EVALUATION OF ANTHELMINTIC ACTIVITIES OF*ACANTHUS MONTANUS*(ACANTHACEAE)LEAF EXTRACTS AGAINST EXPERIMENTAL *HELIGMOSOMOIDES BAKERI* INFECTION IN MICE

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FACULTY OF VETERINARY MEDICINE

AHMADU BELLO UNIVERSITY, ZARIA
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DEPARTMENT OF VETERINARY PARASITOLOGY AND ENTOMOLOGY
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

FEBRUARY, 2014

DECLARATION

I declare that the work in this Thesis entitled, “Evaluation of anthelmintic activities of *Acanthus montanus*leaf extracts against experimental *Heligmosomoidesbakeri* infection in mice”, has been carried out by me in the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.The information derived from literatures has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other institution.

David Omagbe OSHADU

……………………………………. ……………………….

Signature Date

CERTIFICATION

This thesis entitled, "EVALUATION OF ANTHELMINTIC ACTIVITIES OF *ACANTHUS MONTANUS* (ACANTHACEAE)LEAF EXTRACTS AGAINST EXPERIMENTAL *HELIGMOSOMOIDES BAKERI* INFECTION IN MICE" by David Omagbe OSHADUmeets theregulation governing the award of the degree ofMaster of Science degreeinVeterinary Parasitology ofAhmadu Bello University, Zaria, and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

I dedicate this Thesis to my beloved cousin **EDWARD OBIABO AGUDU** from whom I got the encouragement to enroll into a Masters' programme immediately after my undergraduate studies. Brother, I appreciate every encouragement, prayer, support and provisions both in cash and kind to me particularly in the course of my M.Sc. programme in Zaria. May the good Lord, the Rewarder of good deeds reward your kind gesture and generosity. Thank you.

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ABBREVIATIONS

ABZ	-	Albendazole
AQ	-	Aqueous portion (fraction)
BALB/c	-	Bagg albino/genotype c
BUT	-	Butanolportion (fraction)
BZs	-	Benzimidazole/probenzimidazoles
CEE	-	Crude ethanol extract
CHL(O)	-	Chloroform portion (fraction)
EPG	-	Egg per gram
FEC	-	Faecal egg count
H & E	-	Haematoxylin and Eosin
Hb	-	Haemoglobin
IL	-	Interleukins
LE	-	Leaf extract
MCC	-	Maximum convenient concentration
MCD	-	Maximum convenient dose
MCV	-	Maximum convenient volume
MLs	-	Macrocyclic lactones
PGE	-	Parasitic gastroenteritis
PI	-	Post infection
Rf	-	Retention factor/retardation factor/rate of flow
Th	-	Thymus-helper cells
TLC	-	Thin Layer Chromatography

ABSTRACT

*Acanthus montanus*Nees T. Anderson (Acanthaceae)has been employed in folk medicine for treatment of different kinds of ailment, but there is dearth of documented information on its folkloric and pharmacological activities against parasites.This study was designed to evaluate its anthelmintic property with the specific objectives of determining the phytochemical components, the maximum convenient concentrations (MCCs), maximum convenient doses (MCDs) and the lethal dose 50 (LD_{50}); as well as evaluate the activity of the different portions of the leaf extract of the plant.In this study, pulverized*Acanthus montanus*leafwas extracted and solvent-partitioned, yielding 25.58%, 31.42%, 11.58% and 3.00% weight by weight *(w/w)* of crude ethanol extract (CEE), aqueous (AQ), *n*-butanol (BUT) and chloroform (CHLO) portions respectively. All the portions excluding the chloroform portion were administered to worm-infected mice*per os* at dose rates of 1.2 g/kg, 1.4 g/kg, 1.7 g/kg and 2.0g/kg each for five successivedays and faecal samples collected for those days and analyzed. Mice were euthanized and the adult worm counted for rates of deparasitization. Analysis of variance was used to analyze the data obtained. In mice experimentally infected with *Heligmosomoidesbakeri,* the aqueousportion did not cause significant deparasitization even at the highest dose rate of 2.0 g/kg compared to the distilled water-treated control group. The crude ethanol extract caused significant ($p<0.05$)deparasitization rates of 64.44% and 72.35% at the 1.7 g/kg and 2.0 g/kg dose levels respectively. The *n*-butanolportion caused significant deparasitization rates at all the four dose levels compared to figures from distilled water-treated control mice as well as those from mice treated with the aqueous or crude ethanol portion. The deparasitization rates obtained in *n*-butanol-treated mice were 67.06%, 86.17%,

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TABLE OF CONTENTS

Page

Title Page…………………………………………………………………………………... ii
Declaration……………………………………………………………………………… iii
Certification…………………………………………………………………………….... iv
Dedication……………………………………………………………………………….. v
Acknowledgements……………………………………………………………………… vi
Abbreviations……………………………………………………………………………. viii
Abstract………………………………………………………………………………… ix
Table of Contents ……………………………………………………………………….. xi
List of Tables…………………………………………………………………………….. xvi
List of Figures………………………………………………………………………….... xvii
List of Plates……………………………………………………………………………… xviii
List of Appendices……………………………………………………………………….. xix

1.0 CHAPTER 1: INTRODUCTION --1

1.1 General Background to the Study --1

1.2 Statement of Research Problem --3

1.3 Justification ---4

1.4 Research Questions ---6

1.5 Research Hypothesis ---6

1.6 Aim and Objectives of the Study ---6

2.0 CHAPTER 2:LITERATURE REVIEW --8

2.1 Importance of Traditional and Ethno Veterinary Medicines ---------------------------8

2.2 Importance of Helminth Parasites --10

2.3 Synthetic Anthelmintics and Resistance --12

2.4 Some Plants Used as Anthelmintics and their Active Principles -------------------------14

2.5 The Plant Acanthus montanus --18
2.5.1 General characteristics ---18
2.5.2 Origin and geographical distribution of *A. montanus* --20
2.5.3 Scientific classification of *A. montanus* --20
2.5.4 Common names for *Acanthusmontanus*--21
2.5.5Vernacular names for *Acanthusmontanus* --21
2.5.6 Propagation and management of *Acanthusmontanus*--22
2.6 Ethnomedical Uses of *Acanthusmontanus* --23
2.7 Biological Activities of the Plant *Acanthusmontanus* -------------------------------------24
2.7.1 Cardiovascular diseases ---25
2.7.2 Respiratory diseases ---25
2.7.3 Gastrointestinal diseases --25
2.7.4 Inflammatory and infectious diseases ---26
2.7.5 Obstertics and gynaecology ---26
2.8 Pharmacological Activities of Extracts from *A. montanus* --------------------------------27
2.8.1 Antimicrobial, anti-inflammatory and immunological activity ---------------------------27
2.8.2 Analgesic activity --28
2.8.3 Antipyretic activity --28
2.8.4 Anti-abortifacient activity--29
2.8.5 Anticonvulsant activity ---29
2.8.6 Antispasmodic activity --30
2.8.7 Hepatoprotective activity --30
2.9 Helminthiasis in Livestock ---31
2.9.1 Aetiology --31
2.9.2 Clinical signs and pathology ---34
2.9.3 Diagnosis of gastrointestinal helminths ---35
2.9.4 Control of gastrointestinal parasites --39
2.9.4 Treatment --42
2.10 The Laboratory Mouse --45
2.11 Helminths of Rodents ---46

2.12 Effects of Intestinal Helminths in Laboratory Rodents ----------------------------------**47**

2.13*Heligmosomoidesbakeri*--48

2.13.1 Scientific classification ---51

2.13.2 Life cycle of *Heligmosomoidesbakeri* ---51

3.0 CHAPTER 3: MATERIALS AND METHODS --53

3.1 Research Design ---53

3.2 Plant Material Collection and Identification --53

3.3 Preparation and Preservation of Extracts --54

3.4 Qualitative Phytochemical Screening of Crude Ethanol Leaf Extracts of *Acanthus montanus* --56

3.4.1 Test for carbohydrates *(Molisch Test)* --56

3.4.2 Test for glycosides *(Ferric chloride Test)* --56

3.4.3 Test for free anthracene derivatives *(Bontrager's Test)* --------------------------------56

3.4.4 Test for combined anthracene derivatives *(Modified Bontrager's Test)* -------------------57

3.4.5 Test for unsaturated steroid and triterpenes*(Liebermann-Bucchard Test)* ----------------57

3.4.6 Salkowski test for unsaturated sterols --57

3.4.7 Test for cardiac glycoside *(Keller-kiliani Test)* --58

3.4.8 Test for saponin glycoside *(Frothing Test)* --58

3.4.9 Test for tannins *(Ferrric chloride Test)* --58

3.4.10 Test for flavonoids --59

3.4.11 Test for alkaloids ---59

3.5 Thin Layer Chromatography --60

3.6 Experimental Animals Used in Toxicity and Anthelmintic Studies ---------------------61

3.7 Determination of MCCs and MCDsof Extracts Used in Toxicity and Anthelmintic Studies ---63

3.8 Preliminary Acute Toxicity Studies --63

3.9 Postmortem Examination of Organs ---64

3.10 Deparasitization of Mice ---65

3.11 The Parasite *Heligmosomoidesbakeri*--65

3.12 Anthelmintic Screening (Study Design) ---66

3.12.1 Faecal sample collection, coprocultureand recovery of infective $L_3$of *H. bakeri* ------66

3.12.2 Enumeration of recovered L_3 of *H. bakeri* ---71

3.12.3 Experimemental infection of mice with *H. bakeri*L_3 --------------------------------------71

3.12.4 Treatment groups --71

3.12.5 Faecal egg counts --74

3.12.6 Postmortem worm count ---74

3.12.7 Anthelmintic Evaluation --75

3.13 Statistical Analysis --75

4.0 CHAPTER 4:RESULTS ---77

4.1 Extractive and Solvent-Partitioned Yield of Plant Material ------------------------------77

4.2 Solubility of Extracts in Water ---79

4.3 Maximum Convenient Concentrations (MCCs) (g/ml) and Maximum Convenient Doses (MCDs) (g/kg) of Different Portions of Leaf Extracts of *A. montanus*----------------79

4.4Phytoconstituents of *A. montanus*---81

4.5 Thin Layer Chromatography ---83

4.6 Toxicity Studies ---85

4.7 Postmortem Findings --87

4.7.1 Gross pathologic examination of organs --87

4.7.2 Histopathologic findings ---87

4.8Anthelmintic Effects of Various Doses of Leaf Extracts of *A. montanus* ----------------96

4.8.1 Effect of extracts on EPG of faeces --96

4.8.2 Effect of extracts on adult *H. bakeri* --104

5.0 CHAPTER 5: DISCUSSION --107

6.0 CHAPTER 6: CONCLUSIONANDRECOMMENDATIONS ------------------------117

6.1 Conclusion --117

6.2 Recommendations ---117

REFERENCES --119

APPENDICES ---141

LIST OF TABLES

Table 2.1: Summary of investigations on anthelmintic properties of some plants……………16

Table 2.2:Examplesofnematode parasites that parasitize different regions of the gastro-intestinal tract in domestic animals, humans and rodents…….………………………………33

Table 3.1:Summary of anthelmintic screening of the various portions of *A. montanus*in mice infected with *H. bakeri*..…………………………………………………………………...73

Table 4.1:Extract yield (% dry weight) from *A. montanus*leaf………………………………78

Table 4.2: Solubility, MCC (g/ml) and MCD (g/kg) of various portions of leaf extractof *A. montanus*….…………………………………………………………………………………...80

Table 4.3: Results of qualitative phytochemical screening of CEE of *A. montanus*…………..82

Table 4.4: Results of preliminary acute toxicity studies of CEE of *A.montanus in* mice………………………………………………………………………………………....86

Table 4.5: Mean (±SEM) EPG of mice infected with 150 L_3 of *H. bakeri*and orally treated for 5 consecutive days with varying doses of *A. montanus*leaf extracts, distilled water or albendazole 19 days post infection.…………………………………….…………………..101

Table 4.6: Daily and mean percentage (%) decrease in FEC/EPG in mice infected with 150 L_3 of *H. bakeri*and orally treated for 5 consecutive days with varying doses of *A. montanus*leaf extracts, distilled water or albendazole 19 days post infection………………………………103

Table 4.7: Mean (± SEM) worm count in mice infected with 150 L_3 of *H. bakeri*and orallytreated for 5 consecutive days with varying doses of *A. montanus*leaf extracts, distilled water or albendazole 19 days post infection………………………………………………..........105

Table 4.8: Percentage deparasitization (%DP) in mice infected with 150 L_3 of *H. bakeri*and orally treated for 5 consecutive days with varying doses of *A. montanus*leaf extracts, distilled water or albendazole 19 days post infection…………………………………………………106

LIST OF FIGURES

Figure 3.1 Flow chart of solvent partitioning of pulverized leaf of *A. montanus.*………...55

Figure 4.1: Pattern of egg per gram (EPG) of faeces in mice infected with 150 L_3 of *H. bakeri*and orally treated for 5 consecutive days with 1.2 g/kg of *A. montanus*leaf extracts, distilled water or albendazole 19 days post infection……………………………………................97

Figure 4.2: Pattern of egg per gram (EPG) of faeces in mice infected with 150 L_3 of *H. bakeri*and orally treated for 5 consecutive days with 1.4 g/kg of *A. montanus*leaf extracts, distilled water or albendazole 19 days post infection…………………………………….................98

Figure 4.3: Pattern of egg per gram (EPG) of faeces in mice infected with 150 L_3 of *H. bakeri*and orally treated for 5 consecutive days with 1.7 g/kg of *A. montanus*leaf extracts, distilled water or albendazole 19 days post infection…………………………………….................99

Figure 4.4: Pattern of egg per gram (EPG) of faeces in mice infected with 150 L_3 of *H. bakeri*and orally treated for 5 consecutive days 2.0 g/kg of *A. montanus*leaf extracts, distilled water or albendazole 19 days post infection……………………………………………………….100

LIST OF PLATES

Plate I:*Acanthus montanus*plantin the wild……………………………………………………19

Plate II: Adult *Heligmosomoidesbakeri*(male and female)…..50

Plate III: Mice housed in plastic cage……………….…………………………………………62

Plate IV: Egg of *H. bakeri*..…………………………………...……………………………68

Plate V: Infective (L_3) larvae of *H. bakeri*…………………………………………………69

Plate VI: Adult *H. bakeri*…………………………………………………………….......70

Plate VII: TLC Plates showing chromatogram of different portions of leaf extracts of *Acanthus montanus* under visible light………………………………………………………...............84

Plate VIII: Photomicrograph of a section of liver of a mouse exposed to distilled water at 5 ml/kg.……………………………………………………………………………………..88

Plate IX: Photomicrograph of a section of liver of a mouse after 48 hours' exposure to crude ethanol extract of *A. montanus*at 10,000 mg/kg….……………………………………………..89

PlateX: Photomicrograph of a section of liver of a mouse 3 weeks after exposure to crude ethanol extract of *A. montanus* at 10,000 mg/kg……………………………………………90

PlateXI: Photomicrograph of a section of the heart of a mouse exposed to distilled water 5 ml/kg……………………………………………………………………………………...91

Plate XII: Photomicrograph of a section of the heart of a mouse 48 hours after exposure to crude ethanol extract of *A. montanus* at 10,000 mg/kg……………………………………..92

Plate XIII: Photomicrograph of a section of the heart of a mouse 3 weeks after exposure to crude ethanol extract of *A. montanus* at 10,000 mg/kg……………………………………..93

Plate XIV: Photomicrograph of a section of the kidney of a mouse 3 weeks after exposure to crude ethanol extract of *A. montanus* at 10,000 mg/kg……………………………………94

Plate XV: Photomicrograph of a section of the intestine of a mouse 3 weeks after exposure to crude ethanol extract of *A. montanus* at 10,000 mg/kg……………………………………95

LIST OF APPENDICES

Appendix I: Effect of different portions of leaf extracts of *Acanthus montanus*on faecal egg count (FEC) of*Heligmosomoidesbakeri*……………………………………………….....141

Appendix II:Working formula for determination of egg per gram (EPG) of faeces ……..…142

Appendix III: Effect of different portions of leaf extracts of *Acanthus montanus*on adult *Heligmosomoidesbakeri*…………………………………………..…………………....143

Appendix IV: Necropsy result of preliminary acute toxicity studies oncrude ethanol leaf extract of *A. montanus*in mice……………………………………………………………144

Appendix V: Result of histopathology of preliminary acute toxicity studies on crude ethanol leaf extract of*A. montanus*in mice……………………………………………..................145

ABSTRACT

*Acanthus montanus*Nees T. Anderson (Acanthaceae) has been employed in folk medicine for treatment of different kinds of ailment, but there is dearth of documented information on its folkloric and pharmacological activities against parasites. This study was designed to evaluate its anthelmintic property with the specific objectives of determining the phytochemical components, the maximum convenient concentrations (MCCs), maximum convenient doses (MCDs) and the lethal dose 50 (LD_{50}); as well as evaluate the activity of the different portions of the leaf extract of the plant. In this study, pulverized *Acanthus montanus*leaf was extracted and solvent-partitioned, yielding 25.58%, 31.42%, 11.58% and 3.00% weight by weight *(w/w)* of crude ethanol extract (CEE), aqueous (AQ), *n*-butanol (BUT) and chloroform (CHLO) portions respectively. All the portions excluding the chloroform portion were administered to worm-infected mice *per os* at dose rates of 1.2 g/kg, 1.4 g/kg, 1.7 g/kg and 2.0g/kg each for five successive days and faecal samples collected for those days and analyzed. Mice were euthanized and the adult worm counted for rates of deparasitization. Analysis of variance was used to analyze the data obtained. In mice experimentally infected with *Heligmosomoidesbakeri,* the aqueousportion did not cause significant deparasitization even at the highest dose rate of 2.0 g/kg compared to the distilled water-treated control group. The crude ethanol extract caused significant ($p<0.05$) deparasitization rates of 64.44% and 72.35% at the 1.7 g/kg and 2.0 g/kg dose levels respectively. The *n*-butanol portion caused significant deparasitization rates at all the four dose levels compared to figures from distilled water-treated control mice as well as those from mice treated with the aqueous or crude ethanol portion. The deparasitization rates obtained in *n*-butanol-treated mice were 67.06%, 86.17%,

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CHAPTER ONE

INTRODUCTION

1.1 General Background to the Study

Livestock are an important and sometimes overlooked element of the livelihood strategies of the poor. As much as 70% of the rural poor depend on livestock to some degree. Livestock holdings are diverse and include cattle, goats, sheep, pigs, poultry, horses, camels, yaks, and llamas. An estimated 600 million poor people, including 150 million landless poor, own livestock (Delgado *et al.,* 1999; Thornton *et al.,* 2002; WRI, 2005).

Livestock are a crucial source of financial capital for the rural poor. For many, livestock ownership is the only form of savings available. In fact, for pastoralists and often for poor women, livestock are the most important tangible asset they own. Livestock provide a critical reserve against emergencies and decrease vulnerability to financial shocks from ill health, crop failures, and other risks. They yield direct benefits in the form of food, wool, or hides, and can raise farm productivity by providing manure and draught power (PPLPI, 2003).

In spite of the enormous benefits accruing from livestock; the livestock industry is still largely faced with a lot of challenges that bring about reduced production and economic losses. Parasitic diseases especially helminthiasis is being reported to cause serious setback in livestock production, bringing about immense reduction in yield, increase in cost of

production, and eventual colossal loss of the affected animals (Soulsby, 1982; Hammond *et al.,* 1997; Perry and Roudoph, 1999).

Treatment and control of intestinal nematodes in livestock is largely through systemic synthetic anthelmintics. Over the years, there have been reports of anthelmintic resistance (Kaplan, 2004), making helminth control difficult and challenging even with the seemingly potent drugs. Besides, there is threat of residues in animal products such as meat, milk and eggs, posing potential hazard to public health (Tsiboukis *et al.,* 2013). In most cases, the availability and affordability of these systemic medicaments to smallholder farmers and pastoralists is a major problem in many developing countries, bringing animal health to jeopardy. It is with these concerns in view and bid to attempt to overcome these helminth-related setbacks that this study was designed, to evaluate anthelmintic potential of *Acanthus montanus* which may serve as alternative source of helminth control.

The plant *Acanthus montanus* (Nees) T. Anderson (Acanthaceae) is a vigorously thinly branched ground cover perennial shrub with basal clusters of oblong to lance-shaped glossy, dark green leaves reaching up to 12 inches (30 cm) long (Huxley, 1992). This plant whose leaves have silver marks and wavy margins modified to spines grows up to 2 m tall and spikes of pale pink flowers appear during rainy season. This striking small shrub grow wild in grasslands, woods, scrub and rocky hills of the Balkans, Romania, Greece, Eastern Mediterranean and Africa (Huxley, 1992). It is known by different common names including “Bear’s breeches”, “Mountain thistle”, “Alligator plant” (Huxley, 1992) and “Thorny pigweed” (Sanganuwan, 2009).

Acanthus montanus is well known and called by different names by many ethnic groups in Nigeria. It is known as "Ahon Ekon" in Yoruba, "Agameebu" or "Agamsoso" in Ibo (Igoli *et al*., 2005; Sanganuwan, 2009). The Hausas call it "Gautar Fadama". In Benue State, the Igedes call it "Elele-nyijuo", "Idumngbe" in Etulo and "Shishaikyor", "Ityoukibua" or "Pevkyekye" in Tiv (Agishi, 2004).

1.2 Statement of Research Problem

Acanthus montanus has been employed in folk medicine for treatment of different kinds of ailments such as respiratory, gastrointestinal cardiovascular and urinary diseases (Igoli *et al.,* 2005). Noiarsa *et al.* (2010) reported that its leaves or whole plants are used in African countries for treatment of several ailments such as cough, gastritis, epilepsy, urinary disorders and rheumatic pains. In the Democratic Republic of Congo, the leaves are pounded in water with those of *Ananas comosus* and *Costus* spp and used to treat urogenital infections, urethral pains, endometritis, urinary diseases, cystitis and leucorrhoea (Didie, 2005). In Nigeria, the roots are also used for bathing to relieve aches and pains (Ibe and Nwofo, 2005). The root poultice is popularly used by the Igede people of Benue State and Enugu-Ezike community of Enugu State to treat furuncles (Igoli *et al*., 2005).

Positive results seem to be associated with the folkloric use of the leaves of this plant by the Etulo natives in Benue State, Nigeria, in the treatment and control of gastrointestinal worms in children and adults (Agishi, 2004; Oshadu, 2009). However, the anthelmintic efficacy of this plant has not been scientifically tested *in vivo*. Adamu *et al.* (2010) however, demonstrated that the aqueous leaf extract of *Acanthus montanus* produced dose-

dependent inhibition of egg-hatch and larvae of trichostrongylid nematodes of small ruminants *in vitro*. This suggests that the extract might possess some possible anthelmintic properties.

This study, evaluates the *in vivo* anthelmintic effect of the leaf extracts of the plant in an experimental infection of mice with *Heligmosomoides bakeri*, a laboratory model trichostrongyloid nematode, with the view to determining the fraction(s) with significant anthelmintic activity.

