most useful in blending to reduce moisture content. It contains over 6 percent more honey solids than a product of 18.6 percent moisture. In the 490 samples of honey analyzed in the Department's Technical Bulletin 1261, the average moisture content was 17.2 percent. Samples ranged between 13.4 and 22.9 percent, and the standard deviation was 1.46. This means that 68 percent of the samples (or of all U.S. honey) will fall within the limits of 17.2 ± 1.46 percent moisture (15.7 - 18.7); 95.5 percent of all U.S. honey will fall within the limits of 17.2 ± 2.92 percent moisture (14.3 - 20.1). In the same bulletin, a breakdown of average moisture contents by geographic regions is shown. These values (percent) are North Atlantic, 17.3; East North Central, 18.0; West North Central, 18.2; South Atlantic, 17.7; South Central, 17.5; Intermountain West, 16.0; and West, 16.1 (White and Doner, 1980). No consistent differences have been reported between the water contents of honey from nectar and honeydew (Crane, 1990). Increase in sugar content reduces moisture content therefore sugar feed honeys show lower water content compared to other non sugar feed honey samples (Abu-Tarboush *et al*., 1993).

The water content of honey, usually measured by its specific gravity is of great importance to its keeping qualities. Honey when fully ripened by the bees, is safe from fermentation as the water content is reduced to 17.4% or less, giving a specific gravity of 1.4212gm/cc at 20^{o}C or more, if the water content is above 17.4% there is danger of the honey fermenting, particularly when it

granulates. Among the groups of honey used differences were not found in water content (Čelechovská and Vorlova, 2001). Moisture content is affected by climate, season and moisture content of original plant nectar. Sources of honey showed significant differences in the amount of moisture content ($p<0.01$). Moisture content is a physico-chemical property that depends upon the flower types used by honeybees for nectar (Vikas *et al*., 2003).

Moisture content of some Indian honeys varied from 17 to 22.6%. Two samples had 17.0 and 17.6% moisture respectively. Singh and Bath (1997) in Anupama *et al.* (2003) reported moisture content of 18.7–21.8% for Indian honey from three different floral sources. Other scientists reported moisture content in the range of 15.1–18.3%. The commercial samples examined in Anupama *et al.* (2003) study had higher moisture contents than reported values. The samples showed variations in moisture. They had mean value for moisture of 20.05% with standard deviation of 1.92 and variance of 3.69.

2.12 PHYSICO-CHEMICAL PARAMETERS OF HONEY

Recently, much work has been done to find chemical components of honeys originating in the nectar or produced by the bee through biochemical transformation of nectar compositions that could be used as markers for the floral origin of honeys. Some honeys are featured by a key descriptor or component. Methyl anthranilate and caffeine are characteristic constituents of orange honey. Others such as honeydew honeys have high values for electrical conductivity and trisaccharides, *Acacia* honeys exhibit high levels of fructose. Physicochemical parameters such as pH, moisture, sugar composition, colour properties and mineral content were studied and multivariate analysis showed that the best discriminant parameters were fructose, isomaltose and

melezitose for the unifloral honeys studied. The inclusion of mineral profile did not lead to a better performance in the characterization of the honey classes. Principal component analysis showed that the five honeys were not well distinguished by their physicochemical properties though the honeydew honeys were perfectly differentiated from the rest honeys since they had lower values (Terrab *et al*., 2003). Anupama *et al*. (2003) concluded that the viscosity, percent acidity and sucrose content were the major physico-chemical variables contributing to the grouping of honey samples.

Variables such as free acidity, water, invertase, total sugars, solids and electrical conductivity are considered as clear parameters of botanical origin for the honey. Diastase, fructose, glucose and water activity are indicators of maturity of honey, glucose oxidase is an indicator of honey deterioration and proline a parameter associated with botanical origin (Serrano *et al*., 2004). Oddo and Piro (2004) recorded these range of values for physicochemical parameters for some unknown honeys; electrical conductivity (S/m) 0.08 – 2.17, free acidity (meq/kg) 2.3 – 51.4, fructose (F) (g/100g) 25.3 – 47.3, glucose (G) (g/100g) 18.8 – 45.6, F+G (g/100g) 46.6 – 85.7, F/G ratio 0.81 – 1.84. The limits prescribed by the European Directive concerning honey (2001) are as follows; water (g/100g) < 20, exception Calluna honey < 23, F+G (g/100g) >60 exception Honeydew >45, sucrose (g/100g) <5 exception *Eucalyptus* <10, Citrus <10 and Borago <15, electrical conductivity (mS/cm) < 0.8 and honeydew >0.8, exceptions *Eucalyptus* 0.4 -0.6, Tilia 0.3 -0.9. Free acidity <50, hydroxymethylfurfural (HMF) <40 exceptions; tropical honeys <80, Diastase (Schade units) >8 (Oddo and Piro, 2004). The result of the analysis of pure honey from a geographical location (Kaduna State) in Nigeria showed that it contains; levulose (41.0%),

dextrose (35.0%), sucrose (1.9%), dextrin (1.5%), minerals (0.2%), and water (17.0%) (Fadare, 2001).

2.12.1 Mineral and Ash Content

When honey is dried and burned, a small residue of ash invariably remains, which is the mineral content. It varies from 0.02 to slightly over 1% for a floral honey, averaging about 0.17% for the 490 samples analyzed. Honeydew honey is richer in minerals, so much so that its mineral content is said to be a prime cause of its unsuitability for winter stores. Most of the metals in honey are present in only minute amounts called trace elements. The minerals originate in the soil and get into the honey via the plants and the materials bees collect. The average values of mineral contents of light and dark honeys gave mineral elements such as Potassium 205,1676, Chlorine 52,113, Sulphur 58,100, Sodium 18,76, Calcium 49,51, Phosphorus 35,47, Magnesium 19,35, Silicon 9,14, Iron 2.4,9.4, Manganese 0.3,4.1, and Copper 0.3,0.6 parts per million (ppm) in light and dark honeys respectively. Trace elements such as Chromium, Lithium, Nickel, Lead, Tin, Zinc, Osmium, Beryllium, Vanadium, Zirconium, Silver, Barium, Gallium, Bismuth, Gold, Germanium, and Strontium were observed in normal honeys. Minerals affect honey colour, very light coloured honeys often contain little mineral matter and dark honeys may well contain much. The total weight of the mineral elements (total ash) varies from 0.02% to 1% per 100g of the honey. It is commonly 0.1-0.3%. Other factors that make honey dark are related to the plant source of the honey are; high amino acid content and the presence of polyphenolic compounds (Crane, 1990). Mineral content of honey is highly dependent on the type of flower used by bees for nectar (Vikas *et al*., 2003).

Among the groups of honey studied significant differences in the amount of mineral substances were found. Concentrations of risk elements; Cadmium (Cd), lead (Pb), mercury (Hg), copper (Cu) and zinc (Zn) (Cd 0.5-77.4µg/kg, Pb 0.02-1.0mg/kg, Hg 0.67-2.93µg/kg, Cu 0.06-1.55mg/kg and Zn 0.2-22.9mg/kg) were far below the allowable limits and showed statistically significant differences ($p< 0.01$) for Cd, Hg Cu in blossom, blends and honeydew honey (Čelechovská and Vorlova, 2001). Average concentrations of mineral content of honey samples showed the ranges for; Cd 0.008-0.027mg/kg, Zn 4.17-22.3mg/kg, and Pb 0.025-0.071mg/kg. The mean values for Zn, Cd and Pb were 7.76, 0.015 and 0.048mg/kg respectively. Few investigated samples were free of heavy metals. More cadmium was found in honeydew honey and more lead in linden honey. Some mineral contents exceeded acceptable limits especially zinc in floral and heather honey (Przybylowski and Wilczynska, 2001). Cadmium, lead and mercury are major contaminants of food supply and may be considered the most important problem to our environment while others like iron, zinc and copper are essential for biochemical reactions in the body (Sobukola *et al*., 2010).The contents of essential trace elements also participate in the nutritional value of honey. The differences in copper content reached high significance ($p<0.01$) between honeydew (1.5± 0.4mg/kg) and blossom-multifloral (0.7± 0.5mg/kg) or blossom-rape honey (0.3 ± 0.2mg/kg) samples, and ($p<0.05$) between blossom-multifloral and blossom-rape honey samples. Honeydew contained higher concentrations of essential and risk elements. The content of the essential trace elements (Iron (Fe), Manganese (Mn), Zn, and Cu) varied for each group; however, only the Cu content showed significant differences. The concentrations of metals in blossom-multifloral and blossom-rape honey samples were lower than the concentrations in honeydew samples. The concentrations of potentially toxic elements (Pb, Cd, and Hg) were very low (median Pb: 0.053mg/kg; Cd: 3.3µg/kg; Hg: 1.2µg/kg). Statistically

significant differences ($p<0.05$) were found in the content of Cd and Hg between honeydew and blossom-multifloral honey samples, and between blossom-rape and honeydew samples (Vorlová and Čelechovská 2002). Among the sources of honey used by Vikas *et al.* (2003) significant differences in the amount of mineral substances were found ($p<0.01$). The levels of sodium, potassium, calcium, iron, copper, zinc varied from 163.3–304.31, 489.52–932.56, 32.6–84.63, 8.86–13.25, 1.74–2.9, and 2.5–16.77mg/kg, respectively. Potassium was the most abundant of the elements present in all honey types while copper was present in the lowest amount.

Trace and minor elements in Slovenian honey were analysed by total reflection X-ray fluorescence spectroscopy. Up to 16 elements (K, Cl, S, P, Ca, Mn, Rb, Cu, Fe, Ni, Cr, Br, Ti, Pb, Sr and As) were detected, in a range of average content from 1.24mg/kg for Strontium Sr to 2590mg/kg for Potassium K. Statistically significant differences were established between the different types of honeys (*Acacia*, floral, lime, chestnut, spruce, fir, forest and *Metcalfa prunosa* honeydew honey). The highest content of elements was determined in forest honey and the lowest in *Acacia* honey. Total elemental content and the content of S, Cl, K and Rb in honeydew honey were statistically significantly higher than that in nectar honeys. Chestnut honey differed statistically with its significantly higher contents of Rb and Ca from nectar and honeydew honeys. The year of honey production proved to have no statistically significant influence on elemental content (Golob *et al*., 2005).

The North Indian honey had lower ash content than that reported from South Spain commercial Spanish honey and Galicia (North-West Spain). The range of values for ash content (0.12–0.28%) fell within the limit allowed for floral honey (0.6%) and indicated the cleanliness of

honey samples and possibly the lack of adulteration with molasses (Vikas *et al*., 2003). The mineral content of 43 honey samples from the middle regions of Turkey was investigated. Minerals were determined by inductively coupled plasma-atomic emission spectrophotometer. Calcium, potassium, sodium and phosphorus were the most abundant of the elements in all the studied honeys, with average concentrations ranging between 40.13 and 189.69mg/kg for calcium, 161.01 and 598.62mg/kg for potassium, 9.34 and 45.9mg/kg for sodium, and 460.11 and 3,776.96mg/kg for phosphorus (Bagci *et al*., 2007). The mineral content and trace element in honey samples could give an indication of the geographical origin of honey (Vikas *et al*., 2003).

2.12.2 pH and Acidity

The active acidity of a material is expressed as pH; the larger the number the lower is the active acidity. The lactone is a newly found component of honey. Lactones may be considered to be reserve acidity, since by chemically adding water to them (hydrolysis) an acid is formed (White and Doner, 1980). The acids of honey account for less than 0.5 percent of the solids, but this level contributes not only to the flavor, but is in part responsible for the excellent stability of honey against microorganisms. Several acids have been found in honey, gluconic acid being the major one. It arises from glucose through the action of an enzyme called glucose oxidase. Other acids in honey are formic, acetic, butyric, lactic, oxalic, succinic, tartaric, maleic, pyruvic, pyroglutamic, a-ketoglutaric, glycollic, citric, malic, 2- or 3-phosphoglyceric acid, a- or B-glycerophosphate, and glucose 6-phosphate (White and Doner, 1980). Honeydew honey contains more free acid than nectar honey with 33.5 and 22.4meq/kg respectively (Crane, 1990).

Among the groups of honey studied by Čelechovská and Vorlova (2001), significant differences in the pH, ($p < 0.01$) and free acidity ($p<0.05$) were found. Among the seven different sources of honey used by Vikas *et al.* (2003) statistically significant differences ($p<0.01$) were found in free acid and lactonic acid. Free acid, lactone, and total acidity are the physico-chemical properties that depend upon the flower types used by honeybees for nectar. The sources of honey showed significant differences in the total acidity ($p<0.01$) however, total acidity and free acid were highest in citrus honey (Vikas *et al*., 2003). Significant correlation ($p<0.05$) was shown to exist between pH and total sugar, between free acid and proline, between lactonic acid and solids (Serrano *et al*., 2004).

Singh and Bath (1997) in Anupama *et al.* (2003) reported pH of 4.10–4.76 and acidity of 29.5–41.5 meq/kg for Indian honey from three different floral sources. Anupama *et al.* (2003) samples varied in pH from 3.62 to 4.00 except for one sample which had a pH of 5.46 and the values were comparable to that reported by Esti *et al.* (1997) in Anupama *et al.* (2003) who had reported pH in the range of 3.05–4.50, Mateo and Bosch-Reig (1998) and Lopez *et al.* (1996) in Anupama *et al.* (2003) reported pH in the range of 3.61–4.97.

2.13 USES OF HONEY

Nowadays a major part of the world's honey production is used as a sweetener. Domestic use includes liquid sauce, honey and cream cheese icing for cakes. Honey is used in confectionery industries for producing chocolate, in some caramel and boiled sweets. It is also used to coat nuts and breakfast cereals, make biscuits and cookies and preserve fruits. In Ethiopia much honey is fermented to make beer an alcoholic drink. Honey is used to preserve the Bulls semen and

human cornea for transplanting. Honey treats various veterinary ailments. Low grade honey is used as feed for cattles and pigs. About 90% of local production is consumed directly as honey (Crane, 1990). About 10% of the honey produced is used variously for dressing of wounds, as an antibiotic, anti-diarrhoea drug, in the manufacture of pharmaceutical drugs, and in the manufacture of cosmetics (Molan, 2001).

2.14 ANTI-MICROBIAL ACTIVITY OF HONEY

That honey has antibacterial properties has been known for more than a century (Dustmann, 1979). Although it has been used as a medicine since ancient times in many cultures (Majno, 1975), in its ancient usage there was no recognition of its antibacterial properties - it was just known to be an effective remedy. This is not surprising considering that it is only since the latter part of the last century that it has become known that many ailments are the result of infection by microorganisms. Now, it can be seen that there is effectiveness of honey in many of its medical uses and is probably due to its antibacterial activity. It is well established that honey inhibits a broad spectrum of bacterial species. There are many reports of bactericidal as well as bacteriostatic activity. There have also been reports of honey having antifungal activity (Molan, 1992). The collation of data by Molan (1992) shows that honey is active against a wide range of bacteria and fungal species, many of which cause infections. However, there are ailments which may be treated with honey which have not had the infectious agents tested for their sensitivity to the anti-microbial activity of honey. Also, there has not been much distinction made in the different types of anti-microbial activity in honey to which the various microbial species are sensitive. For serious consideration to be given to the use of honey as a therapeutic agent it is necessary that these aspects be further investigated.

The numerous reports of investigations which have established the nature of the anti-microbial factors in honey are; osmotic effect, honey is a saturated or super-saturated solution of sugars, 84% being a mixture of fructose and glucose. The water content is usually only 15-21% by weight. The strong interaction of these sugar molecules with water molecules leaves very few of the water molecules available for microorganisms. Although some yeast can live in honey that has a high water content, causing spoilage of the honey, the water activity of ripened honey is too low to support the growth of any species, no fermentation occurs if the water content is below 17.1%. Inhibition by the osmotic (water-withdrawing) effect of dilute solutions of honey obviously depends on the species of bacteria (Molan, 2006, http://bio.waikato.ac. nz/honey_intro.shtml). Retrieved on 5/17/2011. Honey is characteristically quite acidic, its pH being between 3.2 and 4.5, which is low enough to be inhibitory to many animal pathogens. The optimum pH for growth of these species normally falls between 7.2 and 7.4. The minimum pH values for growth of some common wound-infecting species is: *Escherichia coli*, 4.3; *Salmonella* sp., 4.0; *Pseudomonas aeruginosa*, 4.4; *Streptococcus pyogenes*, 4.5. Thus in undiluted honey the acidity is a significant antibacterial factor. But if honey is diluted, especially by body fluids which are well buffered, the pH will not be so low and the acidity of honey may not be an effective inhibitor of many species of bacteria (Molan, 2001). Hydrogen peroxide the major antibacterial activity in honey has been found to be due to hydrogen peroxide produced enzymatically in the honey. The glucose oxidase enzyme is secreted from the hypopharyngeal gland of the bee into the nectar to assist in the formation of honey from the nectar. The hydrogen peroxide and acidity produced by the reaction below serves to preserve the honey:

Glucose + H_2O+ O_2 --> gluconic acid + H_2O_2

The hydrogen peroxide produced would be of effect as a sterilising agent only during the ripening of honey. Full-strength honey has a negligible level of hydrogen peroxide because this substance is short-lived in the presence of the transition metal ions and ascorbic acid in honey which catalyse its decomposition to oxygen and water. The enzyme has been found to be practically inactive in full-strength honey; it gives rise to hydrogen peroxide only when the honey is diluted. This is because the acidity produced in the action of the enzyme drops the pH to a point which is too low for the enzyme to work any more. On dilution of honey the activity increases by a factor of 2,500 - 50,000, thus giving a "slow-release" antiseptic at a level which is antibacterial but not tissue-damaging (Molan, 2006, http://bio.waikato.ac. nz/honey_intro.shtml). Retrieved on 5/17/2011. The evidence for the existence of other antibacterial factors is mainly that the peroxide-generating system does not account for all of the observed antibacterial activity, but there have also been some reports of isolation of antibacterial substances from honey that are not hydrogen peroxide. Furthermore, it has been found that heating honey, which inactivates the glucose oxidase, causes loss of activity against some species whilst it is retained against others. Although the stability of the enzyme varies in different honeys, there have been reports of honeys with stability well in excess of this variation, showing that there must be an additional antibacterial factor involved. The most direct evidence for the existence of non-peroxide antibacterial factors in honey is seen in the reports of activity persisting in honeys treated with catalase to remove the hydrogen peroxide activity. Several chemicals with antibacterial activity have been identified in honey by various researchers: pinocembrin, terpenes, benzyl alcohol, 3,5-dimethoxy-4-hydroxybenzoic acid (syringic acid), methyl 3,5-dimethoxy-4-hydroxybenzoate (methyl syringate), 3,4,5-trimethoxybenzoic acid, 2-hydroxy-3-

phenylpropionic acid, 2-hydroxybenzoic acid and 1,4-dihydroxybenzene. However, the quantities of these present were far too low to account for any significant amount of activity (Weston *et al*., 2000; Molan, 2002; Aljadi and Yusoff, 2003).

In almost all reports on the medical use of honey as an antibacterial agent no consideration is given to the selection of type of honey for therapeutic use. Aristotle, c.350 B.C. 6, and Dioscorides, c.50 A.D. 7, recommended that honey collected in specific regions and seasons (and therefore presumably from different floral sources) be used for the treatment of particular ailments, but in modern medicine clinical practitioners have not heeded these views nor the laboratory findings of large differences in the antibacterial potency of different honeys. It was recognised more than 40 years ago that there are differences in the antibacterial activity of different honeys, and a method was devised to determine the "inhibine number" of honeys as a measure of their antibacterial activity. The "inhibine number" is the degree of dilution to which a honey will retain its antibacterial activity, representing sequential dilutions of honey in steps of 5% from 25% to 5% (25%, 20%, 15%, 10% and 5%). Studies measuring the "inhibine number" of honeys report activity to range over the five-fold difference in concentration in the dilution series, and studies using a wider range of dilutions report the minimum inhibitory concentrations of the honeys tested to range from 25 to 0.25%, >50 to 1.5%, 20-0.6%, and 50-1.5%. The data showed activities to be fairly well spread over these ranges. A study of 345 samples of New Zealand honey found a large number with low activity (36% of the samples had activity near or below the level of detection), the rest having almost a Gaussian distribution over a twenty-fold range of activity. The major variations seen in overall antibacterial activity are due to variation in the level of hydrogen peroxide that arises in honey and in some cases to the level of non-

peroxide factors. Hydrogen peroxide can be destroyed by components of honey: it can be degraded by reaction with ascorbic acid and metal ions, and by the action of the enzyme catalase which comes from the pollen and nectar of certain plants, more from the nectar. Also, very large differences have been found between honeys from different floral sources in the thermal stability of their glucose oxidase content, and in the sensitivity of this hydrogen peroxide-producing enzyme to denaturation by light because of a photosensitizing component that comes from some floral sources (Molan, 2006; http://bio.waikato.ac. nz/honey_intro.shtml). Retrieved on 5/17/2011.

Although it appears that the honey from certain plants has better antibacterial activity than that from others, there is not enough evidence for such definite conclusions to be justified because the data are from small numbers of samples. However, honeys from some sources have been studied in large enough numbers or have been included in enough different studies for some trends to be noted. Honeydew honey from the conifer forests of the mountainous regions of central Europe has been found to have particularly high antibacterial activity. Also, honey from manuka (*Leptospermum scoparium*) in New Zealand has been found to have a high activity, about half of this type of honey having an exceptionally high level of non-peroxide activity. Manuka honeys exhibit notable anti-microbial activity which cannot be attributed to the peroxide present (Allen *et al*., 1991). It has been documented that the pronounced antibacterial activity of Manuka honey directly originates from the methylglyoxal (MG) it contains (Mavric *et al*., 2008)

Full strength honey has negligible amounts of hydrogen peroxide which is not sufficient to inhibit bacteria growth (Bogdanov, 1997). Molan (2002) Stated that the peroxide system does

not account for all the observed activity in manuka honey, rather, he proposed that a unique plant derived product was responsible for the non-peroxide antibacterial activity. Aljadi and Yusoff (2003) further stated that hydrogen peroxide may be destroyed by tissue catalase, therefore there is a non-peroxide activity of honey which is important to know what it is. The non-peroxide antibacterial activity of honey is usually attributed to the presence of organic components originating from floral sources. A number of organic ingredients with antibacterial activity have been extracted from honey using different solvents. Most of the components are phenolics (flavonoids or phenolic acids) in nature and are of plant origin (Aljadi and Yusoff, 2003). Honeydew honeys have a higher antibacterial activity than nectar honeys (Crane, 1990).

Weston *et al.* (2000) stated that the phenolic fraction of manuka honey had no significant antibacterial activity and instead suggested the activity was associated with the carbohydrate fraction. If at all the phenolic components of manuka honey might contribute to, but does not account for the observed non-peroxide antibacterial activity of manuka honey. Weston (2000) hypothetically concluded that the non- peroxide activity of honey could be interpreted as residual hydrogen peroxide activity which is probably due to the absence of plant derived catalase from honey. Hydrogen peroxide may be destroyed by tissue catalase when honey is used as a wound dressing. Therefore the non-peroxide activity of honey becomes very important because of honey as a whole and of its phenolics as antibacterial factors against antibiotic-resistant bacteria (Aljadi and Yusoff, 2003). Aljadi and Yusoff (2003) identified phenolic acids with antibacterial activity from honey. The phenols were caffeic acid, ferulic acid, vanillic acid, cinnamic acid and benzoic acid. Chromatographic comparisons revealed that both honey samples exhibited similar phenolic profiles but their relative amounts were different. Gelam honey contained higher levels than

Coconut honey, however gallic acid, caffeic acid and benzoic acids were present in both honeys in different quantities but cinnamic acid and ferulic acid were detected only in Gelam honey. Unknown compounds were present in both honeys with two unknown compounds in Coconut honey and one unknown compound in Gelam honey. Disc diffusion assay using Whatman paper containing 0.65, 1.3, 1.95, 2.6 and 3.2mg of phenolic extracts which are equivalent to honey dilution series from 5% (v/v) to 25% (v/v), were assayed against *Escherichia coli*, *Staphylococcus aureus*, Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Methicillin-Sensitive *Staphylococcus aureus* (MSSA). Results showed that phenolic extracts of honeys used exhibited various degree of activity against the organisms. The inhibition zone measured in diameter ranged from 9.6-22mm for Coconut honey while Gelam honey had a range of 9.9-26.5mm with *E. coli* been the most resistant strain to the activity of both honeys. The variation in the antibacterial activity of these honeys could be attributed to their phenolic contents. A linear relationship (r=0.9) was obtained by plotting log concentrations of the phenolic extract of both honey types against inhibition zone diameters. The assay showed that Gelam honey extract had a more pronounced activity than the Coconut honey extract. The phenolic antibacterial activities were assumed to be different from each other due to the different floral sources (Aljadi and Yusoff, 2003).

It is important that when honey is to be used as an anti-microbial agent it should be selected from honeys that have been assayed in the laboratory for anti-microbial activity. It is also important that honey for use as an anti-microbial agent be stored at low temperature and not exposed to light, so that none of the glucose oxidase activity is lost. Although all honey will stop the growth of bacteria because of its high sugar content, when the sugars are diluted by body fluids this

antibacterial action is lost. The additional antibacterial components then become important (Molan, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 HONEY SAMPLES COLLECTION SITES

Honey samples were obtained from three different geo-vegetational locations in Nigeria. These locations were chosen based on beekeepers acceptance to partake in the harvest of honey from the hive. Honey samples were collected from Adamawa State (Mubi) and Jigawa State (Hadejia) in the Sudan savanna, Kaduna State (Kachia) and Plateau State (Pankshin) in the Guinea savanna and Ondo State (Ido-Ani) and Oyo State (Ago- Are) from the rain forest (Figure 3.1).

3.2 COLLECTION AND PREPARATION OF HONEY SAMPLES

Sealed honey comb harvested directly from the hive were collected and transported to the laboratory in labelled sealed containers. At the laboratory, the samples were homogenized by stirring thoroughly at least for three minutes with a glass rod (IHC, 2002).

Clinical strains of *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Salmonella typhi* were obtained from the department of Microbiology, Ahmadu Bello University, Zaria. Honey samples were used to test microorganism's resistance and susceptibility, by measurement of Zone of inhibition (ZOI).

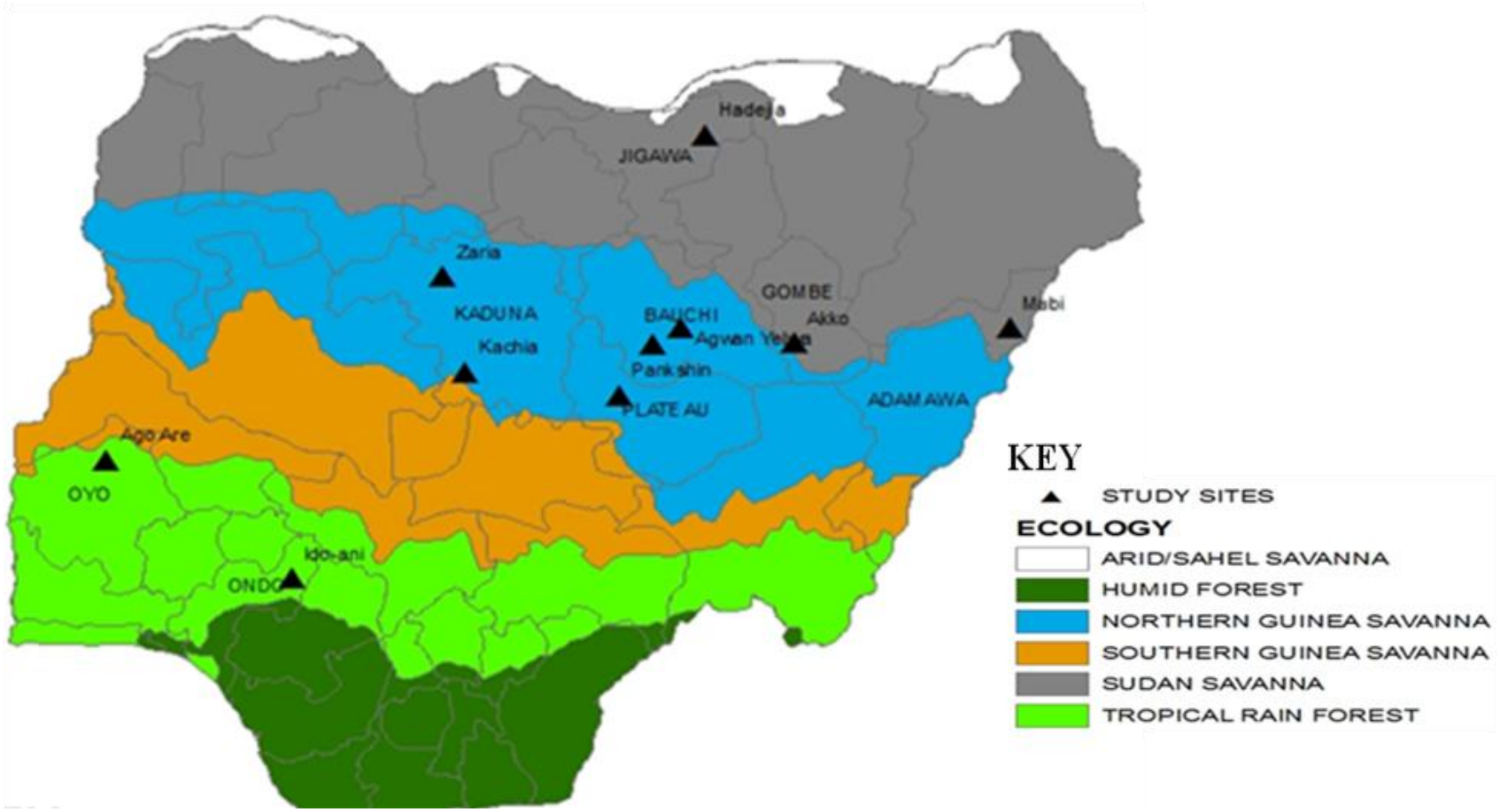


Figure 3.1: Vegetation Map of Site States

3.3 MELISSOPALYNOLOGICAL ANALYSIS OF HONEY

3.3.1 Pollen Characterization

The procedure recommended by Louveaux *et al*. (1970) with modifications was used. The modification includes the option of acetolysis. Pollen sediment of the honey was acetolysed (Erdtman, 1968) and mounted on slides with glycerin jelly and viewed under the microscope using x40 objective lens. Ten gram of honey was dissolved in 30ml of warm water (not more than 40˚C) weak solution of sulphuric acid (acid: water)= 1:200 and agitated at 5000 rotations for 10 minutes with the clear supernatant liquid decanted into a wash basin. Distilled water was added to the residue and mixed using an electric mixer and poured into a calibrated centrifuge tube, then centrifuged and the supernatant decanted. Ten millilitre of glacial acetic acid was added to the residue, stirred well, centrifuged and decanted. Ten millilitre of acetolysis mixture (acetic anhydride: sulphuric acid (9:1)) was added to the residue placed in a water bath at 70˚C for 5 minutes. Samples were then removed from the bath, centrifuged for 5 minutes and decanted. Ten millilitre distilled water was added to wash the samples for 3 times. 50% glycerol was added to the sample residue and allowed to stand for at least 30 minutes, centrifuged and the supernatant decanted. The final volume of residue was noted and a known volume of 100% glycerol was added and mixed and samples poured into labelled storage vials which were used for mounting on slide for observation.

The pollen grains were counted to determine the relative frequency of the different pollen types (Appendix II) in the honey samples under light microscope at X40 objective lens (Olympus CH 30). All grains encountered were counted according to Von der Ohe *et al*. (2004) to consider the geographic and botanical identity of the

honey types. Identification of the pollen in the honey sample was done by comparing the recovered pollen with 3,600 reference pollen slides collection and photomicrographs at the Palynology Laboratory, University of Ibadan.; relevant literature such as Moore and Webb (1978) and Palynology Laboratory, Department of Anthropology, Texas University, U.S.A.

3.4 DETERMINATION OF PHYSICO-CHEMICAL PARAMETERS

3.4.1 Determination of Water Content

Moisture content was determined using the method of IHC (2002). Homogenized prepared samples of honey collected from different locations were placed into a labelled 50ml flask. The flask was air tightly closed and placed in a water bath at less than 40^{o}C until all the sugar crystals dissolved. The honey solution was cooled to room temperature and stirred again. Directly after homogenization a digital refractometer at 20^{o}C was placed in the solution. After six minutes, the refractive index (RI) with the corresponding moisture content was read and recorded.

3.4.2 Determination of Ash Content

The ash dish was heated in the electrical furnace at ashing temperature of 600^{o}C, cooled in desiccators to room temperature and weighed (m_2). To the ash dish 10g of each honey sample was poured and weighed. This became (m_0). Ashing commenced at 50 to 70^{o}C heat rising to 350-400^{o}C by use of a hot plate. After the preliminary ashing, the dish was placed in the preheated furnace and heated for at least one hour. The ash dish was cooled in the desiccators and weighed. This procedure of one hour ashing was done until constant weight was reached (m_1). The proportion of ash W_A in g/100g was calculated using the formula below

$$W_A = \frac{(m_1 - m_2)}{m_0} \times 100$$

where:

* = multiplication sign

m_0 = weight of honey taken

m_1 = weight of dish + ash

m_2 = weight of dish

Result was expressed as a percentage by weight (AOAC, 1990; IHC, 2002).

3.4.3 Determination of Mineral and Heavy Metal Content

Five millilitre of 0.1 N HCl was added to each of the ashed samples and the mixture stirred on a hot plate to almost complete dryness. Ten millilitre of HCl (0.1N) was added to the almost dry mixture and the solution brought up to 50ml with distilled water. For calcium and magnesium determination 1.5ml of Lanthanum (0.1% as chloride) was added in order to suppress other elements. Mineral content (mg/100g) for each sample was then determined using an Atomic Absorption Spectrophotometer (AOAC, 1990; IHC, 2002; Downey *et al.*, 2005).

3.4.4 Determination of pH and Acidity

From a prepared honey sample, 10g of honey was dissolved in 75ml of carbon dioxide-free water in a 250ml beaker. It was stirred with the vortex Mixer (Bibby Stuart SA7) instead of using a magnetic stirrer and the pH electrodes (HANNA Instruments pH 210 microprocessor pH meter) immersed in the honey solution. The pH was read and recorded.

To the above solution, using titrimetric method, 0.05M (0.05N) NaOH was added through titration and stopped at pH 8.50 (free acidity). The volume used was noted. Free acidity was expressed as milliequivalents or millimoles acid per kilogramme honey = 0.1 M NaOH X 10. Immediately 10ml of 0.05M NaOH was pipetted and without delay back titrated with 0.05M HCl to pH 8.30 (Lactone acidity). Total acidity results were gotten from adding free acidity plus lactonic acidity. Result was expressed as meq/kg (AOAC, 1990; IHC, 2002).

3.4.5 Determination of Glucose by Benedict's Method

Two grams of anhydrous sodium trioxo carbonate VI (Na_2CO_3), an anti bump was added to Benedict's reagent and boiled in a beaker. The honey sample in the burette was run into the beaker until the blue colour disappeared. The quantity at which the reagent colour became neutralized by the sample was noted and the glucose content calculated.

Given that 100ml of glucose solution contains 0.5g glucose

Then Z (quantity of honey sample used) contains Xg of glucose

Substitute the value; this gives the amount of glucose in the 0.5% honey sample.

3.4.6 Determination of Reducing Sugar

To approximately 2 ml of test solution, an equal quantity of Benedict's reagent was added, shaken, and heated for a few minutes at 95°C in a water bath. To a weighed clean filter paper the test tube content was filtered. The reducing sugar remained on the paper. It was then dried and weighed. The difference in weight was used to calculate the reducing sugar.

3.4.7 Determination of Sucrose by Lyon's Method

To 10ml of 0.5% honey sample was added 7ml concentrated HCL and 3 drops of phenolphthalein into a beaker. In the burette was 5 M NaOH, which was titrated till it showed a purple colour and was made up to 100ml. The content in the beaker was poured into a clean burette and titrated with 10ml Fehling's reagent in the beaker, the end point was taken when the colour changed from blue to brick red. The difference between total reducing sugar after hydrolysis and total reducing sugar before hydrolysis multiplied by 0.95 equals to percentage sucrose.

3.4.8 Determination of Maltose Content

Samples were treated with 2ml concentrated Tetraoxosulphate (VI) acid for one hour followed by filtration and then boiling of the filtrate with 1 N NaOH (caustic soda) solution. Maltose content was then measured colorimetrically. One point five gram of honey sample was weighed into a 10ml Pyrex test- tube and 5ml of warm distilled water (30˚C) was added. The test tube was covered and shaken vigorously to homogenize the suspension. The test tubes were placed in Gallenkemp Flask Shaker at the rate of 20 agitations per minute for 1 hour at room temperature. During this period, if the enzyme amylase was present in the sample it hydrolysed starch into maltose. After 1 hour the test tubes were removed and 2ml of concentrated H_2SO_4 was added and stirred vigorously. The suspension was filtered through Whatman filter paper placed in a glass funnel. The filtrate which contained maltose was collected in a beaker and allowed to stand for 30 minutes, thereafter 3ml aliquot was pipetted into 20ml test tube and 1ml NaOH solution added and mixed together. The test tubes were then placed in a water bath with boiling water for 5 minutes until the colour changed

from yellow to dark brown. The tubes were then removed from the boiling water bath and allowed to cool in another water bath at room temperature. The absorbance of each sample was measured colorimetrically at 550nm using a spectronic 20 spectrophotometer with distilled water as the blank. Maltose content was then calculated using the equation below

$$\% \text{ Maltose} = A_{550} * 6.734 + 0.0609 \text{ (AOAC, 1990).}$$

Where:

* = multiplication sign

3.4.9 Determination of Protein

Two grams of honey was weighed in 100ml Kjeldahl digestion flask and about 1g of catalyst mixture (K_2SO_4 + anhydrous $CuSO_4$) was added to speed up the reaction. 25ml of concentrated sulphuric acid was added to the flask and the content heated slowly until when ferritin subsided and then moved rigourously with occasional rotation of the flask to ensure even digestion and avoid over heating of the content. The heating continued until a clear solution was obtained. After cooling, the solution was transferred into 100ml volumetric flask and diluted to mark with distilled water. Ten millilitre of the diluted digest was pipetted into Markham semi macro nitrogen still and 10ml of 40% sodium hydroxide solution was added. The sample was distilled and librated ammonia which was trapped in a 100ml conical flask containing 10ml of 40% Boric acid and 2 drops of methyl red indicator. Distillation continued until pink colour indicator turned green. The control was titrated with 40% Boric acid with end point indicated by a change from greenish to pink colour.

The volume of the acid for each sample distillate was noted as well as that of the blank. Protein content was then calculated using the equation below.

$$\% \text{ Total N per sample} = \frac{V_1 - V_0 * M * 14 * 100 * 100}{0.2 * 1000 * 10 * 1}$$

Where:

* = multiplication sign

N = molar concentration of Nitrogen

V_0 = volume of HCl required for blank

V_1 = volume of HCl required for 10ML sample solution

M = molarities of acid (0.1M)

14 = atomic weight of Nitrogen

100 = total volume of digest

100 = % conversion

10 = volume of distillate

0.2 = amount of sample taken in gram

1000 = to convert to litre

Therefore, the crude protein = 6.25 * N (AOAC, 1990).

3.4.10 Determination of Fat by Geber's Method

The butyrometer was placed on the butyrometer stand with open mouth upwards. Ten millilitres of sulphuric acid (H_2SO_4), Ten point nine four millilitres honey and 1ml of fat free amyl alcohol was pipetted into the butyrometer making sure they did not mix together. The tube was closed with a stopper and the content thoroughly mixed till red colour was obtained and immediately agitated at 1100 rotations for 4 minutes. The butyrometer was taken from an upright position with the stopper end downwards from the stand and transferred into a hot water bath at (60˚ C) for at least 3 minutes. The fat

column which appeared clear and yellowish within the graduation was adjusted with the help of key and the reading taken from bottom of the fat column to lower border of the meniscus on the scale (AOAC, 1990).

3.4.11 Determination of Hydroxymethylfurfural (HMF)

Five gram of honey was weighed into a 50ml beaker. The sample was dissolved in approximately 25ml of water and transferred quantitatively into a 50ml volumetric flask. 0.5ml of Carrez solution I was added and mixed. 0.5ml of Carrez solution II, was added and mixed and made up to the mark with water (a drop of ethanol was added to suppress foam). It was then filtered through paper; rejecting the first 10ml of the filtrate. 5.0ml was pipetted in each of the 2 test tubes. 5.0ml of water was added to one of the test tubes and mixed well. Again, 5.0ml of sodium bisulphite solution (0.2%) was added to the second test tube and mixed well (the reference solution). The absorbance of the sample solution against the reference solution at 284 and 336nm in 10mm quartz cells were determined within one hour.

Results were calculated and expressed as:

HMF in mg/kg = $(A_{284} - A_{336}) * 149.7 * 5 * D/W$

Where:

* = multiplication sign

A_{284} = absorbance at 284nm

A_{336} = absorbance at 336nm

$149.7 = \frac{126 * 1000 * 1000}{16830 * 10 * 5}$ = Constant

126 = molecular weight of HMF

16830 = molar absorptivity ε of HMF at λ= 284nm

1000 = conversion g into mg

10 = conversion 5 into 50ml

1000 = conversion g of honey into kg

5 = theoretical nominal sample weight

D = dilution factor, in case dilution is necessary

W = Weight in g of the honey sample

Results were expressed in mg/kg (IHC, 2002).

3.5 ANTI-BACTERIAL ASSAY

3.5.1 Agar Well Diffusion: Honey samples were screened for anti-bacterial activity against clinical isolates of *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Salmonella typhi* and *Pseudomonas aeruginosa* using the agar well diffusion method (Allen *et al*., 1991) in replicates. Solidified Mueller Hinton Agar (MHA) plates were separately flooded with 0.1ml of 0.5ml McFarland standard of the different test organisms grown in sterile nutrient broth at 37^{o}C for 24hr. The plates were allowed to dry and wells of 6mm diameter bored into the agar with a cork borer. From the 100% pure honey 2ml was taken and 2ml sterilized distilled water added, this gave 50% concentration of honey, from the 50% concentration 2ml was taken and 2ml of distilled water added, this gave 25%, 2ml was taken and 2ml distilled water added, this gave 12.5%. Solutions containing 12.5, 25, 50 and 100% (v/v) honey samples in sterile distilled water were separately placed in the different wells and incubated at 37^{o}C for 24hr for bacteria. The diameters of the zones of inhibition were measured with ruler, the average taken and recorded in millimeter. The interpretation of zones of inhibition results; less than 15mm was considered resistant, 16-20mm intermediate and greater than or equal to 21mm susceptible (CLIS, 2007).

3.5.2 Minimum Inhibitory Concentration (MIC): The broth dilution technique was used; honey samples that showed significant antibacterial activity were selected for the determination of MIC using Mueller Hinton Broth (MHB). To 7.5ml of distilled water, 2.5ml of honey sample was added, this formed the stock solution. Based on the result from agar well diffusion most honey samples showed no zone of inhibition as from 25% honey concentration. From the stock solution 2ml of honey sample solution was taken with a sterile syringe and serially diluted to the 5^{th} labeled test tube containing 2.5ml MHB. A loopful of 0.5ml McFarland standard of each test organism was dispensed into each test tube aseptically. All content in the test tubes were incubated at 37^{o}C for 24hr. After the incubation, the test tube with the least concentration showing no visible growth was recorded as MIC (CLIS, 2007).

3.5.3 Minimum Bactericidal Concentration (MBC): From the test tube showing no visible growth from MIC determination, cultures were inoculated into sterile MHA plates by streaking and the plates incubated at 37^{o}C for 24hrs. The least concentration that did not show growth on the agar plate was considered as the MBC (CLIS, 2007).

3.6 CONSUMERS' ACCEPTANCE OF HONEY

Questionnaire was administered to buyers of honey. The data generated was used to determine consumers' acceptance of honey (Appendix I).

3.7 STATISTICAL ANALYSIS

ANOVA was used to test if there were significant differences (at $p<0.05$) in the honey samples collected from the three vegetation zones and the concentration of honey used against the different organism. Duncans multiple range test (DMRT) was used to separate the means. Bar chart was used to show the frequency of plant families occurring in the honey from the different locations. Percentage was used to show pollen grain frequency in honey samples. Pearson's rank correlation analysis and Chi square was used to test consumers' acceptance.

CHAPTER FOUR

4.0 RESULTS

4.1 POLLEN SPECTRA OF HONEY SAMPLES

Pollen of 69 plant species from 21 plant families were identified. The percentages of pollen types from the three vegetation zones are presented in Tables 4.1-4.4. The identified species were herbs, shrubs, grass but majorities were trees. The most important pollen grain plant families are presented in figures 4.1 – 4.8, while photomicrographs of some of the identified pollen are presented in appendix III.

4.1.1 Pollen Spectra of Honey Samples from Sudan Savanna Zone

The honey sample from Adamawa State recorded 31 pollen types while Jigawa State recorded 18 pollen types (Table 4.1). The total number of pollen grains counted and dominant pollen types for each honey sample was 35,915 and 1,339; *Combretum* sp. (90%) and *Parkia biglobosa* (61%) for Adamawa and Jigawa States respectively. Also present in the honey sample from Jigawa were pollen grains from *Diospyros mespiliformis*, Sapotaceae, *Adenolichos paniculata*, *Bridellia* sp., and *Borreria.*

Honey from Adamawa State recorded 90% of the pollen grain from the family Combretaceae followed by 5% of Fabaceae (Figure 4.1). Plants from the family Combretaceae were mostly visited by the bees. Honey sample from Jigawa State (Figure 4.2) had pollen grains majorly from the family Fabaceae (63%), Ebenaceae (29%) and Sapotaceae (5%).

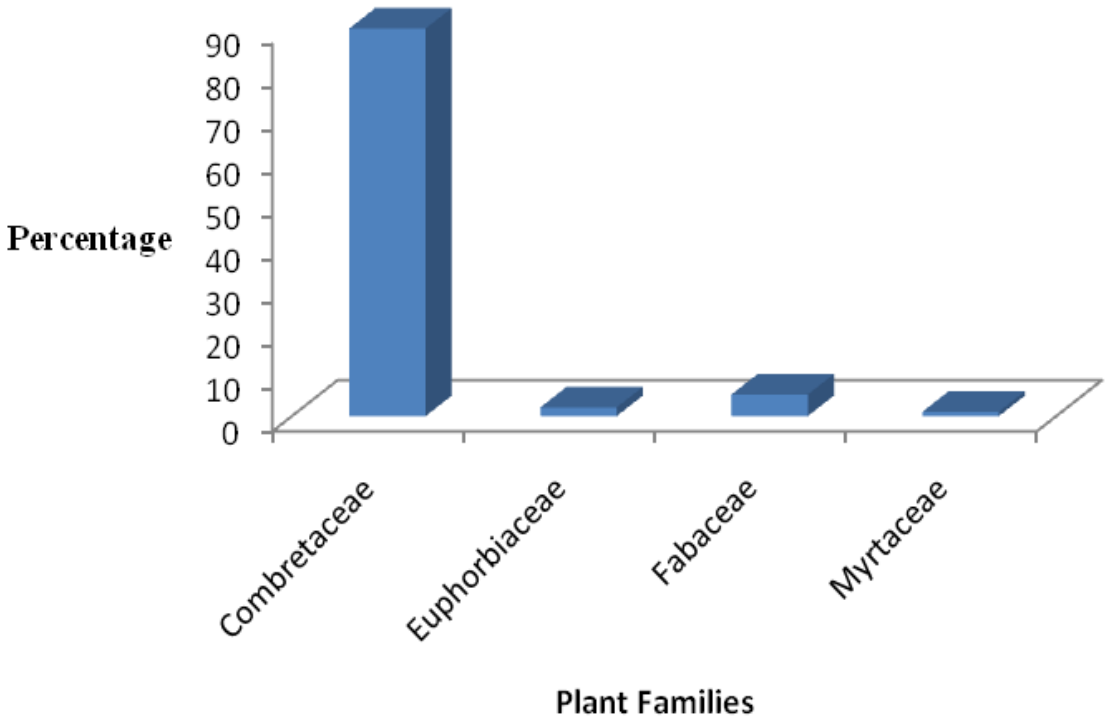


Figure 4.1: Percentage distribution of major pollen plant family from Adamawa State.

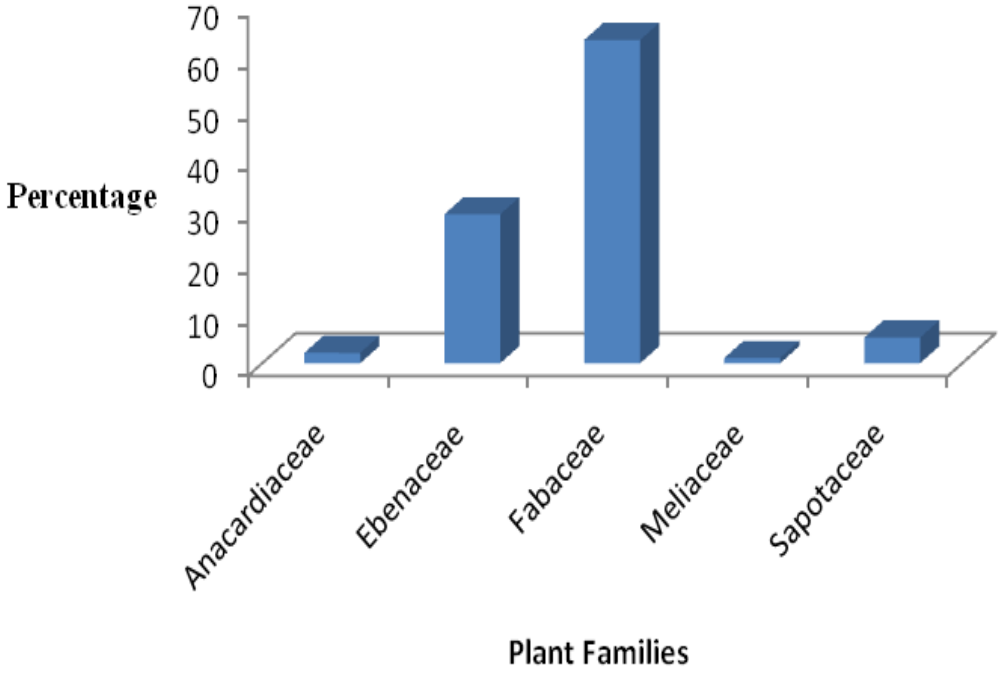


Figure 4.2: Percentage distribution of major pollen plant family from Jigawa State

Table 4.1: Plant Taxa Identified and Their Pollen Percentage Occurrence in Nigerian Sudan Savanna Zone Honey

Plant taxa	Family	Percentage occurrence by State	
		Adamawa	Jigawa
Adenodolichos paniculata (Harms)	Fabaceae	-	1 (8)
Adhatoda robusta (C.b. Clarke)	Acanthaceae	0 (48)	-
Alchornea Species (Schum and Thonn.)	Euphorbiaceae	1 (530)	0 (2)
Anacardium occidentale (Linn.)	Anacardiaceae	0 (49)	-
Annonidium mannii (Oliv.)	Annonaceae	0 (46)	-
Borreria sp. (G. Mey)	Rubiaceae	-	0 (2)
Bridellia sp. (Willd.)	Euphorbiaceae	-	0 (2)
Senna sp. (Linn.)	Fabaceae	-	0 (2)
Fabaceae	Fabaceae	1 (358)	-
Combretum sp. (*R.Br.*exGDon)	Combretaceae	90 (32,155)	-
Cyperaceae	Cyperaceae	0 (43)	-
Daniellia ogea (Harms)	Fabaceae	1 (230)	-
Daniellia oliverii (Rolfe)	Fabaceae	1 (184)	-
Delonix regia (Hooks)	Fabaceae	0 (1)	-
Detarium sp. (Juss.)	Fabaceae	-	0 (2)
Diospyros mespiliformis (Linn.)	Ebenaceae	-	29 (384)
Euphorbia hirta (Linn.)	Euphorbiaceae	0 (51)	-
Entada abyssinica (Steud.)	Fabaceae	-	0 (6)
Gossypium sp. (Linn.)	Malvaceae	0 (83)	-
Hymenocardia acida (Tul.)	Hymenocardiaceae	0 (76)	-
Lannea sp. (A. Rich)	Anacardiaceae	-	1 (8)
Loranthaceae	Loranthaceae	0 (1)	-
Mangifera indica (Linn.)	Anacardiaceae	-	0 (6)

Table 4.1 continues

Plant taxa	Family	Percentage occurrence by State	
		Adamawa	Jigawa

Meliaceae	Meliaceae	0 (24)	1 (8)
Myrtaceae	Myrtaceae	1 (252)	0 (2)
Oldenlandia corymbosa (Linn.)	Rubiaceae	0 (75)	-
Parinari kerstingii (Engl.)	Rosaceae	2 (874)	-
Parkia biglobosa (Jacq.)	Fabaceae	0 (2)	61 (814)
Phyllanthus discoideus (Baill)	Euphorbiaceae	0 (101)	-
Piliostigma thoningii (Schum.)	Fabaceae	0 (177)	0 (2)
Poaceae	Poaceae	-	0 (2)
Rubiaceae	Rubiaceae	0 (51)	-
Sapotaceae	Sapotaceae	0 (47)	5 (70)
Uapaca acuminata (Hutch)	Euphorbiaceae	1 (251)	-
Ulmaceae	Ulmaceae	0 (53)	-
Unidentified1		0 (92)	0 (3)
Unidentified2		0 (25)	-
Unidentified3		0 (21)	-
Vernonia amygdalina (Dell.)	Asteraceae	0 (2)	-
Zea mays Linn.	Poaceae	0 (12)	-
Others (each < 1%)		0 (1)	
Total Pollen Grains		**35,915**	**1,339**
Total Pollen Types		**31**	**18**

Key: cf: looks like, 0 Present but less than 1% - absent, number of pollen in bracket

The higher the *P. biglobosa* content in honey the lesser the number of pollen types recorded (Table 4.1). The predominant plants from this vegetation zone were mostly trees. Bees at Adamawa made use of *Parinari kerstingii* (2%) and *Alchornea* sp.