1.3 Justification

Helminths are a major cause of reduced production in livestock in many countries, particularly the tropics (Hammond *et al.*, 1997). Among the three main classes of helminths (Cestoda, Trematoda and Nematoda) that exist, the Class Nematoda contains the most pathogenic helminths of livestock and companion animals, hence a threat to successful and sustainable livestock production worldwide (Soulsby, 1982; Perry and Randolph, 1999). These helminth infestations cause direct and indirect losses. Direct losses are due to drop in production and death of animals, while indirect losses are due to cost incurred on control strategies such as cost of drugs, labour and drenching equipment. Other helminths-related setbacks include delay in achieving target weights, reduced quality of carcass and predisposition to other diseases (Soulsby, 1982; Kassai, 1999; Mc Gaw and Eloff, 2008).

Systemic anthelmintics have long been considered the most effective way of controlling helminth infestations, to minimize losses. However, the threats of anthelmintic resistance

and risk of residue in meat and milk are of concern. The availability and affordability of systemic anthelmintics to small holder farmers and pastoralists is a major problem in many developing countries. These setbacks justify the need for alternative control methods (Baker *et al.,* 1992, Wanyangu *et al.,* 1996; Waller, 1997). Options such as biological control (Chandrawathani *et al.,* 2003), vaccination (Bain, 1999) and the use of traditional medicine plants (Githiori, 2004) are being explored in different parts of the world. The screening and proper evaluation of medicinal plants could offer possible alternatives that may both be sustainable and environmentally acceptable (Eguale *et al.,* 2007).

In this research, *Heligmosomoides bakeri* of the Subfamily Heligmosominae, which is closely related to species of economic importance contained in the Subfamily Trichostrongylinae (Schnitzer and Hawking, 1963; Flynn, 1973) will be used to experimentally infect laboratory mice, *Mus musculus* (Schwiebert, 2007) to assess the *in vivo* anthelmintic efficacy of *Acanthus montanus*. *Heligmosomoides bakeri* has been used widely as a screening organism for evaluating anthelmintic properties (Dobson, 1961; Thompson *et al.,* 1962; Githiori *et al.,* 2003; Stepek *et al.,* 2005). It is also frequently used in studies of host-parasite relationships especially in immunology (Dobson, 1961, 1962; Lepak *et al.,* 1962; Parker and Inchley, 1990) and in tests of new anthelmintics (Melbey and Altman, 1974; Githiori *et al.*, 2003; Gradé *et al.,* 2008). *H. bakeri* is a convenient parasite to conduct experimental work because of its short and direct life cycle (Melbey and Altman, 1974).

1.4 Research Questions

This research is seeking to answer the following questions:

1. What are the major phytochemical constituents in *Acanthus montanus* leaf?

2. At what level (dose) is *A. montanus* leaf extract toxic to mice?

3. What are the effects of the leaf extracts of *A. montanus* on faecal egg output and adult worm count in mice experimentally infected with *Heligmosomoides bakeri*?

1.5 Research Hypothesis

Leaf extracts of *Acanthus montanus* have no modulatory effects on experimental *Heligmosomoides bakeri* infection in mice.

1.6 Aim and Objectives of the Study

The main aim of this study is to evaluate anthelmintic properties of *Acanthus montanus* leaf against experimental *Heligmosomoides bakeri* infection in mice.

The specific objectives therefore, include:

I. To determine the phytochemical constituents in the leaf of *Acanthus montanus.*

II. To determine the maximum convenient concentrations (MCCs), maximum convenient doses (MCDs) and LD_{50} of leaf extracts of *A. montanus* in mice.

III. To evaluate anthelmintic activity of leaf extracts of *A. montanus* in *H. bakeri.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Importance of Traditional and Ethno Veterinary Medicines

Medicinal plants are extensively used almost all over Africa for human and animal diseases (Lewis, 1981). Plants have sustained mankind not only as a source of food but also as medicine and poisons utilized in various forms for varied purposes (Abdu-Aguye, 1997). In a search to combat old and emerging diseases, plant medicines have given hope to man where many orthodox drugs have failed. Medicines of plant origin are said to be safer and better for human health than synthetic drugs since the ingredients in plants such as proteins, carbohydrates, lipids, vitamins and minerals are part of the body composition (Kilhan, 1999).

The World Health Organization (WHO) 1997, reviewed medical situation in many developing countries and suggested that locally available plants be utilized for drugs. A good number of herbal preparations have stood the test of time particularly for the treatment of the allergic, metabolic and degenerative diseases (Gupta, 1994). It has been estimated by World Health Organization that about 80% of the world's inhabitants rely mainly on traditional medicine for their primary health care needs while plants also play important roles in the health care system of the remaining 20% of the population who reside in the developed world (Hoareau and DaSilva, 1999).

Presently, focus on plant research is on the increase globally and substantial evidence has been collected to show the immense potentials of medicinal plants used in various traditional systems (Mbaya *et al.,* 2007; Adamu *et al.,* 2009). However, investigations on the risks associated with prolonged and improper use of these plant preparations reveal a potential for toxicities (WHO, 1997).

Ethnoveterinary medicine is the scientific term for traditional animal health. Research into Ethnoveterinary medicine is often undertaken as part of community-based approach that serves to improve animal health and provide basic veterinary services in rural areas (Lans *et al.,* 2007). Ethnoveterinary medicine considers that traditional practices of veterinary medicines are legitimate and seeks to validate them (Köhler-Rollefson and Brauing, 1998). Many non-Western traditions of veterinary medicines exist, such as acupuncture and herbal medicine in China, Tibetan veterinary medicine, Ayurveda in India, etc. These traditions have written records that go back thousands of years, for example the Jewish sources in the Old Testament and Talmud and the Sri Lankan 400-year old palm leaf fronds records of veterinary treatments. Since colonial times, scientists had always taken note of indigenous knowledge of animal health and diagnostic skills before implementing their Western technology projects (Köhler-Rollefson and Brauing, 1998). Stock owners continue to utilize ethnoveterinary medicine until better alternatives in terms of efficacy; low cost, availability and ease of administration are found.

Ethnoveterinary medicine and human ethnomedicine practices overlap in several parts of the world. Parallels between medicinal practices in human and animal ethonomedicine not only include the types of resources used and the prevalence of use of those wildlife

resources, but also in the modes of administration of those remedies and ethnomedical techniques employed (McCarkle and Martins, 1998; Souto *et al.,* 2011 a, b).

2.2 Importance of Helminth Parasites

Parasitic diseases remain a major constraint to livestock productivity across all agro-ecological zones and production systems in Africa, and gastrointestinal (GI) nematodes remain a major economic importance in domesticated livestock throughout the world (Prichard, 1994). Helminthiasis, especially parasitic gastroenteritis (PGE) constitutes a serious health problem to the productivity of small ruminants in Nigeria. PGE of small ruminants is a complex of diseases contributed to by many nematode parasites especially species of the genera *Haemonchus, Trichostrongylus, Oesophagostomum* and *Strongyloides* (Soulsby, 1982; Chiejina, 1986; Nwosu *et al.,* 1996). The disease is associated with enormous economic losses due to the associated morbidity, mortality and cost of treatment and other control measures (Schillhorn van Veen, 1973; Akerejola *et al.,* 1979; Chiejina, 1987).

Nematode infections not only cause clinical diseases and mortalities but also affect production. Some of these effects have been reported to include impairment of normal physiological behaviour of the animals and reduced feed intake and nitrogen retention leading to decreased efficiency of utilization of feed, resulting in decreased performance in terms of reduced growth rates by up to 30% or more, low fertility of ewes and cows, low birth weight, low weight gain of lambs and calves, reduced milk and wool production, and a decrease in the percentage of ewes rearing lambs to weaning (Provost, 1989; Meyer,

1991; Agei, 1993; Githigia *et al.,* 1995). These insidious effects of chronic helminthosis have important implications for attainment of maximum productivity in livestock (Chiezey, 1998).

Intestinal nematodes are extremely important pathogens of domestic livestock, especially of sheep, goats and cattle. Collectively, they are responsible for severe losses to livestock agriculture throughout the world. It has been calculated that, in the U.K., intestinal worms constitute the most important disease-related cost of farming sheep, being responsible for an estimated annual loss to the industry of £83 million (Nieuwhof and Bishop, 2005). In developing countries of the world, intestinal worm infections are perceived to be the single most important threat to economic success, as was made dramatically clear in a recent review of the attitudes and concerns of small holder farmers in Africa (Perry *et al.,* 2002).

Annually, a huge amount of money (300 million US dollar) is spent worldwide to combat helminth parasites in livestock (Cole, 2005). Schillhorn van Veen (1975) reported an annual loss of 14 million US dollars in Nigeria. Four years later, Akerejola *et al.,* (1979) reported a loss of over 60 million US dollars. In some other African countries, an estimated 700 million Ethiopian Birr (ETB) was lost due to helminths (Habte-Silasie *et al.,* 1991).

Intestinal nematodes are also important pathogens of humans, with a range of pathologies and consequences for human health (Bethony *et al.,* 2006). Four species dominate: *Ascaris lumbricoides*, *Trichuris trichiura* and the two hookworms, *Ancylostoma duodenale* and *Necator americanus*. Global estimates of disability adjusted life years lost to infection are

almost 5 million with some 3 billion people around the world believed to carry some of the species involved (Bethony *et al.,* 2006; Horton, 2003).

2.3 Synthetic Anthelmintics and Resistance

The treatment of intestinal nematode infections in the 21st century is largely through the use of modern synthetic anthelmintics. Three classes of these anthelmintics dominate the market, each mediating its effect through a different mode of action on the target nematodes. The group 1 anthelmintics include the benzimidazoles and these were introduced in the early 1960s for use in livestock but resistance (in this case to thiabendazole) was detected after only 4 years of usage in the U.S.A. The group 2 anthelmintics, the nicotinic acetylcholine agonists such as pyrantel, levamisole, morantel etc., were introduced in the early 1970s and resistance was detected for the first time in 1977 in Australia. The macrocyclic lactones (e.g. ivermectin), which form the group 3 anthelmintics, were first licensed for use in the early 1980s and resistance became apparent again within seven years and was first reported in South Africa (Kaplan, 2004).

Since then, resistance has spread around the globe, particularly in species affecting sheep, but it is also a significant problem in the husbandry of horses, especially with respect to the cyathostomins (Little *et al.,* 2003; Kaplan, 2004; Kaplan *et al.,* 2004), and is an increasing problem in cattle and pigs (Gerwent *et al.,* 2002; Suarez and Cristel, 2007). Of particular concern is the discovery of nematodes which are resistant to the three groups of anthelmintics, and cannot therefore be easily controlled by any of the three classes of drugs. This was first detected in South Africa in sheep, and then in Scotland among Angora goat flocks (Coles *et al.,* 1996), but is now known to be more widely distributed (Wrigley

et al., 2006). Nematotodes in this category include *Haemonchus contortus* and *Trichostrongylus* spp.

There are also some indications that human hookworms, notably *Necator americanus* and *Ancylostoma duodenale* are becoming less sensitive to the benzimidazoles and to pyrantel, respectively (De Clercq *et al.,* 1997; Reynoldson *et al.,* 1998; Flohr *et al.,* 2007). Studies in communities in Tanzania have shown that the efficacy of mebendazole has declined significantly in areas where the drug was given routinely to school aged children in a national programme to reduce gastrointestinal (GI) nematode infections in the population and to give the children a good start in life (Albonico *et al.,* 2002; Albonico *et al.,* 2003). Currently, there are a number of new programmes being implemented in developing countries based on mass treatment of populations with ivermectin or praziquantel, each combined either with mebendazole or albendazole, and these have already made a significant impact on the prevalence of intestinal parasitic infections in these regions (Savioli *et al.,* 2004; Hotez *et al.,* 2006; Hotez *et al.,* 2007). Nevertheless, veterinary parasitologists have repeatedly warned those working in the medical profession that where mass delivery of anthelmintics is being implemented, the lessons from the veterinary experience should be taken on board by the medical profession, so as to preserve the efficacy of the available drugs into the future (Coles, 1995; Geerts and Gryseels, 2000).

Although there are signs that novel synthetic drugs are being developed (e.g. nitazoxanide, cyclic depsipeptides, octadepsipeptides such as emodepside, tribendimidine, diketopiperazines such as paraherquamides, amino-acetonitrile derivatives {AADs}), no new drugs that operate through a different mode of action to the Class 1, 2 and 3

anthelmintics have become available on the market for the treatment of either livestock or humans (Geary *et al.,* 1999; Harder *et al.,* 2005; Xiao *et al.,* 2005; Cappello *et al.,* 2006; Kaminsky *et al.,* 2008). However, there is an exception for companion animals because emodepsides (a product of fungi associated with the leaves of *Camellia japonica*) are being marketed already for treatment of worms in cats (Bayer Health Care under the trade name Profender). Although it is now 32 years since the avermectins appeared, we are still largely constrained by having to rely on just three classes of anthelmintics. Thus, despite the promise of new agents in the pipeline, the range of effective agents is extremely limited. There is therefore a pressing need for new/additional solutions to the problem of controlling intestinal worm infections (Behnke *et al.,* 2008). The obvious route to follow is to develop novel natural drugs. The screening and proper evaluation of medicinal plants could offer this possible alternative that should be both sustainable and environmentally acceptable (Eguale *et al.,* 2007). Such drugs may be easily and cheaply available to the resource-poor farmers of the developing countries. Moreover, most of these potential medicinal plants are adapted to most climatic zones of the world and are within the range of most livestock farmers.

2.4 Some Plants Used as Anthelmintics and their Active Principles

Modern synthetic medicines are very effective in curing diseases but also causing a number of side effects. Crude drugs are less efficient for curing, but are relatively free from side effects (Mali and Mehta, 2008). A large number of medicinal plants are claimed to possess anthelmintic properties in traditional systems of medicine and are also utilized by ethnic groups worldwide. Following the folk claims, several medicinal plants have been

scrutinized for this activity using various *in vitro* and *in vivo* methods (Mali and Mehta, 2007). A summary of investigations on anthelmintic properties of plants is presented in Table 2.1.

Table 2.1: Summary of investigations on anthelmintic properties of some plants.

S/N	Plant species	Plant part(s)	Extract type	Active ingredient(s)	Activity spectrum	Reference(s)
1	*Ocimum sanctum* Linn.	Whole plant	-	Eugenol, β-caryophyllene	Antinematodal	Handa & Kapoor (1988); Asha *et al.* (2001);
2	*Piliostigma thonningii* Schum.	Stem bark	Ethanol, methanol	D-3-O-methylchiroinosol	Antinematodal	Asuzu & Onu (1994); Asuzu *et al.* (1999)
3	*Melia azedarach* Linn.	Fruit	Ethanol	-	Anticestodal, antiannelidal	Szwezuk *et al.* (2003)
4	*Punica granatum* Linn.	Stem bark	Alcohol	Alkaloids, pelletierine	Antinematodal	Prakash *et al.* (1980); Pradhan *et al.* (1992)
5	*Moghania vestita* Kuntze	Root tuber	Crude extract	-	Antinematodal	Yadav *et al.* (1992); Roy and Tandon (1996); Tadon *et al.* (1997)
6	*Mimosops elengi* Linn.	Stem bark	Crude alcoholic + ethyl acetate & *n*-butanol fractions	Phenolic compounds & tannins	Antiannelidal, antinematodal	Mali *et al.* (2007a); Thompson and Geary (1995)
7	*Calotropis procera* (Ait) R. Bri	Latex, flower	Aqueous	Latex	Antinematodal	Al-Qarawi *et al.* (2001); Iqbal *et al.* (2005); Shivkar and Kumar (2003)
8	*Neolarmarckia cadamba* (Roxb.) Bosser	Stem bark	Aqueous, ethanol	Triterpenoids, alkaloids, saponins	Antiannelidal, anticestodal, antinematodal	Kaushik and Dhiman (1999); Gunasekaran *et al.* (2006)
9	*Capparis deciduas* Edgew. *C. spinosa* Linn.	Root bark	Ethanol	Spermidine alkaloids	Antiannelida	Mali *et al.* (2004); Ahmed *et al.* (1992); Mali *et al.* (2005)
10	*Xylopia aethiopica* A. Rich	Seed	Crude methanol	Tannins, flavonoids, terpenoid	Antinematodal	Suleiman *et al.* (2005)
11	*Butea monosperma* (Lam.) Kuntze	Seed	-	Palasonin	Antinematodal, antitrematodal	Ramanan (1960); Garg and Mehta (1958); Raj and Kurup (1967); Rao *et al.* (1977); Sabir *et al.* (1977); Satyanaryarao

						& Krishnaiah (1982).
12	*Gynandropsis gynandra* (Linn.) Briq.	Leaf, stem	Methanol	-	Antitrematodal, anticestodal, antinnelidal	Ajaiyeoba *et al.* (2001);
13	*Evolvulus alsinoides* Linn.	Whole plant	Ethanol	-	Antiannelidal	Dash *et al.* (2002)
14	*Centratherum anthelminticum* Kuntze	Seed	Alcohol, aqueous, methanol	-	Antitrematodal, antinematodal, anticestodal,	Singh *et al.* (1985); Iqbal *et al.* (2006a)
15	*Carica papaya* Linn.	Seed	-	Benzyl isothiocyanate	Antinematodal	Dhar *et al.* (1965); Kumar *et al.* (1991); Sharma *et al.* (1987); Kermanshai *et al.* (2001)
16	*Nigella sativa* Linn.	Whole plant	-	Thymoquinone, dithymoquinone-cymene, α-pinene	Antiannelidal, anticestodal, antinematodal	Nadkarni (1960)
17	*Semecarpus anacardium* Linn. f.	Nut	-	Anacardic acid	-	Chattopadhyaya (1969)
18	*Piper longum*	Fruit	-	Essential oil	Antinematodal	D'Cruz *et al.* (1980)
19	*Conimiphora mukul* (Hook. ex stocks) Engl.	Oleo-gum resin	Alcohol	Essential oil	Antinematodal, anticestodal,	Kakrani and Kalyani (1984)
20	*Trachyspermuma mmi*	Seed	Crude aqueous, methanol	-	Antinematodal	Lateef *et al.* (2006); Jabbar *et al.* (2006)
21	*Ficus insipida* Willd	Latex	-	Ficin	Antinematodal, anticestodal	Phillips (1990); De Morin *et al.* (1999); Hansson *et al.* (2005)
22	*Cucurbita maxima* Duch.	Seed	Aqueous alcohol, ethereal	-	Antitrematodal, anticestodal, antinematodal	Srivastava and Singh (1967)
23	*Nicotiana tabacum* Linn.	Leaf	Crude aqueous, methanol	-	Antinematodal,	Iqbal *et al.* (2006b)
24	*Cleome icosandra* Linn.	Seed	Alcohol, aqueous	-	Antiannelidal, antinematodal	Mali *et al.* (2007b)
25	*Cannabis sativa* Linn.	Leaf	Crude extract	-	Antitrematodal	Roy and Tandon (1997)
26	*Trifolium repens* Linn.	Aerial shoot	-	-	Anticestodal	Tangpu *et al.* (2004)
27	*Strobilanthes discolor* T. Anders.	Leaf	Methanol	-	Anticestodal,	Tangpu *et al.* (2006)
28	*Acacia auriculaeformis* A. Cunn	Funicle		Acasiaside A&B (saponins)	Anticestodal	Sinha Babu (2005)

2.5 The Plant *Acanthus montanus*

2.5.1 General characteristics

Acanthus montanus (Nees) T. Anderson (Plate I) is a member of the Acanthaceae family (Hutchinson and Dalziel 1937; Burkill 1985). The genus *Acanthus* has about 30 species of flowering plants. The generic name is derived from the Greek word ακανθος (*acanthos*), meaning "thorny" (Umberto, 2000). It is known and variously called by different names in Nigeria and across the world. It is an erect, prickly perennial shrub that can grow up to 2 m tall (Plates I). The stem is stout woody and sparsely branching. Leaves are opposite, glossy and papery in texture, deeply pinnately-lobed, up to 20 cm long and 10 cm wide. The lobes have spines and the upper surface is glossy dark-green in colour. The inflorence consist of a cylindrical spike up to 20 cm long. Flowers are showy, pinkish-white with large bracts having spiny teeth. The upper bract is larger than the lower. Calyx is bilabiate, the upper being larger. The corolla is unilabiate, the upper one being rudimentary. The stamen is didynamous; anthers are unilocular with bearded margins; the base of the style is a hairy sheath. The fruit is a capsule, 2.5 cm long.

Plate I: *Acanthus montanus* plant in the wild.

2.5.2 Origin and geographical distribution of *A. montanus*

Acanthus montanus (Nees) T. Anderson is a native of West Africa and has been introduced to the rest of the world as an ornamental plant. A Consultant Botanist at the University of Lagos, Professor Toyin Ogundipe described the plant as a prickly semi-woody herb growing to nearly two metres height, of the high-forest in Dahomey to West Cameroons and Fernando Po, and to the Congo Basin and Angola. In Malaysia it has become feral and seems to colonize riverside areas in the North-west of Peninsular Malaysia. This

herbaceous perennial grow wild in the grasslands, woods, scrub and rocky hills of the Balkans, Romania, Greece, eastern Mediterranean, and Africa (Huxley, 1992). These plants, known as Bear's Breeches, need winter protection if grown in the North.

2.5.3 Scientific classification of *A. montanus*

According to Germplasm Resources Information Network (GRIN, 2009) *Acanthus montanus* can be scientifically classified as follows:

Kingdom: Plantae

(Unranked): Eudicots

(Unranked): Asterids

Order: Lamiales

Family: Acanthaceae

Tribe: Acantheae

Genus: *Acanthus*

Species: *A. montanus*

Binomial nomenclature: *Acanthus montanus*

2.5.4 Common names for *Acanthus montanus*

Acanthus montanus is known and called by the following common names: African mountain acanthus, Leopard’s tongue or False thistle (Akobundu and Agyakwa 1987; Etukudo 2003), Bear’s breech, Mountain thistle, Alligator plant and Thorny pigweed (Huxley, 1992; Sanganuwan, 2009).

2.5.5 Vernacular names for *Acanthus montanus*

Commonly called false thistle, *Acanthus montanus* belongs to the plant family Acanthaceae (Akobundu and Agyakwa 1987, Etukudo 2003). Malaysians call it “Jeruju”. In Africa, *A. montanus* is called by different names by various tribes and people groups. “Lidjeke”, “Metchieke” (Villi); “Digandou”, “Mpepelo” (Loumbou); “Mpinpelo” (Pygmees Ibongo); “Kpete pela” (Sierra Leone); “Bari tsari”, “Bali tsari” (Congo); “Bangambale” (Gabon); “Nsumelab” (Cameroon) (Umberto, 2000; Hecketsweiler and Ikonga, 1991).

In Nigeria, the Edos call it “agamobo”, Igbo (Asaba) “agameebu”, Igbo (Awka) “agameebu”, Igbo (Onitsha) “agameebu” or “aga” (needle), Igbo (Owerri) “agameebu”, “Ijo-Izon edule imemein” (leopard's claws), Yoruba “ahon ekun” (leopard's tongue), “ahon ekun dudu” (leopard's black tongue), “eekun-arugbo” (old man's knee). The Hausa in Northern Nigeria call it “gautan fadama” (Igoli *et al*., 2005; Okoli *et al.,* 2008). In Benue State, the Igedes call it “elele-nyijuo”, “idumngbe” in Etulo and “shishaikyȏ r”, “ityoukibua” or “pevkyekye” in Tiv (Agishi, 2004).

2.5.6 Propagation and management of *Acanthus montanus*

A. montanus is propagated by seeds and seedlings. Seed is sown at the soil surface, where a layer of mulch can be applied. It can be propagated from root cuttings taken in early spring (rainy season in Nigeria). Plants are evergreen in warm winter locations, but leaves will show considerable damage in colder winters near the northern edge of the growing range.