(1%), *Piliostigma thonningii*, *Uapaca*, *Hymenocardia acida*, *Daniellia oliveri* and even *Daniellia ogea* while at Jigawa State other plants of importance identified to the family level belonged to Sapotaceae (5%) and Fabaceae (2%). The pollen of *Diospyros mespiliformis*, *Adenodolichos paniculata*, *Borreria* and *Bridellia* sp. were obtained from the Jigawa honey sample. Generally the bees in this region made use of majorly one plant as their major source of forage. Honey from Adamawa and Jigawa are unifloral honey of *Combretum* species and *Parkia biglobosa* respectively.

4.1.2 Pollen Spectra of Honey Samples from Guinea Savanna Zone

The sample from Kaduna recorded 31 pollen types while Plateau State sample recorded 23 pollen types with 2 unidentified (Table 4.2). The total number of pollen grains counted for each honey sample was 27,415 and 82,762 for Kaduna and Plateau States respectively. Dominant pollen types in this region were Myrtaceae (95%) and *Pavetta* species (77%) for Plateau and Kaduna respectively. The *Parkia biglobossa* content in most honey samples from this region was very low (Table 4.2). Honey sample from Kaduna State recorded 77% of the pollen grain from the family Rubiaceae followed by Anacardiaceae 9% (Figure 4.3). Plants from the family Rubiaceae were mostly visited by the bees. Honey sample from Plateau State (Figure 4.4) was mostly from the family Myrtaceae (95%).

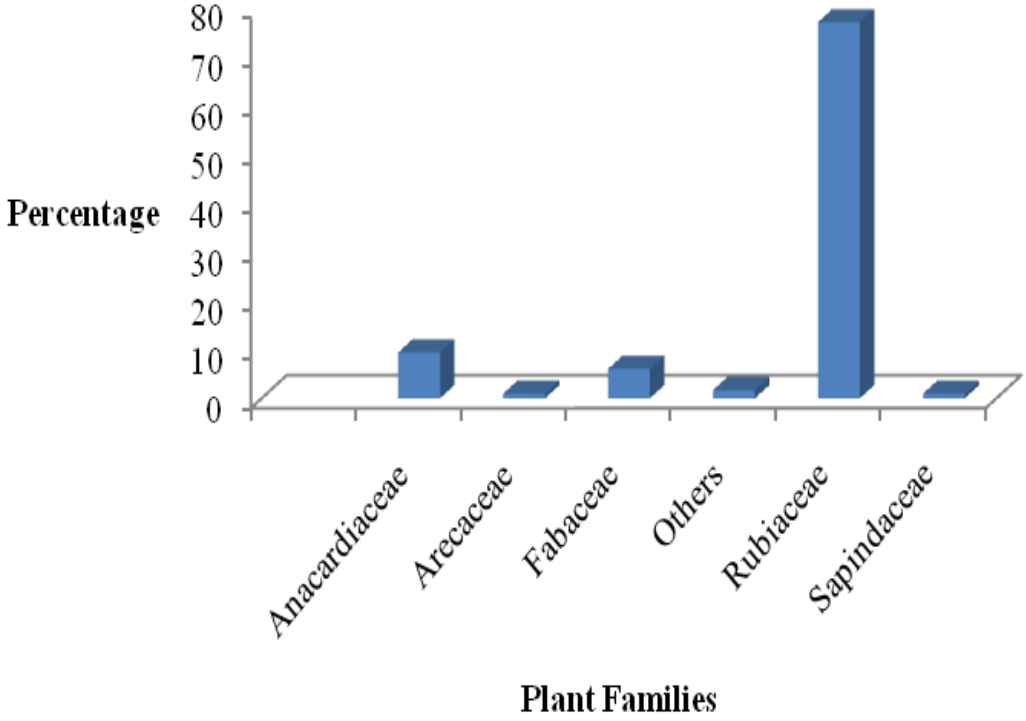


Figure 4.3: Percentage distribution of major pollen plant family from Kaduna State

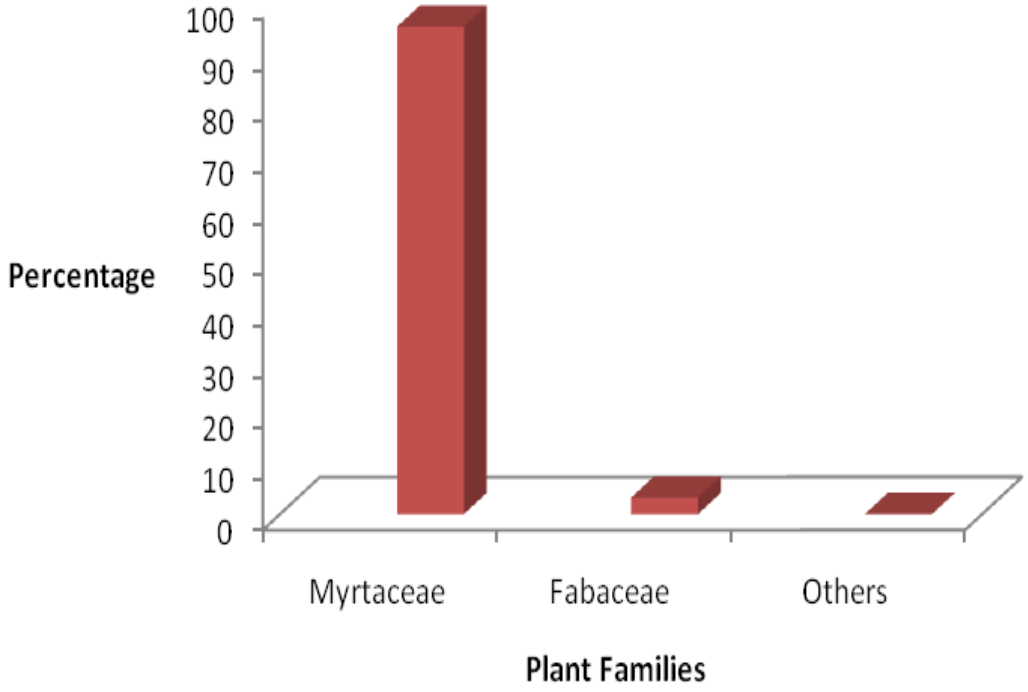


Figure 4.4: Percentage distribution of major pollen plant family from Plateau State

Table 4.2: Plant Taxa Identified and Their Pollen Percentage Occurrence in Nigerian Guinea Savanna Zone Honey

Plant taxa	Family	Percentage occurrence by State	
		Kaduna	Plateau
Adenia cissampeloides	Passifloraceae	0 (84)	0 (12)
Adenodolichos paniculata	Fabaceae	2 (551)	0 (6)
Acacia dudgeon	Fabaceae	-	-
Afzelia africana	Fabaceae	-	0 (13)
Anacardiaceae	Anacardiaceae	9 (2,576)	-
Annonidium mannii	Annonaceae	0 (50)	-
Arecaceae	Arecaceae	-	0 (18)
Asteraceae	Asteraceae	0 (73)	-
Basilicum sp.	Lamiaceae	-	0 (6)
Berlinia grandiflora	Fabaceae	1 (400)	0 (12)
Blighia sapida	Sapindaceae	1 (252)	-
Bombax buonopozense	Bombaceae	0 (75)	-
Borassus aethiopum	Pandanaceae	-	0 (3)
Bridellia sp.	Euphorbiaceae	0 (24)	-
Senna tora	Fabaceae	0 (25)	-
Combretum sp.	Combretaceae	0 (112)	0 (6)
Daniellia ogea	Fabaceae	0 (58)	-
Daniellia oliveri	Fabaceae	0 (28)	-
Elaeis guineensis	Arecaceae	1 (252)	-
Entada abyssinica	Fabaceae	0 (84)	0 (12)
Flabellaria paniculata	Malpighiaceae	0 (56)	-
Hymenocardia acida	Hymenocardiaceae	0 (20)	0 (6)
Lantana camara type	Verbenaceae	-	0 (1)
Loranthaceae type2	Loranthaceae	-	0 (6)
Loranthus cf. *mwereonsis*	Loranthaceae	-	0 (3)
Macaranga sp.	Euphorbiaceae	0 (56)	-
Malvaceae	Malvaceae	-	0 (1)
Manihot utilissima	Euphorbiaceae	0 (28)	-
Mimusops sp.	Sapotaceae	0 (56)	-
Myrtaceae	Myrtaceae	-	95 (11797)
Fabaceae	Fabaceae	0 (24)	3 (398)
Parinari kerstingii	Chrysobalanaceae	0 (56)	-
Parkia biglobosa	Fabaceae	2 (575)	-
Pavetta sp.	Rubiaceae	77 (21,168)	-
Periscopsis sp.	Fabaceae	-	0 (31)

Table 4.2 continues

Plant taxa	Family	Percentage occurrence by State	
		Kaduna	**Plateau**
Phyllanthus discoideus	Euphorbiaceae	-	0 (12)
Piptadeniastrum africana	Fabaceae	0 (116)	-
Poaceae	Poaceae	0 (27)	-
Protea sp.	Protaceae	0 (22)	-
Pterocarpus sp.	Fabaceae	-	0 (12)
Quassia sp.	Fabaceae	0 (19)	-
Rubiaceae	Rubiaceae	0 (21)	-
Syzygium guineense	Myrtaceae	0 (22)	-
Tabernaemontana crassa	Apocynaceae	-	0 (19)
Verbenaceae	Verbenaceae	2 (50)	-
Unidentified	Others	0 (455)	2 (6)
Total Pollen grains		**27,415**	**12, 380**
Total Pollen Types		**31**	**23**

Key: cf: looks like, 0: Present <1% - absent, number of pollen in bracket

4.1.3 Pollen Spectra of Honey Samples from the Rain Forest Zone

Ondo State had 21 pollen types and 1 unidentified with a total of 23, 883 pollen grains while Oyo State had 20 pollen types and 409 pollen grains. Honey sample from Oyo State seemed to be very low in pollen grain (Table 4.3). Honey sample from Ondo State was a mixed floral honey of *Chromolaena odorata* cf and *Milicia excelsa* cf. while Oyo honey was low in pollen content < 1,200 therefore cannot be considered as a floral honey. This honey might be named honeydew honey of *Tridax procumbens*. Honey sample from Ondo State recorded 35% of the pollen grain from the family Asteraceae, 31% from Moraceae, 18% from Arecaceae and 6% from Sapindaceae (Figure 4.5) while Oyo State recorded Asteraceae 44%, Fabaceae 40% and Combretaceae 2% (Figure 4.6).

4.1.4 Abundance of Plant Families Foraged by Bees

The plant families mostly foraged by honey bees from this study were; Fabaceae with a total pollen grain count of 35,657, Myrtaceae (27,384), Combretaceae (26,243), Rubiaceae (22,535), Sapotaceae (13,692), Asteraceae (9,984), Moraceae (8,842), Ebenaceae (8,272), Arecaceae (5,420) and Anacardiaceae (4,849) (Figure 4.7).

4.1.5 Pollen Spectrum of Honey from Bauchi State

Bauchi State (Figure 4.8) had pollen grains majorly from Anarcardiaceae (80%), Combretaceae (11%) and Fabaceae (6%). The honey sample from Bauchi State showed 20 pollen types (Table 4.4) while the total number of pollen grains counted for the honey sample was 50,110. Dominant pollen type was *Mangifera indica* (80%). *Parkia biglobosa* content in honey was very low, less than 1%. The predominant plant from this State was Mango which is a cultivated plant.

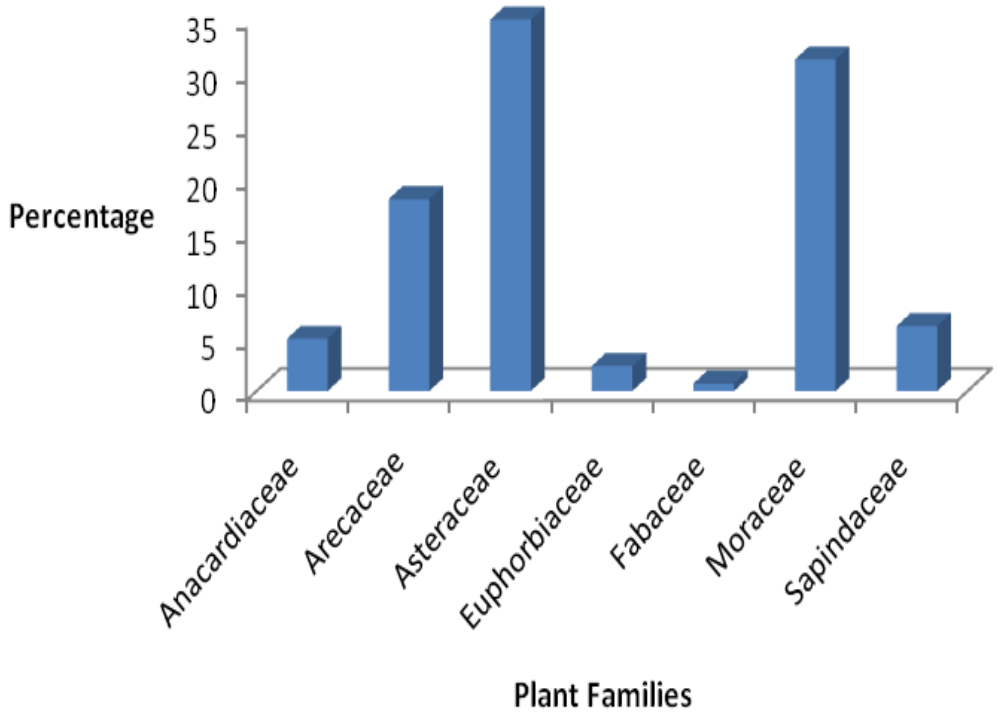


Figure 4.5: Percentage distribution of major pollen plant family from Ondo State

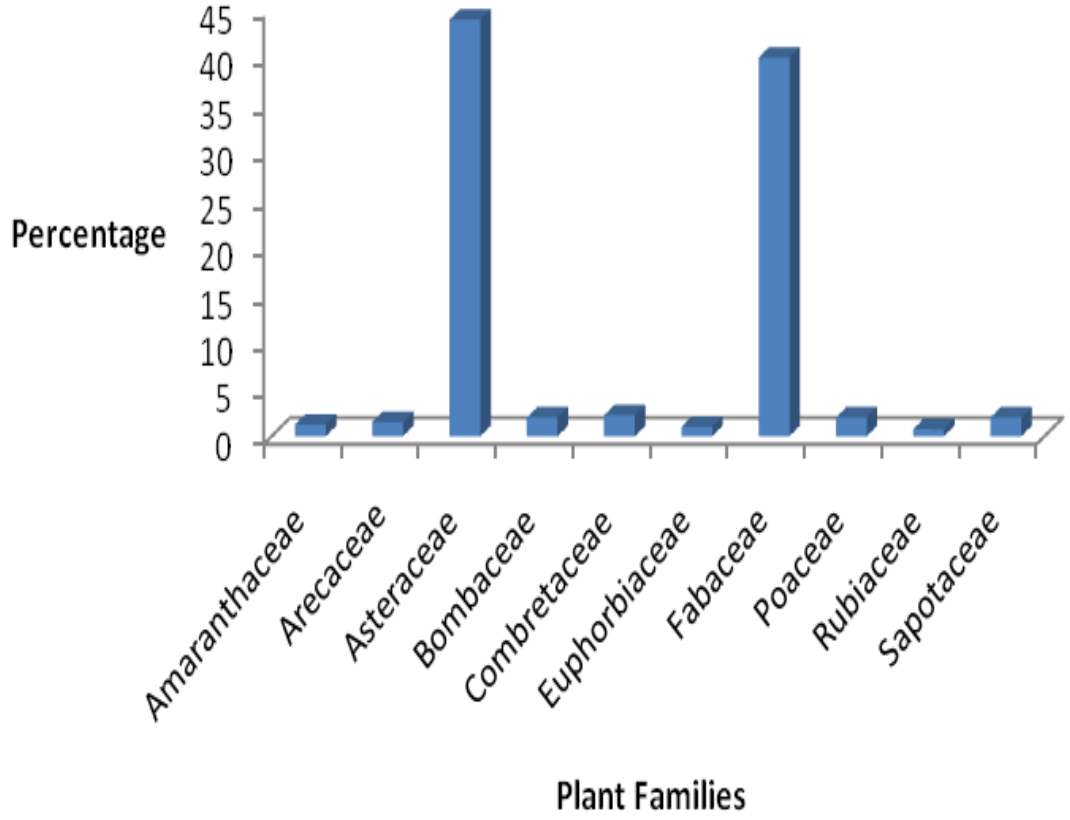


Figure 4.6: Percentage distribution of major pollen plant family from Oyo State

Table 4.3: Plant Taxa Identified and Their Pollen Percentage Occurrence in Nigerian Rain Forest Zone Honey

Plant Taxa	Family	Percentage occurrence by State	
		Ondo	Oyo
Acanthaceae	Acanthaceae	0 (4)	-
Adenodolichos paniculata	Fabaceae	1 (168)	3 (14)
Afzelia africana	Fabaceae	0 (8)	-
Alchornea cordifolia	Euphorbiaceae	1 (336)	1 (4)
Amaranthaceae	Amaranthaceae	-	1 (5)
Asteraceae	Asteraceae	-	1 (4)
Blighia sapida	Sapindaceae	6 (1456)	-
Bombax buonopozense	Bombaceae	0 (28)	-
Borassus aethiopum	Pandanaceae	0 (2)	-
Borreria sp.	Rubiaceae	-	1 (3)
Brachystegia eurycoma	Fabaceae	0 (2)	-
Senna tora	Fabaceae	0 (6)	6 (24)
Ceiba pentandra	Bombaceae	-	2 (8)
Chromolaena odorata cf	Asteraceae	35 (8344)	-
Combretum sp.	Combretaceae	0 (112)	2 (9)
Cyperaceae	Cyperaceae	-	0 (1)
Daniellia oliveri	Fabaceae	0 (110)	-
Diospyros sp.	Ebenaceae	-	0 (2)
Elaeis guineensis	Arecaceae	18 (4312)	1 (6)
Hildergardia barteri	Sterculiaceae	0 (38)	-
Hymenocardia acida	Hymenocardiaceae	0 (2)	-
Lamiaceae	Lamiaceae	-	0 (2)
Mangifera indica	Anacardiaceae	5 (1176)	0 (2)
Milicia excelsa cf.	Moraceae	31 (7448)	-
Myrtaceae	Myrtaceae	0 (50)	-
Oldenlandia corymbosa	Rubiaceae	0 (53)	-
Fabaceae	Fabaceae	-	31 (128)
Parkia biglobosa	Fabaceae	-	0 (1)
Phyllanthus discoideus	Euphorbiaceae	1 (224)	-
Piptadeniastrum africanum	Fabaceae	-	0 (2)
Poaceae	Poaceae	-	2 (8)
Rutaceae	Rutaceae	-	0 (2)
Sapotaceae	Sapotaceae	-	2 (8)
Tridax procumbens cf	Asteraceae	-	43 (176)
Unidentified		1 (2)	-
Vernonia cf. amygdalina	Asteraceae	0 (2)	-
Total Pollen grains		**23,883**	**409**
Total Pollen types		**21**	**20**

Key: cf: looks like, 0: Present < 1%, - absent, number of pollen in bracket

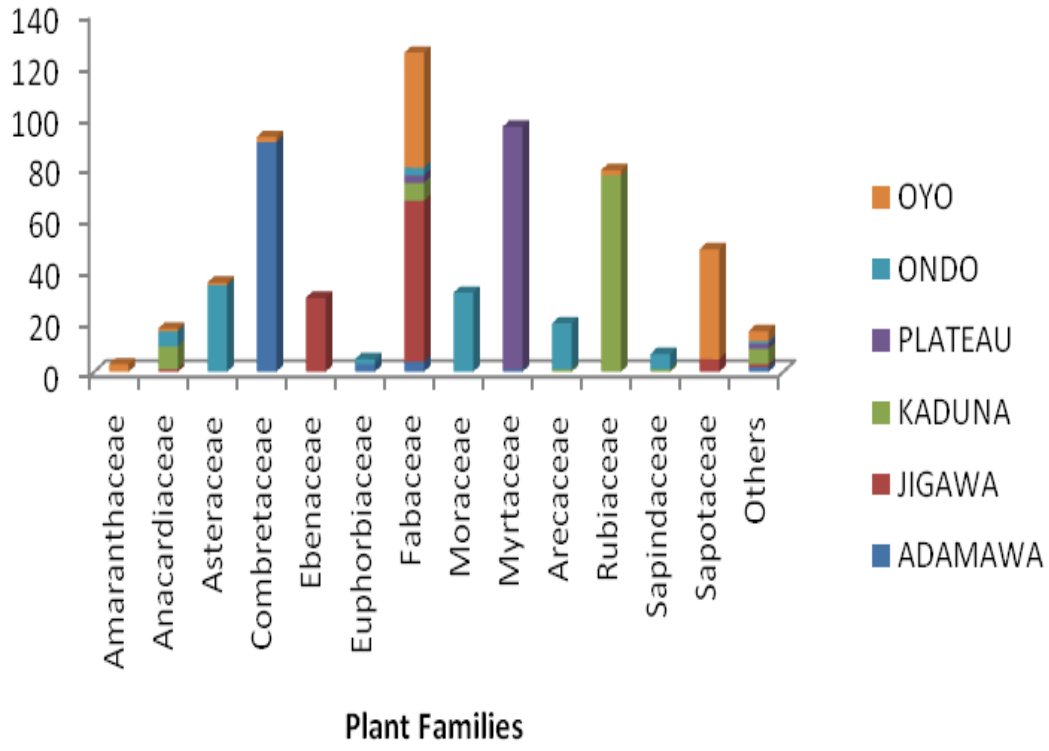


Figure 4.7: Comparative abundance of each plant family from six honey samples

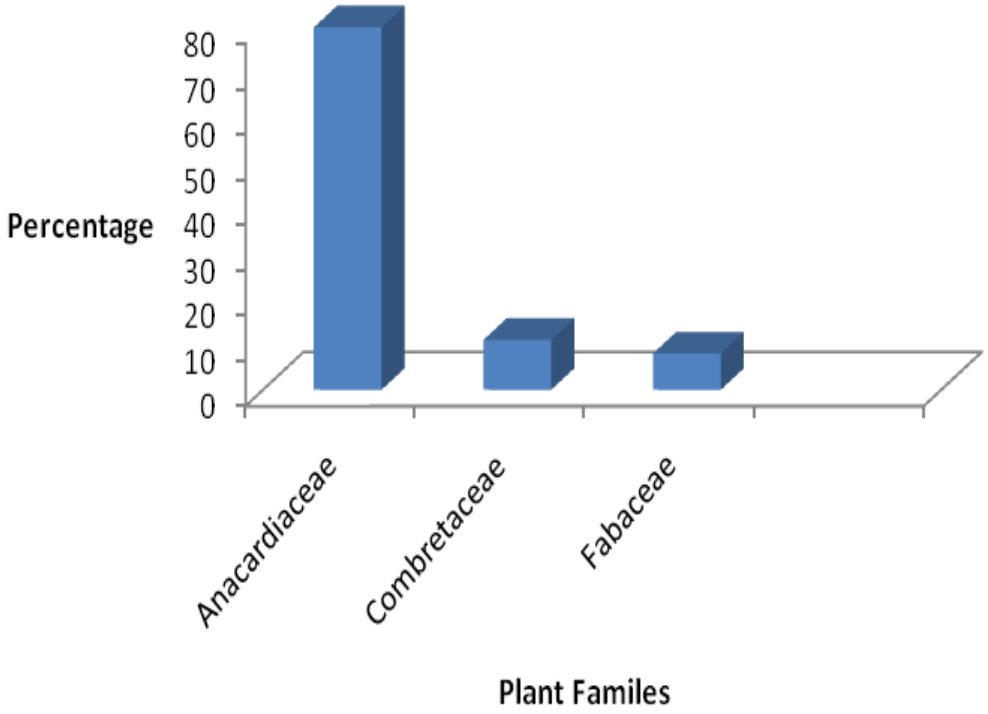


Figure 4.8: Percentage distribution of major pollen plant family from Bauchi State

Table 4.4: Plant Taxa Identified and Pollen Percentage Occurrence in Bauchi State Honey

Plant Taxa	Family	Percentage occurrence
Alchornea Species (Schum and Thonn.)	Euphorbiaceae	0 (84)
Aubrevillea kerstingii (Harms)	Fabaceae	0 (12)
Senna sp. (Linn.)	Fabaceae	0 (4)
Combretum sp. (R.Br.exGDon)	Combretaceae	11 (5,572)
Daniellia oliverii (Rolfe)	Fabaceae	0 (12)
Entada abyssinica (Steud.)	Fabaceae	2 (1,120)
Gardenia ternifolia (J. Ellis)	Rubiaceae	0 (4)
Gossypium sp. (Linn.)	Malvaceae	0 (142)
Hildergardia barteri (Mast.)	Sterculiaceae	0 (6)
Khaya senegalensis (Desr.) A. Juss	Meliaceae	0 (4)
Liliaceae	Liliaceae	0 (16)
Mangifera indica (Linn.)	Anacardiaceae	80 (40,152)
Parkia biglobosa (Jacq.)	Fabaceae	0 (8)
Scorodophloeus cf. Zenkeri (Harms)	Fabaceae	6 (2,869)
Thunbergia alata (Bojer ex sims)	Acanthaceae	0 (20)
Unidentified (3)	Others	0 (72)
Others (each < 1%)		0 (13)
Total Pollen Grains		**50,110**
Total Pollen Types		**20**

Key: cf: looks like, 0: Present but less than 1% - absent

Bees from Bauchi State foraged *Scorodophloeus zenkeri cf.* (6%) and *Entada abyssinica* (2%). Honey from Bauchi was a unifloral honey of *mangifera indica*.

4.2 PHYSICO-CHEMICAL ANALYSIS

There was a significant ($p<0.05$) difference in the physico-chemical parameters of the honey samples (Table 4.5 -4.9).

4.2.1 Moisture, Ash, pH and Acidity of Honey Samples

There was a significant difference in the moisture content (Table 4.5) of honey samples from the different States ($p<0.05$). Honey from Adamawa State recorded the highest moisture content

(15.03±0.01%) followed by Ondo. Moisture content of honey from Plateau, Jigawa, Kaduna and Oyo State were not significantly ($p\geq0.05$) different from each other. All samples were within the Standard limit of ≤20% for moisture content.

A breakdown of the average moisture content by vegetation zones was; Sudan savanna 14.60±0.01%, Guinea savanna 14.19±0.01% and Rain forest 14.30±0.02%. Bauchi State recorded the highest moisture content (15.05%). There was a significant difference in the ash content of honey from various States (Table 4.5). Honey from Kaduna and Plateau had a significantly ($p<0.05$) higher ash content than the honey from Jigawa, Oyo, Ondo and Adamawa States.

Table 4.5: Moisture, Ash Content, pH and Acidity of Honey Samples from States within Three Vegetation Zones in Nigeria

State	Moisture (%)	Ash (%)	pH	Free acidity (Meq)	Lactonic acidity (Meq)	Total acidity (Meq)
Bauchi	15.05±0.01	0.34±0.01	4.32±0.03	53.85±0.75	11.25±2.00	65.00±3.75
Adamawa	15.03±0.01^{a}	0.18±0.01^{d}	4.53±0.10^{c}	149.25±7.75^{a}	27.50±0.00^{a}	176.75±7.75^{a}
Jigawa	14.17±0.01^{c}	0.26±0.01^{bc}	4.96±0.03^{a}	11.25±1.75^{d}	23.50±2.00^{ab}	34.75±3.75^{d}
Plateau	14.21±0.01^{c}	0.32±0.02^{a}	4.59±0.02^{ab}	9.75±4.25^{d}	17.50±0.00^{bc}	27.25±4.25^{d}
Kaduna	14.17±0.01^{c}	0.30±0.02^{a}	4.27±0.02^{c}	69.50±3.50^{bc}	15.50±1.50^{c}	84.50±2.00^{b}
Ondo	14.42±0.02^{b}	0.22±0.01^{c}	4.44±0.02^{bc}	46.00±2.00^{c}	13.00±1.00^{c}	59.00±1.00^{c}
Oyo	14.17±0.01^{c}	0.26±0.01^{bc}	4.02±0.04^{d}	82.00±2.00^{b}	14.25±0.25^{c}	96.25±2.25^{b}
Standard	**≤20**	**≤0.6% nectar ≤1.2% honeydew**	**3.5-5.5**	**≤40meq honey bakers honey ≤80meq**	**≤40meq**	**≤40meq**

$^{a-d}$ Means with the same letter(s) in a column are not significantly different p≥0.05

A break down of the average ash content by vegetation zones are; Sudan Savanna (0.22±0.01%), Guinea Savanna (0.31±0.02%) and Forest (0.24±0.01%). All states ash content was within the standard limit of ≤0.6% for nectar honey. Honey from Bauchi State had an ash content of 0.34±0.01%.

The pH value for this study (Table 4.5) was between 4.02 and 4.96. Though the pH range in this study was within standard limit (3.5-5.5), the optimum pH range (4.5) was exceeded by samples from Jigawa, Plateau, Kaduna and Ondo States. Honey from Oyo State was significantly more acidic than honeys from Kaduna, Adamawa, Ondo, Plateau and Jigawa States.

Free acidity had a range of 9.75-149.25meq. Free acidity for Jigawa (11.25) and Plateau (9.75meq/kg) were very low. Honey from these two States met the European Union Standard of ≤40meq. The free acidity of Ondo State was 46meq/kg. Samples from Ondo, Bauchi and Kaduna met the standard for bakers honey (≤80meq). The free acidity of honey sample from Oyo and Adamawa States were high. Lactonic acidity recorded was 13.00 – 27.50meq/kg, all States were within the standard range of ≤40meq/kg while 27.25 – 176.75meq/kg was recorded for total acidity. Only honey samples from Jigawa and Plateau were within the standard range of ≤40meq for total acidity. Honey from Adamawa State had the highest free, lactonic and total acidity which was significantly different from the honeys from other States.

4.2.2 Carbohydrate Content of Honey Samples

Plateau State had significantly the highest glucose content followed by Jigawa, Kaduna, Ondo, Adamawa and Oyo States (Table 4.6). Honey from Jigawa and Plateau States exceeded the standard limit of glucose. Jigawa State had the highest fructose content followed by Adamawa, Plateau, Kaduna, Ondo and Oyo States.

Table 4.6: Carbohydrate Content of Honey Samples

State	% Glucose	% Fructose	% Sucrose	% Maltose	Fructose/ Glucose	Total Reducing Sugar
Bauchi	30.97±0.01	38.50±0.02	1.21±0.01	1.66±0.01	1.24	69.47±0.03
Adamawa	30.79±0.01[d]	40.01±0.01[a]	1.34±0.01[d]	1.82±0.01[a]	1.30	70.95±0.02[c]
Jigawa	33.71±0.01[b]	40.04±0.02[a]	1.83±0.01[a]	1.81±0.01[a]	1.19	73.74±0.03[a]
Plateau	34.02±0.01[a]	39.11±0.01[b]	1.87±0.01[a]	1.81±0.01[a]	1.15	73.13±0.00[b]
Kaduna	29.02±0.01[e]	38.31±0.01[c]	1.76±0.00[b]	1.82±0.02[a]	1.32	70.91±0.04[e]
Ondo	30.97±0.01[c]	37.91±0.01[d]	1.51±0.01[c]	1.83±0.01[a]	1.22	68.88±0.01[d]
Oyo	28.30±0.01[f]	37.81±0.01[e]	1.21±0.01[e]	1.83±0.01[a]	1.34	66.11±0.02[f]
Standard	31	38.5	<5			>60

[a – f] Means with the same letter (s) in a column are not significantly different p≥0.05

Honey from Adamawa, Jigawa and Plateau States exceeded the standard limit for fructose. Plateau State had the highest sucrose content followed by Jigawa, Kaduna, Ondo, Adamawa and Oyo States. The sucrose content and total reducing sugars (Fructose + Glucose) of honeys from all States were within their standards. Honey sugars from Kaduna, Ondo and Oyo States were within standard limits. The maltose content in the honey samples from all States was not significantly ($p \geq 0.05$) different. The honey from Bauchi State had the lowest maltose content and all its sugars were within the standard limits.

4.2.3 Protein, Fat, Dextrin and Hydroxymethylfurfural (HMF) Content of Honey Samples

Two significantly different groups occurred for the protein content (Table 4.7). Honey sample from Oyo State had the lowest protein content a category on its own, while the other States fell into another category. Only honey sample from Oyo State was within the limit of 0.20% for protein by National Honey Board. The other States exceeded the standard limit. Honey sample from Bauchi State showed the highest protein content (0.31 ± 0.01) in this study. There was no significant ($p > 0.05$) difference between the fat and Dextrin content of honey samples though Kaduna and Ondo had no appreciable amount of fat and were within the Standard limit while other States were approximately zero and therefore within the standard limit also. Although there was no significant difference in the dextrin content of all honey samples, Kaduna State recorded the highest dextrin content followed by Oyo, Ondo and Jigawa States. Kaduna and Oyo States recorded the highest HMF content followed by Jigawa, Plateau, Adamawa and Ondo States. All the honey samples from the different States recorded HMF content within the standard limit. Bauchi State recorded the highest HMF content from this study (Table 4.7).

Table 4.7: Protein, Fat, Dextrin and Hydroxymethylfurfural (HMF) Content of Some Nigerian Honeys

State	% Protein	% Fat	Dextrin	HMF (mg/kg)
Bauchi	0.31±0.01	0.03±0.02	1.13±0.01	20.81±0.01
Adamawa	0.27±0.01[a]	0.05±0.01[a]	0.98±0.02[a]	20.17±0.01[c]
Jigawa	0.27±0.01[a]	0.02±0.02[a]	1.17±0.01[a]	20.45±0.01[b]
Plateau	0.27±0.01[a]	0.01±0.01[a]	1.07±0.01[a]	20.17±0.01[c]
Kaduna	0.27±0.01[a]	0.00±0.00[a]	1.75±0.01[a]	20.75±0.01[a]
Ondo	0.27±0.01[a]	0.00±0.00[a]	1.17±0.01[a]	20.15±0.01[c]
Oyo	0.21±0.01[b]	0.01±0.01[a]	1.25±0.01[a]	20.71±0.01[a]
Standard	**0.20**	**0**	**Nil**	**40**

[a – c] Means with the same letter(s) in a column are not significantly different $p \geq 0.05$

4.2.4 Heavy Metal Composition of Honey Samples

Copper (0.02 – 0.29ppm), cadmium (was negligible in all samples), nickel (0.05 -0.20ppm), zinc (0.10 – 0.82ppm), chromium (1.92 – 3.88ppm) and lead (0.04 – 0.82ppm) were detected from the honey samples from all the States (Table 4.8). Copper, cadmium, nickel and zinc from all States were within standard limits while only Jigawa State exceeded the standard limit for lead. Jigawa State had the highest copper and Zinc content while Kaduna and Oyo State had the highest nickel content.

Honey samples generally had a high chromium content compared with other metals from this study though Ondo had the highest chromium content. Honey from Bauchi State had 0.18, 0.79 and 0.39ppm copper, zinc and lead respectively.

4.2.5 Mineral Composition of Honey Samples

Jigawa State honey sample had the highest iron content (Table 4.9). Calcium was present in high concentration in all samples available. Magnesium had a range of 0.18 – 8.45ppm with Kaduna State honey sample having the highest concentration.

4.3 ANTI-BACTERIAL ACTIVITY OF HONEY SAMPLES

All honey samples showed inhibitory activity against all bacteria species at different concentrations. Result of susceptibility test of honey samples against clinical strains of *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi* using agar well diffusion method is shown in Table 4.10-4.19.

Table 4.8: Heavy Metal Composition of Honey Samples from Parts of Nigeria

	Elements (ppm)						
State	**Copper**	**Cadmium**	**Nickel**	**Zinc**	**Chromium**	**Lead**	**Total**
Adamawa	0.06	-	0.06	0.16	3.34	0.15	3.77
Jigawa	0.29	-	-	0.82	-	0.82	1.99
Plateau	0.02	-	-	0.24	3.25	-	3.51
Kaduna	0.08	-	0.20	0.15	1.92	0.16	2.51
Ondo	0.05	-	0.05	0.12	3.88	0.04	4.14
Oyo	0.04	-	0.08	0.10	2.21	0.15	2.58
Bauchi	0.18	-	0.06	0.79	-	0.39	1.42
Standard	**10ppm**	**0.05**	**1000 µg/l**	**20ppm**	**35 µg/day**	**0.5ppm**	

Key: - Negligible

Table 4.9: Mineral Composition of Honey Samples from Parts of Nigeria

	Minerals (ppm)			
State	**Calcium**	**Magnesium**	**Iron**	**Total**
Adamawa	10.37	5.98	0.41	16.76
Jigawa	-	0.18	0.72	0.90
Plateau	13.34	3.30	0.36	17.00
Kaduna	15.35	8.45	1.44	25.24
Ondo	13.86	3.92	0.53	18.31
Oyo	12.22	1.48	0.10	13.80
Bauchi	-	1.97	4.81	6.78
Standard	1,000mg/day	420mg/day	18mg/day	

Key – Negligible

4.3.1 Anti-bacterial Activity of Honey sample from Adamawa State

The susceptibility pattern for Honey sample from Adamawa State shows that the higher the concentration the more the zone of inhibition. Adamawa honey (Table 4.10) showed anti-microbial activity that was significantly higher in the zone of inhibition than that of the control for *Escherichia coli,* the same range of inhibitory activity was recorded for the control and 100% concentration honey against *Salmonella typh*i. *Bacillus cereus* showed inhibitory activity at 50-100% honey concentration only. *E. coli* and *Salmonella typh*i were susceptible to Adamawa honey at all concentrations while *B. cereus, Staphylococcus aureus* and *Pseudomonas aeruginosa* were resistant at 50% downward and showed intermediate activity at 100% for *S. aureus* and *P. aeruginosa* Adamawa honey showed the highest zone of inhibition against *P. aeruginosa* at 100% compared to honey from the other States.

4.3.2 Anti-bacterial Activity of Honey sample from Jigawa State

There was a significant decrease in the anti-microbial activity of the honey from Jigawa State (Table 4.11) at 100% compared with the control for *E. coli.* For the remaining organisms the control had a significantly higher zone of inhibition than the honey. *E. coli*, *B. cereus*, *S. aureus* and *P. aeruginosa* were resistant against Honey sample from Jigawa State at all concentrations used. Only *S. typhi* was susceptible to this honey at all concentrations.

4.3.3 Anti-bacterial Activity of Honey sample from Kaduna State

The pattern of susceptibility for Honey sample from Kaduna State (Table 4.12) was the higher the concentration the higher the zone of inhibition. Kaduna States honey inhibitory activity was similar to that of the control at 100% for *E. coli* and *S. typhi*. The control was significantly higher in activity than the honey from Kaduna State against other bacteria

Table 4.10: Anti-bacterial Activity of Honey from Adamawa State

Test organisms	Zone of inhibition (mm) in concentrations % of honey				
	100.00	50.00	25.00	12.50	Control Cipro®10µg
Escherichia coli	36.00±0.00[a]	35.00±0.00[a]	30.00±0.00[b]	22.00±3.00[c]	12.75±0.09[d]
Bacillus cereus	33.75±0.09[a]	19.00±1.00[b]	14.00±0.00[c]	0.00±0.00[d]	0.00±0.00[d]
Staphylococcus aureus	17.00±1.00[b]	13.00±0.00[c]	0.00±0.00[d]	0.00±0.00[d]	31.50±0.18[a]
Salmonella typhi	39.00±1.00[a]	33.00±0.00[b]	32.00±0.00[b]	27.00±0.00[c]	39.50±0.18[a]
Pseudomonas aeruginosa	17.00±0.00[b]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]	37.75±0.09[a]

Key: [a–d] Means with the same letter (s) on the same row are not significantly different (p≥ 0.05). Cipro®: Ciprofloxacin 10µg

Table 4.11: Anti-bacterial Activity of Honey from Jigawa State

Test organisms	Zone of inhibition (mm) in concentrations % of honey				
	100.00	50.00	25.00	12.50	Control Cipro®10µg
Escherichia coli	15.50±2.50[a]	21.00±0.50[b]	13.75±0.25[c]	9.50±0.00[c]	12.75±0.14[ab]
Bacillus cereus	17.55±0.50[b]	13.00±1.00[c]	0.00±0.00[d]	0.00±0.00[d]	33.75±0.14[a]
Staphylococcus aureus	12.00±0.00[b]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]	31.50±0.29[a]
Salmonella typhi	38.50±1.50[a]	35.00±1.00[b]	32.50±0.50[b]	26.75±0.75[c]	39.50±0.29[a]
Pseudomonas aeruginosa	17.00±3.00[b]	12.00±1.00[c]	0.00±0.00[d]	0.00±0.00[d]	37..75±0.14[a]

Key: [a –d] Means with the same letter (s) on the same row are not significantly different (p≥ 0.05)

Table 4.12: Anti-bacterial Activity of Honey from Kaduna State

	Zone of inhibition (mm) in concentrations % of honey				
Test organisms	**100.00**	**50.00**	**25.00**	**12.50**	**Control** Cipro®10µg
Escherichia coli	15.50±2.50[a]	21.00±0.50[b]	13.75±0.25[c]	9.50±0.00[c]	12.75±0.14[ab]
Bacillus cereus	17.55±0.50[b]	13.00±1.00[c]	0.00±0.00[d]	0.00±0.00[d]	33.75±0.14[a]
Staphylococcus aureus	12.00±0.00[b]	0.00±0.00 [c]	0.00±0.00[c]	0.00±0.00[c]	31.50±0.29[a]
Salmonella typhi	38.50±1.50[a]	35.00±1.00[b]	32.50±0.50[b]	26.75±0.75[c]	39.50±0.29[a]
Pseudomonas aeruginosa	17.00±3.00[b]	12.00±1.00 [c]	0.00±0.00[d]	0.00±0.00[d]	37..75±0.14[a]

Key: $^{a-d}$ Means with the same letter (s) on the same row are not significantly different ($p \geq 0.05$)

species used at all concentrations. *B. cereus, S. aureus* and *P. aeruginosa* were resistant to 12.50-50% honey concentrations, though *S. typhi* was susceptible at all concentrations to honey sample from Kaduna State.

4.3.4 Anti-bacterial Activity of Honey sample from Plateau State

The pattern of antibacterial activity of Plateau honey (Table 4.13) shows that the higher the honey concentration the higher the zone of inhibition for most organisms. The honey was significantly more effective than the control at 25-100% concentration for *E. coli.* The control was more effective than the honey for the remaining bacteria species. *E. coli* and *S. typhi* were susceptible to this honey at most concentrations. *B. cereus* was susceptible at 100% and resistant at 50% downwards. This honey had the highest zone of inhibition recorded in this study for *B. cereus. S. aureus* was susceptible at 100% only while *P. aeruginosa* was resistant to all concentrations of Plateau honey.

4.3.5 Anti- bacterial Activity of Honey sample from Ondo State

The honey from Ondo State (Table 4.14) was significantly more effective on *E. coli* with a ZOI of 20.00± 0.00 - 37.00 ±1.00mm than the control (12.75±0.09mm) at all concentrations. The control was significantly more effective than the honey for the remaining bacteria species. *E. coli* and *S. typhi* were susceptible to this honey at all concentrations. *B. cereus* and *P. aeruginosa* were resistant at all concentrations. *S. aureus* was intermediate at 100% only while it was resistant at the remaining concentrations.

4.3.6 Anti- bacterial Activity of Honey sample from Oyo State

Honey from Oyo State (Table 4.15) was significantly more inhibitory than the control for *Escherichia coli* at all concentrations while the inhibitory activity of the honey at 100.00%

Table 4.13: Anti-bacterial Activity of Honey from Plateau State

Test organisms	Zone of inhibition (mm) in concentrations % of honey				
	100.00	50.00	25.00	12.50	Control Cipro®10µg
Escherichia coli	33.00±0.00[a]	30.00±1.00[a]	25.00±0.00[b]	16.25±3.75[c]	12.75±0.09[d]
Bacillus cereus	27.00±0.00[b]	13.00±0.00[c]	0.00±0.00[d]	0.00±0.00[d]	33.75±0.10[a]
Staphylococcus aureus	26.00±0.10[b]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]	31.50±0.19[a]
Salmonella typhi	35.00±0.25[b]	30.00±0.30[c]	27.00±0.20[d]	23.00±0.15[e]	39.50±0.18[a]
Pseudomonas aeruginosa	15.00±0.00[b]	12.00±0.00[c]	0.00±0.00[d]	0.00±0.00[d]	37..75±0.10[a]

Key: [a – e] Means with the same letter(s) on the same row are not significantly different (p≥0.05)

Table 4.14: Anti-bacterial Activity of Honey from Ondo State

Test organisms	Zone of inhibition (mm) in concentrations % of honey				
	100.00	50.00	25.00	12.50	Control Cipro®10µg
Escherichia coli	37.00±1.00[a]	33.00±1.00[b]	27.00±1.00[c]	20.00±0.00[d]	12.75±0.09[e]
Bacillus cereus	15.00±0.00[b]	12.00±1.00[c]	0.00±0.00[d]	0.00±0.00[d]	33.75±0.10[a]
Staphylococcus aureus	15.00±0.00[b]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]	31.50±0.19[a]
Salmonella typhi	35.50±0.50[b]	34.50±0.50[b]	29.00±1.00[c]	31.00±2.00[c]	39.50±0.18[a]
Pseudomonas aeruginosa	13.75±0.25[b]	10.00±0.05[c]	0.00±0.00[d]	0.00±0.00[d]	37..75±0.10[a]

Key: [a –e] Means with the same letter (s) on the same row are not significantly different (p≥ 0.05)

Test organisms	Zone of inhibition (mm) in concentrations % of honey				
	100.00	50.00	25.00	12.50	Control Cipro®10µg
Escherichia coli	36.00±0.00[a]	35.00±1.00[a]	31.00±1.00[b]	25.00±0.00[c]	12.75±0.09[d]
Bacillus cereus	17.50±2.50[b]	17.50±2.50[b]	0.00±0.00[c]	0.00±0.00[c]	33.75±0.10[a]
Staphylococcus aureus	18.00±0.00[b]	12.00±0.00[c]	0.00±0.00[d]	0.00±0.00[d]	31.50±0.19[a]
Salmonella typhi	42.00±0.50[a]	40.00±0.50[b]	36.00±1.00[c]	31.00±0.50[d]	39.50±0.18[a]
Pseudomonas aeruginosa	15.00±1.00[b]	0.00±0.00[c]	0.00±0.00[c]	0.00±0.00[c]	37..75±0.10[a]

Key: [a –d] Means with the same letter (s) on the same row are not significantly different (p≥0.05)

Table 4.15: Anti-bacterial Activity of Honey from Oyo State

concentration for *S. typhi* and the control was similar. *P. aeruginosa* was resistant at all concentrations used. *B. cereus* and *S. aureus* were intermediate at 100% while at 50% *S. aureus* was resistant.

4.3.7 Anti- bacterial Activity of Honey sample from Bauchi State

All bacteria species were resistant to Bauchi honey at 25-100% concentration (Table 4.16) but at 12.50% all organisms were susceptible (Plate 1). Bauchi's honey inhibitory activity was significantly higher than that of the control which was resistant for *E. coli* at 12.50%. Though the control inhibitory activity was significantly higher for the other bacteria, the

bacteria were all susceptible to the honey sample. Only Bauchi honey showed inhibitory activity and susceptibility strength against *B. cereus*, *S. aureus* and *P. aeruginosa* at 12.50%v/v. Bauchi State (Soro) was treated as an outlier because it was the only honey sample that showed a different susceptibility pattern differing from the expected; higher zones of inhibition at lower concentration.

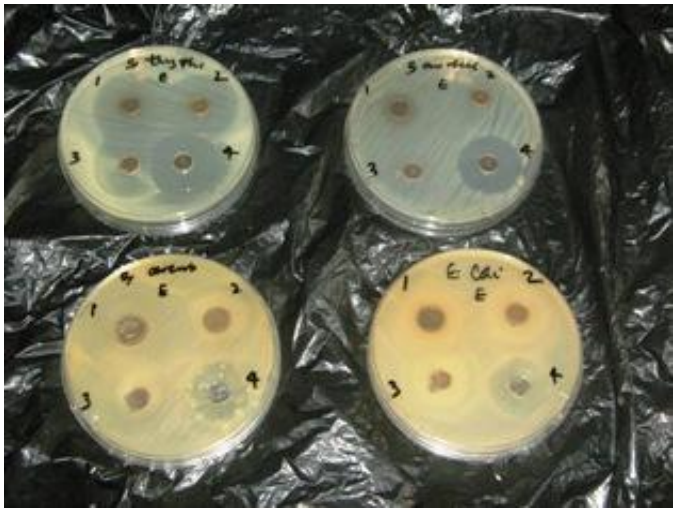
4.3.8 Organisms Reaction to Honey from Different States of Nigeria

Result of susceptibility test of clinical strains of *B. cereus*, *E. coli*, *P. aeruginosa*, *S. aureus* and *S. typhi* using honey samples by agar well diffusion method at different concentrations is presented in Table 4.17 a-d. There was a significant difference in the susceptibility of the micro-organisms to the same honey sample at different concentrations for most of the States. *S. typhi* showed a higher zone of inhibition to honey sample from Jigawa State compared to all the other test organisms used at all concentrations (Table 4.17 a-d). *S. typhi* was susceptible to all honey samples at all concentrations used (Table 4.17 a-d). The honey from Adamawa, Plateau and Ondo States reacted similarly against most organisms; *E. coli* and

Table 4.16: Anti-bacterial Activity of Honey from Bauchi State

Test organisms	Zone of inhibition (mm) in concentrations % of honey				
	100.00	50.00	25.00	12.50	Control Cipro®10µg
Escherichia coli	11.75±0.75^{c}	0.00±0.00^{d}	0.00±0.00^{d}	22.50±0.50^{a}	12.75±0.75^{b}
Bacillus cereus	13.75±0.25^{c}	11.75±0.25^{d}	0.00±0.00^{e}	23.75±0.25^{b}	33.75±0.08^{a}
Staphylococcus aureus	13.7±0.75^{c}	0.00±0.00^{d}	0.00±0.00^{d}	28.00±1.00^{b}	31.50±0.15^{a}
Salmonella typhi	12.50±1.50^{c}	0.00±0.00^{d}	0.00±0.00^{d}	31.50±0.50^{b}	39.50±0.15^{a}
Pseudomonas aeruginosa	11.00±1.00^{c}	0.00±0.00^{d}	0.00±0.00^{d}	27.50±0.50^{b}	37.75±0.08^{a}

Key: $^{a-d}$ Means with the same letter (s) on the same row are not significantly different ($p \geq 0.05$)





(A) **(B)**

Plate I**:** Zones of inhibition of honey from Bauchi State against different bacterial species

(A) The lowest concentration (4= 12.50%) showed the highest zone of inhibition for *S. typhi, S. aureus*, *B. cereus* and *E. coli.* **(B)**The lowest concentration (4= 12.50%) showed the highest zone of inhibition for *P. aeruginosa.* (1= 100%, 2=50%, 3=25% and 4=12.50% honey concentration).

Table 4.17a: Mean Inhibitory Zones of Honey against Test Organisms at 100%

State	Test Organism				
	Escherichia coli	*Bacillus cereus*	*Staphylococcus aureus*	*Salmonella typhi*	*Pseudomonas aeruginosa*
Jigawa	11.50±0.50[b]	14.00±0.00[b]	14.50±1.50[b]	34.50±0.50[a]	11.25±0.25[b]
Adamawa	35.50±.05[b]	33.75±0.09[b]	17.00±1.00[c]	39.00±1.00[a]	16.50±0.50[c]
Plateau	32.50±0.5[b]	26.50±.05[c]	25.50±0.05[c]	34.50±0.50[a]	14.75±0.25[d]
Kaduna	15.50±2.50[b]	17.55±0.50[b]	12.00±0.00[c]	38.50±1.50[a]	17.00±3.00[b]
Ondo	37.00±1.00[a]	15.00±0.00[b]	14.50±0.50[b]	35.50±0.50[a]	13.75±0.25[b]
Oyo	35.75±0.25[b]	17.50±2.50[c]	17.00±1.00[c]	41.00±1.00[a]	14.50±0.50[c]

a –d Means with the same letter(s) on a row are not significantly different (p≥0.05)

Table 4.17b: Mean Inhibitory Zones of Honey against Test Organisms at 50%

State	Test Organism				
	Escherichia coli	*Bacillus cereus*	*Staphylococcus aureus*	*Salmonella typhi*	*Pseudomonas aeruginosa*
Jigawa	0.00±0.00^{c}	0.00±0.00^{c}	13.50±0.50^{b}	35.75±0.00^{a}	0.00±0.00^{c}
Adamawa	34.50±0.50^{a}	13.75±0.25^{c}	12.75±0.25^{c}	32.75±0.25^{b}	0.00±0.00^{d}
Plateau	29.50±0.5^{a}	12.650±.05^{b}	0.00±0.00^{c}	29.75±0.25^{a}	12.00±0.00^{b}
Kaduna	21.00±0.50^{b}	13.25±0.25^{c}	0.00±0.00^{d}	34.75±0.25^{a}	12.00±0.50^{c}
Ondo	33.00±1.00^{a}	12.00±1.00^{b}	0.00±0.00^{c}	34.50±0.50^{a}	10.50±0.50^{b}
Oyo	35.00±1.00^{b}	17.50±2.50^{c}	12.25±0.25^{d}	39.50±0.50^{a}	0.00±0.00^{e}

a –d Means with the same letter(s) in a column vertically are not significantly different (p≥0.05)

Table 4.17c: Mean Inhibitory Zones of Honey against Test Organisms at 25%

State	Test Organism				
	Escherichia coli	*Bacillus cereus*	*Staphylococcus aureus*	*Salmonella typhi*	*Pseudomonas aeruginosa*
Jigawa	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	31.50±0.50[a]	0.00±0.00[b]
Adamawa	30.00±0.00[b]	0.00±0.00[c]	0.00±0.00[c]	31.75±0.25[a]	0.00±0.00[c]
Plateau	25.00±0.00[b]	0.00±0.00[c]	0.00±0.00[c]	26.50±0.50[a]	0.00±0.00[c]
Kaduna	13.75±0.25[b]	0.00±0.00[c]	0.00±0.00[c]	32.50±0.50[a]	0.00±0.00[c]
Ondo	27.00±1.00[a]	0.00±0.00[b]	0.00±0.00[b]	29.00±1.00[a]	0.00±0.00[b]
Oyo	31.00±1.00[b]	0.00±0.00[c]	0.00±0.00[c]	36.00±1.00[a]	0.00±0.00[c]

a –c Means with the same letter(s) in a column vertically are not significantly different (p≥0.05)

Table 4.17d: Mean Inhibitory Zones of Honey against Test Organisms at 12.50%

	Test Organism				
State	*Escherichia coli*	*Bacillus cereus*	*Staphylococcus aureus*	*Salmonella typhi*	*Pseudomonas aeruginosa*
Jigawa	0.00±0.00[b]	0.00±0.00[b]	0.00±0.00[b]	25.50±0.50[a]	0.00±0.00[b]
Adamawa	22.00±3.00[a]	0.00±0.00[b]	0.00±0.00[b]	26.50±0.50[a]	0.00±0.00[b]
Plateau	16.25±3.75[b]	0.00±0.00[c]	0.00±0.00[c]	23.00±0.15[a]	0.00±0.00[c]
Kaduna	9.50±0.00[b]	0.00±0.00[c]	0.00±0.00[c]	27.13±0.63[a]	0.00±0.00[c]
Ondo	20.50±0.50[b]	0.00±0.00[c]	0.00±0.00[c]	31.00±2.00[a]	0.00±0.00[c]

Oyo	25.00±0.50[b]	0.00±0.00[c]	0.00±0.00[c]	31.00±0.50[a]	0.00±0.00[c]

a –c Means with the same letter(s) in a column vertically are not significantly different (p≥0.05)

S. typhi were most susceptible to these honeys than *B. cereus, S. aureus* and *P. aeruginosa. B. cereus, S. aureus* and *P. aeruginosa* were resistant to all honey samples as from 12.5-50% honey concentration. *E. coli* showed varying zones of inhibition to different honey types depending on the concentration of honey used.