Plants can spread invasively by creeping rootstocks, particularly in loose soils. Unwanted spread can be addressed by root barriers

Acanthus plant requires the humid tropical climate to thrive. Moist and well drained soil is ideal for the plant. A mixture of compost will help to maintain soil water holding or draining capacity. A layer of mulch will help to maintain soil moisture and studies have shown that mulched plants grow faster than non-mulched plants.

Full sunlight is needed for the plant to assume its full potential. Many of these plants will do fine with a little less sunlight although they may not flower as heavily or their foliage as vibrant.

Young plants need extra phosphorus to encourage good root development. Annuals and perennials may be fertilized using:

(i) Water-soluble, quick release fertilizers;

(ii) Temperature controlled slow-releasing fertilizers; or

(iii) Organic fertilizers such as fish emulsion.

Water soluble fertilizers are generally used every two weeks during the growing season or per label instructions. Controlled, slow-releasing fertilizers are worked into the soil usually only once during the growing season or per label directions. For organic fertilizers such as fish emulsion, label directions should be followed as they may vary per product. For monthly application, water-soluble fertilizers should be continued through the end of summer (late rainy season in Nigeria)

2.6 Ethnomedical Uses of *Acanthus montanus*

Shoots, leaves, stem, stembark and roots of *Acanthus montanus* are used for various purposes. Documented uses of *Acanthus montanus* according to the “Useful Plants of West Africa” by Burkill (1985) include the leaves used in Southern Nigeria for the treatment of a sort of boil on the fingers called, mlelonko by the Igbos from Asaba. Similarly in Congo, the central portion of the twigs or the leaves is applied as a hot poultice to mature abcesses.

The plant has various medicinal uses in Nigeria, chiefly as a cough remedy medicine for women and children. Use against cough is recorded in Gabon and Congo either as a leaf-infusion or cooked with vegetable. In Cameroon, it is used for cough and chest complaints. A decoction of leafy-twigs is taken in Congo as a purgative. A leaf-macerate is given to children in Gabon as an emetic and fresh young growths are taken for heart-troubles. Also, the young shoots cooked with groundnuts or the kernel-butter of *Irvingia gabonensis* of family Ixonanthaceae are taken to settle upset-tummy and to counteract 'morning sickness' in pregnant women. The Ijaw of Southern Nigeria takes the pounded leaves cooked with pepper and salt to eat with fish for rheumatism. Men also chew the stems for this same purpose.

Diuretic action is claimed in Congo where the plant is pounded up with a stem of *Costus* and a young pineapple fruit and then soaked in palm wine. This is held to be a good remedy for urethral discharge. A shoot-macerate is used in Gabon for treatment for syphilis, and the leaf-spines are used to make scarifications in treating an area of rheumatic

pain, which precedes yaws. Some alkaloids have been detected in the leaves of Nigerian material.

The Yorubas of South-western Nigeria invoke the plant to rescue people attacked by witches. In Congo a few drops of sap from crushed leaves put on the eyebrows are believed to protect one from devils, and along with other plants a mixture is made for use in ceremonies of purification and exorcism.

2.7 Biological Activities of the Plant *Acanthus montanus*

A. montanus has been used in manners similar to *Acanthus illicifolius* Linn. and *Acanthus ebracteatus*. Many of the uses of this plant are almost similar to their uses by various societies in the continent of Africa. *A. montanus* have been reported to show activities against the following disease conditions:

2.7.1 Cardiovascular diseases

In various countries of West Africa such as Cameroon, Congo and Angola where this plant is indigenous, the leaves of *A. montanus* have been used in the treatment of hypertension and cardiac dysfunctions. Nigerians use the leaves while the Geviya people of Gabon make use of the young shoots eaten with salt to treat their heart diseases. Hypertension is treated by giving the patient a decoction of the leaves (Bagchi, 2008; Van der Veen and Bodinga-bwa-Bodinga, 2002; Igoli *et al.,* 2005).

2.7.2 Respiratory diseases

The leaves of *A. montanus* in the form of tea are used by the people of Gabon and those of South-eastern Nigeria to treat cough (Van der Veen and Bodinga-bwa-Bodinga, 2002). The Igede people of Nigeria advocate the use of the leaves and the bark of the stem for treatment of cough-related ailments (Igoli *et al.*, 2005).

2.7.3 Gastrointestinal diseases

The macerated leaves of *A. montanus* are used to induce vomiting in children amongst the Geviya tribe of Gabon. Women with stomach-ache and nausea are given young shoots cooked with peanut butter which is called "mo-dika" to relief pain (Van der Veen and Bodinga-bwa-Bodinga, 2002). Abdominal pains are relieved by drinking the decoction of the leaves. The leaves and stems had been used to ease the pains of acute gastritis and is believed to be an antacid (Iwu, 1993; Jiofack *et al.,* 2008, 2009). Leaves are also used in the treatement of hepatitis and hepatosplenomegaly in certain areas of Africa (Boullard, 2001).

2.7.4 Inflammatory and infectious diseases

Acanthus montanus is used in the treatment of inflammatory conditions by scarification using the thorns. This is done in similar manners by the Geviya people of Gabon (Van der Veen and Bodinga-bwa-Bodinga, 2002) and the people of Aguambu-Bamumbu of the Cameroon (Focho *et al.,* 2009). The same group of people makes use of parts of the plant to treat gonorrhoea and syphilis. In this case the macerated stems are given to the patient to

take. The roots are highly acclaimed by various societies in Africa as an effective remedy for abscesses. To the people of South-eastern Nigeria, the roots are macerated and applied over boils (Igoli *et al.,* 2005).

2.7.5 Obstertics and gynaecology

The people of Aguambu-Bamumbu of the Cameroon treat menstrual irregularities by taking the macerated leaves of *A. montanus* orally (Focho *et al.,* 2009). Those of the Upper Nyong valley forest in the Cameroon advocate the use of the leaves to treat dysmenorrhoea (Focho *et al.,* 2009). The traditional women of South-eastern Nigeria make use of the roots instead to treat dysmenorrhoea (Nwosu, 2000). In Cameroon, the leaf extracts had been used to treat cases of threatened abortion (Asongalem *et al.,* 2004).

2.8 Pharmacological Activities of Extracts from *A. montanus*

2.8.1 Antimicrobial, anti-inflammatory and immunological activity

Some African societies have used the leaves in the treatment of various inflammatory conditions. Asongalem *et al.* (2004) reported that the aqueous extract of the leaves indeed has significant anti-inflammatory activity. This is evidenced by the significant reduction in oedema induced by carrageenan (organic substance capable of inducing inflammation) within 30 minutes of application of the extract at a dose of 200 mg/kg (Asongalem *et al.,* 2004).

The root is popularly used in the treatment of boils in Nigeria. This has initiated Okoli *et al.* (2008) to conduct experiments into the antimicrobial, anti-inflammatory and possible

immunological properties of the roots of *A. montanus*. They found that the aqueous extracts of the roots (hot water maceration of the root powder) could inhibit the growth of *Pseudomonas aeruginosa* and *Streptococcus aureus* moderately. They also observed that this extract significantly reduced and suppressed artificially induced acute oedema in rat ears and paws and inhibited vascular permeability and haemolysis of red blood cells. Haematological studies showed an increase in total leukocyte and neutrophil counts and a significant dose-related increase in the macrophage population. There was also a significant increase in the phagocytic activity as evidenced by increase in the number of macrophages with ingested *Candida albicans.* It was thus concluded that the anti-inflammatory activity of the root extracts of the plant is due to the mobilization of leukocytes to the site of infection and the activation of phagocytic activity together with the suppression of exacerbated immune response by the constituents. These antimicrobial and anti-inflammatory activities are also a player in the mechanism of healing (Okoli *et al.,* 2008).

The methanol extract of leaves of *A. montanus* showed significant inhibitory effects on the growth of *Helicobacter pylori* (Ndip *et al.,* 2007). This could possibly substantiate the use of the leaves as an antiulcer treatment as practiced by some traditional healers in Africa (Igoli *et al.,* 2005; Okoli *et al.,* 2008).

2.8.2 Analgesic activity

Adeyemi *et al.* (2004) found that the methanolic extract of the leaves has analgesic effects which could possibly be both centrally and peripherally mediated (Adeyemi *et al.,* 2004).

Asongalem *et al.* (2004) reported similar findings using the aqueous extracts of the leaves, although the aqueous extracts did not show any centrally mediated analgesic properties.

2.8.3 Antipyretic activity

The leaves of *A. montanus* had been advocated in the treatment of fever in traditional medical practices by various communities globally. The aqueous extracts of the leaves was able to reduce fever within 6 hours at doses greater than 100 mg/kg (Asongalem *et al.*, 2004).

2.8.4 Anti-abortifacient activity

In Cameroon, the leaves of *A. montanus* have been used to treat threatened abortion. Asongalem *et al.* (2004) observed that even though the methanol/methylene chloride leaf extract of the plant caused abnormal foetal growth in rats within five days of gestation, the effects were reversed later on and there were no maternal or organ toxicity observed in the experimental rats. They also found that β-sitosterol was a major component in the extract and believed that this compound could be instrumental to the effects observed (Nana *et al.,* 2008).

The same investigators studied the effect of *A. montanus* aqueous extract on the estrous cycle pre- and post-implantation in rats and its mechanism of action. They found that irrespective of the dose this extract reversibly prolonged the metestrous and occasionally the diestrous stages of the estrous cycle. It did not alter the uterine wet weight or deciduoma count, suggesting a lack of estrogenic and progestational effects. At a dose of

1000 mg/kg/day it was observed that there were an appreciable pre-implantation losses of 36.8±6.5%, however, there were no post-implantation losses. They also noted a delay in foetal growth (Asongalem *et al.,* 2008).

2.8.5 Anticonvulsant activity

In the process of screening 6 medicinal plants for their anti-convulsant and sedative activities Bum *et al.* (2009) observed that *A. montanus* protected 66.6% of mice against maximal electroshock (MES), picrotoxin (PIC), and strychnine (STR)-induced convulsions and also protected 83.3% of mice from pentylenetetrazol (PTZ)-induced convulsions.

2.8.6 Antispasmodic activity

It was observed that the methanolic extract of the leaves of *A. montanus* was able to relax and inhibit the contraction of smooth muscles of the gut of rabbits and guinea pigs. This effect of the extract was not blocked by propranolol (3 x $10^{(-7)}$ M) but partially antagonised by phentolamine ($10^{(-6)}$ -3 x $10^{(-6)}$ M), procaine ($10^{(-3)}$ M) and methylene blue ($10^{(-5)}$ M) (Adeyemi *et al.,* 1999).

2.8.7 Hepatoprotective activity

The leaves of *A. montanus* have been used in the treatment of hepatitis and hepatosplenomegaly by the Africans. Patrick-Iwuanyanwu and Wegwu (2008) looked into this liver protective property of the leaves and stems. In the pre-treated group of rats it was observed that the aqueous extract of stem of *Acanthus montanus* showed a marked decrease in the levels of Serum L-alanine aminotransferase (L-ALT), L-aspartate amino

transferase (L-AST) and alkaline phosphatase (ALP) and the alcoholic extract of leaves showed the lowest in the level of lactate dehydrogenase (LDH). Total serum bilirubin also showed a remarkable decrease with aqueous and alcoholic extracts of the stem and leaf. Lipid peroxidation expressed by malondialdehyde (MDA) concentration was significantly reduced. Histopathological examinations of pre-treated rats showed significant improvements in the architecture of rat liver. All these results suggested that the aqueous and methanol extracts of the leaves and stems of *A. montanus* may prevent liver damage induced by CCl_4 in rats (Patrick-Iwuanyanwu and Wegwu, 2008).

2.9 Helminthiasis in Livestock

Prichard (1994) asserted that parasitic diseases remain a major constraint to livestock productivity across all agro-ecological zones and production systems in Africa, and gastrointestinal nematodes remain a major economic importance in domesticated livestock throughout the world.

Helminthiasis, especially parasitic gastroenteritis (PGE) constitutes a serious health problem to the productivity of small ruminants in Nigeria. PGE of small ruminants is a complex of diseases contributed to by many nematode parasites especially species of the genera *Haemonchus*, *Trichostrongylus*, *Oesophagostomum* and *Strongyloides* (Soulsby, 1982; Chiejina, 1986). The disease is associated with enormous economic losses due to the associated morbidity, mortality and cost of treatment and other control measures (Schillhorn van Veen, 1973; Akerejola *et al.,* 1979; Chiejina, 1987).

2.9.1 Aetiology

The term "internal parasites" represent many different types of organisms that live in animals, feeding on tissues or body fluids or competing directly for the animal's food (Pratt, 1985). These parasites have an amazing variety in size and appearance. There are four major groups of endoparasites- nematodes, acanthocephalans, platyhelminths, (trematodes and cestodes) and protozoans (Ballweber, 2001).

Blood and Studdert (1988) defined nematodiasis as a state of infection with nematodes. Nematode refers to roundworm or any individual organism of the Class Nematoda. Important genera in the Class are listed in Table 2.2.

Table 2.2: Examples of nematode parasites that parasitize different regions of the gastro-intestinal tract in domestic animals, humans and rodents.

HOST		SPECIES	
	Stomach	**Small intestine**	**Large intestine**
Sheep	*Teladorsagia circumcincta, Haemonchus contortus, Trichostrongylus axei*	*Trichostrongylus colubriformis, Nematodirus battus*	*Trichuris ovis*
Cattle	*Ostertagia ostertagi Trichostrongylus axei*	*Cooperia* spp, *Nematodirus spathiger*	*Oesophagostomum radiatum, Trichuris globulosa*
Pigs	*Hyostrongylus rubidus*	*Ascaris suum*	*Oesophagostomum* spp.
Humans		*Ancylostoma duodenale, Necator americanus, Ascaris lumbricoides*	*Trichuris trichiura, Enterobius vermicularis*
Rodents	*Protospirura*	*Heligmosomoides*	*Trichuris muris*

(rats/mice)	*muricola*	*bakeri, Nippostrongylus brasiliensis, Trichinella spiralis*	
Horses	*Habronema* spp, *Trichostrongylus axei, Draschia megastoma*	*Parascaris equorum*	*Oxyrus equi, Strongylus* spp/ Cyathostomes*, Strongyloides westeri*
Dogs		*Ancylosoma caninum, Uncinaria stenocephala, Toxacara canis, Toxasaris leonina*	*Trichuris vulpis*

Source: Behnke *et al.* (2008); Ballweber (2001).

2.9.2 Clinical signs and pathology

According to Ballweber (2001), the age most likely to acquire pathogenic numbers of parasites is the first year grazing calves, lambs and kids. Poor nutrition and other stress factors may contribute to clinical diseases in adult animals.

The general clinical signs mostly seen in helminthiasis in young animals during rainy season include diarrhoea, dehydration and weight loss (Ballweber, 2001) as well as oedema of lower limbs and emaciation (Lodha and Raisinghani, 1977). In sheep and goats, acute haemonchosis presents anaemia, dark faeces (melena) and submandibular oedema; while inappetence weight loss or decreased weight gain, anaemia and death due to depleted protein and iron stores are signs of the chronic phase. Pica is associated with haemonchosis and worm infection in camels (Sharma and Satija, 1974). Parasite may affect food utilization, decrease synthesis of protein in skeletal muscles and cause tissue damage, inflicting stress on

the animal. Haematological and clininico-pathological features of helminthiasis in animals include decreased packed cell volume (PCV), eosinophilia, hypoproteinaenia, hypoalbuminaemia, hyperglobunaemia, elevated blood urea, increased serum creatine phosphokinase and lactate dehydrogenase (Arzoun *et al.,* 1983; Qadir *et al.,* 2011; Bordoloi *et al.,* 2012).

Soulsby (1982) and Chiejina (1987) have reviewed the pathological changes which occur in animal suffering from parasitic gastro-enteritis. According to Soulsby (1982), both the adult and 4th stages of *Haemonchus* spp are pathogenic in the abomasum. The morphological changes noted during haemonchosis involve damage to the integrity of the abomasal mucosa which show haemorrhages at points of attachment of worms.

In general, the post-mortem findings in helminth infection are those of anaemia, haemorrhagic gastritis and include pale skin and mucous membranes, watery blood, hydrothorax, hydropericardium, ascites, cachexia, fragile and fatty liver, swollen abomasal mucosae with bile marks and ulcers and reddish brown fluidy ingesta (Soulsby, 1982).

According to Chiejina (1987), non-blood suckers such as *Trichostrongylus axei* in abomasums and *T. columbriformis* and *Cooperia* spp. in the small intestine cause essentially non-proliferative gastropathy, accompanied with catarrhal inflammation and epithelial ulceration. In such cases, post-mortem lesions seen include epithelial shedding and villous atrophy, cellular hyperplasia and metaplasia with the replacement of normal intestinal absorptive cells by immature and non-functional mucus-producing cells.

Oesophagostomum spp which are parasitic in the large intestine have been reported by

Soulsby (1982) to produce nodular lesions which may lead to ulceration with leakage of proteins and varying degrees of peritonitis as sequellae. However, this may vary between those due to blood suckers and other non-blood suckers.

2.9.3 Diagnosis of gastrointestinal helminth infections

According to Ballweber (2001), diagnosis of nematode parasites can be achieved at two levels –Antemortem and Postmortem.

2.9.3.1 *Antemortem diagnosis*

Clinical signs may give a clue for a tentative diagnosis. However, signs often vary and are non-specific. Knowing the grazing history and seasonal occurrence of parasitism in an area may be helpful. Egg detection in faeces provides a basis for confirmatory diagnosis. The eggs, with 16-32 cells- eggs of *Ostertagia*, *Teladorsagia*, *Haemonchus* and *Trichostrongylus* (as well as *Cooperia*, *Oesophagostomum* and *Chabertia*) - are very similar and difficult to differentiate, and are therefore, usually characterized as trichostrongyle type without being specific as to genus or species. Eggs of *Strongyloides* are smaller, each containing fully developed larva. Definitive diagnosis can only be made on identification of infective larvae recovered from coproculture. However, this is usually not performed by the practitioner.

3.9.3.2 Postmortem diagnosis

Diagnosis can be made at postmortem. However, because of their size, most of these

nematodes are missed on gross necropsy. *Haemonchus* is the largest nematode in this group (up to 3.0 cm in length), *Ostertagia* and *Teladorsagia* are mid-sized (up to 1.2 cm in length), and *Trichostrongylus* is the smallest up to 0.8 cm in length). Adult *Haemonchus* have white reproductive tract that spirals around the red (blood-filled) intestine giving a barber's pole appearance (hence the common name barberpoleworm"). The "Moroccan leather" appearance of the abomasum is pathognomonic for *Ostertagia* in cattle; petechial or ecchymotic haemorrhages in the abomasum may indicate *Haemonchus* in cattle, sheep and goats.

The use of a quick and efficient diagnostic procedure is necessary for a precise establishment of prevalence and exposure levels within a population and these are a prerequisite for accurate surveillance and control of parasitic gastro-enteritis in animals. According to Soulsby (1982), Chiejina (1987) and Blood *et al.* (1995), the traditional methods available for the diagnosis of the disease in animals include a good history, clinical examination of affected animals and conducting of appropriate laboratory examination such as faecal egg counts, carrying out faecal culture; identification of infective larvae as well as postmortem examination for pathological signs and total and differential worm counts.

In most field situations, adequate history of the animals and their management are almost always never available. The clinical signs presented during PGE are primarily dependent on the predominant species and the number of worms present in the animal. According to Chiejina (1987), none of these signs is pathognomonic for the disease and a variety of factors can modify the manifested signs. Therefore, only a small proportion of cases can be diagnosed based on clinical signs.

Visual appraisal of body weight and clinical evidence of parasitism is the definition for most situations. Assessment by faecal egg counts has limitations in all but very young animals, as egg counts are modified by immunity, the species present, the consistency of faeces, stages of maturity of the worms and the occurrence of the parturient rise. They do, however, give a direct measurement of the degree of contamination and they correlate sufficiently well with worm burden to allow their use as a diagnostic tool in young weaners (Blood *et al.,* 1995).

The total worm counts, which as with modern methods become an accurate and simple field technique, is superior to a faecal egg count as a measure of the severity of an infestation but does not take into account whether or not the worms are in a high contaminative phase. The method is often pre-empted by the unavailability of a sufficient number of guts for worm count. There should be at least two (Blood *et al.,* 1995). According to Blood *et al.* (1995), in a mixed strongyle infection, counts less than 2,000 adult worms are regarded as light while counts of 10,000-50,000 or more are severe and pathogenic depending on the species dominating. For *H. contortus* they recommended that total counts of more than 1,000 worms in sheep may be considered a heavy infection while counts of 3,000 in lambs and 9,000 in adult sheep usually lead to heavy mortalities.

In view of the obvious limitations of the above traditional diagnostic methods, Chiejina (1987) has used haematological examinations estimating PCV, Hb and RBC values as well as serum gastrin and pepsinogen levels in infected animals in the diagnosis of parasitic gastro-enteritis. However, on their own, these methods have only been partially successful and at best complementary to other diagnostic methods.

Immunodiagnostic techniques and serodiagnostic methods based on antibody assay have been tried especially during prepatent (acute) haemonchosis when eggs may be absent in faeces. However, these methods have had serious limitations in animals due to extensive cross-reactions between various nematode parasites and do not with certainty distinguish between previous and extant infections. Therefore, there is need for further studies to detect and further purify these antigens in order to determine those that could be useful in specific immunodiagnosis of disease due to each of the various nematodes and their larval stages involved in PGE (Thomas *et al.,* 1978; Meshgi and Hosseini 2007).

2.9.4 Control of gastrointestinal parasites

Due to the fact that livestock are often exposed to infected pasture, gastrointestinal helminth infection should be regarded as a normal course of infection since grazing animals are usually exposed to infection as long as there is pasture to graze.

The aim of control is therefore to establish a harmonious balance between the host and its worm burden rather than to produce a worm-free animal even when this is possible. Also, it is necessary to remember that the objective of a parasite control programme must be the achievement of maximum economic gain and this may not be synonymous with total control of infection. The objective may be different with different classes of stock.

Generally, control measures are aimed primarily at protecting the most susceptible group which is the young ones exposed to infection for the first time. Several methods have therefore been proposed for the control of gastrointestinal parasites in grazing animals (Reviewed in Brunden, 1980; Marley and Donald, 1980). However several factors including

the concentration of animals, their nutritional status and the epidemiology of the parasite in that environment influence which control method or combination of methods are adapted for use in any particular circumstance. According to Chiejina (1986), the most commonly used methods and which are of proven practical value are those based on grazing management and hygiene or strategic anthelmintic medication with or without some measure of grazing management.

2.9.4.1 Control by grazing management

According to Chieina (1986), general hygienic practices and adequate knowledge of the epizootiology and binomics of the parasite will contribute to the success of grazing management as a control method. However, since the use of the method entails the possession of limitless land which is not practicable under the predominantly nomadic system of livestock management practiced in Nigeria, Chiejina (1986) considers the method inapplicable in Nigeria.

Moreover, control of gastrointestinal helminths by pasture management became increasingly difficult as stocking densities is raised so that alternate means of control must be sought. Consequently, several modifications of rotational grazing have been advocated for the control of helminthes in animals. According to Chiejina (1986), adult resistant animals could be grazed before young susceptible stock with the expectation that the former would remove most of the infective larvae on pasture without themselves contributing significantly to the pasture contamination. However, since worm burden cannot be reduced to very low numbers, the aim of this method is to keep the burden at tolerable levels by the judicious use

of anthelmintics at strategic periods during the grazing year.