The honey sample from Oyo State showed the largest zone of inhibition as from 12.5-50% honey concentration while Jigawa and Kaduna honeys showed a low zone of inhibition against. All honey samples showed anti-bacterial activity against all organisms used at 100% honey concentration (Table 4.17a).

4.3.9 Effect of Concentration on Microorganisms

Comparing the different organisms reaction to concentration (Table 4.18), at 12.5 - 100% *S. typhi* and *E. coli* were most susceptible while *P. aeruginosa* was the most resistant bacteria at all concentrations used in this study. At 50 and 100%v/v concentration *B. cereus, S. aureus* and *P. aeruginosa* showed similar pattern of inhibition. At 12.5 and 25%v/v *B. cereus, S. aureus* and *P. aeruginosa* were highly resistant giving a low or no zone of inhibition that was not significantly different ($p \geq 0.05$) but was significantly different ($p < 0.05$) from *S. typhi* which was susceptible at 12.50 and 25% and *E. coli* which showed immediate activity at 25%v/v and was resisitant at 12.50%v/v.

4.3.10 Effect of Concentration and Source of Honey on Zone of Inhibition

Excluding the honey from Bauchi State, the honey from Plateau State (Table 4.19) had the highest zone of inhibition (ZOI) at 100% which was significantly higher than the honey from Oyo, Adamawa and Ondo State. Honey from Kaduna and Jigawa were least inhibitory at

Table 4.18: Organisms Response to Different Honey Concentrations

	Zone of inhibition (mm) in concentrations of honey (%)			
Test organisms	100.00	50.00	25.00	12.50
Escherichia coli	33. 83±6.77[a]	25.75±4.60[a]	18.83±4.06[b]	13.88±3.12[b]
Bacillus cereus	18.33±1.32[b]	11.58±1.69[b]	0.00±0.00[c]	0.00±0.00[c]
Staphylococcus aureus	17.08±1.35[b]	8.50±1.82[b]	0.00±0.00[c]	0.00±0.00[c]
Salmonella typhi	37.50±0.81[a]	34.75±0.92[a]	31.33±0.87[a]	27.38±0.91[a]
Pseudomonas aeruginosa	14.79±0.72[b]	5.75±1.75[b]	0.00±0.00[c]	0.00±0.00[c]

[a –c] Means with the same letter(s) within a column are not significantly different (p≥0.05)

Table 4.19: Anti-bacterial Activity of Honey from Different States at Different Honey Concentrations

State	Zone of Inhibition (mm) in Concentrations (%) of honey			
	100.00	50.00	25.00	12.50
Bauchi	10.75±1.20b	2.00±0.07c	0.00±0.00d	22.42±0.78a
Jigawa	17.30±2.99b	10.00±4.70b	6.30±4.20a	5.10±3.40b
Adamawa	25.60±3.27ab	19.00±4.41ab	12.40±5.07a	9.80±4.06a
Plateau	33.90±7.68a	19.00±4.96ab	10.40±4.25a	7.85±3.33ab
Kaduna	20.10±3.20b	14.30±3.80ab	6.50±4.33a	5.35±3.57ab
Ondo	23.25±3.55ab	20.40±3.65ab	11.20±4.58a	10.20±4.33ab
Oyo	25.70±3.71ab	20.90±4.94a	13.40±5.50a	11.20±4.62a

a –d Means with the same letter(s) on a column are not significantly different ($p \geq 0.05$)

100%. At 12.50 and 50% concentration, honey from Oyo State was significantly most effective than that of Jigawa State. At 25% honey from Oyo State was more effective with a
higher ZOI than that of other State, though not significantly ($p \geq 0.05$) different from that of the other States.

At 12.5% concentration honey from Oyo, Ondo, Kaduna and Adamawa States showed a higher anti-microbial activity which was significantly higher than that of the remaining States honey. Bauchi State was treated as an outlier because its pattern of activity was different. It was most effective at the lowest concentration used, followed by 100% and then 50%. The general trend for most of the other States was the higher the concentration the higher the ZOI.

At 25% and 100% concentration there was no significant difference in the zone of inhibition between honey from the Sudan, Guinea and Forest zone. At 50% honey concentration from the forest zone had a significantly ($p<0.05$) higher zone of inhibition than the honey from other regions while that from the Guinea showed a similarity between the Forest and Sudan zone but was significantly higher ($p<0.05$) in inhibitory activity, than the Sudan zone. At 12.50% honey from the Sudan zone showed similar inhibitory activity like the honey from Guinea and forest zones but the honey from the Forest zone was significantly higher in its zone of inhibition compared to the other zones of vegetation. In all zones the higher the concentration the higher the zone of inhibition.

4.4 MINIMUM INHIBITORY CONCENTRATION (MIC)

Honey sample from Adamawa State showed different MIC range for the test organisms. The honey inhibited bacterial growth both at high concentration (12.50%) and at low concentration (3.13%) especially for *B. cereus* and *S. aureus* (Figure 4.9). Honey sample

\

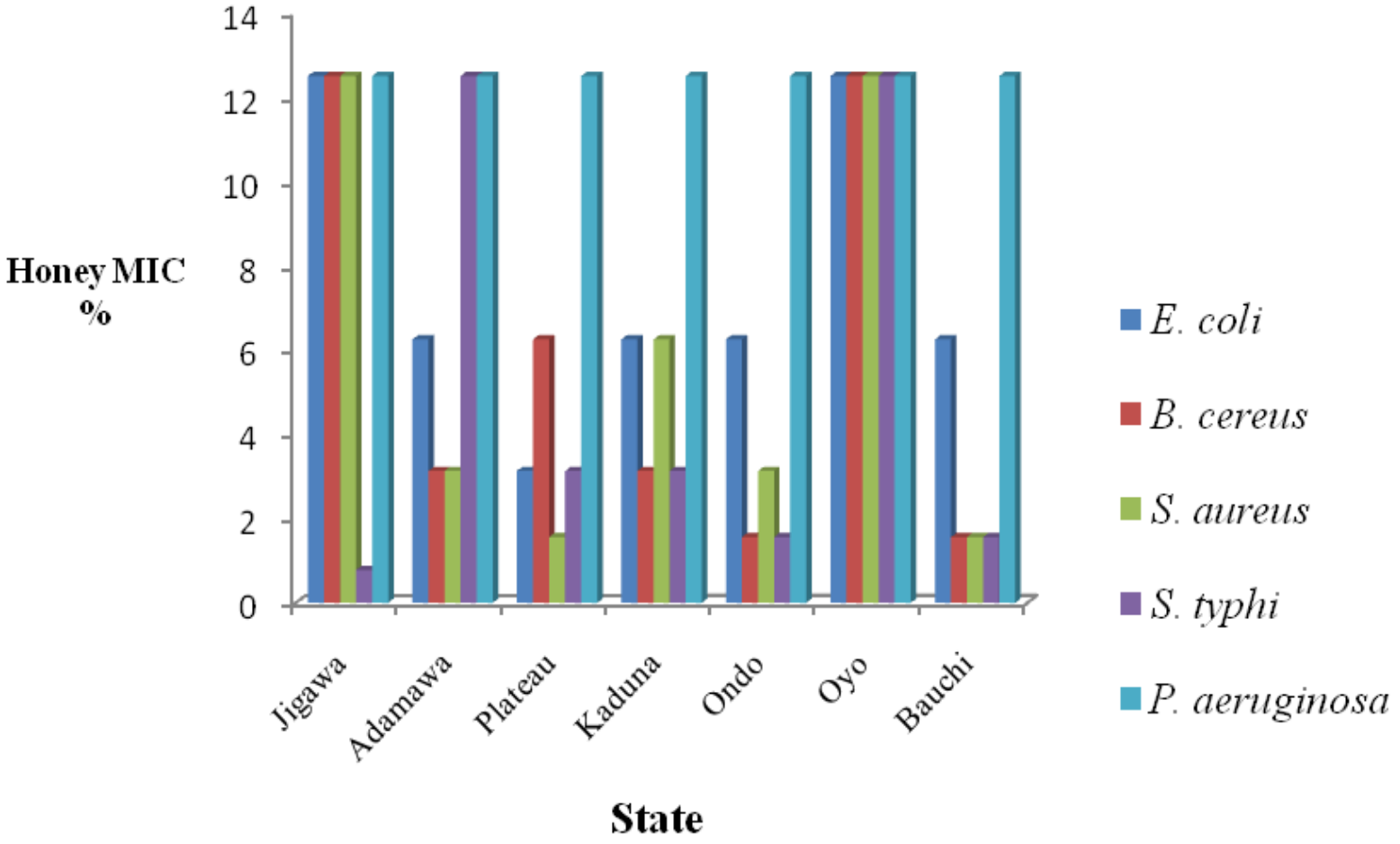

Honey MIC %
14
12
10
8
6
4
2
0
Jigawa
Adamawa
Plateau
Kaduna
Ondo
Oyo
Bauchi
State
E. coli
B. cereus
S. aureus
S. typhi
P. aeruginosa

Figure 4.9: Minimum Inhibitory Concentration of Honey Samples from the Different States to Bacteria Species

from Jigawa State showed an MIC of 12.5% for all organisms except *S. typhi* with 0.78% the lowest MIC in this study.

Honey sample from Kaduna and Plateau State inhibited the growth of all test organisms at low concentrations except for *P. aeruginosa* where it required 12.50% while honey from Ondo State inhibited the organisms both at high and low concentrations and honey from Oyo State showed that a high concentration of honey sample was required to inhibit the growth of all test organisms.

E. coli was inhibited mostly at 12.50% followed by 6.25%. *B. cereus* was inhibited mostly at 1.56 and 12.50%; *S. aureus* was mostly inhibited at 1.56 and 12.50%, *S. typhi* was inhibited at a low concentration for almost all honey samples with honey from Jigawa State inhibiting *S. typhi* with as low as 0.78%, while *P. aeruginosa* was inhibited at all States at the highest concentration used (Figure 4.9**)**.

The honey sample from Bauchi State inhibited the growth of *B. cereus, S. aureus* and *S. typhi* at 1.56% (a low concentration) except for *E. coli* (6.25%) and *P. aeruginosa* (12.50%). Honey sample from Bauchi State had the lowest MIC observed among the honeys from all other States.

4.5 MINIMUM BACTERICIDAL CONCENTRATION OF THE HONEY SAMPLES

Only Honey sample from Adamawa State had bactericidal activity against *E. coli* at 12.50% (Table 4.20). Honey sample from Adamawa and Oyo State killed *B. cereus* at 12.50%. The honey sample from Jigawa and Adamawa State Killed *S. aureus* at 12.50%, none of the

Table 4.20: Minimum bactericidal Concentration (% v/v) of the honey samples

	Organism				
Sample site	*Escherichia coli*	*Bacillus cereus*	*Staphylococcus aureus*	*Salmonella typhi*	*Pseudomonas aeruginosa*
Bauchi	-	-	-	-	-
Jigawa	-	-	12.50	-	-
Adamawa	12.50	12.50	12.50	-	-
Plateau	-	-	-	-	-
Kaduna	-	-	-	-	-
Ondo	-	-	-	-	-
Oyo	-	12.50	-	-	-

Key: - Growth recorded after sub culturing

honeys killed *S. typhi* while honey sample from Bauchi, Kaduna, Plateau and Ondo State killed no organism.

4.6 DEMOGRAPHIC FEATURES OF RESPONDENTS

Questionairre was used to know the criteria for honey acceptance by consumers (Tables 4.21 -4.26). There was a significant (r=0.9) relationship between the quantity of honey purchased and consumers' income level. No significant difference ($p \geq 0.05$) was established between the level of education and the criteria for honey purchase but there was a strong relationship (r=0.09) between the highest level of education and the quantity of honey bought (Table 4.23). The demographic data (Table 4.21) revealed that 92% of the respondents were below 50 years of age while 84% were males.

4.7 REASONS FOR HONEY PURCHASE

About 43% of respondents ranked medical reasons for the purchase of honey, 38% ascribed it to dietary awareness, 11% due to religious or traditional belief of its efficacy while 8% consumed it purely as food item (Figure 4.10).

4.8 FACTORS AFFECTING PURCHASE

Price was ranked as the most important criteria for honey purchase (28%), followed by quality of honey (20%) and the type of honey, colour and flavor were all ranked equally (12%). The type of honey could be white, black, brown or comb honey while the name of honey origin (7%) was less important to most consumers (Figure 4.11).

Table 4.21: Demographic Features of Respondents

Variable	Number of Respondents
1. Age (Years)	
21-30	41
31-40	29
41-50	22
Above 50	8
Total	100
2. Gender	
Male	84
Female	16
Total	100
3. Monthly income (₦)	
No response	12
< ₦10,000	51
11,000-N20,000	7
21,000-N30,000	8
31,000-N40,000	13
41,000-N50,000	9
Total	100
4. Family size	
No response	34
1 – 5	35
6 – 9	9
10 and above	22
Total	100
5. Education Level	
Non formal	26
Quranic	33
Primary	10
Secondary	6
Post Secondary	25
Total	100

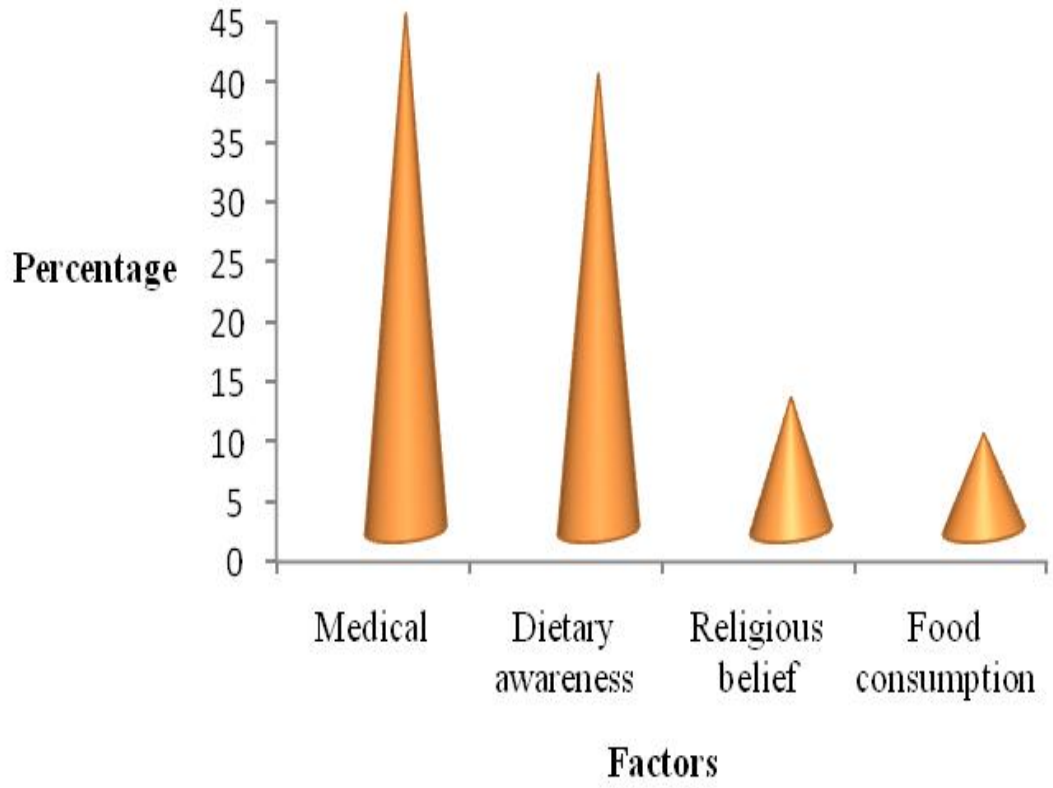


Figure 4.10: Motivators of honey purchase.

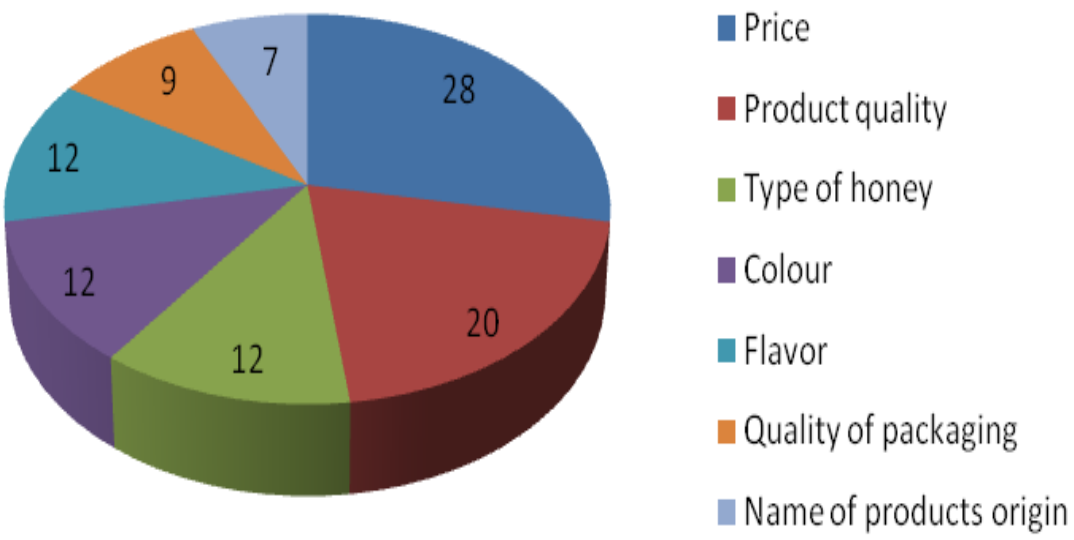


Figure 4.11: Criteria for Honey Purchase.

4.9 RELATIONSHIPS BETWEEN DEMOGRAPHIC FEATURES AND HONEY PURCHASE

4.9.1 Level of Education and Criteria for Honey Purchase

There was no significant relationship in the level of education and the criteria for honey purchase (Table 4.22).

4.9.2 Level of Education and Quantity of Honey Purchased

There was no significant relationship between the level of education and the quantity of honey purchased (Table 4.23). The level of education did not affect the quantity of honey purchased.

4.9.3 Effect of Income on Quantity of Honey Purchased

From Table 4.24 there was a significant relationship in the level of income and the quantity of honey purchased. Those who earned less than ₦20, 000 purchased 1-5 litres majorly while none of those who earned more than ₦20, 000 bought less than 11 litres. The low income earners also purchased comb honey more followed by the people who did not disclose their income. Respondents with the highest income were not the group that purchased 11L and above the most.

4.9.4 Monthly Income and Preference for Imported Honey

There was a significant relationship in the choice of imported honey compared to Nigerian honey by the consumers. Respondents with <=₦10,000 – ₦20,000 preferred to buy Nigerian honey, those who earned ₦21,000 and above preferred to purchase imported honey while respondents who refused to disclose their income preferred to buy any type of honey (Table

4.25). People who earned above ₦21, 000 and above were specific about their choice of honey while others would go for any type.

Table 4.22: Highest Level of Education and Honey Purchase

Criteria for purchase	Highest education level				
	Non formal	Quranic	Primary	Secondary	Post secondary
Product quality	3	6	4	4	10
Price	5	7	0	0	0
Type of honey	5	6	2	1	3
Quality of packaging	3	8	3	0	0
Colour	3	3	1	1	4
Flavour	1	0	0	0	1
Product origin	0	0	0	0	1
Thickness	5	1	0	0	5
Medicinal uses	1	1	0	0	1
No response	0	1	0	0	0
Total	26	33	10	6	25

No significant difference at p≥ 0.05 in the criteria for honey purchase based on the respondents highest level of education

Table 4.23: Highest Level of Education and Quantity of Honey Purchased

	Quantity of honey purchased in Litres			
Highest education level	1 – 5	6 – 10	11 – above	Comb
Non formal	27.30	50.00	26.70	25.00
Quranic	36.40	0.00	43.30	28.60
Primary	27.30	0.00	6.70	8.90
Secondary	9.10	0.00	6.70	5.40
Post secondary	0.00	50	16.50	32.10

No significant differnce at $p \geq 0.05$ in the quantity of honey purchased r=0.09 L = Litre Values are in percentage

Table 4.24: Quantity of Honey Purchased Based On Monthly Income

	Quantity of Honey Purchased (Litres)			
Monthly Income	**1 – 5**	**6 – 10**	**11 – and above**	**Comb**
< ₦ 10,000	81.80	100.00	22.60	58.90
₦11000-₦ 20000	18.20	0.00	0.00	8.90
₦21000-₦30000	0.00	0.00	25.80	0.00
₦31000-₦40000	0.00	0.00	35.50	3.60
₦41000-₦50000	0.00	0.00	12.90	8.90
No response	0.00	0.00	3.20	19.60

alpha level of significance, r<0.9, Significant difference at p< 0.05 was observed among income earners who purchased diverse litres of honey

Table 4.25: Honey Preference Based on Monthly Income

Monthly income	Type of honey preferred			
	Imp.	**Nig.**	**Any type**	**Total**
< ₦10000	1 (3.60)	38 (82.60)	12 (46.20)	51
₦11000-₦20000	1 (3.60)	4 (8.70)	2 (7.70)	7
₦21000-₦30000	8 (28.60)	0 (0.00)	0 (0.00)	8
₦31000-₦40000	11 (39.30)	2 (4.30)	0 (0.00)	13
₦41000-₦50000	**7** (25.00)	2 (4.30)	0 (0.00)	9
No response	0 (0.00)	0 (0.00)	12 (0.00)	12

Imp.- imported, Nig.- Nigerian, Percentage in brackets. A significant ($p < 0.05$) relationship ($r<0.9$) was observed in the choice of honey preferred.

4.9.5 Level of Income and Opinion about Honey Price

There was a significant relationship (Table 4.26) between respondents classification of honey sample as per price. A higher percentage of low income earners (<₦10, 000.00- ₦30, 000) considered honey to be very cheap or cheap. Respondents who earned ₦31, 000 – ₦40, 000 considered honey to be expensive while ₦41, 000.00 and above considered the honey as very cheap.

Table 4.26: Respondents Opinion about Price of Honey Purchased Based on Their Monthly Income

Monthly Income	Opinion about Price					
	Very cheap	Cheap	Expensive	I do not know	Undecided	Total
< ₦10000	12 (44.40)	11 (64.70)	3 (23.10)	3 (50.00)	22 (59.50)	51
₦11000-₦20000	1 (3.70)	4 (23.50)	1 (7.70)	1 (16.70)	0 (0.00)	7
₦21000-₦30000	5 (8.50)	0 (0.00)	2 (15.40)	0 (0.00)	1 (2.70)	8
₦31000-₦40000	3 (11.10)	0 (0.00)	6 (46.20)	1 (16.70)	3 (8.10)	13
₦41000-₦50000	5 (18.50)	0 (0.00)	1 (7.70)	0 (0.00)	3 (8.10)	9
No response	1 (3.70)	2 (11.80)	0 (0.00)	1 (16.70)	8 (21.60)	12

A significant difference $p < 0.05$ in respondents opinion about price of honey was observed, $r<0.9$, Percentage in bracket

CHAPTER FIVE

5.0 DISCUSSION

5.1 POLLEN SPECTRA OF HONEY SAMPLES

The study revealed that the honey samples contained numerous pollen types from plants characteristic of the Sudan, Guinea and Forest ecological zones (Keay, 1989). Results showed that though the pollen of plant variety in the Sudan honey was large, predominance of some few species was high. The reason might be that the bees preferred the nectar of particular plants than others or were more specific for forage preference. Most of the honey samples from Guinea and Sudan savanna showed that bees depended on few plants for their pollen meal. *Combretum* sp. (*molle*) (R.Br. ex.G. Don) was predominant in honey from the Sudan region but it is not a good ecological indicator, this is because it can be found in Guinea savanna and in some Rain Forest zones, therefore it can not be used as an ecological marker for Sudan savanna honey. However, the occurrence of *Piliostigma thoningii* (Schum.) Milne-Redhead, *Entada abyssinica* (Stued.) and *Lannea* sp. (A. Rich) along with other pollen that had a distinct ecological habitat confirms that the honey is from Sudan savanna. The occurrence of *Parkia biglobosa* (Jacq.) R. Br. ex. G. Don in all Sudan zone honey samples agrees with the work of Agwu and Okeke (1997), who studied honey from three savanna zones of Northern Nigeria. The reason for *P. biglobosa* occurrence in this zone might be that the plant is highly visited by bees when flowering due to its sweet nectar. The plant is also well spread over the savanna of Nigeria but the low percentage of occurrence in Adamawa State might be due to a long distance of the plant from the hive or this may reflect the importance of this plant to bees themselves as a bee forage plant. *Combretum* species was the

major bee forage plant at Adamawa State, this agrees with Mbuya *et al.* (1994) and Mutsaers (1991) who stated that plants from the family combretaceae are major bee forage plants. The bees might have preferred this plant due to its high sugar composition. The fructose plus glucose value in Adamawa State was 70.80% greater than the 60% minimum value required for honey, while its sucrose content was 1.34%. Bees prefer nectar with high sucrose and fructose (Percival, 1965). One of the samples with high fructose content was the honey sample from Adamawa State, this might account for the bees visit to the predominant pollen (*Combretum* species) in the honey. The presence of *Alchornea* sp. (Schum & Thonn.) and *Uapaca acuminata* (Hutch) plants adapted to river banks and swamps suggests the presence of a water body but the presence of *Entada abyssinica* (Steud.), *Hymenocardia acida* (Tul) and *Daniellia oliveri* (Rolfe) confirms the honey is from an area with savanna type vegetation. The presence of *Daniellia ogea* (Harms) a plant of a typical forest environment shows that the honey sample may be from a forest relic in the savanna zone. The presence of *Parinari kerstingii* (Engl.) a tree found by fringing forest in savanna regions suggests the honey sample to be from the savanna zone.

Jigawa State with the lowest number of pollen types for this zone, recorded the highest quantity of *Parkia biglobosa* (60.79%), this agrees with the work of Agwu and Okeke (1997) whose sample from Gombe *Parkia biglobosa* (49.5%) recorded the lowest number of pollen types. A high presence of *Parkia biglobosa* might have an effect on the bees' reduction in preference for other plants. The sucrose content of this honey was high 1.83%, which was mainly from *Parkia biglobosa,* this might account for the low number of pollen types from this State honey sample. *Diospyros mespiliformis* (Linn.) a tree found in the Sahel and Sudan region in fringing forests, riverbanks and rocky hills Keay (1989) confirms the honey from Jigawa to be consistent with the plants found in this zone. The presence of Asteraceae, an herbaceous plant or weed asociated with human activities and

Mangifera indica (Linn.) suggests cultivation activities taking place at the site of the bee hive. The presence of *Bridellia* sp. (Willd.) usually found in the sudan and guinea savanna, fringing forest and fallow land; the absence of *Daniellia ogea* which is a forest vegetation marker shows the sample was from savanna vegetation, the presence of *Adenodolichos paniculata* (Harms) confirms this. Two types of honey were found in the Sudan savanna zone. The presence of *Mangifera indica* in Bauchi indicates anthropogenic activities i.e. cultivation of this plant. The leaves and flower of *Mangifera indica* is used medically to treat dysentery, scurvy, insomnia, diabetes, wounds, sore throat and tooth decay (Arbonnier, 2004) and is also a good bee forage plant.

Pavetta species was the most preferred bee forage for Kaduna State. Though this plant was not recorded by Agwu and Okeke (1997) and Ebenezer and Olugbenga (2010) for Kaduna, it belonged to the plant family Rubiaceae known to be a favorite bee forage Crane (1990), Mbuya *et al.* (1994) and Mutsaers (1991). The presence of *Syzygium guineense* (Willd.) found by fringing forest by streams and rivers in savanna regions; *Blighia sapida* found in drier forest areas and forest outliers in savanna regions; *Elaeis guineensis* and *Macaranga* sp. markers for secondary regrowth forest Keay (1989) suggests the honey is from the savanna zone with a forest type of vegetation. In the honey sample from Plateau State, the plant from the family myrtaceae was the most dominant and had the highest sucrose content of 1.87% in this study. This might be the reason bees foraged this plant so much. The presence of *Pavetta* species, *Afzelia africana, Daniellia oliverii* and *Hymenocardia acida* from the Guinea savanna habitat (NEST, 1991) confirms the honey to be from this vegetation zone. Though *Chromolaena odorata* and *Tridax procumbence* had the highest percentage occurrence of pollen grains in Ondo and Oyo honey respectively the honey was from the family Moraceae and Fabaceae for the two States respectively. At Ondo State, *Elaeis guineensis* was also dominant; this agrees with NEST (1991) who stated that forest lands have been converted to oil palm plantations. The presence of *Milicia excelsia* a large forest tree found in the rain forest and forest outliers in savanna woodland

areas confirms the honey from Ondo to be from the rain forest zone. Bees in this zone visited more than one plant mainly for pollen. They made use of grass pollen a lot and a variety of other plants unlike other zones. The presence of grasses like *Chromolaena odorata* and *Tridax procumbence*, plants like *Alchornea cordifolia, Brachystegia eurycoma, combretum species* and *Ceiba pentandra* confirms the honey to be from the rain forest vegetation zone. The reason the rain forest samples had a high percentage of grasses might be due to human activities such as logging, farming and conversion of forest land to plantations which has reduced the true forest vegetation. This agrees with the findings of NEST (1991) who stated that the vegetation in the forest zone is a mosaic of farms, fallow vegetation or converted land. Different honey types were produced from the different zones, this agrees with the report of Soria *et al.* (2004), that a wide variety of botanical source of honey are associated with different climatic conditions. The families mostly visited from this study were; Anacardiaceae, Combretaceae, Fabaceae, Myrtaceae, Rubiaceae and Sapotaceae, this agrees with the findings of Crane (1990) and Mbuya *et al.* (1994) who recorded all these families as plants bees usually forage. Most beekeepers harvested once a year, this agrees with the findings of Agwu and Njokuocha (2004) who stated that honey production by wild bees (*Apis mellifera*) in Nigeria is moderate and non-seasonal. This may account for the presence of plants that flower at different seasons being present in the honey sample.

5.2 PHYSICO-CHEMICAL PARAMETERS OF HONEY SAMPLES

The moisture content for all States was within the international standard for honey. This agrees with Oddo and Piro (2004). This means that the honey samples are capable of remaining stable and can resist spoilage by yeast fermentation upon storage. There was a significant deference in the water content of the honey samples from the different States.

Variations in the moisture content of honey have been attributed to the composition and floral origin of honey (Nanda *et al*., 2003; Malika *et al*., 2005). Honey sample from Adamawa and Ondo showed a significantly ($p<0.05$) higher moisture content different from honey from other States, the reason might be that the honey from Adamawa and Ondo State had a high number of total pollen grains. The moisture content of honey samples from this study ranged from 14.17 – 15.03%. The values obtained were comparable to values gotten for the U.S. standard honey ≤ 20% (Crane, 1990). The range recorded in this study falls within that reported by Omafuvbe and Akanbi (2009) for some Nigerian honeys (11.47-19.62%). The moisture content of honey samples in this study was lower than that of Adebiyi *et al.* (2004) (16.38-30.82%) and Odeyemi *et al.* (2013) (16.81-21.52%) for some Nigerian honeys. The reason for the low moisture content might be because the samples were collected from the hives sealed while other investigators bought commercial honey. Also, given that honey is hygroscopic in nature and takes up moisture from the environment Hak-Gil *et al.* (1988), during processing, alteration`s and moderations that affect honey quality through exposure to the environment had been excluded. Also, White and Doner (1980) stated that the moisture content of honey after extraction may change depending on conditions of storage. Another reason for the difference might be because of the different sample sites though the commercial sample from Ondo State for Lawal *et al.* (2009) (16%) was higher than this study sample from Ondo State (14.2%). This agrees with Anupama *et al.* (2003) who stated that commercial samples examined had higher moisture contents. The Sudan savanna honey showed the highest water content. The reason might be as a result of the technique of bee keeping which is predominantly local in this zone. The finding of this study agrees with Celechovska and Vorlova (2001) who found no difference in the water content of their honey samples. The moisture content of honey from Jigawa and Oyo State showed no significant difference ($p\geq0.05$), this may be as a result of a low number of total pollen grains counted in

each honey sample. Generally the moisture content of the honey sample studied was lower than that recorded for Indian honey samples 17 -22.6% and 18.7 – 21.8% by Anupama *et al.* (2003) and Singh and Bath (1997) respectively and Nigerian honey samples 16.38-30.82 and 16.61-21.52% by Adebiyi *et al.* (2004) and Odeyemi *et al.* (2013) respectively. Bauchi honey was predominantly from *Mangifera indica* followed by *Combretum* sp. White and Doner (1980) and Nanda *et al.* (2003) stated that the amount of moisture is a function of the factors involved in ripening, weather conditions (climate and season) and the original moisture of the nectar. The major pollen source of Bauchis' honey nectar and fruit was watery and might account for the honeys high moisture content.

This study recorded an ash content of 0.18±0.01-0.32±0.02%. Honey samples from Kaduna and Plateau States had the higher ash content; the reason might be because of the large number of pollen spectra and grain. Though Adamawa State had a wide pollen spectra it had the lowest ash content, it was made majorly from *Combretum* species. This agrees with IHC (2002) that blossom honey (honey from nectar producing plants) have lower ash content. The range obtained in this study is similar to the range (0.095 – 0.518%) recorded by Adebiyi *et al.* (2004) and (0.19 – 0.36%) Omafuvbe and Akanbi (2009) while the range of this study was lower than that obtained by Lawal *et al.* (2009) which was 0.60 – 0.84 % for Nigerian honey. The Guinea savanna and Forest zone had a higher ash content compared to the Sudan savanna zone. The Guinea zone had the highest ash content of 0.62±0.02%. The reason for the high ash content might be because of the pollen grain content of 110,177, the highest in this study. The Rain Forest had had an ash content of 0.48±0.01% with only 24,292 pollen grains and the Sudan 0.44±0.01% ash content with 37,254 pollen grains. The reason for the Sudan savanna though with a higher number of pollen grains than the Forest zone having a

lower ash content may be due to the fact that the pollen grains from the Sudan were majorly from plants while that of the Forests' were majorly from weeds.

All honeys are acidic with pH value lying between 3.5 and 5.5 (Bogdanov *et al*., 2004). The range (4.02±0.04 – 4.95±0.03) obtained in this study showed that all honey samples were acidic and agrees with the report of Adebiyi *et al.* (2004) and that of Lawal *et al.* (2009) who showed a range of 4.31 -6.02 and 4.65 -5.14 respectively. The values of pH gotten from this study were less acidic than that recorded by Bonvehi and Ventura Coll (1993) for French Lavender honey produced in Spain. The reason might be that the honey samples are from different geographic regions, also, the major botanical origin of the honey was different. Honey sample from Oyo State had the lowest acidity value therefore it was the most acidic honey sample and from the anti-bacterial activity of the honey samples. It was the most potent at 12.5 -50%. This affirms that the pH of honey makes honey a very potent anti-bacterial agent. Honey sample from Jigawa State with the highest pH value showed the lowest anti-bacterial activity against all bacteria used. This agrees with the findings of White and Doner (1980) who stated that the larger the pH's number the lower is the active acidity. The reason why honey from Plateau State showed the lowest free acidity might be because of the major plant from the family Myrtaceae. The values (9.75 – 11.25meq/kg) for Plateau and Jigawa States were lower than that recorded by Omafuvbe and Akanbi (2009) (24.00 – 31.00meq/kg) but close to 13.1meq/kg by Soria *et al.* (2004) and within the range (2.3-51.4meq/kg) recorded by Oddo and Piro (2004). The free acidity of Ondo State 46meq/kg was within the limit (27.00 -47.70meq/kg) recorded by Bonvehi and Ventura Coll (1993) and qualified for standard bakers honey.

Honey from Ondo, Bauchi, Kaduna, Oyo and Adamawa failed to attain the European Union standard of ≤40meq/kg. This is in agreement with Kugonza and Nabakabya (2008) who stated that the free acidity levels of honey samples from Ugandan market failed to attain the European Union standard of ≤40meq/kg. Honey samples from Adamawa and Oyo States had high free acidity, this may make the honey to sour quickly, the reason might be due to the large number of pollen grains in Adamawa honey sample while the honey sample from Oyo State had a low number of pollen grains its pollen grains were majorly from grasses/weeds which are known to be high in acids. The results shows that honey samples from Adamawa and Oyo State may not be kept for long if their free acidity value is taken into consideration but since their moisture content was less than 17% these honeys may not ferment easily. Lactonic acidity recorded was 11.25 – 27.50meq/kg and 25.50 – 176.75meq/kg for total acidity. The Lactonic activity of Bauchi, Ondo and Oyo States honey samples showed a similar range (6 -16.25meq/kg) recorded by Omafuvbe and Akanbi (2009). The total acidity of honey samples from Jigawa (34.75) and Plateau State (27.25) fell within the E.U standard and showed similar values to Bonvehi and Ventura Coll (1993) (27.75 – 53.54 meq/kg) though it was a bit lower than that recorded by Omafuvbe and Akanbi (2009) (35.75 – 39.50meq/kg). Samples from other States showed a total acidity range of 59.00 – 176.75meq/kg a value higher than that recorded by other works.

Sugars were the main constituents of all the honey samples studied. This agrees with the findings of White *et al.* (1962), Flodhazi (2004) and Bertoncelj *et al.* (2007). The range percentage glucose, fructose, sucrose, maltose and reducing sugars for this study was 28.30 – 34.25%, 37.81 – 40.03%, 1.21 – 1.87%, 1.66 – 1.83% and 66.11 – 73.74% respectively. This agrees with the values recorded by Oddo *et al.* (1995) and Oddo and Piro (2004) for glucose (18.80-45.60%), fructose (25.30 – 49.20%), sucrose (0 – 3.3%), maltose (0.2 – 3.3%) and

fructose / glucose (0.81-1.84%). The sucrose content of all honey samples were within the range of <5g/100g of Codex Alimentarius (2001). Honey samples from Oyo and Ondo States had the lowest fructose content; the reason might be because the bees in these States visited grasses for pollen. Pollen is not nectar; it is nectar that is sweet due to fructose in it. Jigawa State had the highest fructose content; the reason might be that the honey is majorly from the plant *Parkia biglobosa* whose nectar is very sweet. The glucose, fructose and sucrose content from this study is similar to that recorded by Fadare (2001) for some Nigerian honeys (35, 40 and 1.9%) respectively. Also honey samples from Oyo and Ondo State showed the highest maltose content, this agrees with Louveaux *et al.* (1978) who stated that honeydew honey showed more of secondary sugars than blossom honey. These honeys had their major pollen source from grass. Any honey with >3% Honey Dew Element is called honeydew honey. The grass pollen in these honey samples exceeded 3%. Honey sample from Bauchi State with the least maltose content behaved significantly differently from all other honeys at lower concentrations. The low maltose content of the honey sample from Bauchi State might have accounted for the honeys anti-microbial activity at low concentrations and shows that the honey is truly from a nectar producing plant which was *Mangifera indica* from this study. Maltose content of this study (1.66 – 1.83) was within the range (0.48-9.19) recorded by Terrab *et al*. (2003). The reducing sugar (glucose + fructose) value was within the Codex Alimentarius (2001) value (> 60g/100g). The value recorded in this study was similar to that of Meda *et al.* (2005) (67.30± 1.90 – 96.20± 6.40g/100g) for Burkina Faso honey and Oddo and Piro (2004) for European honey (46.60-85.70%). In agreement with White and Doner (1980), floral honey (Bauchi, Jigawa, Adamawa, Plateau and Kaduna) from this study had higher simple sugars, lower disaccharides and dextrin than honeydew honey from Oyo State. The fructose over glucose value ranged from 1.11 – 1.34%, this is within the range (0.81-1.84%) recorded by Oddo and Piro (2004) for European honey. A low F/G ratio indicates that

the honey will granulate rapidly. The values gotten were not low for F/G ratio; it therefore means that the honey samples studied will not granulate on time.

*Pollen is the major source of protein while nectar contains little protein (Percival, 1965). Percival (1965) reported that there was no great difference in the protein content of anemophilous and entomophilous pollen. Though two significantly different groups occurred for the protein content in this study, there was really no great difference in the protein content for all the samples (0.21 – 0.27%). Adamawa, Jigawa, Plateau, Kaduna and Ondo States fell into another category while Oyo into the second category. Though the honey sample from Oyo State was from pollen majorly but the total number <500 was very small. This might have accounted for the significant difference in its low protein content, given that pollen is majorly made up of protein, it therefore means that, the more the pollen the higher the protein content. The reason for the high protein content that exceeded the standard limit for protein from other States might be accounted for by the large number of pollen grains and or the pollen types. Looking at the trend, honey majorly from anemophilous pollens (from Oyo State) was distributed into a different category thus disagreeing with the statement of Percival (1965) that there was no great difference in the protein content of anemophilous and entomophilous pollen. The reason for honey sample from Bauchi State having highest protein content might be due to the sources of pollen visited by Bees in this State to make honey. There was no significant difference between the fat and Dextrin content of honey samples. States with 0 or 0.01 fat mostly used modern hives while those with more than 0.01 used local hives. Honey sample from Kaduna State recorded the highest HMF contentfrom this study. HMF is a substance that develops in honey from its sugars, at ordinary temperature honey gets richer in this substance (Bianchi, 1977). HMF is an indicator of freshness

(Downey *et al*., 2005). All honey samples HMF were within standard limit. All samples from this study were fresh and well below the standard recommended for any honey in the world.

Generally the metals in the honey samples were present in small amounts this agrees with Crane (1990) who stated that most metals in honey are present in only minute amounts called trace elements. Most States met the minimum requirement for each metal except Jigawa for lead content. When compared with other States honey samples from Jigawa and Bauchi State had a high content of copper, Zinc and lead. Result shows that most honeys were less exposed to industrial or farming pollution and the honey sample from Kaduna was the richest in minerals and Ondo the richest in heavy metal composition while the honey sample from Jigawa State had the least mineral and heavy metal composition. Though the average content of iron 0.42mg by Bertoncelj *et al.* (2007), White and Doner (1980) was exceeded by most samples, honey sample from Oyo State was very low in Iron content. The values recorded for calcium (10.34-13.86ppm) and Magnesium (1.48-5.98ppm) from this study was lower than that recorded by Crane (1990) for honey from the U.S.A. Only iron in Bauchi honey had a value higher than that reported by Crane (1990). The reason for low calcium and magnesium content may be due to depletion or and reduction of minerals in the soil from the different States. The value for copper (0.02 – 0.29ppm) and zinc (0.10 – 0.82ppm) was similar to that recorded by Downey *et al.* (2005) (0.10-0.23 and 0.16-2.25ppm respectively) for honey purchased and lower than that recorded by Morse and Lisk (1980) (0.68 – 1.22 and 2.00 -17.2ppm) from honey taken from storage barrels from different countries. The cadmium content was negligible in all samples; the reason might be due to the location of the bee hives which were mostly far from major road sides. The honey sample from Bauchi State was the least in heavy metal composition compared to honey samples from all other States.

Calcium quantitatively was the most abundant mineral found in the honey samples except in Bauchi and Jigawa State. Magnesium levels in this study were similar to those found by Downey *et al.* (2005). Iron level from Bauchi State was higher than that recorded by Downey *et al*. (2005) but was similar to Terrab *et al.* (2003). All values of mineral element recorded were much lower than recorded for Spanish honey by Terrab *et al.* (2004). Metals such as iron and zinc differ greatly in total concentration in soils, but are only available to plants in large amounts when the soil in the plant root zone becomes acidic (pH 6.0). The magnitude of iron absorption by plants is also influenced by the concentration of a number of other metals in the soil solution (Morse and Lisk, 1980). Honey sample from Jigawa Sate had the highest iron content from the Sudan zone and also recorded the highest concentrations for copper, Zinc and lead, thus agreeing with the observation of Morse and Lisk (1980). The lead content of this study (0.04-0.82ppm) was similar to the range (0.02-1.00 and 0.02-0.071mg/kg) obtained by Celechovska and Vorlova (2001); Pryzylowski and Wilczynska (2001). The zinc content of the honey samples was much lower than that recorded by both authors.

5.3 ANTI-BACTERIAL ASSAY

5.3.1 Agar Well Diffusion

All honey samples showed inhibitory activity against all bacteria species at different concentrations. This agrees with the report of Omafuvbe and Akanbi (2009) who obtained their samples from Ewu-Esan Edo, Enugu-Ezike Enugu, Osogbo Osun and Saki Oyo State and, the findings of Braide *et al.* (2012) using honey from Elu-orie Enugu. Most samples showed high inhibitory effect against *Escherichia coli* and *Salmonella typh*i. This agrees with the report of Adebolu (2005) whose study recorded a high zone of inhibition (ZOI) (20mm) against *E. coli* and Mohapatra *et al.* (2011) who reported that honey inhibited the growth of *E. coli* and *S. typh*i at 25±11 and 12.50±2.7µg/ml and gave a ZOI of 26.49±6mm and

34.39±4mm for methanol extract of raw honey. The control drug - ciprofloxacin gave a ZOI of 16.67±3.5mm and 14.75±1.7mm for the organisms respectively.

The inhibitory activity exhibited by these honey samples may be due to the high sugar content and low water content which prevents bacterial growth and variation in the concentration of hydrogen peroxide in honey samples or non peroxide factors related to floral source (Mohapatra *et al.,* 2011). Honey samples from this study had a greater inhibitory activity on two gram-negative bacteria *E. coli* and *S. typh*i but not *Pseudomonas aeruginosa* than on the gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus*.

Escherichia coli and *S. typh*i were more susceptible than other test organisms. This is similar to the report of Mohapatra *et al.* (2011) with methanol extract of raw and processed honey from India and in constrast to the findings of Muli *et al.* (2008) with Kenyan pure honey at 25, 50, 75 and 100% honey concentrations.

5.3.1.1 Anti-bacterial assay for Adamawa State Honey Sample

Honey sample from Adamawa State was significantly more effective against *Escherichia coli* than the control at all concentrations used. The pattern of the higher the concentration the larger the zone of inhibition exhibited by honey sample from Adamawa State agrees with the findings of Omafuvbe and Akanbi (2009) who recorded a higher zone of inhibition for a higher concentration of honey. The inhibition of *Bacillus cereus* at only high concentrations (50-100%) of Honey sample from Adamawa State agrees with Omafuvbe and Akanbi (2009) who recorded inhibitory activity only at 75- 100% for most of their honey samples.

Honey sample from Adamawa State exhibited inhibitory activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* though the bacteria were either intermediate (17mm) or resistant (≤ 13mm) to this honey, this disagrees with the report of Muli *et al.* (2008) who recorded no ZOI at 25, 50, 75 and 100% honey concentrations for *S. aureus* and only 6.8mm for *P. aeruginosa* at 100% and, Omafuvbe and Akanbi (2009) whose honey samples were not active against *S. aureus* and *P. aeruginosa* at all concentrations but agrees with the findings of Mohapatra *et al.* (2011) who recorded a ZOI range of 8.5±3 and 35.95±11 for *S. aureus* using methanol extract of raw Indian honey. The ZOI (13mm) recorded by honey sample from Adamawa State for 50% for *S. aureus* was greater than that recorded by Nzeako and Hamdi (2000) (9-12mm) at 50% for black forest (Germany), orange flower, black forest (Langaneza), forest (Langaneza), summer flowers and Turkish honeys while the ZOI (17mm) at 100% was lower than that of black forest, orange flower and black forest (Langeneza) honeys. Honey sample from Adamawa State was only effective at 100% against *P. aeruginosa*, this agrees with the values of orange flower and forest honeys, other honey types were more effective and showed some form of activity up to 50% (Nzeako and Hamdi, 2000). The reason for the low effectiveness of honey sample from Adamawa State on *P. aeruginosa* might be due to the ability of *P. aeruginosa* to form biofilm. The honey sample from Adamawa and Plateau State had the highest zone of inhibition (ZOI) against *B. cereus*, the reason might be the high fructose content recorded, HMF content of 20.17±0.01mg/kg and a similar quantity of zinc, magnesium and lead content. The significant antibacterial activity by the honey sample from Adamawa State may be due to the low pH, low water content and osmolarity along with the non peroxide factor related to its floral source which was majorly from *Combretum* species.

5.3.1.2 Anti-bacterial assay for Jigawa State Honey Sample

The reason for most of the organisms used been resistant to honey sample from Jigawa State and *Salmonella typhi* been susceptible agrees with Molan (1999) and Aljadi and Yusoff (2003) who stated that all honeys show some form of anti-microbial activity. The reason for the low activity observed for this honey might be due to a low heavy metal and mineral content and the predominant pollen type which was *Parkia biglobosa*. This agrees with Allen *et al.* (1991) who stated that a large number of samples of honey from different floral sources were found to be of low activity. It might also be due to the pH (4.95) value of the honey, it had the highest pH in this study. The higher the pH the lesser the acidity. It therefore means that this honey was the least acidic, therefore it could not inhibit most organisms due to its weak acidity. This agrees with Todar (2009) who stated that for most bacteria there is an orderly increase in growth rate between the minimum and the optimum pH and a corresponding orderly decrease in growth rate between the optimum and the maximum pH. Also it was the only sample with high lead content that was above the standard limit, negligible amount of calcium and Magnesium. The high lead content might have accounted for the honeys low antibacterial activity, since lead is known to be toxic in small concentrations and can lead to gradual paralysis in the muscles and death. Though this honey showed the lowest activity in this study, it was still effective. This agrees with the report of Mandal and Mandal (2011) who stated that microbial resistance to honey has never been reported.

5.3.1.3 Anti-bacterial assay for Kaduna State Honey Sample

Similar susceptibility pattern as recorded by Omafuvbe and Akanbi (2009) was shown by Kaduna honey; the higher the concentration the higher the zone of inhibition, i.e. as honey concentration increased the antibacterial effect of the honey samples on the organisms

increased. This agrees with Odeyemi *et al.* (2013). *B. cereus*, *S. aureus* and *P. aeruginosa* were resistant to all concentrations of honey sample from Kaduna State. This agrees with Muli *et al.* (2008). Honey sample from Kaduna State showed intermediate activity to *B. cereus* at 100% and was ineffective at 50% downwards. This agrees with Mohapatra *et al.* (2011) who recorded a ZOI of 11.11±6 for methanol extract of raw Indian honey. The reason might be due to the presence of *B. cereus* in honey Omafuvbe and Akanbi (2009) but was in contrasts to the findings of Odeyemi *et al.* (2013) who recorded a ZOI range of 10 -32mm for honey samples from Ado-Ekiti, Ekiti State. *S. typhi* was susceptible to all concentrations of honey used. The susceptibility of *S. typhi* agrees with the study of Mohapatra *et al.* (2011) (28-38mm). The reason for *S. typhi'*s susceptible to honey at all concentrations might be due to a mechanism in honey that attacks this organism's defence mechanism and the botanical sources of the honey. It was only the *Mangifera indica* (Bauchi) honey at high concentrations that did not inhibit *S. typhi*.

5.3.1.4 Anti-bacterial assay for Plateau State Honey Sample

The anti-bacterial activity of honey sample from Plateau State was the higher the concentration the higher the zone of inhibition. This is similar to the observation of Agbagwa and Frank-Peterside (2010) and Odeyemi *et al.* (2013). The honey from Plateau and Ondo States were significantly more effective than the control at all concentrations used for *E. coli.* This is similar to the findings of Mohapatra *et al.* (2011) whose honey samples showed a higher zone (26.46±6mm) of inhibition than ciprofloxacin (16.67+3.5mm) the control also used in this study. *B. cereus* was susceptible at 100% and resistant at 50% downwards. The reason might be that, the anti-bacterial activity of the honey sample from Plateau State might have been due to the high sugar content and the osmotic power of the honey, therefore once the honey was diluted the sugar and osmotic strength reduced and that might explain its

pattern of anti-bacterial activity. This agrees with findings of Odeyemi *et al.* (2013), also, the strong interaction of sugar in honey with water molecules may decrease the water available for microorganisms at high honey concentrations, thus the low moisture content of honey also plays an important part of the system which protects honey degradation and gives it anti-bacterial activity. *S. aureus* was susceptible at 100% only while *P. aeruginosa* was resistant to all concentrations of the honey sample from Plateau State. This agrees with Cooper *et al.* (2002) who stated that *S. aureus* was more susceptible to honey of similar antibacterial potency than *P. aeruginosa.*

5.3.1.5 Anti-bacterial assay for Ondo State Honey Sample

E. coli and *S. typhi* were susceptible to the honey from Ondo State at all concentrations. This agrees with the findings of Mandal and Mandal (2011) who demonstrated that Manuka honey being effective against *E. coli, S. aureus* and *P. aeruginosa* with a ZOI of 15, 23 and 16mm respectively. *B. cereus* and *P. aeruginosa* were resistant at all concentrations. This agrees with the report of Omafuvbe and Akanbi (2009). *S. aureus* was intermediate at 100% only; this disagrees with the findings of Omafuvbe and Akanbi (2009) while it was resistant at the remaining concentrations. This agrees with the findings of Omafuvbe and Akanbi (2009). At 50%, honey sample from Ondo and Oyo States recorded a ZOI of 12±0.01 and 17.50±2.50mm. The antibacterial activity of the honey sample from Ondo State might have been influenced greatly by the presence of *C. odorata* a weed with anti-microbial activity (Charaborty *et al*., 2011).