2.9.4.2 Control by strategic anthelmintic medication

This method describes a system whereby anthelmintics are administered at carefully selected periods in a grazing year. When the existing knowledge of the epizootiology of PGE in the area indicates that herd dosing will remove existing burdens thereby preventing pasture contamination and outbreaks of clinical disease. Effective use of this control method entails that the epizootiology of the worms involved in PGE in the area must be previously and adequately established (Chiejina, 1986).

New device of anthelmintic administration which makes the strategic movement of treated flock to new pastures unnecessary has been developed and found to be highly effective in controlling PGE; this device is an intra-ruminal implantation which slowly releases the anthelmintic morantel over a period of 90 days. Chiejina (1986) has suggested its possible usefulness in controlling PGE of livestock in the Nigerian savannah.

This control measure will achieve prevention of potentially dangerous number of eggs being passed on pasture and also remove existing worm burden before they affect the health of the herd.

2.9.4.3 Control by general hygiene

The aim of good hygiene measure as a way of control is geared towards avoidance of excessive localised contamination. Feeding and water troughs should be frequently moved and areas surrounding them kept cleaned and dry. Also, feeding and water troughs should be

mounted above ground levels on concrete slabs to avoid contamination of feed and water by faecal matter. Animals on range pasture should be denied access to marshes, wet areas around troughs and where surface water collects. These marshy areas should be denied or fenced up where practicable.

2.9.4.4 Use of vaccines

Current mathematical models on worm control have shown that a vaccine yielding 60% protection in 80% of the herd, or flock, would be a highly valuable control tool (Barnes *et al.,* 1995). Following the success of the irradiated larval vaccine against bovine lungworm, *Dictyocaulus viviparus,* a similar approach using irradiated *H. contortus* L_3 was found to consistently offer good protection in sheep older than six months (Gray, 1997). However, hopes for commercial production of an irradiated *H. contortus* vaccine disappeared when it became clear that high levels of protection could not be achieved in young lambs, and useful protection only developed if sheep were worm-free prior to vaccination (Gray, 1997; Bain, 1999). Current research on helminth vaccines has generally concentrated on the production of synthetic or recombinant vaccines using either natural or hidden (concealed) antigens (Schallig *et al.,* 1997; Smith *et al.*, 2003). A number of reviews have been published on possible recombinant vaccines developed against nematode parasites of ruminants (Smith, 1999; Knox and Smith, 2001; Claerebout *et al.,* 2003; Dalton *et al.*, 2003; Meeusen and Piedrafita, 2003; Newton and Meeusen, 2003). Irrespective of the progress that has been made, and the promise for the future, it will take a long time before these vaccines have a place in control of *H. contortus* in the flocks of pastoralists and small holder farmers

(Githiori, 2004).

2.9.4 Treatment

Protective dosing with anthelmintics has become an important part of most preventive programmes against clinical or subclinical parasitic diseases. Two classes of treatment are usually programmed: strategic treatments are carried out at the same time each year, or at the same stage in the management programme, with the specific purpose of reducing contamination. Tactical treatments are added to the strategic programme, particularly in pastured animals, to abort outbreaks when abnormal climatic or nutritional conditions arise (Blood *et al.,* 1995). A good deal of controversy arises on the question of how frequently and when strategic and tactical treatments need be applied.

Most naturally acquired helminth infection are multiple, the incidence of individual species are rare. The emphasis in treatment therefore is to use broad spectrum anthelmintics rather than those with narrow spectrum of activity. The uses of broad spectrum anthelmintics, which remove parasites in different stages of development within the host species, are the cornerstone of parasite control in GI nematode infections (Githiori, 2004). Although recommendations on the choice of drugs for each helminth species are made, it is not possible to lay down rigid rules of their order of preference. The range of parasites to be controlled may affect the selection, as may cost, the severity of the infection and the physical status of the animals relative to possible toxic effects of the drug. In an effort to standardize dose recommendations, the most favoured calibration of mg/kg body weight and the dose rates by the manufacturers are more generally to be preferred (Blood *et al*., 1995).

Because a diagnosis of parasitic disease is a diagnosis of a herd or flock problem, it is customary to treat all animals in the group to reduce environmental contamination to the minimum. It is necessary in many instances to repeat the treatment after several weeks to remove recently matured worms which were in an immature or more resistant stage at the first treatment. Modern anthelmintics appear to remove such stages. The assessment of treatment used to be measured in terms of changes in egg counts or total worm burdens. It is becoming more common to accept a level of infection that does not interfere with economic performance. It then becomes rational to assess the result of treatment in terms of increased productivity relative to the value of treatment (Blood *et al.*, 1995). Anthelmintic resistance has been reported from a number of areas and this may be attributed to the use of an insufficient dose or incorrect anthelmintics (Blood *et al.,* 1995).

The major classes of synthetic anthelmintics used for control of GI nematode parasites of ruminant livestock are:

• The benzimidazole/probenzimidazoles (BZs) group. The mode of action of BZs is by interference with polymerization of microtubules (Harder, 2002). These drugs bind to the protein tubulin of the parasite, therefore causing death by starvation (Roos, 1997). Examples of anthelmintics in this group include albendazole, mebendazole, thiabendazole, fenbedazole, and flubendazole.

• The tetrahydropyrimidines/imidazothiazoles group. These drugs affect acetylcholine neuro-transmission by interfering with nicotinic acetylcholine receptors (Roos, 1997; Harder, 2002). Examples of such drugs include levamisole, pyrantel, morantel and oxantel.

• The macrocyclic lactones (MLs) or avermectins/milbemycins group. The MLs are thought to interact with chloride channels on helminth gamma-aminobutyric acid (GABA) receptor complexes, and also inhibit pharyngeal pumping (and hence feeding), motility and fecundity in susceptible nematodes, resulting in paralysis and ultimately elimination from the host (Harder, 2002; Yates *et al.,* 2003). Examples of drugs in this category include ivermectin, abamectin, doramectin, eprinomectin, selamectin, milbemycin oxime and moxidectin.

There are other anthelmintics referred to as narrow spectrum compounds, which have activity against fewer species of parasites and/or lack high levels of efficacy against all stages of the parasites (Bowman *et al.,* 2003). Examples of these anthelmintics include naphthalophos, salicylanilides and substituted phenols (closantel, oxyclozanide and nitroxynil), and triclabendazole.

2.10 The Laboratory Mouse

BALB/c Mouse, *Mus musculus* is derived from albino mice stocks originally disseminated by Bagg (in 1913) to Snell in 1932. It has an albino coat with genotype A, b, c. The BALB/c mouse develop splasmacytomas upon injection with mineral oil, which form the mouse cell component of the mouse-human hybrid cells that are used in the production of monoclonal antibodies. This strain exhibits excellent breeding performance and a long lifespan which contribute to its being one of the most highly utilized of all available mouse strains (Medical Dictionary on-line).

The advantages of mice as research animals are many. Their genetic characterization, the large number of strains available, and the large list of catalogued mutant genes provide

animals suited for a number of different areas of research. Mice are easy to care for and handle, and are relatively inexpensive compared to other species. A high reproductive performance with a large litter size and a short gestation means that many generations can be produced in a relatively short period of time (one million descendants after 425 days!). The disadvantages of mice as research animals include their small size, which limits the procedures that may be performed as well as the sample volume size that can be obtained from an individual animal. To overcome the latter limitation, samples from several animals may be pooled for research analysis and statistical significance (Schwiebert, 2007).

The use of the mouse as a research animal has resulted in many scientific advancements. Much of our early understanding of the immune system was derived from studies done in the mouse. The use of the mouse continues to be an important part of various research endeavours including aging, embryology, cancer induction, pharmacological and toxicological testing, and infectious diseases research. Transgenic and knockout mice have become important tools for investigating the relationship of genetic make-up to disease states as well as elucidating pathways of normal mammalian development (Schwiebert, 2007).

2.11 Helminths of Rodents

Studies conducted by Pulido-Flores *et al.* (2005) in Mexico among forty-two rodents, representing 3 murid species (*Mus musculus*, *Peromyscus maniculatus*, and *Rattus rattus*), revealed that 13 species of helminths were present. These include 10 helminths in nematode taxa (*Syphacia obvelata*, *Syphacia muris*, *Syphacia peromysci*, *Aspiculuris* sp., *Gongylonema* sp., *Trichuris muris*, *Trichinella* sp., *Nippostrongylus brasiliensis*, *Capillaria*

gastrica, and *Carolinensis carolinensis*) and 3 cestode taxa (*Rodentolepis nana*, *Taenia taeniaeformis*, and *Taenia* sp.). *Nippostrongylus brasiliensis* was most prevalent. *Aspiculuris* sp. was the most intense and abundant, followed by *R. nana*.

2.12 Effects of Intestinal Helminths in Laboratory Rodents

The most common endo-parasites of rodents include *Hymenolepis nana*, *Taenia taeniaformis*, *Entrobius* sppand *Trichuris* spp. (Rafique *et al.,* 2009). These are mostly observed in *Rattus rattus*, *R. norvegicus* and *Mus musculus.* Others include *Rictularia* spp. *Vampirolepis* spp. *Protospirura* spp.

In experimental animal models, the pathology associated with infection is characterized by villus atrophy, crypt hyperplasia, goblet cell hyperplasia and infiltration of the mucosa by a variety of inflammatory cells, of which eosinophils and mast cells are prominent (Manson-Smith *et al.,* 1979; Wang *et al.,* 1990 and Garside *et al.,* 2000). A requirement for gross intestinal pathology in the expulsion of nematodes has been widely accepted not only because the two phenomena are usually coincident in immunologically normal hosts, but it has been shown in concurrent infections that the expulsion of one species of nematode can bring about the loss of antigenically unrelated species (Behnke *et al.,* 1992). It is also true that, in concurrent infections, *H. polygyrus*, which is known to down regulate intestinal changes, can suppress the expulsion of *T. spiralis* (Behnke *et al.,* 1978; Dehlawi *et al.,* 1987). Two explanations for the association between expulsion and pathology have been proposed. First, those pathological changes create an unfavourable environment for the parasites, which are then forced from their preferred niches, or that the local inflammatory

response (through increased permeability) results in increased exposure to components of the systemic immune system (Wakelin, 1978). Studies in nude, thymectomized or cyclosporin A-treated mice have established the T-cell dependency of parasite expulsion and much of the accompanying intestinal pathology (Manson-Smith *et al.,* 1979; Wang *et al.,* 1990). Second, cell transfer studies further demonstrated that protection against gastrointestinal nematodes is mediated by $CD4^+$ T helper cells (Grencis *et al.,* 1985; Riedlinger *et al.,* 1986). More recently, protective responses have been associated with production of Th2 cytokines, including IL-3, IL-4, IL-5, IL-9 and IL-13 and antiparasite IgE and IgG1 antibody responses (Grencis *et al.,* 1991; Else *et al.,* 1994). Th1-mediated events appear unimportant or antagonistic in terms of protection (Urban Jr. *et al.,* 1993; Else *et al.,* 1994; Ishikawa *et al.,* 1998).

2.13 *Heligmosomoides bakeri*

A popular mouse trichostrongyle for exploring the host-parasite relationship of gastrointestinal nematodes is commonly known as *Heligmosomoides polygyrus bakeri*. Recently, this parasite was raised to full species level as *H. bakeri*, to distinguish it from a close relative, *H. polygyrus sensu stricto*, the dominant intestinal nematode of wood mice (*Apodmus* spp) in Western Europe, which is unable to infect laboratory mice (*Mus* sp.) without the aid of powerful immunosuppressants (Behnke *et al.,* 2009; Behnke and Harris, 2009; 2010). They are 5–20 mm in length and bright red due to the pigmentation of their tissues. They are usually heavily coiled, with the female having 12–15 coils and the male 8–12. The male can be distinguished from the female by a prominent copulatory bursa and two long, thin spicules at the posterior end (smaller worm to the right) (Plate II). Eggs can be

seen in the posterior third of the female adult (larger worm to the left). In the early literatures, this parasite was known as *Nematospiroides dubius* and *Heligmosomoides polygyrus* (Spurlock, 1943, Ehrenford, 1954; Bryant, 1973).

Plate II: Adult *Heligmosomoides bakeri* (male and female).

2.13.1 Scientific classification

Kingdom: Animalia

Phylum: Nemathelminthes

Class: Nematoda

Order: Rhabditida

Family: Heligmosomidae

Genus: *Heligmosomoides*

Species: *H. bakeri*

Binomial name: *Heligmosomoides bakeri*
Source: Soulsby (1982); Behnke *et al.* (2009).

2.13.2 Life cycle of *Heligmosomoides bakeri*

Heligmosomoides bakeri is a trichostrongylid nematode found in small rodents, the albino mice, *Mus musculus.* Its life cycle is direct and involves both free-living and parasitic stages (Bryant, 1973).

2.13.2.1 Larvae

Eggs contain fully developed larvae 28 hours after being laid. The larvae can be observed moving vigorously within the egg shell. The eggs hatch after 36-37 hours giving rise to first stage larvae. After 28-29 hours of hatching, the L_1 larvae molt to give rise to the L_2 larval stage. A partial molt occur 17-20 hours later giving rise to ensheathed L_3 infective stage larvae. The L_3 are active but non-feeding (Bryant, 1973).

2.13.2.2 Parasitic stages

Within 24 hrs of infection, no larvae could be found in the intestinal lumen indicating that the larvae had shed their sheath and penetrated the intestinal mucosa. The fourth larval molt takes place about 90-96 hrs after infection. The next molt takes place about 144-166

hrs after infection. By 191 hours most of the worms have passed from the mucosa into the intestinal lumen. Worms were observed *in copula* and the first eggs detected in the host faeces by 240 hours of infection. The life cycle, from egg to egg, takes about 325 hrs or 13.5 days (Bryant, 1973). In mice, gross pathology associated with *H. bakeri* infection includes white nodules on the intestine which are presumably reactions to larvae in the submucosa. They could be apparent for some time after the larvae have matured into adults and migrated back into the lumen of the intestine.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research Design

The study design for this research involved collection and processing of *Acanthus montanus* leaf, determination of its phytochemical components, and toxicity and anthelmintic study/evaluation in mice models. Extraction, solvent-partioning, phytochemical screening and thin layer chromatography of extracts were carried out at the Postgraduate Laboratory in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria; while the determination of maximum convenient concentrations (MCCs) and maximum convenient doses (MCDs), acute toxicity and anthelmintic studies/evaluation of extracts in mice were carried out at the Teaching and Research Laboratory in the Department of Veterinary Parasitology and Entomology, Faculty Veterinary Medicine, Ahmadu Bello University, Zaria.

3.2 Plant Material Collection and Identification

Literatures on ethnomedical uses and the folkloric claims warranted the choice of the plant materials. Fresh leaves of *Acanthus montanus* (Plate I) with stalks were collected/ harvested in the months of March and April along a stream in northern part of Katsina-Ala township of Katsina-Ala Local Government Area of Benue State. Katsina-Ala town is located on latitude 7º 10□ N and longitude 9º 19□ E in the middle belt (Guinea Savannah)

of Nigeria. A sample of the plant was brought to Zaria and was identified/authenticated by a plant taxonomist at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, Zaria, where a voucher specimen was deposited. It was assigned a voucher number 7037 of 5th May, 2011.

3.3 Preparation and Preservation of Extracts

The harvested plant materials were air-dried at room temperature until a constant weight was obtained, then pounded in a wooden mortar with pestle. The pulverised product weighing about 6 kg was stored in an air-tight nylon bag under cool, dark conditions at room temperature until use.

During extraction, 1 kg (250 g per separating funnel) of the pulverized product was soaked in 95% ethanol (EtOH) in the ratio 1:6 *w/v* in four separating funnels (pre-plugged with cotton wool) for 72 hours. The extracted solution collected in excess of the solvent was transferred in evaporating dishes and concentrated to dryness over waterbaths at 60ºC. The dried extract obtained after concentration was then weighed to determine the percentage yield. Thereafter, about 100 g of the mixture was suspended in 300 ml of 17.65% methanol (MeOH) in a large beaker. The solution was then partitioned with chloroform in equal volume in a separating funnel to yield chloroform (CHLO) and aqueous (AQ) portions. Lastly, the aqueous portion was further partitioned with *n*-butanol in equal volume also, to obtain final *n*-butanol (BuOH/BUT) and aqueous fractions. In each partitioning step, the mixtures were vigorously shaken to re-suspend the particles. Impurities were pooled together in a separate beaker and discarded. The different portions collected in separate

conical flasks were again concentrated to residue over the waterbath at 60ºC and weighed to determine percentage yield in terms of the mass of the crude ethanol extract (CEE). These different fractions of leaf extracts of *A. montanus* were packed in clean air-tight glass bottles and stored at room temperature in the laboratory hood until used (Figure 3.1).

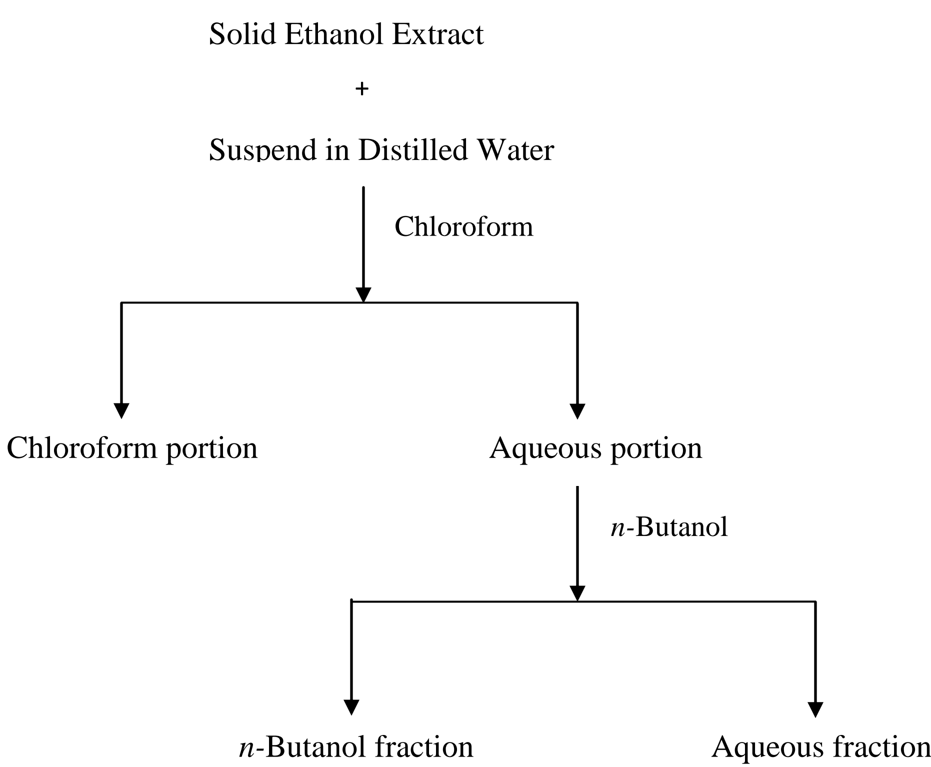

Figure 3.1 Flow chart of solvent partitioning of pulverized leaf of *A. montanus*.

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3.4 Qualitative Phytochemical Screening of Crude Ethanolic Leaf Extracts of *Acanthus montanus*

About 1 g of crude ethanol leaf extracts of *A. montanus* was dissolved in about 100 ml of distilled water in three test tubes and subjected to qualitative phytochemical screening employing standard screening tests (Evans, 2002).

3.4.1 Test for carbohydrates *(Molisch Test)*

To 2 ml of the extract solution in a test tube, was added a few drops of Molisch reagent, followed by addition of concentrated (100%) sulphuric acid down the side of the test tube to form a lower layer. A reddish coloured ring at the interphase indicates presence of carbohydrates (Evans, 2002).

3.4.2 Test for glycosides *(Ferric chloride Test)*

To 2 ml of the extract solution, 5 ml of 10% sulphuric acid was added and boiled on water bath for 10-15 minutes. This was then cooled and neutralized with equal volume of 20% KOH. It was then divided into two portions. The first was discarded. To the second portion, about 3 ml of 5% aqueous ferric chloride solution was added. A green to blue colour if produced indicated the release of phenolic aglycones due to hydrolysis (Evans, 2002).

3.4.3 Test for free anthracene derivatives *(Bontrager's Test)*

To 2 ml of extract solution in a dry test tube, 5 ml of chloroform was added and was shaken for at least 5 minutes. This was filtered and the filtrate shaken with equal volume of

10% ammonia solution. Appearance of a bright pink colour in the aqueous (upper) layer indicates the presence of free anthraquinones (Evans, 2002).

3.4.4 Test for combined anthracene derivatives *(Modified Bontrager's Test)*

Two (2 ml) of the extract solution in a test tube was boiled with 5 ml of 10% hydrochloric acid for 2-3 minutes. This would hydrolyse the glycoside to yield aglycones, which are soluble in hot water. This was then filtered to remove any coarse particle and the filtrate was cooled and extracted with 5 ml of benzene. The benzene layer was pipetted off and shaken gently in a test tube with half of its volume of 10% ammonium hydroxide. The change of colour from rose pink to cherry red in lower ammonia layer was indicative that the mixture contained anthraquinones derivative (free or in combined state) (Evans, 2002).

3.4.5 Test for unsaturated steroid and triterpenes *(Liebermann-Burchard Test)*

To 2 ml of the extract solution, an equal volume of 0.1 M acetic acid anhydride was added and mixed gently. One (1) ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Colour changes were observed immediately and over a period of one hour. Blue to blue-green colour in the upper layer and a reddish, pink or purple colour indicated the presence of triterpene (Evans, 2002).

3.4.6 Salkowski test for unsaturated sterols

To 2 ml of the extract solution, 2-3 drops of concentrated sulphuric acid were added at the side of the test tube. Immediate colour changes at the interphase of the extract and

sulphuric acid was noted. Colour changes over one hour period were also noted (cherry red coloration usually indicates the presence of unsaturated sterols) (Evans, 2002).

3.4.7 Test for cardiac glycoside *(Keller-kiliani Test)*

Two (2 ml) of the extract solution was dissolved in 1 ml of 0.1 M glacial acetic acid containing traces of 1% ferric chloride solution. This was then transferred into a dry test tube and 1 ml of concentrated sulphuric acid was added down the side of the test tube to form a layer at the bottom and the interphase carefully observed for purple-brown ring. This colour change indicated the presence of desoxy sugars while a pale green colour in the upper acetic acid layer indicated the presence of cardiac glycosides (Evans, 2002).

3.4.8 Test for saponin glycoside *(Frothing Test)*

About 10 ml of distilled water was added to 0.5 g of the extract and shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 minutes. A honeycomb froth that persisted for 10-15 minutes indicated the presence of saponins (Evans, 2002).

3.4.9 Test for taninns *(Ferrric chloride Test)*

To 5 ml of the extract solution, 3-5 drops of 10% ferric chloride solution was added. A green-black precipitate indicated the presence of condensed tannins while blue or brownish-blue precipitate indicated the presence of hydrolysable tannins (Evans, 2002).

3.4.10 Test for flavonoids

3.4.10.1 Shinoda Test

About 0.5 g was dissolved in 1-2 ml of 50% methanol and then heated. Metallic magnesium chips and a few drops of concentrated hydrochloric acid were added. Appearance of red colour indicated the presence of flavonoids (Evans, 2002).