5.3.1.6 Anti- bacterial assay for Oyo State Honey Sample

Honey sample from Oyo State was significantly more effective than the control against *E. coli* at all concentrations used while *S. typh*i was significantly more effective than the control at the highest concentration (100%) used. The inhibition of *B. cereus* at only high

concentrations (50-100%) for honey sample from Oyo State agrees with the report of Omafuvbe and Akanbi (2009) who recorded inhibitory activity only at 75- 100% for most of their honey samples. Honey sample from Oyo State showed intermediate inhibitory activity against *S. aureus* at 100% and was resistant to *P. aeruginosa*, this finding disagrees with the report of Muli *et al.* (2008) who recorded no ZOI at 25 - 100% honey concentrations for *S. aureus* and only 6.8mm for *P. aeruginosa* at 100%. The honey sample from Oyo State was very poor in pollen content and the predominant pollen was from *Tridax procumbence* (looks like cf) a weed. This might have affected the ZOI recorded. Though the honey sample had a low protein content compared to samples from other States, it still showed antibacterial activity, it therefore shows that a combination of pollen spectra and physico-chemical properties account for the antibacterial activity observed. Honey sample from Adamawa and Oyo State showed anti-bacterial activity against *B. cereus* at 50% higher than all other samples, this may be as a result of a similar lead content of 0.15ppm and the highest free acidity content.

5.3.1.7 Microorganisms Response to Honey Samples

The reason for the significant difference in the susceptibility pattern of various microorganisms to honey sample from the same State may be due to the observation of Odeyemi *et al.* (2013) who stated that the type of test organism determines the potency of honey as an anti-microbial agent and Molan (2006) who stated that the inhibition by the osmotic effect of dilute solutions of honey depends on the species of bacteria. Bauchi State an outlier in this study showed no significant difference in the susceptibility pattern for all the organisms used, the reason might be accounted for by the statement of Mundo *et al.* (2004) and Molan (2006) that honey shows a broad spectrum antibacterial activity. The reason for honey sample from Oyo State being most effective with the highest ZOI against *E. coli* and *S.*

typhi while *B. cereus*, *S. aureus* and *P. aeruginosa* as compared to the reaction of organisms to honeys from different States been similar might be because of the anti-bacterial activity of honey which could be attributed to factors like pH (the lowest), acidity, osmotic effect of honey, presence of hydrogen peroxide and honey phytochemical components (Molan, 2002; Mundo *et al*., 2004) interacting with the organism and its defence mechanism (Allen *et al*., 1991). Also from this study, honey sample from Oyo State was honeydew honey (honey mainly from non nectar producing plants) and had a low pollen content, while Crane (1990) states that the honeydew honeys have a higher antibacterial activity than nectar honeys.

5.3.1.8 Response of Microorganisms to Different Concentrations of Honey

The reason for the antibacterial activity observed at all concentrations for *S. typhi* and *E. coli* and the resistance of *P. aeruginosa* at all concentrations used means that *E. coli* and *S. typhi* infectious load can be reduced by pure honey as well as diluted honey. Generally, the trend of the higher the concentration the higher the activity was true for all six honey samples in this study. This agrees with the findings of Omafuvbe and Akanbi (2009) that the higher the concentration the higher the zone of inhibition. The response of *P. aeruginosa* a gram negative bacterium was similar to that of *B. cereus* and *S. aureus* gram positive bacteria while *E. coli* and *S. typhi* both gram negative reacted similarly. The reason for *P. aeruginosa* acting like the gram positive bacteria in this study may be due to *P. aeruginosa*'s known resistance to honey samples. The gram positive bacteria were more resistant to the honey samples used.

5.3.1.9 Comparison of the Anti- bacterial Activity of Honey Samples from Different States

The significant difference in the honey from different States at different concentrations confirms the reports that different honey vary substantially in their potency of antibacterial activity depending on their plant source (Aljadi and Yusoff, 2003; Flodhazi, 2004; Molan, 2006; Taormina *et al*., 2001).

From this study pure (Full strength) honey from Plateau State was most effective at 100% and Oyo at 12.50-50% while Bauchi honey as an outlier was most effective at 12.50%. This could be due to the variation of a multiple pathway mechanism in honey to kill microbes or could be accounted for by regional vegetational differences that might have influenced the amount of hydrogen peroxide (Osato *et al*., 1999) and the antibiotic properties of the honey samples.

At 50% concentration there was a significant difference ($p<0.05$) between honey from the Forest zone and the Sudan Savanna and the Guinea Savanna showed a relationship between the Forest and Sudan Savanna zones. The difference in anti-bacterial activity of the vegetation zones agrees with the statement of Taormina *et al.* (2001) who stated that physical property along with geographical distribution and different floral source may play an important role in the anti-microbial activities of honey. Physically, the Guinea savanna is the link with the Forest and Sudan zone, the similarity between the two zones is consistent with Nigerian vegetation and agrees with the statement of Louveaux *et al.* (1978) who stated that different geographical areas present particular floral associations and the greater the climatic difference the more conspicuous the variation in the floral association. Sudan savanna showed the lowest potency at all concentrations. This agrees with the report of Flodhazi (2004) who stated that compositional differences arise in honey due to the plants bee visit for forage which is dependent on the type of soil and the nutrients available in the soil of that region. From this study it was observed that the concentration of honey used affected the zone of inhibition pattern of the honey from the different vegetation zones.

5.3.1.10 Anti-bacterial assay for Bauchi State Honey Sample

All organisms were resistant at 25-100% and susceptible at 12.5% to Bauchi honey sample. This shows that the honey sample is a broad spectrum antibacterial agent. This agrees with the findings of Molan (1992; 2006) who stated that honey inhibits a broad spectrum of

bacteria species. The similar trend for all organisms shows that there may be little or no interference or interaction between the honey and the organisms. In contrast to the findings of Lurlina and Fritz (2005) and Omafuvbe and Akanbi (2009) whose honey samples stopped inhibitory activity at 25% concentration. Honey sample from Bauchi State showed inhibitory activity against *E. coli*, *B. cereus*, *S. aureus*, *S. typhi* and *P. aeruginosa* at 12.5% while the honey sample from Elu-orie Enugu State by Braide *et al.* (2012) showed inhibitory activity against *P. aeruginosa* and *S. aureus* at 10% though at a very low ZOI (6.5mm). The reason for this observed honey activity might be due to the high iron content of this honey. It had the highest iron content in this study, as well as high maltose content, its anti-microbial activity may be due to release of hydrogen peroxide given that most types of honey generate hydrogen peroxide when diluted (Bogdanov, 1997; Al-Jabri, 2005; Molan, 2006); or due to the major plant, *Mangifera indica* foraged by bees to produce the honey. This agrees with the report of Mohapatra *et al.* (2011) who stated that the major variations seen in honey antibacterial activity were due to changes in the level of hydrogen peroxide achieved and in some cases to the level on non peroxide factors which is obviously related to the floral source and sometimes may account for the major part of antibacterial activity in honey.

Also, the general non inhibitory activity at full strength honey and then inhibitory activity upon dilution of honey shows that the antibacterial agents of this honey majorly may be hydrogen peroxide. This agrees with the statement of Molan (2006) that full strength honey has a negligible level of hydrogen peroxide because glucose oxidase enzyme is practically inactive in full strength honey but gives rise to hydrogen peroxide only when the honey is diluted. On dilution the activity increases by a factor of 2,500-50,000, thus making honey an antiseptic which is antibacterial but not tissue damaging. Honey sample from Bauchi State had the highest value of 5.78ppm for transistion metals. This was the highest recorded in this

study. The implication was that the honey at full strength was less active, upon dilution the activity increased. This agreed with the findings of Molan (2006) who stated that the presence of transistion metal ions in honey makes water to be present negligibly in full strength honey. The honey from Bauchi State may have been peculiar in activity due to a low maltose, high protein and high iron content. The predominant pollen grain was *Mangifera indica* a plant known to possess antibacterial activity. Honey sample from Bauchi State was significantly higher in inhibitory activity than that of the control at 12.50% for all organisms. This agrees with Molan (2006) who stated that some honeys are more effective than antibiotics.

5.3.2 Minimum Inhibitory Concentration

The honey sample from Jigawa State showed an MIC of 12.50% for all organisms minus *S. typhi*, it needed the lowest concentration (0.78%) to inhibit *S. typhi*. The reason for the inhibitory activity against *S. typhi* shows that there may be an interaction between the honey and the organisms' defense mechanism that it required such a low quantity to inhibit the organism. Also the botanical origin of this plant might be responsible for the kind of activity observed. This agrees with Molan (2006) who stated that inhibition by osmotic effect of dilute solutions of honey obviously depends on the species of bacteria. The reason for the honey sample from Jigawa State's antibacterial activity may majorly be its osmotic effect. Honey sample from Oyo State showed that a high concentration (12.50%) of honey sample was required to inhibit the growth of all test organisms. This concentration was much lower than the MIC values recorded by Mandal *et al.* (2010) for *S. aureus* (25.00%), *P. aeruginosa* (35.00%) *and E. coli* (40.00%) for Nigilis honey. This honey required a higher concentration to be more inhibitory in action. It is therefore best to use this kind of honey *in-vitro* as more dilute concentrations will cause bacteria growth. Honey sample from Adamawa State

inhibited *B. cereus* (3.13%) and *S. aureus* (3.13%) at low concentration and others at high concentrations. This means that honey sample from Adamawa State can reduce the growth of *B. cereus* and *S. aureus.* The ability of honey sample from Adamawa State to inhibit both at high and low concentration agrees with Bogdanov (1997) who stated that different organisms react differently to honey. Honey sample from Plateau State inhibited the growth of all test organisms at low concentrations except for *P. aeruginosa* where it required 12.50%. This agrees with the report of Mandal *et al.* (2010) who stated that, *P. aeruginosa* is less susceptible to honey of similar anti-microbial activity compared to Staphylococci, *S. aureus* and Enterococcus species. The reason for the low concentration for most organisms might be due to its high sucrose content. All the organisms were inhibited differently; the reason for the difference in the organisms reaction might be accounted for by the pollen spectra of the honey samples, its physico-chemical parameters or there might have been an interaction between the honey, its components and the different bacteria defence mechanism which might be the reason for the different organism requiring different concentration(s) to inhibit their growth.

The honey sample from Bauchi State inhibited the growth of all the organisms at low concentration (1.56%) except for *E. coli* (6.25%) and *P. aeruginosa* (12.50%). The result of 12.50% obtained in this study was within the range also obtained by Mandal *et al.* (2010) for Manuka honey against *P. aeruginosa and E. coli.* Although in this study, honey sample from Bauchi had a lower MIC of 6.25% for *E. coli*. The MIC range for Bauchi honey was 1.56% - 12.50%. This range was higher than that recorded for *Eucryphia cordifolia* honey (3.10%-6.30%) (Mandal *et al.*, 2010) but similar to 0.60 -20% (Molan, 2006). Honey sample from Bauchi State had the lowest MIC observed among the honey from all States. The result affirms the agar well diffusion assay result that Bauchi honey shows more inhibitory activity

at lower concentrations. The behavior of Bauchi honey agrees with the observation of Molan (2006), that the major variation seen in overall antibacterial activity are due to variation in the level of hydrogen peroxide that arises in honey. The reason for *P. aeruginosa* needing a high concentration might be due to the organisms' resistance to honey and the production of biofilms by this organism.

5.3.3 Minimum Bactericidal Concentration

Honey sample from Adamawa State was the most bactericidal in anti-microbial activity of all the honey samples used. It killed three organisms namely *E. coli*, *B. cereus* and *P. aeruginosa*. Honey samples from Jigawa and Oyo States showed bactericidal activity. All organisms were killed except *S. typhi*. This agrees with the findings of Wahdan (1998) who stated that honey is more bactericidal than bacteriostatic. The honey from Bauchi, Kaduna, Ondo and Plateau did not show bactericidal activity. This shows that the ability of honey to inhibit the growth of an organism at a small concentration does not mean it can kill the organism entirely. The value gotten from this study was 12.50% which was within the range recorded by Mandal *et al.* (2010) (10.00%-40.00%) for *E. coli*, *S. aureus* and *P. aeruginosa.* No State honey killed *S. typhi* this means that these honeys can only reduce the population of these organism at the concentrations used.

5.4 CRITERIA FOR HONEY ACCEPTANCE

5.4.1 Motivators for Honey Purchase

Medical benefit was the major motivator for honey purchase in this study and this agrees with Vanyi *et al.* (2009) who stated that the comsumption of honey had increased due to the emergence of new diseases and Nanda *et al.* (2003) who stated that Indians major use of honey was for medical purposes. Only 8% of the respondents purchased honey for food consumption, this was the lowest

motivator for honey purchase. The reason might be because honey is not considered as a food. This agrees with the finding of Nanda *et al.* (2003) who stated that the consumption of honey as food in India is very low.

5.4.2 Factors Affecting Honey Purchase

Price ranked first for the criteria for honey purchase, like the law of demand, price affected the purchasing capability. The level of education did not affect the criteria for honey purchase, it might mean that both the educated and uneducated purchase honey for the same reason(s).

5.4.3 Relationships between Demographic Features and Honey Purchase

There was no relationship between the highest level of education and the quantity of honey bought. This shows that education does not affect the quantity bought but the income determines what quantity of honey would be bought by the respondents. The significant relationship observed for the level of income and the quantity of honey purchased might be because high income earners had more money available to purchase honey and preferred processed honey while low income earners purchased more comb honey. In Nigeria comb honey is usually sold by hawkers in the open or market places, this is more attractive to the low income earners and might account for the reason why they buy more of comb honey. The highest income earners bought 11 litres and above but not as high as those who earned ₦21,000-₦ 40,000, the reason might be due to other alternatives available and affordable by this group, though within this group they purchased 11L and above more. The trend of higher income earners preferring imported honey instead of Nigerian honey does not agree with Vanyi *et al.* (2009) whose respondents preferred their own country honey. The reason for this might be accounted for by Nigerian factor, that is, the show of class and not necessarily because of the anti-bacterial activity or botanical name of the imported honey. The reason for the significant relationship ($p<0.05$) in the opinion about honey price by the different levels of income earners may be that respondents who earned <=₦10,000-₦30,000 bought more

Nigerian honey which is cheaper than imported honey; while ₦31,000-₦40,000 earners considered honey expensive, the reason may be because they preferred to buy imported honey which is more expensive than Nigerian honey while ₦41,000-₦50,000 Naira earners considered honey to be very cheap and preferred imported honey. The reason might be that they have more money available to them compared to other low income earners. Generally respondents with no response were most likely not to be specific about most things.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

Pollen spectrum was consistent with the three vegetation zones. The issue of season was not emphasized because on the field, most beekeepers harvest honey once a year which covers both the dry and wet season as honey production is a prolonged process. With respect to apiculture, *Combretum* species, *Parkia biglobosa* and *Mangifera indica* honey may be produced in Sudan Savanna zone of Nigeria, *Pavetta* species in the Guinea zone and the grasses should be controlled in the forest zone for the production of honey. Beekeepers in these regions can take advantage of this by carrying their hives to these plants at their flowering period to increase their honey yield. Also the botanical origin of honey influenced the anti-microbial activity of honey.

The anti-microbial activity of honey differed; only Bauchi State's honey antibacterial activity increased upon dilution. Honey sample from Bauchi State was a broad spectrum anti-bacteria honey that could be used *in- vivo* at 1.37- 12.5% v/v to reduce the growth of *Escherichia coli, Bacillus cereus, Salmonella typhi, Pseudomonas aeruginosa and Staphylococus arueus* while honey samples from other States were better used *in-vitro* mostly at 100%. The honey sample from Adamawa and Plateau State were the only honey samples apart from the sample from Bauchi State at 12.5% that showed anti-microbial activity below 100% v/v concentration against *P.aeruginosa. Escherichia coli* was resistant to Cipro® but was susceptible to all honeys except the honey sample from Jigawa State. The response of the same organism to different honey samples varied and the response of different organisms to the same honey sample differed.

Therefore sensitivity test should be carried out for every honey to be used for the treatment of bacterial infections. Honey samples from Adamawa, Jigawa and Oyo States killed *E. coli, B. cereus* and *S. arueus* respectively at 12.5% v/v. None of the honey samples killed *S. typhi* or *P. aeruginosa*.

6.2 CONCLUSION

There was difference in the pollen content of honey samples. Most samples were from one plant majorly. *Pavetta* species according to literature was a new plant added to bee forage for bees in Kaduna State. There was a significant difference between the honey concentrations used and the zone of inhibition of each honey from the different States. There was a significant difference in the response of the organisms to the different honey samples and concentrations. There was a significant difference in the presence of vegetation on the activity of honey at some concentrations. There was a significant difference in moisture, ash, pH and acidity content of honey samples from different locations. There was a significant difference in the carbohydrate content of honey samples from different locations. There was a significant difference in the protein and hydroxymethylfurfural content of honey samples from different States. There was no significant difference in the fat and dextrin content of honey samples from different States. There was a significant relationship between; the quantity of honey purchased and income, between income and the price of honey among respondents. There was no significant relationship between the level of education and the criteria for honey purchase, the level of education and quantity of honey purchased.

6.3 RECOMMENDATIONS

1. For increased honey production bee keepers in the Guinea Savanna should maximize the use of *Pavetta* species; those in the Sudan zone can transfer their hives to *Combretum* species, *Parkia biglobosa* and *Mangifera indica* trees; while bee keepers from the Forest zone should not under estimate the importance of grasses like *chromolaena odorata* and *Tridax procumbence*; and plants such as *Elaeis guineensis*.
2. *Mangifera indica* honey can be used effectively *in- vivo* at ≤12.5%v/v concentration to inhibit the growth of *E. coli, B. cereus, S. arueus, S. typhi* and *P. aeruginosa*.
3. *Combretum* species honey can be used to kill *E. coli, B. cereus* and *S. arueus* at 12.5%v/v.
4. High income earners in Nigeria can patronize honeys produced in the country because they possess anti-bacterial activities (i.e can effectively reduce the population of *E. coli, B. cereus, S. arueus, S. typhi* and *P. aeruginosa*) comparatively more effective than some imported honeys.
5. The honey sample from Jigawa State should be confirmed free from lead poisioning before consumption.

REFERENCES

Abdulla, F. and Abdul-Aziz, M.A. (1998). The prophylactic and curative effect of cedar honey induced ulcers in rabbits. *The Second International Arab Apicultural Conference Amman,* 1: 26-31.

Abu-Tarboush, H.M., Al-Kahtani, H.A. and El- Sarrage, M.S. (1993). Floral type identification and quality evaluation of some honey types. *Food Chemistry,* 46: 13-17.

Adebiyi, F.M., Akpan, I., Obiajunwa, E.I. and Olaniyi, H. B. (2004). Chemical and physical characterization of Nigerian honey. *Pakistan Journal of Nutrition,* 3(5): 278-281.

Adebiyi, A.A. Personal Communication, 2010

Adebolu, T.T. (2005). Effect of natural honey on local isolates of diarrhea- causing bacteria in southwestern Nigeria. *African Journal of Biotechnology,* 4(10): 1172-1174.

Adriano, D.C. (1995). *Trace elements in the terrestrial environment*. Springer, New York. PP. 906-1382.

Agbagwa, O.E. and Frank-Peterside, N. (2010). Effect of raw commercial honeys from Nigeria on selected pathogenic bacteria. *African Journal of Microbiology Research,* 4: 1801-1803.

Agwu, C.O.C. and Njokuocha, R.C. (2004). Pollen analysis of honey and the biological effects of honey as rooting medium. *Nigerian Journal of Botany,* 17: 74-82.

Agwu, C.O.C. and Okeke, G.I. (1997). Pollen analytical and thin-layer chromatographic study of honey from three Savanna Zones of Northern Nigeria. *Nigerian Journal of Botany*, 10: 25-36.

Allen, K.L., Molan, P.C. and Reid, G.M. (1991). A survey of the antibacterial activity of some New Zealand honeys. *Journal of Pharmacy and Pharmacology*, 43: 817-822.

Al-Jabri, A.A. (2005). Honey, milk and antibiotics. *African Journal of Biotechnology,* 4 (13): 1580-1587.

Aljadi, A.M. and Yusoff, K.M. (2003). Isolation and Identification of Phenolic Acids in Malaysian honey with antibacterial properties. *Turkish Journal of Medical Sciences,* 33: 229-236.

Al-Mamary, M., Al-Meeri, A. and Al-Habori, M. (2002). Antioxidant activities and total phenolics of different types of honey. *Nutrition Research,* 22: 1041–1047.

Anupama, D., Bhat, K. K. and Sapna, V. K. (2003). Sensory and physicochemical properties of commercial samples of honey. *Food Research International,* 36 (2): 183–191.

Arbonnier, M. (2004). *Trees, shrubs and lianas of West African dry zones*. CIRAD- MARGRAF-MNHN. PP. 572.

Association of Official Analytical Chemists (AOAC) (1990). *Official Methods of Analysis*, 15th ed. Association of Official Analytical Chemists, Inc., Arlington. PP. 1025-1034.

Bagci, Y., Arslan, D., Oezcan, M.M. and Dursun, N. (2007). Determination of the mineral content of bee honeys produced in Middle Anatolia. *International Journal of Food Sciences and Nutrition,* 58(7): 567-575.

Bianchi, E.M. (1977). Honey: Its importance in children's nutrition. *American Bee Journal,* 117-733.

Bertoncelj, J., Dobersek, U., Jamnik, M. and Golob, T. (2007). Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food Chemistry,* 105: 822–828.

Bogdanov, S. (1997). Nature and origin of the antibacterial substances in honey. *Lebensm-Wissenschaft Und Technologie,* 17: 74-76.

Bogdanov, S., Ruoff, K., Persano, O. L. (2004) Physico-chemical methods for the characterisation of unifloral honeys: a review. *Apidologie,* 35: S4-S17.

Bonhevi, J.S. and Coll, F.V. (1993). Study of the carbohydrate fraction of the principal varieties of Tarragona hazelnuts (*Corylus avellana* L.). Food Chemistry, 46(3): 285-288.

Brady, N., Molan, P. and Bang, L. (2004). A survey of non-manuka New Zealand honeys for antibacterial and antifungal activities. *Journal Apiculture Research,* 43: 47-52.

Braide, W., Oranusi, S.U., Akaluka, C.K., Nwaoguikpe, R.N., Akobundu, C.I. and Ikechukwu, P. (2012). Antibacterial efficacy of crude and diluted honey on four wound isolates. *Global Advanced Research Journal of Microbiology,* 1(1): 1-4.

Brooks, J. and Shaw, G. (1968). Identity of Sporopollenin with older Kerogen and evidence for the possible biological source of chemical in scanning rock. *Nature,* 220: 678-679.

Buldini, P.L., Cavalli, S., Mevoli, A. and Sharma, J.L. (2001). Ion chromatographic and voltammetric determination of heavy and transition metals in honey. *Food Chemistry,* 73: 487-495.

Celechovska, O. and Vorlova, L. (2001). Groups of honey-physicochemical properties and heavy metals. *Acta Veterinary Borno,* 70: 91–95.

Chakraborty, A.K., Ramhade, S. and Patil, U.K. (2011). Chromolaena odorata (L.): An overview. *Journal of Pharmacy Research,* 4(3): 573-576.

Chinaka, C. C. (1995). *Beekeeping Technology for Farmers.* National Agricultural Research Project, (Ed. NAERLS). PP. 33.

Clinical and Laboratory Standards Institute (2007). *Performance standards for antimicrobial susceptibility testing: Seventeenth informational supplement*. CLSI document: M100-S17

Codex Alimentarius (2001). Codex Standard for Honey, FAO, Rome. *Alinorm,* 1: 19 – 26.

Cooper, R.A., Molan, P.C. and Harding, K.G. (2002). Honey and gram positive cocci of clinical significance in wounds. *Journal of Applied Microbiology,* 93: 857-863.

Crane, E. (1975). *History of honey* In Crane, E (ed.) Honey, a comprehensive survey, William Heinemann, London. PP. 439-488.

Crane, E. (1990). *Bees and Beekeeping: Science, Practice and World Resources*. Heinemann Newness, Oxford UK. PP. 614.

Devillers, J., Morlot, M., Pham-Delegue, M.H. and Dore, J.C. (2004): Classification of monofloral honeys based on their quality control data. *Food Chemistry,* 86: 305-312.

Diez, M.J., Andres, C. and Terrab, A. (2004). Physicochemical parameters and pollen analysis of Moroccan honeydew honeys. *International Journal of Food Science Technology,* 39: 167-176.

Dillon, H.C. and Ware, J.C. (1981). Sources and susceptibilities of Staphylococci isolated from children. *American Journal of Disabled Child,* 135: 427-430.

Downey, G., Hussey, K., Kelly, D.J., Walshe, T.F. and Martin, P.G. (2005). Preliminary contribution to the characterization of artisanal honey produced on the Island of Ireland by palynological and physicochemical data. *Food Chemistry,* 91: 347-354

Dustmann, J.H. (1979). Antibacterial effect of honey. *Apiacta,* 14: 7-11.

Ebenezer, I.O. and Olugbenga, M.T. (2010). Pollen characterization of honey samples from North Central Nigeria. *Journal of Biological Sciences,* 10(1): 43-47.

Efem, S.E. (1988). Clinical observations on the wound-healing properties of honey. *British Journal of Surgery,* 75: 679–681.

Erdtman, G. (1968). *Handbook of Palynology. An Introduction to the Study of Pollen Grain and Spore.* Hafner Publishing Co. Inc., New York. PP. 486.

Esti, M., Panli, G., Marconi, E. and Trivisonno, M. C. (1997). Valorization of the honeys from the Molise region through physico-chemical, organoleptic and nutritional assessment. *Food Chemistry,* 58: 125–128.

European Directive. (2001) Council of the European Union *Document relating to honey* (2001/110/CE). *Official Journal of the European Communities,* 12.1.2002: L10/47-L10/52.

Fadare, S.O. (2001). An Economic Analysis of Honey Productions in Kaduna State, Zaria. MSC Thesis. Department of Agricultural Economics and Rural Sociology Ahamadu Bello University, Zaria.

Flodhazi, G. (2004). Analysis and quantification of sugars in honey of different botanical origin using high performance liquid chromatography. *Acta Alimentatia*, 23(3): 299-311

Ghosh, G. K. and Ghosh, S. (1999). *Honey as food, Medicine, Cosmetics and Honey Recipes*. Sankar Prasad Nayak Printers. Dinendra Street Calculta, India.

Golob, T., Dobersek, U., Kump, P. and Necemer, M. (2005). Determination of trace and minor elements in Slovenian honey by total reflection X-ray fluorescence spectroscopy. *Food Chemistry,* 91: 593–600.

Hak-Gil, C., Myung-Kyoo, H. and Jae-Gil, K. (1988). The chemical composition of Korean honey. *Korean Food Science and Technology,* 20: 631-636.

http://bio.waikato.ac.nz/honey_intro.shtml. Retrieved on 5/17/2011.

International Bee Research Association, (IBRA) (1997). *The management of African honeybees including the design of low cost hives*, IBRA, UK. PP. 4 -14.

International Honey Commission, (IHC). (2002). Revised harmonized methods of the European honey commission. *Apidologie,* (Extra Issue): 1-59.

Irish, J., Blair, S. and Carter, D.A. (2011). The antibacterial activity of honey derived from Australian flora. *Plos One,* 6(3): e18229

Jones, G.D., Bryant, J., Goodman, D.K. and Clarke, R.T. (2001). *Alcohol dilution of honey*. 9th International Palynological Congress Houston PP. 453-458.

Keay, R.W.J. (1989). *Trees of Nigeria*. Clarendon Press, Oxford.

Keay, R.W.J., Onochie, C.F.A. and Stanfield, D.P. (1964). *Nigerian Trees Volume I and II*. Printed at the Nigerian National Press Limited and published by Department of Forest Research, Ibadan.

Kugonza, D. R. and Nabakabya, D. (2008). Honey quality as affected by handling, processing and marketing channels. *Tropiculture,* 26(2): 113-118.

Lawal, R.A., Lawal, A.K. and Adekalu, J.B. (2009). Physico-Chemical studies on adulteration of honey in Nigeria. *Pakistan Journal of Biological Sciences,* 12(15): 1080-1084.

Lopez, B., Latorre, M., Fernandez, M., Garcia, M., Garcia, S. and Herrero, C. (1996). Chemometric classification of honey according to their type based on quality control data. *Food Chemistry,* 55: 281-287.

Louveaux, J., Maurizio, A. and Vorwohl, G. (1970). Methods of melissopalynology. *Bee World,* 59: 139-157.

Louveaux, T., Maurizio, A. and Vorwohl, G. (1978). Methods of melissopalynology. *Bee World*, 59: 139-157.

Lurlina, M.O. and Fritz, R. (2005). Characterization of microorganisms in Argentinean honeys from different sources. *International Journal of Food Microbiology*, 105: 297-304.

Majno, G. (1975). *The Healing Hand: Man and Wound in the Ancient World*. First Havard University Press, United State of America. PP. 553.

Malika, N., Mohamed, F. and Chakib, E. (2005). Microbiological and physico - chemical properties of Moroccan honey. *International Journal of Agricultural Biology,* 7(5): 773- 76.

Mandal, S., Deb Mandal, M., Pal, N.K. and Saha, K. (2010). Antibacterial activity of honey against clinical isolates of Escherichia coli, *Pseudomonas aeruginosa* and *Salmonella enterica serovar typhi*. *Asian Pacific Journal of Tropical Medicine* (Accepted).

Mandal Deb, M. and Mandal, S. (2011). Honey: its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine,* 11: 154-160.

Mateo, R. and Bosch-Reig, F. (1998). Classification of Spainish unifloral honeys by discriminant analysis of electrical conductivity, colour, water content, sugars and pH. *Journal of Agricultural and Food Chemistry,* 46: 393-400.

Mavric, E., Wittmann, S., Barth, G. and Henle, T. (2008). Identification and quantification of methylglyoxal as the dominant antibacterial constituent of manuka (*Leptospermum scoparium*) honeys from New Zealand. *Molecular Nutrition and Food Research,* 52(4): 483-489.

Mbuya, L. P., Msanga, H.P., Ruffo, C.K., Birnie, A. and Tengnas, B. (1994). Useful Trees and Shrubs for Tanzania : Identification, Propagation, and Management for Agricultural and Pastoral Communities. Nairobi, Kenya: Regional Soil Conservation Unit, Swedish International Development Authority, Technical handbook (Nairobi, Kenya), no. 6.

Meda, A., Lamien, C.E., Romito, M., Millogo, J. and Nacoulma, O.G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Faso honeys as well as their radical scavenging activity. *Food Chemistry,* 91: 571–577.

Mohapatra, D.P., Thakur, V. and Brar, S.K. (2011). Antibacterial efficacy of raw and processed honey. *Biotechnology Research International,* 10: 4061-4066.

Molan, P.C. and Russell, K.M. (1988). Non-peroxide antibacterial activity in some New Zealand honeys. *Journal Apiculture Research,* 27: 62-67.

Molan, P.C. (1992). The antibacterial activity of honey. *Journal of Apicultural Research,* 216: 178-184.

Molan, P. C. (1999). The role of honey in the management of wounds. *Journal of Wound Care,* 8: 415–418.

Molan, P.C. (2001). Why Honey is Effective as a Medicine. *Bee World,* 82 (1): 22-40.

Molan, P. C. (2002). Re-introducing honey in the management of wounds and ulcers - theory and practice. *Ostomy and Wound Management,* 48(11): 28-40.

Molan, P.C. (2006). Using honey in wound care. *International Journal of Clinical Aromatherapy,* 3 (2): 21-24.

Moore, P.D. and Webb, J.A. (1978). *An illustrated Guide to Pollen Analysis*. 1st Edition, Hodder and Stoughton, London.

Morse, R. A. and Lisk, D. J. (1980). Elemental analysis of Honeys from several Nations. *American Bee Journal,* 120(7): 522-533.

Muli, E.M., Maingi, M. and Macharia, J. (2008). Anti-microbial Properties of Propolis and Honey from the Kenyan Stingless bee, Dactylurina Schimidti, *Apiacta,* 43: 49-61.

Mundo, M.A., Padilla-Zakour, O.I. and Worobo, R.W. (2004) Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. *International Journal of Food Microbiology,* 97: 1-8.

Musher, D.M. and McKenzie, S.O. (1977). Infections due to *Staphylococcus aureus*. *Medicine Baltimore,* 56: 383 `

Mutsaers, M. (1991). Bees in their natural environment in Southwestern Nigeria. *The Nigerian Field,* 56: 3-18.

Nagia, T., Inoue, R., Kanamori, N., Suzuki, N. and Nagashima, T. (2006). Characterization of honey from different floral sources, its functional properties and effects of honey species on storage of meat. *Food Chemistry*, 97 (2): 256-262.

Nanda, V., Sarkar, B. C., Sharma, H. K. and Bawa, A. S. (2003). Physico-chemical properties and estimation of mineral content in honey produced from different plants in Northern India. *Journal of Food Composition and Analysis,* 16: 613-619.

National Honey Board, *Honey, Health and Therapeutic Qualities.* Lashley Street Longmont, (2003), [http://www.nhb.org].

Nigerian Environmental Study Team. (1991). *Nigeria's Threatened Environment, A National Profile*. Intec Printers Limited Ibadan, Nigeria.

Nwali, L. (1996). Agric Panorama, Media Extension, NAERLS, Ahmadu Bello University, Zaria.

Nzeako, B.C. and Hamdi, J. (2000). Anti-microbial potential of honey on some microbial isolate. *Medical Sciences*, 2: 75-79.

Oddo, P.L. and Piro, R. (2004). Main European unifloral honeys: descriptive sheets. *Apidologie,* 35: S38-S81.

Oddo, P.L., Piazza, M.G., Sabatini, A.G. and Accorti, M. (1995). Characterization of unifloral honeys. *Apidologie,* 26: 453-465.

Odeyemi, A.T., Adefemi, S.O. and Adebayo, A.A. (2013). Anti-microbial and proximate properties of some processed honey in Ado-Ekiti. *International Journal of Aquatic Science,* 4(1): 36-43.

Ogunsanya, T.L, Rotimi, V.O. and Adenuga, A. (1994). A study of aetiological agents of children diarrhoea in Lagos, Nigeria. *Journal of Medical Microbiology,* 40: 10 – 14.

Omafuvbe, B.O. and Akanbi, O. O. (2009). Microbiological and physico-chemical properties of some commercial Nigerian honey. *African Journal of Microbiology Research,* 3(12): 891-896.

Osato, M.S., Reddy, S.G. and Graham, D.Y. (1999). Osmotic effect of honey on growth and viability of *Helicobacter pylori*. *Digestive Diseases and Sciences,* 44(3): 462-464.

Oztasan, N., Altinkaynak, K., Akcay, F., Gocer, F. and Dane, S. (2005). Effects of mad honey on blood glucose and lipid levels in rats with streptozocin-induced diabetes. *Turkish Journal of Vetenary and Animal Science,* 29: 1093–1096.

Paterson, P.D. (2006). *Beekeeping*. Macmillian Publishers Limited Towns Road, Oxford, UK. PP. 118.

Percival, M.S. (1965). *Floral Biology*. Pergamon Press Limited, Headington Hill Hall, Oxford 4 and 5 Fitzory Square, London. PP. 66 – 191.

Phuapradit, W.W. and Saropala, N. (1992). Topical application of honey in treatment of abdominal wound disruption. *Australian and New Zealand Journal of Obstetrics and Gynaecology,* 32 (4): 381–384.

Przybylowski, P. and Wilczynska A. (2001). Honey as an environmental marker. *Food Chemistry,* 74: 289-291.

Sawyer, R. (1988). Honey Identification. Cardiff Academic Press, Cardiff. PP. 350

Serrano, S., Villarejo, M., Espejo, R. and Jodral, M. (2004). Chemical and physical parameters of Andalusian honey: classification of Citrus and Eucalyptus honeys by discriminant analysis. *Food chemistry,* 87: 619–623.

Shinefield, H.R. (1976). *Staphylococcal infections.* In : Remington, J.S.and Klein, J.O. (Eds) Infectious disease of the fetus and newborn infant. Philadelphia, Saunders, WB and Co. PP. 979-1019.

Singh, N. and Bath, P.K. (1997). Quality evaluation of different types of Indian honey. *Food Chemistry,* 58: 129-133.

Sobukola, O. P., Adeniran, O. M., Odedairo, A. A. and Kajihausa, O. E. (2010). Heavy metal levels of some fruits and leafy vegetables from selected markets in Lagos, Nigeria. *African Journal of Food Science,* 4(6): 389 – 393.

Soria, A.C., Gonzalez, M., de Lorenzo, C., Martinez-Castro, I. and Sanz, J. (2004). Characterization of artisanal honeys from Madrid (Central Spain) on the basis of their melissopalynological, physicochemical and volatile composition data. *Food Chemistry,* 85: 121-130.

Sowunmi, M.A. (1976). The potential value of honey in paleopalynology and archaeology. *Review of Palaeobotany and Palynology,* 21: 171-185.

Sowunmi, M.A. (2001). Microscopic Analysis of Honey. *The Nigerian Fields,* 66: 125-133.

Taormina, P.J., Niemira, B.A. and Beuchat, L.R. (2001). Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. *International Journal of Food Microbiology,* 69: 217-225.

Terrab, A., Diez M. J. and Heredia, F. J. (2003). Palynological, Physicochemical and Colour Characterisation of Moroccan Honeys. I. River Red Gum (*Eucalyptus camaldulensis* Dehnl.) Honey. *International Journal of Food Science and Technology,* 38: 379-386.

Terrab, A., Recamales, A.F., Hernanz, D. and Heredia, F.J. (2004). Characterisation of Spanish thyme honeys by their physicochemical characteristics and mineral contents. *Food Chemistry,* 88 (4): 537-542.

Todar, K. (2005). Todar's Online *Textbook of Bacteriology*. URL:http//textbook of bacteriology.net/ Staph_5.html

Todar, K. (2009). Todar's Online *Textbook of Bacteriology*. URL:http//textbook of bacteriology.net

Vanyi, G. Á., Zsolt, C. and Laszlo, K. (2009). *Evaluation of consumers' honey purchase habits in Hungary*. Paper presented at the 113th EAAE seminar "A resilient European food industry and food chain in a challenging world" Crete, Greece. PP. 12.

Vikas, N., Sarkar, B.C., Sharma, H.K. and Bawa, A.S. (2003). Physico-chemical properties and estimation of mineral content in honeyproduced from different plants in Northern India. *Journal of Food Composition and Analysis,* 16(5): 613-619.

Von der ohe, W., Oddo, L.P., Piana, M.L., Morlot, M. and Martin, P. (2004). Hamonized methods of mellissopalynology. *Apidologie*, 35: S18-S25.

Vorlová, L. and Celechovská O. (2002). Activity of Trace Element Content in Bee Honey. *Acta Veternary Borno,* 71: 375–378.

Wahdan, H.A. (1998). Causes of the anti-microbial activity of honey. *Journal of Infection,* 26: 167-176.

Weston, R. J., Mitchell, K. R. and Allen, K. L. (1999). Antibacterial phenolic components of New Zealand manuka honey. *Food Chemistry,* 64: 295-301.

Weston, R.J. (2000). The contribution of catalase and other natural products to the antibacterial activity of honey: a review. *Food Chemistry,* 71: 235 - 239.

Weston, R.J., Brocklebank, L.K. and Yinrong, L. (2000). Identification and quantitative levels of antibacterial components of some New Zealand honeys. *Food Chemistry,* 70(4): 427-435.

White, A. and Brooks, G.F. (1977). *Furunculosis, Pyoderma and Impetigo*. In: Hoeprich PD (ed) Infectious diseases, 2nd edn. Hangerstown, Harper and Row. PP. 785-793.

White, J.W. and Doner, L.W. (1980). *Honey Composition and Properties*. In Beekeeping in the United States agriculture Handbook number 335: 82-91, Washington D.C., Science and Education, United State Department of Agiculture.

White, J.W., Riethof, M.L., Subers, M.H. and Kushnir, I. (1962). *Composition of American Honeys*. Technical Bulletin U.S. Department of Agriculture. NO. 1261, PP. 124.

Zet-Sche, B. (1932). Pollen grains. *Review of Paleobotany and Palynology,* 20: 151-160.

APPENDIX I

QUESTIONNAIRE

Department of Biological Science,
Faculty of Science
Ahmadu Bello University, Zaria

Dear Respondent,
I am a post graduate student of the Department of Biological Science, Faculty of Science, Ahmadu Bello University, Zaria. I am conducting a study on the consumer-acceptance of honey.

Your absolute honesty and sincerity will be appreciated, as all the information is for academic purpose and will be treated with utmost confidentiality.

Thank you.

Yours faithfully,

Ibegbulem Judith A.

BIODATA
QUESTIONNAIRE
PART A:

Instruction please tick as appropriate

1. Name: …….
2. Sex: (a) Male ( ) (b) Female ( )
3. Age: ( )
4. Nationality: (a) Nigerian ( ) (b) Others ( )
5. Tribe: ( )
6. Marital Status: (a) Single ( ) (b) Married ( ) (c) Widow/Widower ( )
7. Main Occupation: (a) (b) Secondary Occupation
8. What is your income per month…….
9. Highest education level: (a) Non formal
   (b) Quaranic
   (c) Primary
   (d) Secondary
   (e) Post secondary
10. Place of residence_____
11. House hold size/Number of children ( )

PART B

Please tick as appropriate

(1) What motivates your purchase of honey? (rank them)

Medical benefits _____ Dietary awareness _____ Religious belief _____

Food consumption _____

(2) What are your criteria when purchasing honey? (rank them 1 - 3)

Product quality_____ Price _____ Type of honey_____ Quality of packaging, _____ Colour_____ Flavor_____ Name of producer or origin _____

Thickness _____

(3) What kind of honey do you buy? (list at least 3 different kinds of honey by name that you most often buy) 1. _____, 2. _____ 3. _____

(4) How often do you buy honey and what quantity? (a) weekly _____litre (b) Monthly _____ litre (c) Occasionally _____ litre (d) Never _____litre

(5) If never, why?

_____

(6) Do you prefer imported honey to Nigerian honey?

_____

(7) Where do you buy honey?

_____

(8) What is your opinion about the price of honey available

(a)Generally cheap ( ) (b) Generally acceptable ( ) (c) Generally expensive ( ) (d) I do not know ( )

(9) What factors could increase the quantity of honey you purchase? (rank 1 - 2)

_____ Price discount _____ Emphasis on health life style _____ Improved quality of packaging _____ Increased availability

(10) In what form do you buy honey? (a) Strained (b) Crude/Comb honey (c) Table honey

(11) Which honey colour is more preferable by you for consumption

(a) Dark brown (b) Deep yellow (c) light yellow (d) others list_____

(12) What are the factors that govern the price of the honey in your locality? (rank)

(a) Season of the year (b) colour and taste of honey (c) distance from market (d) traditional ceremonies (e) Other

(13) How do you evaluate the local market price?

(a) High (b) Medium (c) Low

(14) For how long do you store honey? …….

a. I don't store.
b. One to Six months c. seven to twelve month
d. One year to two years e. more than two years.

(15) If your honey is granulated or crystallized, did you change it to viscous honey?
1. Yes 2. No

(15.1) If yes, what methods do you use? a. Direct heating using fire
b. Putting in boiled water c. Using sun light d. Others ----------

(16) How do you rate the quality of your honey?
a. by color b. by smelling c. by its odor
d. by testing e. by its thickness f. other method specify

APPENDIX II

PERFORMANCE OF MICROSCOPICAL EXAMINATION OF HONEY SAMPLES

The determination of geographical origin was based on the identification and counting of pollen grains and other particles in honey. Identification was made by reference to literature and to comparative preparations. Complete analysis which involved identifying, as far as possible, all pollen grains or other microscopic constituents in the sediment was carried out.

The degrees of accuracy in counting microscopic elements in the sediment are distinguished:

- Estimate: Count 100 pollen grains and honeydew elements corresponding to 100 grains.
- Determination of frequency classes: Count 200-300 pollen grains and the corresponding honeydew elements. If the pollen is from only a few species, it is sufficient to count 200 grains.
- Counts expressed in percentages: Presentation of frequencies as percentages is permissible only if 1200 pollen grains are counted. Make counts on two slides, prepared independently, from the same honey.

Note: Any pollen of wind-pollinated or nectarless plant was separated. Important wind-pollinated plants in this context are: Poaceae (wild grasses, cereals, Maize), Cyperaceae (sedges), *Rumex* sp. (sorrel), *Cannabis* (hemp), *Quercus* (oaks), Amaranthus (pigweed). Pollens of conifers *Abies*, *Pinus*, *Picea*, *Juniperus*, *Larix*, *Betula* sp. (birch), *Fagus* sp. (beech), *Carpinus* sp. (hornbean), *Populus* sp. (poplar), *Alnus* sp. (alder), *Corylus* sp. (hazel) are not important in honey, nor are pollens of *Urtica* sp. (nettles), *Typha* sp. (reedmace) or Juncaceae (rushes). The following plants are known to be nectar less but more or less entomophilous: *Papaver* sp. (poppies), *Plantago* sp. (plaintains), *Thalictrum* sp. (meadow-rues), Chenopodiaceae (goosefoot family), *Ambrosia* sp. (ragweeds), Artemisia sp. (worm- woods). The status of Cistaceae and *Filipendula* sp., which in general produce little or no nectar, is doubtful (Louveaux *et al*., 1978)

.

EXPRESSION OF RESULTS

Where pollen grains could be identified as far as the genus or species level, names were used. Otherwise, a note was added after the scientific name, to indicate that the term is used in a wider meaning, e.g. *Trifolium repens* s.l. (*sensu lato*), or *Trifolium repens* group (pollens which are identical with or very similar to *T. repens* in shape and morphological characteristics, but which may belong to another species e.g. *T. hybridum*, *T.resupinatum*, *T. arvense*). Here I used cf. Looks like. If detailed knowledge was not available, or if lack of time did not allow a closer identification, pollens were associated with larger groups (forms or types), e.g. *Teucrium*-form (a 3-colpate Lamiaceae pollen grain with opercula), or *Symphytum*-type (stephano- colporate Boraginaceae pollen grain) (Louveaux *et al*., 1978).

The examination under the microscope was carried out at magnifications of 400 to 1000. The relative frequencies of each pollen type was determined by identifying and counting pollen grains in groups of 100 following five parallel equidistant lines uniformly distributed from one edge of the cover slip to the other until 500 grains were counted. If 500 pollen grains were not sufficient for interpretation 1000 following another five parallel lines suituated between the first five were counted. In the case of honey with low pollen content a complete sequence of successive fields of vision was counted. The relative frequencies with respect to the total number of pollen grains counted were calculated for each pollen type. 500 to 100 pollen grains were counted for the determination of relative frequencies. For the determination of the botanical origin of the honey, the relative frequency excluding pollen from nectarless plants was recalculated (Von der Ohe *et al*., 2004).

PRESENTATION OF FREQUENCIES

The following terms were used for frequency classes: 'Predominant pollen' (more than 45% of the pollen grains counted); 'Secondary pollen' (16-45%); 'Important minor pollen' (3-15%); 'Minor pollen' (less than 3%). Where 1,200 or more pollen grains were counted, the frequencies were expressed in percentages with an accuracy of 1%. Pollens whose frequencies were less than 1% were quoted as "present" (Louveaux *et al*., 1978 ; Von der Ohe *et al*., 2004).

INTERPRETATION

Geographical Origin

Occasionally geographical origin can be established by the presence of characteristic pollens that are limited to a certain region. More often, the region in which the honey was produced can be

determined from the presence of certain pollen combinations (honey types). The pollen spectrum of a honey depends on the floral agricultural and forest conditions where it is produced. Microscopical data indicate the geographical provenance of honey rather than its country of origin (Louveaux *et al*., 1978).

Botanical Origin

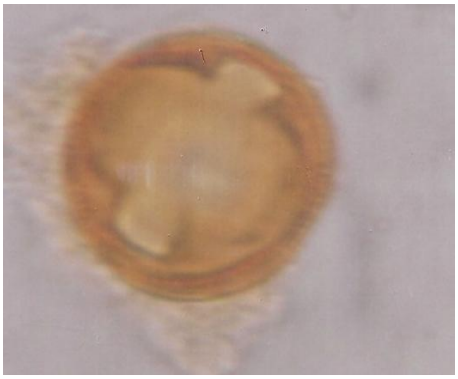
The extent to which a given honey sample is derived from different plant sources can be deduced from the frequencies of the pollens and honeydew elements in it. In general, honey has been produced mainly from one plant (unifloral honey) if the pollen of that plant is predominant: pollens of anemophilous and nectarless plants are excluded when calculating the percentages. This rule is valid only if the honey contains few honeydew elements (HDE/P <1). The percentage of pollens from nectarless plants is usually higher in honeydew honey than in flower honey (Louveaux *et al*., 1978).

Special case

The botanical origin of honeys which contain a high percentage of unknown pollen grains must be treated with reserve, because the degree of representation of these pollen grains will be unknown (Louveaux *et al*., 1978). Monofloral honey is derived from one particular plant source and may be more valuable than bifloral honey from two species of bee pollinated plants, heterofloral/multifloral honey also known as plurifloral honey and wild honey derived from the nectar of several plant species (Zet-Sche, 1932). No honey produced by bees flying free is entirely unifloral, unless the bees are reared in apiaries and exposed to only one particular species of bee pollinated plant (Terrab *et al*., 2003).

APPENDIX III

POLLEN MICROGRAPHS OF SOME PLANTS



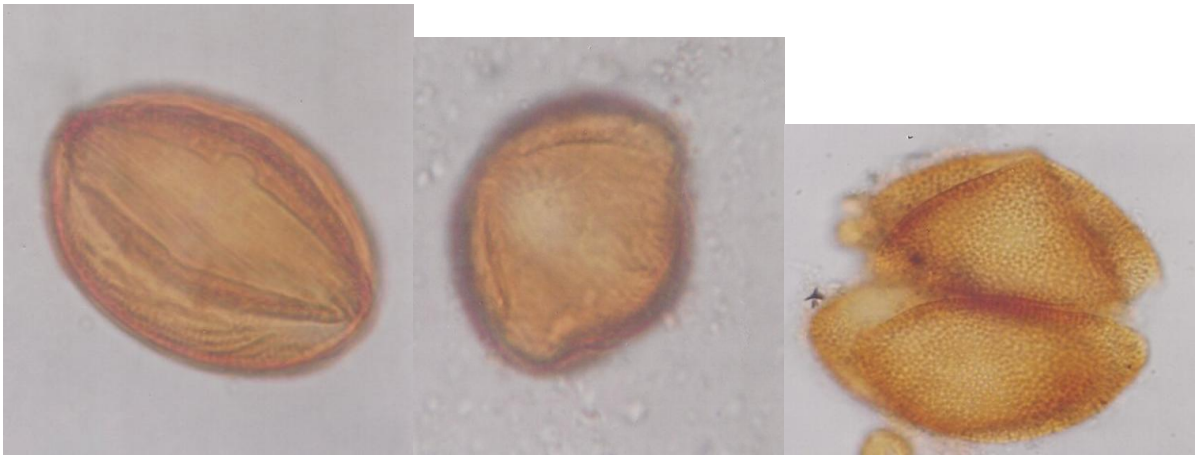
Mangifera indica



Thunbergia alata

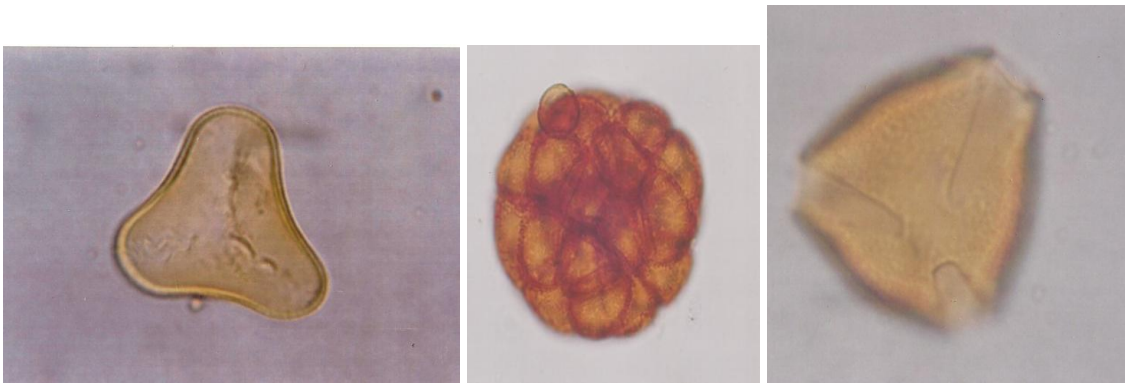


Poaceae

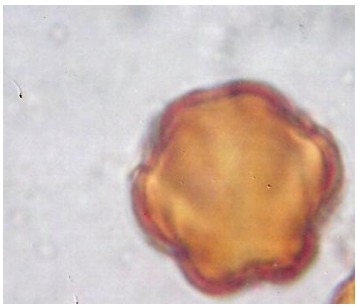


Berlinia grandiflora *Scorodophloeus zenkeri* *Anonidium manni*

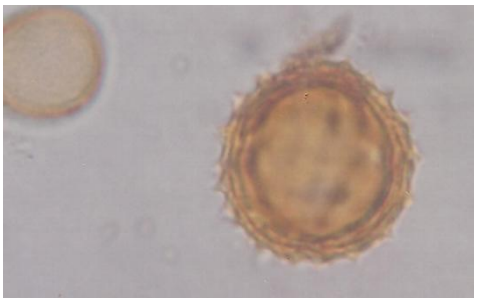
Plate 2a: Some of the Identified Pollen Types



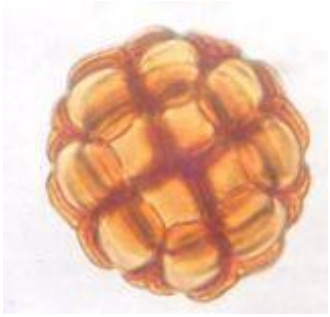
Elaeis guineensis *Parkia biglobosa* *Adenodolichos paniculatus*



Combretum sp.



Chromoleana odorata



Acacia dudgeoni

Plate 2b: Some of the Identified Pollen Types

APPENDIX IV

PLATE FOR CONTROL AGAINST TEST ORGANISMS



Plate 3: Zone of Inhibition of Ciprofloxacin on Test Organisms