3.4.10.2 Sodium hydroxide Test

Few drops of 10% sodium hydroxide were added to 2 ml of extract solution in a test tube. Yellow coloration indicated the presence of flavonoids (Evans, 2002).

3.4.11 Test for alkaloids

About 0.5 g of extract was stirred in 5 ml of 1% aqueous HCl on water bath and then filtered. Equal portions of the filtrate were taken individually in three test tubes and the following tests conducted.

3.4.11.1 Mayer's Test

To the first portion of the extract solution, a few drops of Mayer's reagent were added. A creamy white precipitate indicated the presence of alkaloids (Evans, 2002).

3.4.11.2 Wagner's Test

Few drops of Wagner's reagent were added to the second portion of the extract solution. The observation of reddish brown precipitate indicated the presence of alkaloids (Evans, 2002).

3.4.11.3 Draggendoff's Test

To the third portion of the extract solution, a few drops Dragendoff's reagent was added. An orange brown precipitate indicated the presence of alkaloids (Evans, 2002).

3.5 Thin Layer Chromatography

Thin Layer Chromatography (TLC) was carried out on the crude ethanol leaf extracts of *A. montanus* and the different fractions thereof using the method of Mehta (2012). Little quantities of the Crude Ethanol, *n*-Butanol, Chloroform and Aqueous portions of *A. montanus* leaf extracts were fetched with a spatula and dissolved in about 5 ml their respective solvents in well labeled Bijou bottles. These were used to spot 10 cm x 5 cm TLC plates (already coated with 0.25 mm silica gel $60F_{254}$ that were marked at about 1.0 cm intervals and 1.5 cm from the bottom) using capillary tubes and were allowed to air-dry. The plates were then placed (the spotted portion downwards) in an air-tight TLC tank that was already charged with eluting solvent (chloroform-ethyl acetate-formic acid, 5:4:1). The respective plant constituent(s) were allowed to drag upward along the stationary phase from the spots by capillary action until the solvent front was seen reaching the top. The plates were then removed, marked, air-dried and sprayed with the detecting (spray)

reagents (1% $FeCl_3$, Dragendoff's and Anisaldehyde-H_2SO_4 in 10% ethanol- Harborne, 1980) in the fume hood to develop the chromograms. Retention factor *(Rf)* value of the compound(s) was calculated by dividing the distance travelled by the compound (solute) by that travelled by the solvent front (Evans, 2002; Mehta, 2012).

3.6 Experimental Animals Used in Toxicity and Anthelmintic Studies

Ninety (5-10 weeks) apparently healthy albino mice, *Mus musculus* of both sexes weighing between 15 and 25 g were purchased from the Animal House of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, and a commercial mice and rat Dealer in Zaria, Nigeria. The animals were maintained in the Animal House of Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. Major work done on the animals was carried out in the Helminthology Teaching and Research Laboratory of the same Department. The animals were housed in well fenestrated plastic cages lined with chopped wood shavings as litter under standard conditions (Plate III) and allowed free access to standard feed (poultry growers' mash, Grand Cereals Ltd® mixed with maize bran) and tap water. The litter was routinely changed every three days and cages mopped clean with diluted antiseptic (Dettol®). Water bottles too were washed routinely and fresh water given daily. Within the two weeks of acclimatization, faecal pellets obtained *per rectum* from the group set aside for anthelmintic studies were analysed and the mice treated with broad spectrum anthelmintic, albendazole at 10 mg/kg for deparasitization.

Plate III: Mice housed in plastic cage.

3.7 Determination of MCCs and MCDs of Extracts Used in Toxicity and Anthelmintic Studies

The maximum convenient concentrations (MCCs) of crude ethanol and the various fractions of *A. montanus* leaf extracts were prepared as described by Ibrahim (1984). One gramme (1g) of extracts was thoroughly mixed with a given volume (in ml) of distilled water (0.05 ml at a time) until the solution could be delivered through an 18 gauge needle at room temperature. From the volume of the solvent used, the MCC of extract in g/ml was determined. Thus, the maximum convenient doses (MCDs) for the extracts were prepared

(Ibrahim, 1984). The maximum convenient volume (MCV) that could be administered to mice by the oral route (gavage) is 5ml/kg (Loomis, 1978; Ibrahim, 1984). The MCDs (g/kg) were thus calculated by multiplying the MCCs (g/ml) by the MCV (ml/kg).

3.8 Preliminary Acute Toxicity Studies

Fifteen mice of both sexes were randomly assigned to five groups of three mice each and the amount of CEE that would cause 0-100% death when administered orally was determined using [Lorke's method (1983) as modified by Dzenda *et al.,* 2004]:

Group 1 received 10,000mg/kg body weight

Group 2 received 1,000mg/kg body weight

Group 3 received 100mg/kg body weight

Group 4 received 10mg/kg body weight

Group 5 (the control group) were given 5ml distilled water/kg body weight.

The stock solution was prepared in four well labeled test tubes as previously described, by serial dilution. Briefly, 10,000 mg of CEE was thoroughly dissolved in 1 ml of distilled water (vehicle) to obtain 10,000 mg/ml. one (1) ml of this was transferred into the second test tube containing 9 ml of distilled water and vigorously mixed to obtain 10 ml, giving 1,000 mg/ml of the solution. Again, 1 ml of this solution was transferred to the third test tube containing 9 ml of water and thoroughly mixed to obtain 10 ml of 100 mg/ml solution. Lastly, 1 ml of this solution was dissolved in the fourth test tube containing 9 ml of distilled water to obtain 10 ml of 10 mg/ml.The actual amount of these solution administered to each mice was calculated based on the MCV (5 mg/ml) and the body

weight of each mouse. Mice were deprived of water about 12 hours prior to extract administration and about 3 hours after. Treatments were administered by gavage *(per os)* directly into the oesophagus using a blunted tip, slightly curved 18 gauge needle mounted on tuberculin (1 ml) syringe. Mice were observed closely for any change in behaviour and signs of toxicity for 48 hours. Thereafter, one mouse from each group was sent to the Department of Veterinary Pathology, Ahmadu Bello University, Zaria for necropsy.

3.9 Postmortem Examination of Organs

The mice were euthanized in chloroform chamber and necropsied. Vital internal organs *viz:* the liver, lungs, heart, kidneys, spleen and intestine were observed for gross pathologic changes by a Pathologist, and recorded. Thereafter, tissues taken from each of these organs were fixed in 10% buffered neutral formalin for Histopathology. After 21 days during which no death occurred in any group, one out of the two mice remaining in the group that took the highest dose was necropsied and the vital organs studied as described earlier. Tisssues were trimmed and processed in tissue mation embedded in paraffin wax, cut at 5μ. The processed tissues were stained with Haematoxylin and Eosin (H & E) stains (Luna, 1960).

3.10 Deparasitization of Mice

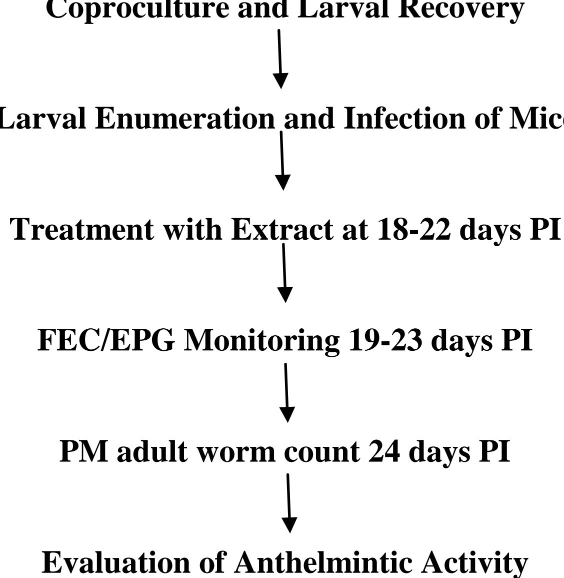
Albendazole (ALBIDOL® Animal Care, Batch No. 90028) -150 mg (10 mg/kg) purchased in a nearby Agro-vet shop was crushed to powder using ceramic mortar and pestle in the lababoratory. This was carefully packed into 50 ml test tube and thoroughly mixed with 15 ml of distilled water to obtain 10 mg/ml MCC solution. As described before, the calculated

amount of the solution was given to each mouse by gavage three consecutive times (one week apart to ensure complete deworming) until all the mice were confirmed by faecal flotation to be worm-free.

3.11 The Parasite *Heligmosomoides bakeri*

The different stages in the life cycle of this helminth are shown on Plates IV, V and VI. The larvae of this parasite were obtained from Helminthology Laboratory of the Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, courtesy of Dr. L.A. Ngongeh. Both infected mice and infective (L_3) larvae were transported to Ahmadu Bello University, Zaria. Efforts were immediately made to multiply and maintain the parasite in more mice under laboratory conditions.

3.12 Anthelmintic Screening (Study Design)



3.12.1 Faecal sample collection, coproculture and recovery of infective L_3 of *H. bakeri*

The modified method of Burren (1980) was used to obtain clean infective larvae used for anthelmintic trial. Briefly, infective source mice were placed in clean cages for 12 hours to shed faecal pellets. Drinking water but not food was left in the cages. Faecal pellets were scrapped using a small spatula into 50 ml centrifuge tubes and mashed into smooth paste with applicator sticks. The tubes were then filled with distilled water and spun at 250 rpm (11 g) for 2 minutes. After decanting the supernatant, the resuspended slurry was filtered into other 50 ml tubes through a double layer of gauze and centrifuged as described previously. The sediments were plated in Petri dishes that were lined with moistened Whatman 40 filter paper. The cultures were moistened with distilled water where necessary. After 7 days, a few drops of distilled water were sprinkled on and underneath the filter paper and the water suspending the recovered infective (L_3) pippetted into a clean 30 ml tube to allow the larvae to sediment under laboratory conditions. All procedures were under standard laboratory conditions.

Plate IV: Egg of *H. bakeri* (x 400).

Plate V: Infective (L_3) larvae of *H. bakeri* (x 400).

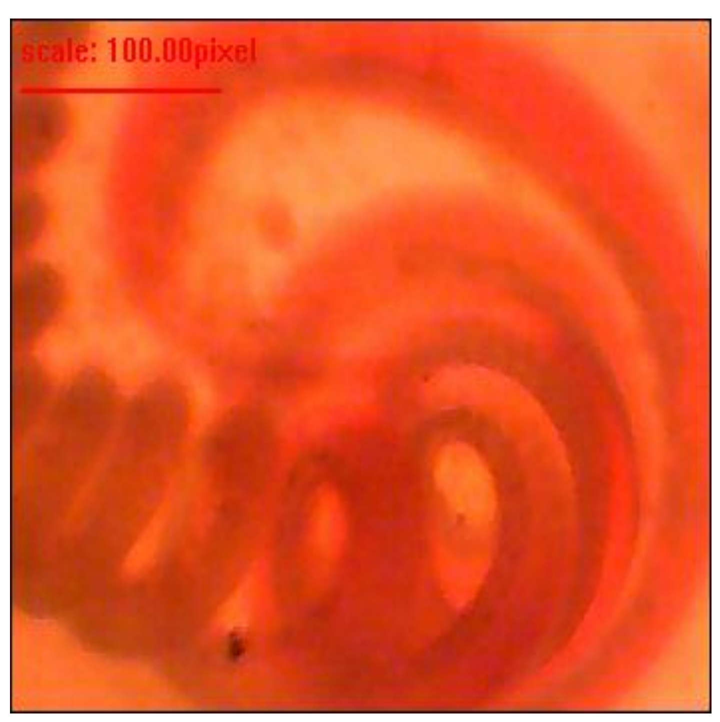


Plate VI: Adult *H. bakeri.*

3.12.2 Enumeration of recovered L_3 of *H. bakeri*

The larval suspension was allowed to stand on the bench for one hour. The supernatant fluid was carefully aspirated using Pasteur pipette. To ensure an even distribution of the larvae, the the suspension was mixed by gentle shaking of the tube. Thereafter, an aliquot of 0.1 ml was drawn onto a clean microscope slide and larvae counted under x4 objective. This was repeated four more times, after which mean count obtained was used to determine the number of larvae/ml of the suspension.

3.12.3 Experimemental infection of mice with *H. bakeri* L_3

Experimental infection of mice was by oral route (gavage). Mice were well restrained at the scruff and 0.1 ml of the larval suspension inoculated right into the oesophagus via a blunted tip slightly curved 18 gauge needle mounted on tuberculin (1 ml) syringe. One hundred and fifty (150) infective larvae (L_3) of *H. bakeri* were inoculated into each worm-free mouse.

3.12.4 Treatment groups

Seventy (70) mice positive for ova of *H. bakeri* at 15th day post infection (PI) were randomly randomly assigned into 14 experimental (treatment) groups of five mice each. Twelve (12) groups were administered with four (4) graded doses of CEE, BUT and AQ fractions. The remaining two (2) groups were controls, i.e. positive control (Albendazole, ABZ 10 mg/kg) and negative control (Distilled water, DW 5 ml/kg) (Table 3.1). Each of the estimated dose of the various fractions of *A. montanus* were administered to mice

(according to grouping previously described) for five consecutive days i.e., on the 18th, 19th, 20th, 21st and 22nd days post infection (PI) (Lai, 2006). Doses were chosen at a common logarithmic interval of 0.08 (Suleiman, 2002) for all extract-treated groups. All treatments were administered orally as explained previously. Each mouse in the groups was later tagged with identification number at the tail base with adhesive tape.

Table 3.1: Summary of Anthelmintic Screening of the various portions of *A. montanus* in mice infected with *H. bakeri.*

Group	Substance used	No. of mice	Route of administration	Log Dose	Log^{-1} Dose (g/kg)
1	CEE	5	oral	0.08	1.2
2	”	5	”	0.16	1.4
3	”	5	”	0.32	1.7
4	”	5	”	0.64	2.0
1	BUT	5	oral	0.08	1.2
2	”	5	”	0.16	1.4
3	”	5	”	0.32	1.7
4	”	5	”	0.64	2.0
1	AQ	5	oral	0.08	1.2
2	”	5	”	0.16	1.4
3	”	5	”	0.32	1.7
4	”	5	”	0.64	2.0
Control (-)	DW	5	”	-	5 ml/kg
” (+)	ABZ	5	”	-	10 mg/kg
Total		**70**			

5 ml/kg is the MCV
10 mg/kg is the Dose recommended by manufacturer

3.12.5 Faecal egg counts

The *in vivo* anthelmintic efficacy of leaf extract of *A. montanus* was determined by means of quantitative egg count from faecal samples collected from all the groups. Daily faecal pellets were collected from each group (described previously) for five consecutive days starting from the 19th day PI when the faecal egg output is supposed to be at its peak. The modified McMaster technique was used for egg counting (Soulsby, 1982; Hansen and Perry, 1990). Briefly, 1 g of faecal pellets was suspended in 14 ml of saturated sodium chloride/sucrose solution of specific gravity of 1.28 and centrifuged at 250 rpm (11 g) for 10 minutes. Both chambers of Mcmaster slides were filled with well mixed aliquots of the supernatant from each sample, and eggs counted under x10 objective. Egg per gramme (EPG) was calculated by multiplying FEC by 50 (factor) (Appendix II).

3.12.6 Postmortem worm count

On the 23rd day PI, all the mice were deprived of food but not water for 24 hours so as to empty the gastrointestinal tract to make worm counting easier. Mice were euthanized in chloroform chamber and necropsied. Modified method of Ngongeh (2004) was used for worm count. Briefly, the entire intestine of each mouse was opened by cutting along its longitudinal axis with a pair of fine scissors. The adult worms were recovered individually by suspending each intestine with fine threads and dipping into normal saline and incubating at 37ºC for 20 hours. This is to enable the migration of the worms into the solution. At the end of incubation, the suspensions were centrifuged at 250 rpm (11 g) for 2 minutes. The supernatant was discarded and about 5 ml of saturated salt solution was used to resuspend the sediments for 30 minutes. This is to immobilize, disentangle and relax the

tight spiral coils characteristic of the living worms for easy counting. The mixture was poured in Petri dish and viewed using the magnifying lens where the reddish worms were identified, counted (picked with pin) and recorded.

3.12.7 Anthelmintic Evaluation

Anthelmintic efficacies of the extracts were accessed by counting the eggs and worms in the treated animals and comparing with counts from the untreated control mice. The percentage deparasitization, (i.e %DP - reduction in the egg and worm counts) for the various groups was then calculated using the formula:

$$\%DP = \frac{N-n}{N} \times 100$$

Where: N = mean egg/worm count in untreated group.

n = mean egg/worm count in treated groups.

(Ibrahim, 1984; Suleiman, 2002).

Fifty percent (50%) reduction in egg/worm count or more was considered significant at $p<0.05$ (Ibrahim, 1984).

3.13 Statistical Analysis

The raw faecal egg and worm counts data generated were summarized to tables and expressed in percentages (percentage deparasitization) and Microsoft Office Excel 2007 was used to plot the daily egg per gram graph. One-way analysis of variance (ANOVA) and Tukey's Multiple Comparison Post Test (Graphpad Instat) were used for data analysis. The results were expressed as Mean ± SEM. Difference between Means of treated and untreated control groups was considered significant at $p<0.05$.

CHAPTER FOUR

RESULTS

4.1 Extractive and Solvent-Partitioned Yield of Plant Material

The leaves were collected fresh and sample identified with voucher number 7037-5/5/11. Extraction of pulverized leaf of *Acanthus montanus* showed that 1000 g yielded 255.84 g of crude ethanol (EtOH) extract. Solvent partitioning of 100 g of crude (EtOH) extract yielded 31.42 g, 11.58 g and 3.00 g of aqueous (AQ), *n*-butanol (BuOH) and chloroform (CF) fractions respectively. The remaining portion was discarded as residue. The colour and percentage yieds of the plant material is as shown in Table 4.1. Generally, partitioning with water resulted in the highest quantity of crude extract, while CF gave the least quantity.

Table 4.1: Extract yield (% dry weight) from *A. montanus* leaf.

Extract	**Colour**	**Yield (% *w/w*)**
Crude EtOH Extract	Brown	25.58
AQ Fraction	Light-brown	31.42
BuOH Fraction	Dark-brown	11.58
CF Fraction	Dark-green	3.00

4.2 Solubility of Extracts in Water

The crude ethanol extract was readily soluble in water. Of the different fractions, aqueous portion was most soluble (requiring less volume of water) followed by *n*-Butanol. CF was not soluble in water at all. It was however, readily soluble in chloroform and other non-polar solvents (Table 4.2).

4.3 Maximum Convenient Concentrations (MCCs) (g/ml) and Maximum Convenient Doses (MCDs) (g/kg) of Different Portions of Leaf Extracts of *A. Montanus*

One and a quarter (1.25 ml) of distilled water conveniently dissolved (to pass through 18 guage needle) 1 g of crude ethanol extract giving an MCC of 0.8 g/ml. *n*-butanol and aqueous portions required 2 ml and 1 ml respectively of distilled water to conveniently dissolve 1 g the extracts. Chloroform fraction was not soluble in water, however, 1 g of it passed through 18 guage needles at room temperature when 0.8 ml of chloroform solvent was used to dissolve it. The MCDs (g/kg) were obtained by multiplying MCCs (g/ml) by MCV (5 ml/kg) (Table 4.2).

Table 4.2: Solubility, MCC (g/ml) and MCD (g/kg) of various portions of leaf extract of *A. montanus*.

montanus

Portion	MCC (g/ml)	MCD (g/kg)
CEE	0.8	4
BUT	0.5	2.5
CF	1.25	6.25
AQ	1.0	5

4.4 Phytoconstituents of *A. montanus*

The results of qualitative phytochemical screening of CEE of *A. montanus* are as shown in Table 4.3. The major phytochemicals present in *A. montanus* leaf extract include glycosides, unsaturated steroids and triterpenes, saponins, tannins, flavonoids and alkaloids.

Table 4.3: Results of qualitative phytochemical screening of CEE of *A. montanus*.

Constituent(s)	Test	Reference	Observation	Inference
Carbohydrates	Molish	Evans, 2002	Violet ring colour	+
Glycosides	Ferric chloride	”	Dark brown coloration	+
Free Anthracene Derivative	Bontrager’s	”	Light yellow coloration	–
Combined Anthracene Derivative	Modified Bontrager’s	”	Light yellow coloration	–
Unsaturated Steroid	Liebermann-Burchard	”	Yellowish brown ppt	+

Triterpenes	”	”	Reddish coloration	+
Unsaturated sterols	Salkowski	”	Brownish coloration	+
Cardiac glycoside	Keller-kiliani	”	Purple brown coloration	+
Saponin glycoside	Frothing	”	Persistent (honey comb) froth	+
Tannins (condensed)	Ferric chloride	”	Greenish-black ppt	+
Tannins (hydrolysable)	”	”	Greenish-black ppt	−
Flavonoids	Shinoda	”	Dark red coloration	+
”	Sodium hydroxide	”	Yellow coloration	+
Alkaloids	Mayer’s	”	Creamy white ppt	+
”	Wagner’s	”	Reddish brown ppt	+
”	Dragendoff’s	”	Orange brown ppt	+

+ Test substance present; − Test substance absent.

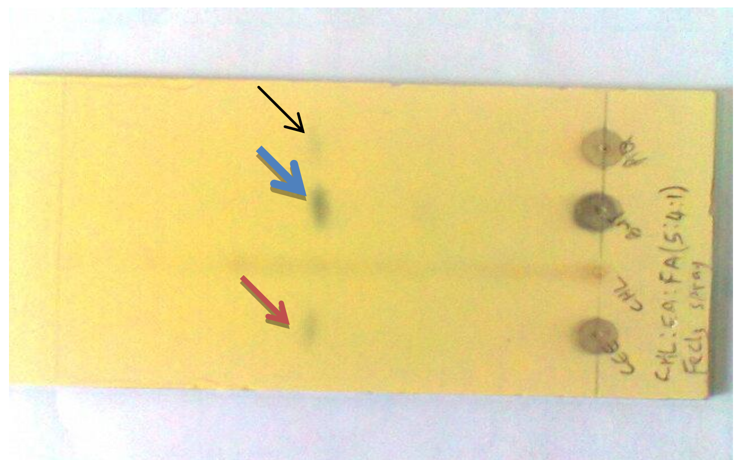
4.5 Thin Layer Chromatography

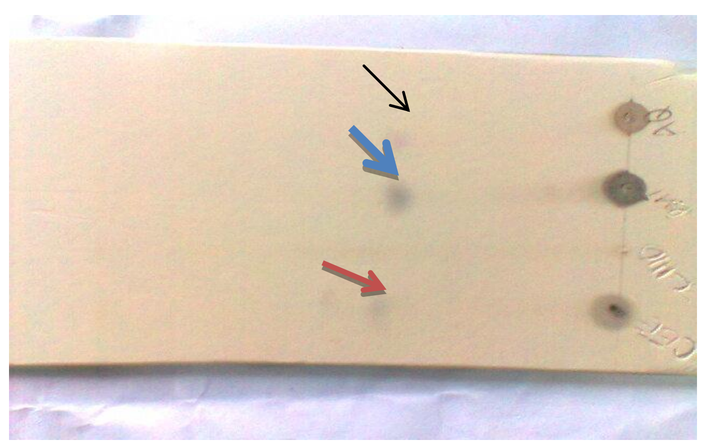
The chromatograms of TLC plates spotted with the various portions of *A. montanus* sprayed with 1% $FeCl_3$ are displayed in the TLC plates below (Plate XIV). *n*-butanol (BUT) portion displayed more conspicuous spot followed by crude ethanol extract (CEE), then the aqueous (AQ) portion. Chloroform [CHL(O)] portion did not display any visible spot (visible light).

Rf value depicted as retardation factor, rate of flow or retention factor for each spot was calculated and the same value got. This indicates a particular compound (solute) being eluted. This result shows that the compound is polar considering the eluting system

(mobile phase) and the stationary phase (silica gel). The colour of the spots is greenish black. This is an evidence of phenolics (likely tannins, because of 1% $FeCl_3$ spray reagent used) eluted.

$$Rf\ (retention\ factor) = \frac{distance\ travelled\ by\ compoud\ (solute)}{distance\ travelled\ by\ solvent\ front} = \frac{3.9\ cm}{7.4\ cm} = 0.53$$





Legend

compound eluted from crude ethanol extract (CEE)

compound eluted from *n*-butanol portion (BUT)

compound eluted from aqueous portion (AQ)

Plate VII: TLC plates showing chromatogram of different portions of leaf extracts of *Acanthus montanus* under visible light.

4.6 Toxicity Studies

The result of toxicity studies is as shown in Table 4.4. No observable clinical signs were displayed by mice treated with the dose range of 10-10,000 mg/kg of crude ethanol leaf extract of *A. montanus*. For each group, mortality was 0% (0/3) from the first 24 hours through 3 weeks post treatment.

Table 4.4: Results of preliminary acute toxicity studies of CEE of *A. montanus* in mice.

Group	Dose (mg/kg)	No. Dead/No. Alive	Clinical sign(s)
1	10,000	0/3	No observable signs
2	1,000	0/3	”
3	100	0/3	”
4	10	0/3	”
5	DW 5 ml/kg	0/3	”

5 ml/kg is the MCV

4.7 Postmortem Findings

4.7.1 Gross pathologic lesions in organs

The gross lesions observed in mouse exposed to crude ethanol extract of *A. montanus* during preliminary acute toxicity studies were those of congestion especially of the liver, lungs, heart and the spleen in the group exposed to 10,000 mg/kg of the extract. There was however no significant gross pathologic changes in the group exposed to 10-1,000 mg/kg and distilled water 5 mg/kg.

4.7.2 Histopathologic findings

The histopathological findings in organs of mice exposed to the vary doses of crude ethanol extract of *A. montanus* and distilled water 5 mg/kg during preliminary acute toxicity studies are as presented in Appendix V and the plates following.

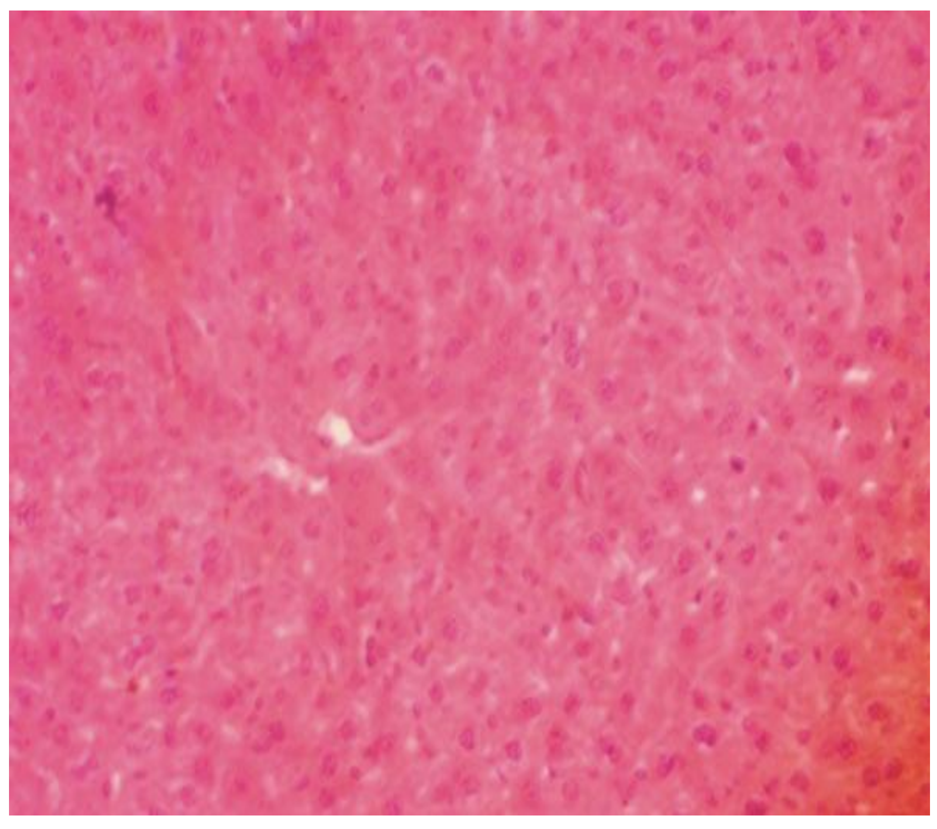
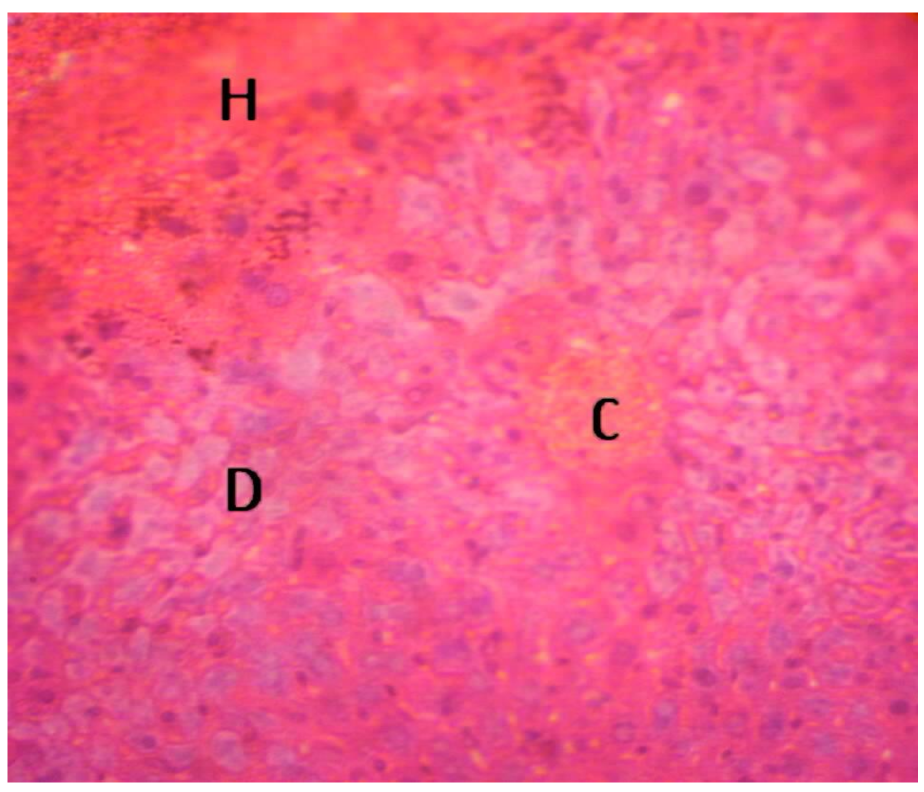


Plate VIII: Photomicrograph of a section of liver of a mouse exposed to distilled water at 5 ml/kg. No obsvervable microscopic lesions (H & E, ×400)



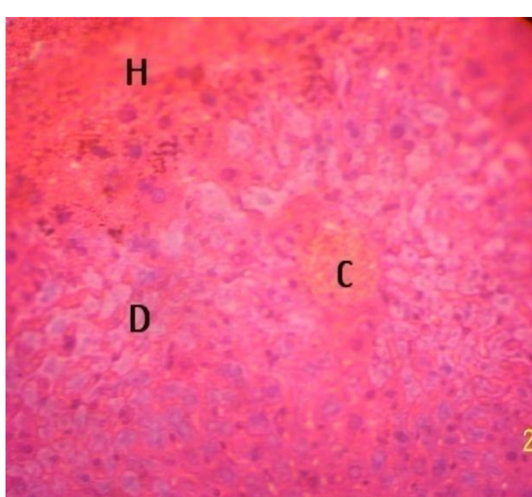


Plate IX: Photomicrograph of a section of liver of a mouse after 48 hours' exposure to crude ethanol extract of *A. montanus* at 10,000 mg/kg. Note the hepatocellular

degeneration (D) around the congested central vein (C) and haemorrhages (H) (H & E, ×400).

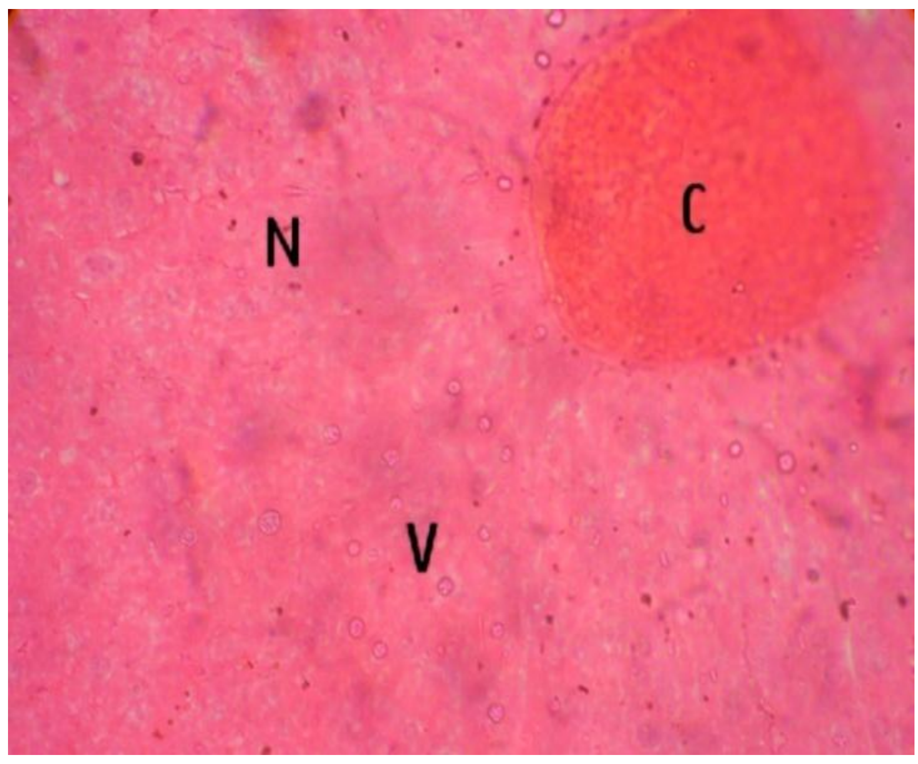


Plate X: Photomicrograph of a section of liver of a mouse 3 weeks after exposure to crude ethanol extract of *A. montanus* at 10,000 mg/kg, showing diffused hepatocellular necrosis (N), congested central vein (C) and vacuolar degeneration (V) (H & E, ×400).

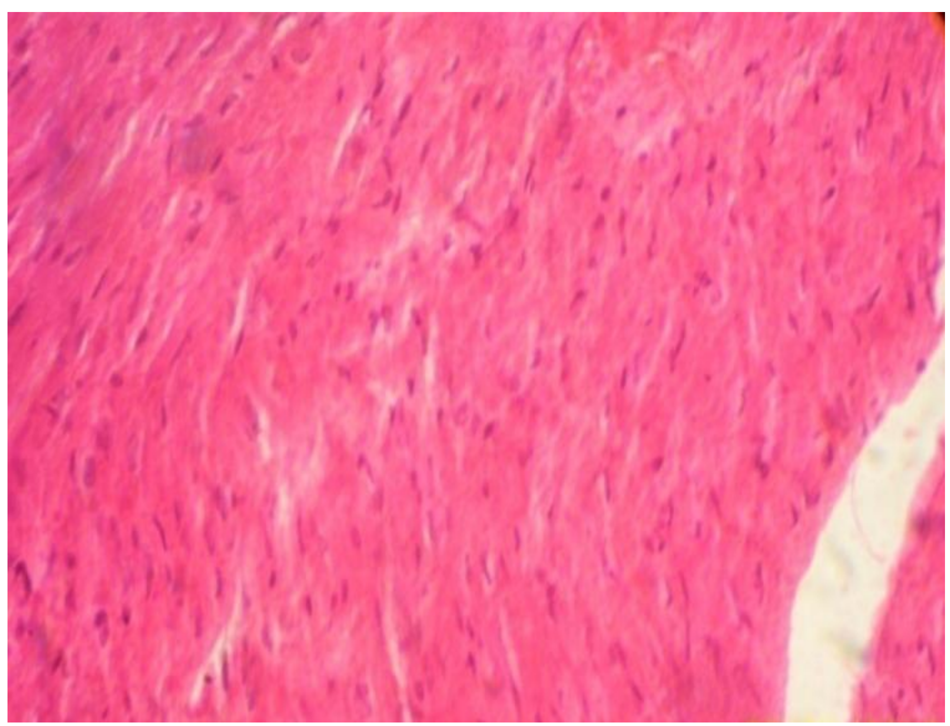


Plate XI: Photomicrograph of a section of the heart of a mouse exposed to distilled water 5 ml/kg. No obsvervable microscopic lesions (H & E, ×400).

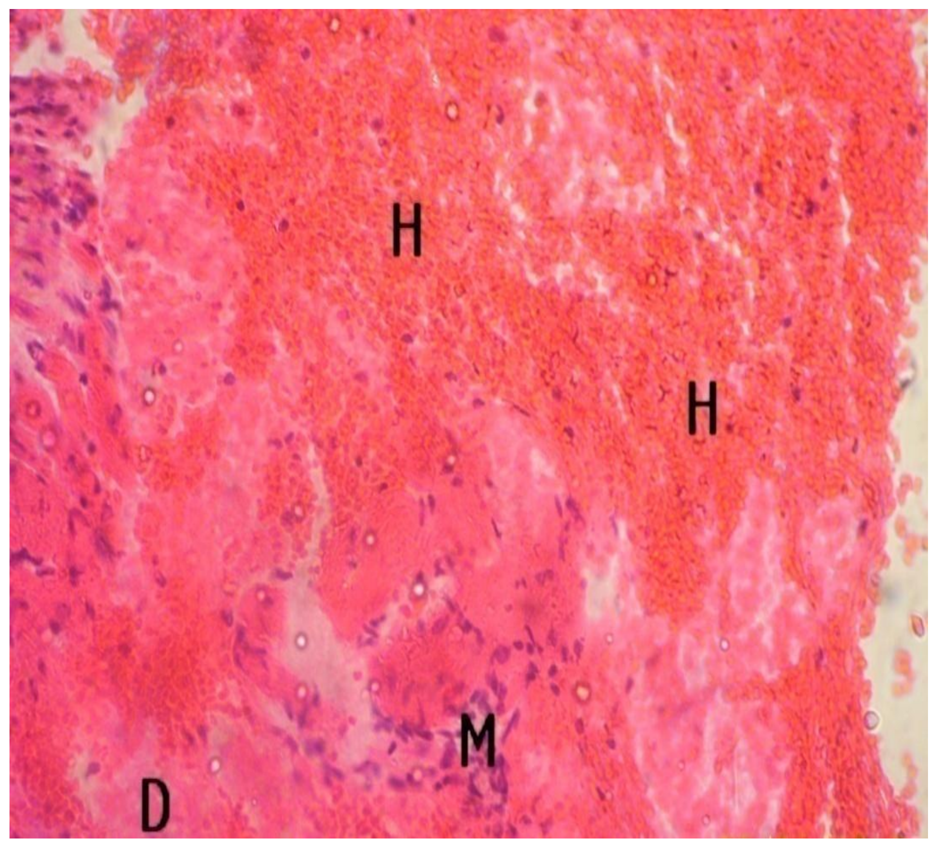


Plate XII: Photomicrograph of a section of the heart of a mouse 48 hours after exposure to crude ethanol extract of *A. montanus* at 10,000 mg/kg, showing massive haemorrhage (H) and myocardial degeneration (D) and foci of mononuclear cellular infiltration (M) (H & E, ×400).

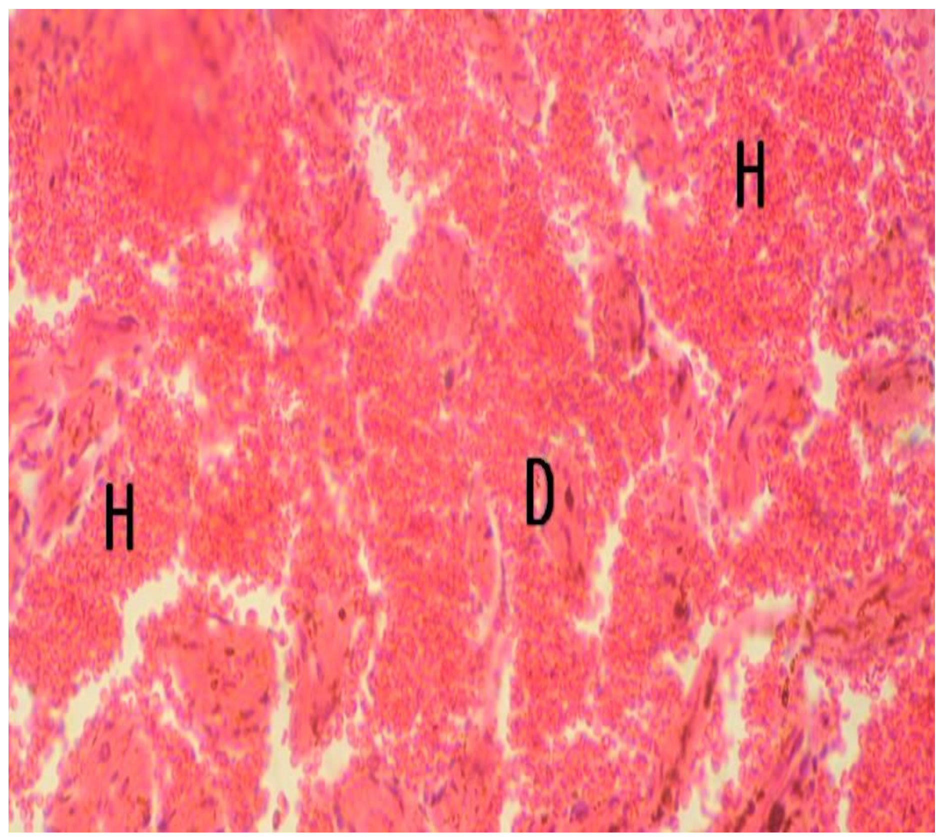


Plate XIII: Photomicrograph of a section of the heart of a mouse 3 weeks after exposure to crude ethanol extract of *A. montanus* at 10,000 mg/kg, showing haemorrhage (H) and myocardial degeneration (D) (H & E, ×400).

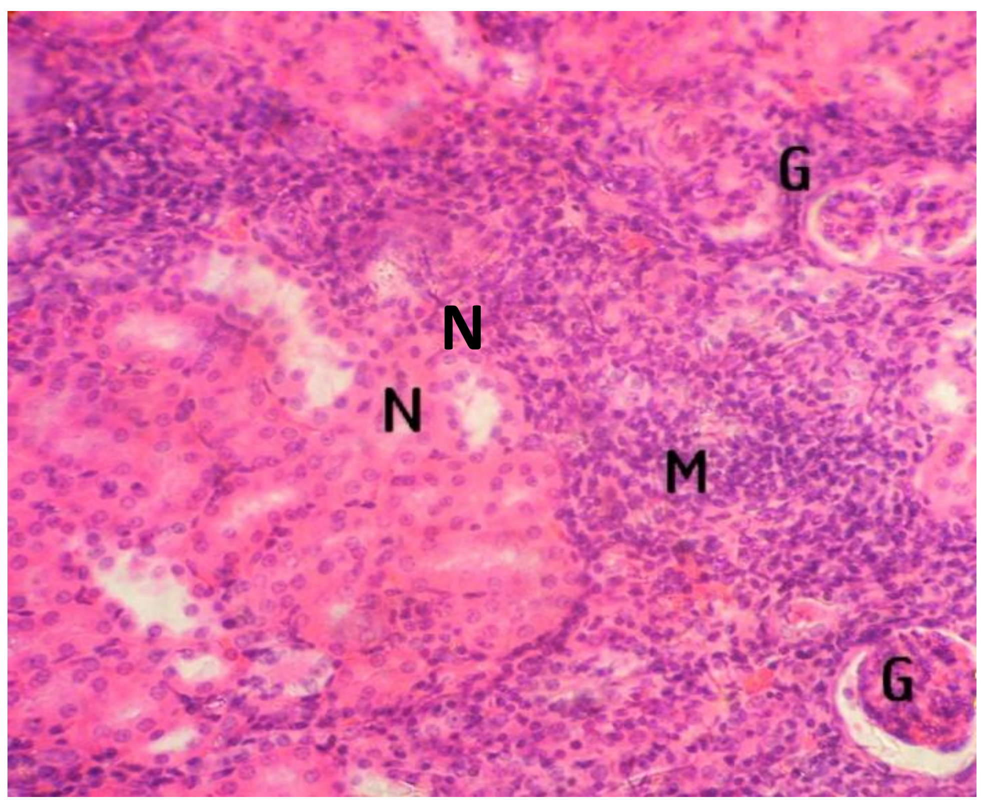


Plate XIV: Photomicrograph of a section of the kidney of a mouse 3 weeks after exposure to crude ethanol extract of *A. montanus* at 10,000 mg/kg, showing renal tubular necrosis (N), glomerular degeneration/sclerosis (G) and mononuclear cellular infiltration (M) of into the interstices (H & E, $\times$400).

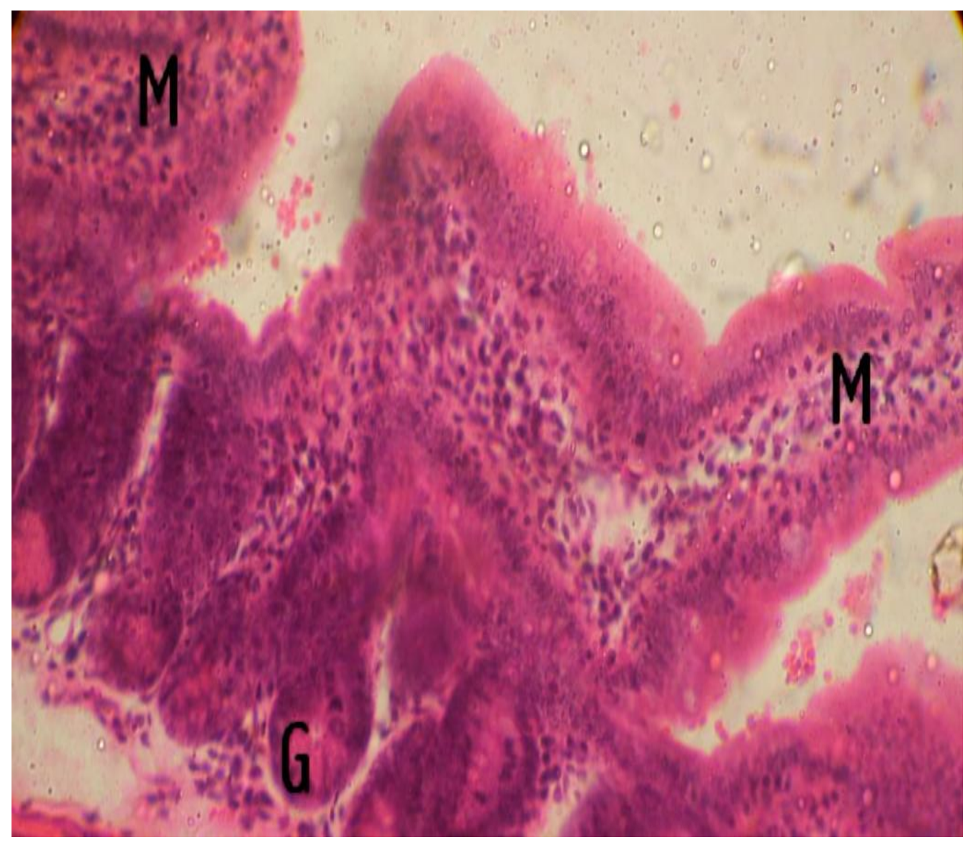


Plate XV: Photomicrograph of a section of the intestine of a mouse 3 weeks after exposure to crude ethanol extract of *A. montanus* at 10,000 mg/kg, showing regeneration of desquamated epithelium (metaplasia), mononuclear cellular infiltration (M) into the villi and hyperactive goblet cells (G) (H & E, ×400).

4.8 Anthelmintic Effects of Various Doses of Leaf Extracts of *A. montanus*

4.8.1 Effect of extracts on EPG of faeces

Generally, a decrease in EPG was seen to be significantly lower in *n*-butanol-treated mice compared to other extract-treated groups. At the dose level of 2.0 g/kg, *n*-butanol produced

mean EPG of 2540±1002.7 ($p<0.001$), while crude ethanol extract and aqueous portion produced 3930±1107.5 ($p<0.01$) and 4760±826.20 ($p<0.05$) respectively compared to distilled water-treated (negative control) group. Likewise albendazole (positive control group) at dose rate of 10 mg/kg produced significant decrease (1060±997.92) in mean EPG ($p<0.001$) in comparison with distilled water control (9410±216.45). There was however, no statistical significant difference ($p>0.05$) in between extract-treated groups (Figure 4.1-4.4 and Table 4.5).

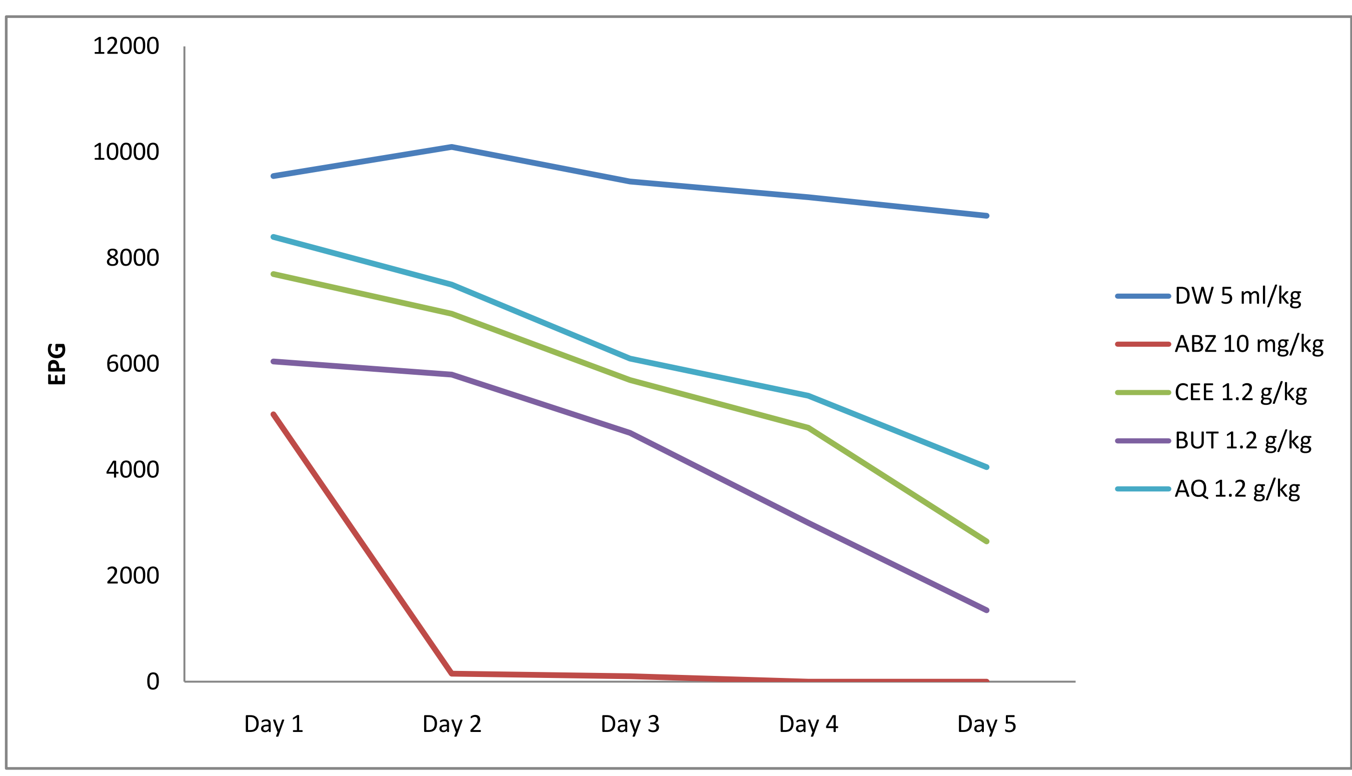


Figure 4.1: Pattern of egg per gram (EPG) of faeces in mice infected with 150 L_3 of *H. bakeri* and orally treated for 5 consecutive days with 1.2 g/kg of *A. montanus* leaf extracts, distilled water or albendazole 19 days post infection.

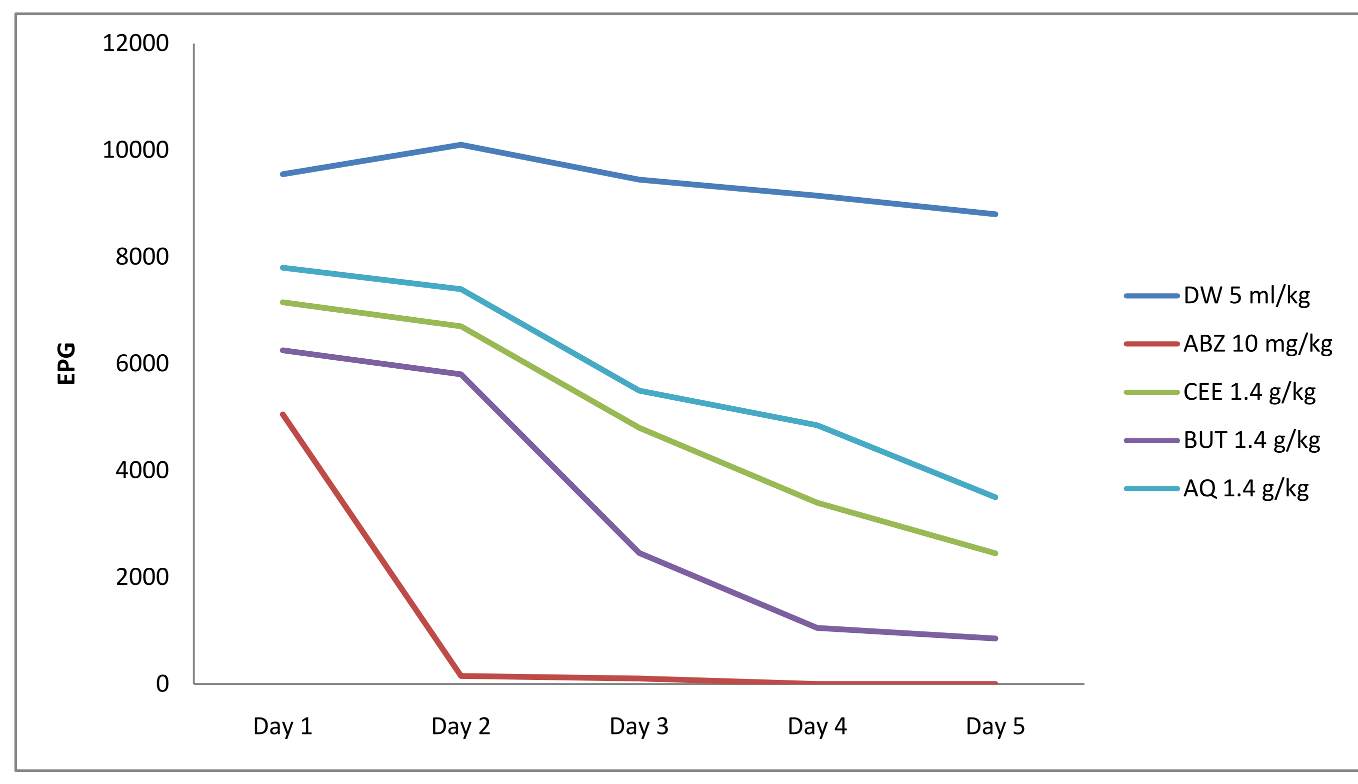


Figure 4.2: Pattern of egg per gram (EPG) of faeces in mice infected with 150 L_3 of *H. bakeri* and orally treated for 5 consecutive days with 1.4 g/kg of *A. montanus* leaf extracts, distilled water or albendazole 19 days post infection.

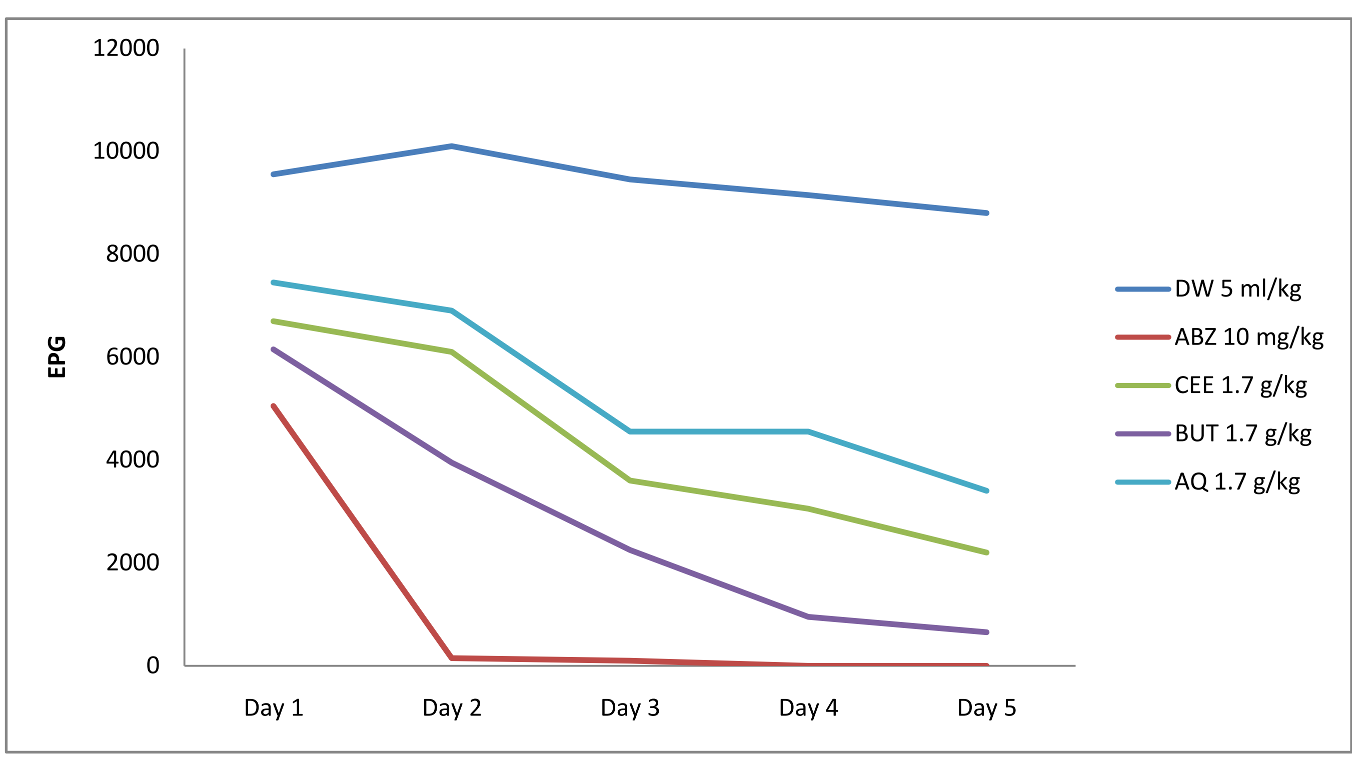


Figure 4.3: Pattern of egg per gram (EPG) of faeces in mice infected with 150 L_3 of *H. bakeri* and orally treated for 5 consecutive days with 1.7 g/kg of *A. montanus* leaf extracts, distilled water or albendazole 19 days post infection.

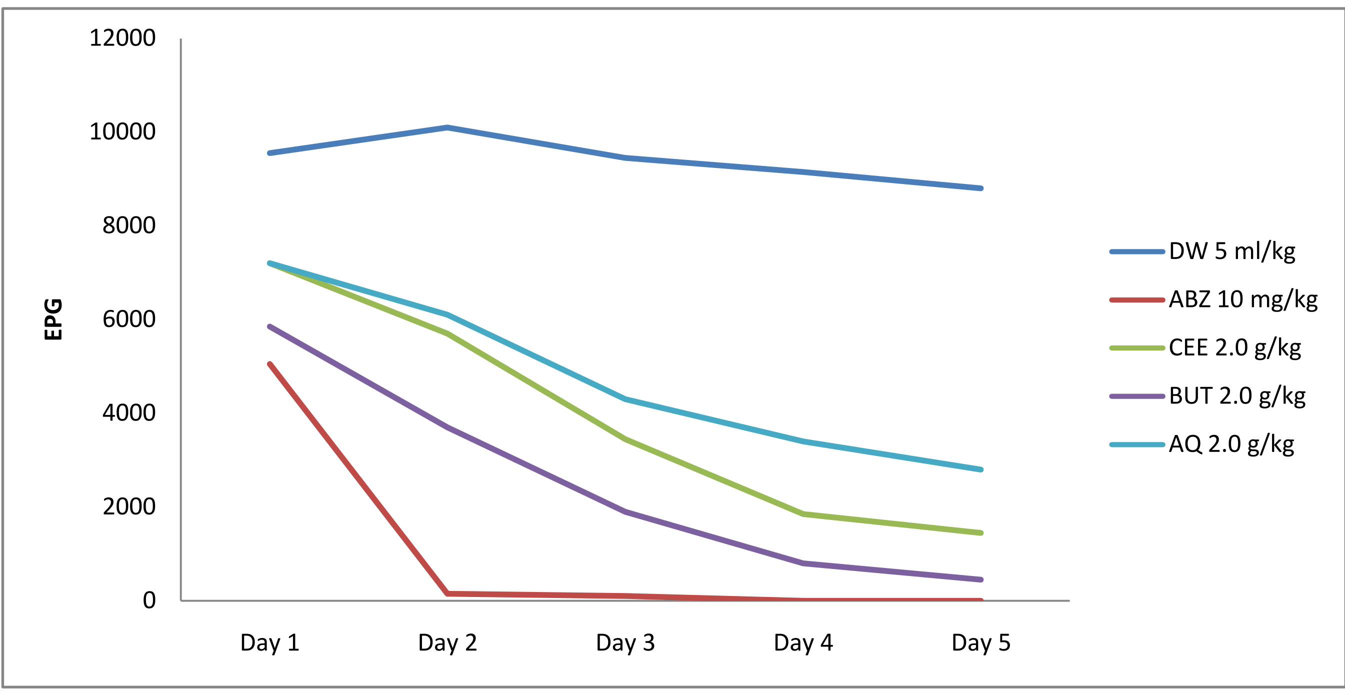


Figure 4.4: Pattern of egg per gram (EPG) of faeces in mice infected with 150 L_3 of *H. bakeri* and orally treated for 5 consecutive days 2.0 g/kg of *A. montanus* leaf extracts, distilled water or albendazole 19 days post infection.

Table 4.5: Mean (±SEM) EPG of mice infected with 150 L_3 of *H. bakeri* and orally treated for 5 consecutive days with varying doses of *A. montanus* leaf extracts, distilled water or albendazole 19 days post infection.

Dose (g/kg)	Substance				
	CEE	BUT	AQ	DW 5ml/kg	ABZ 10 mg/kg
1.2	5560±882.52[a]	4180±888.62[a]	6290±766.88[a]	9410 ±216.45[c]	1060±997.92[b]
1.4	4900±910.08[a]	2950±1014.9[b]	5810±801.31[a]		
1.7	4330±879.15[a]	2790±1022.1[b]	5370±771.14[a]		
2.0	3930±1107.5[b]	2540±1002.7[b]	4760±826.20[a]		

F=5.131 (MStreatment/MSresidual)
5 ml/kg is the MCV
10 mg/kg is the Dose as recommended by manufacturer
a, b, c differ significantly ($p<0.05$) from one another

Albendazole (positive control) at dose rate of 10 mg/kg (88.91±10.45%) and *n*-butanol portion of *A. motanus* at all the doses used produced significant ($p<0.05$) reduction in epg with the highest mean percentage decrease rate of 73.63±10.31% at the dose of 2.0 g/kg. Aqueous portion, however, did not produce significant ($p<0.05$) mean percentage reduction in epg at all the doses used. For crude ethanol extract, only dose rates of 1.7 g/kg and 2.0 g/kg (53.56±9.31% and 59.31±10.89%) produced significant mean percentage reduction in epg. Statistically, there was no significant ($p>0.05$) difference between treatment groups. However, there was significant difference between extract-treated and the distilled water control groups ($p<0.01$) as well as extract-treated and albendazole groups ($p<0.05$) (Table 4.6).

Table 4.6: Daily and mean percentage (%) decrease in FEC/EPG in mice infected 150 L_3 of *H. bakeri* and orally treated for 5 consecutive days with varying doses of *A. montanus* leaf extracts, distilled water or albendazole 19 days post infection.

Substance/Dose (g/kg)	Day 1	Day 2	Day 3	Day 4	Day 5	Mean±SEM decrease in epg (n=5days)
CEE 1.2	19.37	31.19	39.68	53.01	69.89	42.63 ± 8.75^{a}
1.4	25.13	33.66	49.21	62.84	72.16	48.60 ± 8.75^{a}
1.7	24.61	39.60	61.90	66.67	75.00	53.56 ± 9.31^{a}
2.0	26.18	43.56	63.49	79.78	83.52	59.31 ± 10.89^{a}
BUT 1.2	36.65	42.57	50.26	67.21	84.66	56.27 ± 8.76^{a}
1.4	34.55	58.91	74.07	88.32	90.34	69.24 ± 10.35^{b}
1.7	35.98	60.89	76.19	89.62	92.61	71.06 ± 10.41^{b}

	2.0	38.74	63.37	79.89	91.26	94.89	73.63 ± 10.31^{b}
AQ	**1.2**	12.04	25.74	35.45	40.98	53.98	33.64 ± 7.07^{a}
	1.4	18.32	26.73	41.80	46.99	60.23	38.81 ± 7.42^{a}
	1.7	21.99	31.68	51.85	50.27	61.36	43.43 ± 7.20^{a}
	2.0	24.61	39.60	54.50	62.84	68.18	49.95 ± 7.96^{a}
DW 5 ml/kg		0	0	0	0	0	0.000 ± 0.00^{c}
ABZ 10 mg/kg		47.12	98.51	98.94	100	100	88.91 ± 10.45^{b}

F=5.905 (MStreatment/MSresidual)
5 ml/kg is the MCV
10 mg/kg is the Dose as recommended by manufacturer
a, b, c differ significantly ($p<0.05$) from one another

4.8.2 Effect of extract on adult *H. bakeri*

There was a dose-dependent decrease in worm count in mice given each of the three preparations. Thus, for each extract, the highest worm count was seen in mice given the lowest dose of 1.2 g/kg; and the lowest in those given the highest dose of 2.0 g/kg. For each dose level, mice given the *n*-butanol portion had the lowest worm counts, followed by those given the crude ethanol extract; and then those given the aqueous portion. At all the four dose levels, the worm counts from mice that received the *n*-butanol portion were significantly lower than those of the crude ethanol extract or aqueous portions. At all dose levels, the worm counts of the crude ethanol extract-dosed mice were not significantly different from those mice that were dosed with the aqueous extract.

Compared to the negative control, mice given any of the dose-levels of *n*-butanol had significantly lower worm counts. Mice treated with crude ethanol extract at dose levels of 1.7 g/kg and 2.0 g/kg had significantly lower worm counts (7.20±0.58 and 5.60±0.33 respectively) than mice in the negative control groups (20.25±1.11). Also, mice treated with aqueous portion at dose rate of 2.0 g/kg had significantly lower counts (7.50±0.65) than the distilled water-treated control mice (Table 4.7.). The worm counts of the albendazole-treated (0.00±0.00) positive control group was not significantly different ($p<0.05$) from the counts (0.60±0.40) of mice treated with *n*-butanol at the highest dose level of 2.0 g/kg. The degree of deparasitization achieved by dosing with the extracts or albendazole or distilled water is the direct converse of the data on worm count (Table 4.8).

Table 4.7: Mean (± SEM) worm count in mice infected with 150 L_3 of *H. bakeri* and orally treated for 5 consecutive days with varying doses of *A. montanus* leaf extracts, distilled water or albendazole 19 days post infection.

Dose	Substance				
(g/kg)	CEE	BUT	AQ	DW 5 ml/kg	ABZ 10 mg/kg
1.2	10.40 ± 0.51^{a} (5)	6.67 ± 0.33^{a} (3)	12.67 ± 0.88^{a} (3)	20.25 ± 1.11^{c} (4)	0.00 ± 0.00^{b} (4)
1.4	8.20 ± 0.86^{a} (5)	4.20 ± 0.37^{b} (5)	10.80 ± 0.58^{a} (5)		
1.7	7.20 ± 0.58^{a} (5)	1.80 ± 0.49^{b} (5)	8.40 ± 0.51^{a} (4)		
2.0	5.60 ± 0.33^{a} (5)	0.60 ± 0.40^{b} (5)	7.50 ± 0.65^{a} (4)		

F=78.043 (MStreatment/MSresidual)

5 ml/kg is the MCV
10 mg/kg is the Dose at manufacturer's recommendation
a,b,c, differ significantly ($p<0.05$) from one another
Values in parenthesis represent the number of mice in the group.

The *n*-butanol extract gave the highest rate of deparasitization at the four doses that were used. At 2.0 g/kg dose rate, the deparasitization rate achieved through *n*-butanol was 97% compared to 72.4% and 63% for crude ethanol extract and aqueous portion respectively. The deparasitization caused by *n*-butanol at any of the doses was significantly higher compared to those caused by either crude ethanol extract or aqueous portion at such corresponding dose.

Table 4.8: Percentage deparasitization (%) in mice infected with 150 L_3 of *H. bakeri* and orally treated for 5 consecutive days with varying doses of *A. montanus* leaf extracts, distilled water or albendazole 19 days post infection.

Dose	Substance				
(g/kg)	CEE	BUT	AQ	DW 5ml/kg	ABZ 10 mg/kg
1.2	48.64	67.06	37.43	0.000	100.0
1.4	59.51	86.17	46.67		
1.7	64.44	91.11	58.52		
2.0	72.35	97.04	62.96		

5 ml/kg is the MCV
10 mg/kg is the Dose at manufacturer's recommendation

CHAPTER FIVE

DISCUSSION

Acanthus montanus was selected according to earlier reports of anthelmintic efficacy (Oshadu, 2009; Adamu *et al.,* 2010) and folkloric claims. In this study, *Acanthus montanus* leaf was extracted with ethanol and solvent-partitioned to yield chloroform, *n*-butanol and aqueous portions. The portions tested for anthelmintic potential produced deparasitization rate and reduction in faecal egg count/egg per gram (FEC/EPG) that is dose dependent as was seen from adult worm count postmortem and daily faecal analysis in mice.

This result is similar to that of Suleiman (2002) who reported that the butanol extract of *Terminalia avicennoides* at dose rates of 1.3 g/kg, 1.5 g/kg 1.8 g/kg and 2.2 g/kg produced deparasitization rates of 61%, 72%, 76% and 91% respectively, in rats experimentally infected with *Nippostrongylus brasiliensis*. Also, Ibrahim *et al.* (1983) obtained an 89% deparasitization in using methanol root extract of the same plant in rats experimentally infected with *N. brasiliensis* at dose rate of 2.5 g/kg. Jegede *et al.* (2009) reported deparasitization rate of 90% using aqueous ethanol fraction of *Spigelia anthelmia* in experimental infection with *N. brasiliensis* in rats that had the extract at the dose rate of 5.0 g/kg. Studies conducted by Simon *et al.* (1989) on stem bark of *Combretum molle* in experimental infection of rats with *N. brasiliensis* showed that aqueous methanol, chloroform and *n*-butanol fraction at 1,000 mg/kg produced deparasitization of 86.98%, 79.20% and 72.72% respectively.

Partitioning plant extracts is one method of separating plant components based on relative solubilities in solvents used (Harbone, 1984). However, due process must be followed to

obtain reasonable yield. The plant material used in this study was dried to prevent the fluctuations in chemical composition as a result of moisture content. Pounding of dried plant material to powders was to increase the surface area for greater extraction with the extracting solvents (Adeyemi, 2009). Although, the solvent mainly used for herbal preparation in traditional medicine is water and a few preparations utilize water-alcohol for rapid results (Zschocke and Van Staden, 2000; Sparg *et al.*, 2002), chloroform, *n*-butanol and water were used in this study as solvents for partitioning for consistency, and for achievement of total extraction of potential bioactive compounds (Mcgaw and Eloff, 2008). The concentrated crude ethanol extract was suspended in diluted methanol to aid its solubility for solvent partitioning.

Generally, partitioning with water resulted in the highest quantity of crude extract (31.42% *w/w*), while chloroform gave the least quantity (3% *w/w*). This might be due to the high polarity associated with water. Chloroform is non-polar. The high polarity of ethanol (used for extraction) and water (used as solvent for partitioning) probably accounted for the high percentage yield because they are polar and some less polar compounds were extracted (Adeyemi, 2009). The lowest yield (3% *w/w*) was obtained using chloroform, probably due to its inability to extract those biologically active compounds due to its less or non-polar nature. As expected, the extract yield increased when using solvents of increasing polarity in partitioning. The general trend of the yield obtained in ascending order was CF < BuOH < water (H_2O). Although water is more polar and have tendency to extract hydrosoluble compounds, substances like butanol in addition to hydrosoluble substances have tendency to extract lipid substances, alkaloids, and phenols (Ciulei, 1982). This probably explains

why the *n*-butanol fraction had deep coloration, and was pasty and greasy. The greenish coloration of the chloroform fraction is essentially due to chlorophyll (and/or other pigments) which is present in every green plant. Other pigments and/or oils may also probably have been extracted by the chloroform since they are of poor polarity. Thus the extraction of active principles from the medicinal plants for pharmacological evaluation was to some extent dependent on the polarity of the solvents used in the extraction and partitioning.

The crude ethanol extract was readily soluble in water. Of the different fractions, aqueous was most soluble, with MCC and MCD of 1 g/ml and 5 g/kg respectively. This was followed by the *n*-butanol fraction. Chloroform fraction was not soluble in water at all. These findings are similar to those obtained by Suleiman (2002) in his MCC and MCD determination of different portions of *Terminalia avicennoides.* He reported MCCs (g/ml) of 1, 0.5, 1.2 and 0.8 and MCDs (g/kg) of 5, 2.5, 6 and 4 for aqueous methanolic, butanol, chloroform portions and petroleum ether extract. The other portions were soluble in water because of their hydrophilic nature i.e., they contain hydroxyl group (OH^-) (Adeyemi, 2009). CF lacks this property, giving an indication that the constituents of the portion are mainly non-polar. It was however, readily soluble in chloroform and other non-polar solvents.

Phytochemicals generally refer to those chemical compounds that occur naturally in plants that are responsible for colour, and organoleptic properties. They may have biological significance but are not established as essential nutrients (US FDA, 2012). The results of phytochemical screening showed that glycosides, unsaturated steroids and triterpenes,

saponins, tannins, flavonoids and alkaloids are present in leaf extract of *A. montanus.* Separate studies conducted by Anam (1977 a, b) and Okeke *et al.* (2009) on different parts (leaf, stem and roots) of the same plant showed similar results with more concentration of phytoconstituents in the leaf. With some other plants such as *Cyclea peltata* the ethanol leaf extract showed the presence of alkaloids, flavonoids, tannins, diterpenes and saponins (Hullatti *et al.,* 2011). Simon *et al.* (2008) also reported the presence of phytocomponents that have anthelmintic property such as alkaloids, steroids, tannins, flavonoids and glycosides in crude extracts of *Combretum molle* stem bark.

Thin layer chromatography (TLC) is a chromatographic technique used to separate mixtures as those in drugs, plant extracts as well as other chemicals (Lewis and Moody, 1989). Different compounds in a sample travel at different rates due to the differences in their attraction to the stationary phase, as well as differences in solubility in the solvent (Mehta, 2012). To develop the spots of chromatograms, different spray reagents were used: 1% ferric chloride for tannins; anisaldehyde-sulphuric acid for sterols; and Dragendoff's reagent for alkaloids (Harborne, 1984). The TLC results from other plates further showed that the compounds eluted from the TLC plates sprayed with Dragendoff's reagent and Anisaldehyde-sulphuric acid were likely alkaloids and steroids/sterols respectively.

The results of the thin layer chromatography (TLC) in this study showed that *n*-butanol portion contains much of the biologically active compound(s) as evident in the conspicuous spot, followed by the crude ethanol extract, while the aqueous portion contains the least of these (Plate VII). This finding agrees with the report of Coulidiati *et al.* (2011) that butanol fractions of *Combretum* species studied showed the highest content

in total phenolics compared to the other fractions *viz n*-hexane, ethyl acetate and water. The authors also, reported that the majority of the total phenolics in butanol fraction of *C. sericeum* are constituted by tannins, representing 76.61%. The findings of the present study showed that the biologically active compound(s) was/were not contained in the chloroform portion at all with the eluting system used. All of the spots from the different portions of the extract have the same *Rf* value (0.53) (Yamuna *et al.,* 2012) characteristic of a particular compound. The *Rf* value obtained is indicative that the compound eluted is relatively polar due to its tight adsorption to the solid support (silica gel), making it to traverse relatively short distance. Based on the eluting system at the ratios indicated (chloroform-ethyl acetate-formic acid, 5:4:1), the spray reagent (1% $FeCl_3$) and the colour of the spots, the biologically active compound is phenolic. Of the polyphenolic compounds isolated, tannins exhibit these characteristics, since it is only tannins that react with $FeCl_3$ to yield greenish black (dirty green), blue-black, green or blue-green precipitate depending on the type/nature of the tannins (Evans, 2002; Yamuna *et al.,* 2012). Two types of tannins exist: condensed and hydrolysable tannins (McMahon *et al.,* 2000). The phenolic compound so detected is condensed tannins, since hydrolysable ones were absent confirming the qualitative phytochemical screening conducted in this study. There is the possibility that other biologically active compounds might be present in the respective portions of the leaf extracts of *Acanthus montanus;* since $FeCl_3$ that was used is specifically a detecting agent for tannins. These include alkaloids, saponins, flavonoides, steroids and sterols.

For the preliminary acute toxicity studies, all the treated mice were closely examined for signs of toxicity from the time of administration of extracts to more than three weeks. None exhibited any obvious signs of toxicity or change of demeanour at doses ranging from 10-10,000 mg/kg. All the treated mice except those euthanized, remained alive even long after the three weeks of observation. This suggests an oral LD_{50} greater than 10,000 mg/kg. The high LD_{50} value implies a remote risk of acute intoxication and high degree of relative safety (Loomis, 1978; Lorke, 1984) when extract is administered orally. It is therefore considered practically non-toxic. Earlier, Okoli *et al.* (2008) established an LD_{50} greater than 5,000 mg/kg when the aqueous root extract of *A. montanus* was administered orally and intraperitoneally. In an earlier study, Nana *et al.* (2007) demonstrated LD_{50} of the same plant to be greater than 8,000 mg/kg in rats. This probably explains why undetermined amount of aqueous leaf extract is taken locally in folk medicine without side effects. Attempts to grade dose-toxicity relationships of toxic substances under experimental conditions have been made. One such grading according to Loomis (1978), defined < 1 mg/kg as extremely toxic, 1-50 mg/kg as highly toxic, 50-500 mg/kg as moderately toxic, 0.5-5 g/kg slightly toxic, 5-15 g/kg as practically non-toxic and > 15 g/kg as relatively harmless. In this study, the extract tested in mice is therefore regarded as practically non-toxic, since the LD_{50} fall within the range 5-15 g/kg.

Mice treated with dose range of 10-1,000 mg/kg of crude ethanol extract had no detectable pathology in the organs studied, but lesions, mainly congestion, were observed in the lungs, liver, heart and spleen of mice that were dosed with 10,000 mg/kg. After three weeks, a mouse from the group that was dosed with 10,000 mg/kg was necropsied and

internal organs examined for risk of remote toxicity. It was discovered that the congestions seen previously was resolved. This further confirmed the fact that *A. montanus* plant might be practically non-toxic (Loomis, 1978; Lorke, 1984; Okoli *et al.,* 2008).

Histopathologic examination of organs of mice treated with varying doses of crude ethanol extract showed areas of haemorrhages, congestions, necrosis, villar desquamation and in some cases mononuclear infiltration of organs. These microscopic lesions were however, marked in groups exposed to highest dose (10,000 mg/kg). Most organs exposed to lower doses and distilled water were relatively normal. Some organs especially the intestine showed marked signs of villar regeneration as evident in the metaplasia noticed. It can be inferred from this result that the extract may be toxic to mice exposed for a prolonged period to doses above 10,000 mg/kg.

The results of the anthelmintic study indicate that *n*-butanol portion of *A. montanus* leaf produced significantly higher deparasitization followed by crude ethanol and then aqueous portions. Similar result was obtained when butanol portions of *Combretum* species were screened for their antioxidant and antibacterial activities (Coulidiati, *et al.,* 2011). In this study, the efficacy of the *n*-butanol portion, for instance increased with dose, an indication of graded response of the parasite to the drug (Iqbal *et al.,* 2006 a, b). Different classes of anthelmintics are established to show profound effects on the physical activities, generally culminating into loss of mobility and mortality of helminth parasites in a dose-dependent manner (Urrea-Paris *et al.,* 2000; Tippanangkosal *et al.,* 2004; Xiao *et al.,* 2004).

The active principle(s) in extract(s) responsible for this anthelmintic activity might be individual phytocomponents as detected during phytochemical screening or a number of them working in synergy. This higher effect, according to Wabo *et al.* (2011) could be due to secondary metabolites such as tannins, flavonoids, polyphenols, coumarins or alkaloids. This assertion was made from their findings on *in vitro* study on leaf extracts of *Ageratum conizoides* on *H. polygyrus*. These compounds create unfavorable conditions to the survival of the parasites. Suleiman *et al.* (2005) had ealier reported such dose-dependent activity in methanol seed extract of *Xylopia aethiopica* in experimental *Nippostrongylus brasiliensis* infection in rats. According to them, tannins, flavonoids or terpenoids present in the extract were responsible for the observed anthelmintic activity. In the present study, tannins (condensed tannins) were likely responsible for the observed profound anthelmintic activity (Niezen *et al.*, 1995) due to its abundance in the extract screened phytochemically and confirmed by thin layer chromatography.

Phenonlics (such as tannins) were shown to possess anthelmintic activities (Niezen *et al.*, 1995). Chemically, tannins are polyphenolic compounds which are uncouplers of oxidative phosphorylation in helminth parasites (Bate-Smith, 1962). Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide, bithionol etc., are reported to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation (Martin, 1997). It is possible that the large amount of tannins detected in the *n*-butanol leaf extract of *Acanthus montanus* produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tracts of host animal

(Athanasiadou, 2001) or glycoprotein on the cuticle of the parasite (Thompson and Geary, 1995), and may thus cause death.

Polyphenolic compounds which are reported to be present in leaves of mainly dicotyledonous plants are potent anthelmintics. Condensed tannins which are derived from flavonol are soluble in water and are capable of precipitating proteins. They are reported to be found in cell walls or stored in vacuoles, stems, leaves, flowers or seeds (McMahon *et al.,* 2000; Acuna, 2008). Tannins protect the intestine from reinfection by "tanning" proteins in the lining of the gut (intestine) (Wallace, 2012). Consumption of vegetative portions of such plants is advocated. For example, the intestinal worms are paralysed and killed by daily taking of walnut leaf extracts or tea prepared with walnut leaves. Walnut contains abundant tannins. This may explain why the plant (*A. montanus*) aerial parts are used as vermifuge by the Etulo natives of Benue State, Nigeria. It is well known that a high level of UV radiation increases the concentrations of total phenols and the main flavonoids (Garcia-Macias *et al.,* 2007). Previous works have reported high leaf/stem polyphenol proportions in *Platogo* species (Grubesic *et al.,* 2005), thus confirming that leaf function serves as defence mechanisms against UV damage (Harborne and Williams, 2000).

Todorov and Genov (1973) reported that most of the plants tested for anthelmintic activity in Bulgaria had a neurogenous-cholinergic effect. Indeed, the same is true for some synthetic anthelmintics. The benzimidazoles/probenzimidazoles (e.g. albendazole, mebendazole, thiabendazole, fenbedazole, and flubendazole) act by interferring with polymerization of microtubules (Harder, 2002). These drugs bind to the protein tubulin of the parasite, therefore causing death by starvation (Roos, 1997). The

tetrahydropyrimidines/imidazothiazoles group (levamisole, pyrantel, morantel and oxantel) affect acetylcholine neuro-transmission by interfering with nicotinic acetylcholine receptors of nematodes muscle cells and produce contraction and spastic paralysis (Roos, 1997; Harder, 2002). The macrocyclic lactones or avermectins/milbemycins group interact with chloride channels on helminth gamma-aminobutyric acid (GABA) receptor complexes, and also inhibit pharyngeal pumping (and hence feeding), motility and fecundity in susceptible nematodes, resulting in paralysis and ultimately elimination from the host (Harder, 2002; Yates *et al.,* 2003). Examples of drugs in this category include ivermectin, abamectin, doramectin, eprinomectin, selamectin, milbemycin oxime and moxidectin.

The modes of action of anthelmintics are diverse, reflecting the natural differences in the physiology of the parasites and its potential host. It has been firmly established that one of the hallmark effects of any anthelmintic is the destruction of the worm's surface. This is due to the fact that the tegument and/or cuticular structures are the primary parasite-host interface vital for absorption of nutrients and perception of the surrounding micro-environment provided by the host (Williams *et al.,* 2001; Xiao *et al.,* 2004, 2005).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study has demonstrated that the *n*-butanol extract of *Acanthus montanus* leaves has profound anthelmintic activity against experimental *Heligmosomoides bakeri* infection in mice. With the LD_{50} greater than 10,000 mg/kg, it can be inferred that the plant might have a wide margin of safety typical of anthelmintics. The study also showed the major phytochemial components of *Acanthus montanus* to be glycosides, unsaturated steroids and triterpenes, saponins, tannins, flavonoids and alkaloids. Based on the result of this study, null hypothesis that "leaf extracts of *Acanthus montanus* have no modulatory effects on experimental *Heligmosomoides bakeri* infection in mice" is rejected. Also, this study has scientifically established the folkloric claim among the Etulo natives of Benue State, Nigeria, that the plant is a vermifuge.

6.2 Recommendations

1. In addition to crude extraction and solvent partitioning, attempts should be made to quantitatively isolate the phytochemical constituents.
2. Each phytocomponent so isolated should be tested individually with respect to its toxicity, and anthelmintic activity against important ruminant nematodes.
3. Inspite of the seeming low toxity or the non-toxicity as demonstrated in the present study, there is need to conduct detailed chronic toxicity studies especially with the *n*-

butanol portion of *A. montanus* in laborаratory animals and target animals species in order to justify its use in veterinary practice.

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APPENDIX I: Effect of different portions of leaf extracts of *Acanthus montanus* on faecal egg count (FEC) of *Heligmosomoides bakeri.*

Substance/Dose (g/kg)	Day 1	Day 2	Day 3	Day 4	Day 5
CEE 1.2	154	139	114	86	53
1.4	143	134	96	68	49
1.7	144	122	72	61	44
2.0	141	114	69	37	29
BUT 1.2	121	116	94	60	27
1.4	125	83	49	21	17
1.7	123	79	45	19	13
2.0	117	74	38	16	9
AQ 1.2	168	150	122	108	81
1.4	156	148	110	97	70
1.7	149	138	91	79	68
2.0	144	122	86	68	56
DW 5 ml/kg	191	202	189	183	176
ABZ 10 mg/kg	101	3	2	0	0

5 ml/kg is the MCV
10 mg/kg is the Dose as recommended by manufacturer

APPENDIX II: Working formula for determination of egg per gram (EPG) of faeces.

1 g of faecal pellets suspended in 14 ml of flotation medium (saturated NaCl/sucrose solution, specific gravity of 1.28).

NOTE: a) 1 g ≡ 1 ml ≡ 1 cm^3

b) 1 McMaster chamber = 0.15 ml, 2 chambers = $0.15\ ml \times 2 = 0.3\ ml$

→1 g of faecal pellets + 14 ml of flotation medium = 15 ml (total volume).

If $xeggs$ is contained in 0.3 ml (McMaster chambers)

Then, 15 ml will contain $\left(15xeggs \times {}^{1}/_{0.3}\right) = 50xeggs$

∴ 50 is the multiplication factor for Faecal Egg Count (FEC) in this study.

NB: FEC×50 (McMaster egg count) =EPG.

APPENDIX III: Effect of different portions of leaf extracts of *Acanthus montanus* on adult *Heligmosomoides bakeri*.

Substance/Dose (g/kg)	M 1	M 2	M 3	M 4	M 5	Mean	% DP

CEE	1.2	10	9	12	11	10	10.40	48.64
	1.4	8	10	9	9	5	8.200	59.51
	1.7	6	7	8	9	6	7.200	64.44
	2.0	5	6	7	5	5	5.600	72.35
BUT	1.2	6	7	*	7	*	6.670	67.06
	1.4	5	4	5	4	3	4.200	79.26
	1.7	3	2	0	2	2	1.800	91.11
	2.0	0	2	0	0	1	0.600	97.04
AQ	1.2	13	*	*	14	14	12.67	37.43
	1.4	10	12	9	12	11	10.80	46.67
	1.7	*	9	8	10	7	5.800	58.02
	2.0	8	7	6	*	9	7.500	62.96
DW	5 ml/kg	19	23	21	*	18	20.25	0.000
ABZ	10 mg/kg	0	0	*	0	0	0.000	100.0

5 ml/kg is the MCV

10 mg/kg is the Dose as recommended by manufacturer

M =Mouse

* Mouse died before end of experiment. Worm count not observed.

$\%DP = \frac{N-n}{N} \times 100$ where: DP = Deparasitization

N = mean worm count in untreated group (DW).

n = mean worm count in treated groups.

APPENDIX IV: Necropsy result of preliminary acute toxicity studies on crude ethanol leaf extract of *A. montanus* in mice.

AHMADU BELLO UNIVERSITY
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF PATHOLOGY AND MICROBIOLOGY

Necropsy No.: 60-2012

Date: 13.7.2012

Clinic No.:

Animal Mice Species ______ Age Ad. Sex ______ Breed white

Owner Dr David O. Oshadu Clinician ______

History : Exptl. Rx of mice with Acanthus montanus. Rx with 10000mg/kg ⟶ 10mg/kg.

Gross Necropsy Findings : Congested lungs and liver in 10000mg/kg Rx group; Other groups - no significant histopathological findings

Tentative Diagnosis Exptl. Administration of Acanthus montanus

Comments liver, kidney, lungs, spleen, intestines, heart.

Pathologist Dr. B. Mohammed [signature]

APPENDIX V: Result of histiopathology of preliminary toxicity studies on crude ethanol leaf extract of *A. montanus* in mice.

Group	Dose (mg/kg)	Organ	Microscopic lesion(s)

1	10,000	Liver	Hepatocellular degeneration around the congested sinusoides and central vein; haemorrhages.
		Lung	Slide not available.
		Heart	Massive haemorrhage, myocardial degeneration and foci of mononuclear cellular infiltration.
		Kidney	Renal tubular necrosis and mononuclear cellular infiltration of into the interstices.
		Spleen	Diffused splenic cellular necrosis within the trabaculae.
		Intestine	Slide not available.
1 (Follow-up 3 weeks post-treatment)	10,000	Liver	Diffused hepatocellular necrosis, congestion of sinusoides, vacuolar degeneration and foci of mononuclear cellular infiltration.
		Lung	Slide not available
		Heart	Myocardial degeneration, haemorrhages as well as foci of mononuclear cellular infiltration.
		Kidney	Haemorrhage, necrosis/atrophy (sclerosis) of glomerulii and tubules, focal infiltration of mononuclear cells.
		Spleen	Pale area of splenic cellular necrosis.
		Intestine	Massive desquamation (enteritis) and regeneration of villi. Enlarged goblet cells. Note changes in villar epithelium (metaplasia).
2	1,000	Liver	Congested blood vessels, perivascular vacuolar degeneration.
		Lung	Slide not available.
		Heart	Slide not available.
		Kidney	Glomerulonephritis.
		Spleen	Slide not available.
		Intestine	Enteritis, presence of necrotic tissues in the lumen.
3	100	Liver	Congested blood vessels and sinusoides, foci of perivascular degeneration around central vein.
		Lung	Slide not available.
		Heart	No obvious histopathology
		Kidney	Slide not available.
		Spleen	Slide not available.
		Intestine	Mild villi desquamation and regeneration.
4	10	Liver	Congested sinuoides.
		Lung	Focal area of thickened interaveolar tissues.
		Heart	No obvious histopathology
		Kidney	Slide not available.
		Spleen	Slide not available.

		Intestine	Hyperactive goblet cells, normal villi.
5	5 ml/kg Distilled water (Control)	Liver	No observable lesion.
		Lung	No observable lesion.
		Heart	No observable lesion.
		Kidney	No observable lesion.
		Spleen	Slide not available.
		Intestine	Slide not available.

5 ml/kg is the MCV