

**EFFECTS OF CRUDE LEAF EXTRACTS OF *FICUS*
THONNINGII ON GROWTH, GASTROINTESTINAL
MORPHOMETRY AND CLINICAL BIOCHEMISTRY
OF SUCKLING SPRAGUE DAWLEY RATS**

Rachael Dangarembizi

A thesis submitted to the Faculty of Health Sciences, University of
the Witwatersrand, in fulfillment of the requirements for the degree

of

Master of Science in Medicine

Johannesburg, 2012

DECLARATION

I declare that this dissertation is my own work, with the aid of the acknowledged persons. This dissertation is being submitted for the degree of Master of Science in Medicine in the Faculty of Health Sciences at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University. All procedures used in this dissertation were approved by the Animal Ethics screening Committee of the University of the Witwatersrand (AESC number 2011/22/2A).

.....

.....day of.....2012

DEDICATION

To my loving family, who have stood behind me in all my endeavours.

To my supervisors, colleagues and friends: for the tireless support and motivation.

To my late mother and my best friend: This is just one step up, the goal gets nearer and nearer with each coming day.

ABSTRACT

Ficus thonningii is a nutraceutical that is extensively used in ethnomedicine. Nursing mothers use *F. thonningii* leaves as nutritional and medicinal supplements and are at risk of exposing their infants to its constituent phytochemicals. The exposure of the sensitive neonatal gastrointestinal tract (GIT) to these phytochemicals can result in irreversible changes in growth and development. The objectives of this study were to determine the effects of crude *F. thonningii* extracts on; growth, morphology and morphometry of the abdominal viscera and clinical biochemistry of neonatal rats.

Forty, suckling Sprague Dawley rats of either sex were randomly divided into 5 groups. Each group was orally gavaged once daily with either low (50 mg/kg b.w) or high (500 mg/kg b.w) doses of aqueous or methanolic extracts of *F. thonningii*, for 7 days. The control rats received distilled water. The pups were euthanased and tissues were collected and weighed. Samples of the liver, caecum and proximal small intestine were preserved and processed for histology. Plasma biochemical parameters were analysed colorimetrically. Data was presented as means \pm SD.

F. thonningii extracts exhibited trophic effects on the stomach and ceecal mucosa of rats but had no significant growth-promoting effects on the small intestine and visceral organs. Histological analysis of the intestine, liver and caeca revealed no mucosal damage. Clinical chemistry parameters were not abnormally altered. There was a significant decrease ($p < 0.05$, ANOVA) in the plasma concentrations of basal (non-fasting) glucose in the pups

on the high methanolic extracts. However, the triglyceride and cholesterol levels were unaltered by the treatments.

The findings suggest that *F. thonningii* extracts exhibit trophic effects on the mucosal layers of the stomach and caecum. *F. thonningii* extracts also possess glucose-lowering activity. At low doses, *F. thonningii* extracts can be safely used without the risk of any disruption in the structural integrity of the neonatal rat GIT and function of the liver and kidneys.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank the Lord for His guidance and for opening windows of opportunity in my life. I am deeply indebted to my supervisors Mr Eliton Chivandi and Assoc. Professor Kennedy Erlwanger for their guidance, constant encouragement and tireless support. This work wouldn't have been successful without your invaluable assistance.

I am sincerely grateful to my colleagues Davison Moyo, Zinhle Gasa, Trevor Nyakudya and Janine Donaldson for assisting throughout the course of my studies. A heartfelt thanks goes to Margaret Badenhorst for offering her technical help in processing tissues for histology. Special thanks also goes to John and Sandra Burrows, Walter Barker and Donald McCallum for assistance with species identification. I would like to thank the CAS staff; Amelia Rammekwa, Lorraine Setimo and Nico Douths for the technical assistance rendered during my animal studies.

I would like to express my sincere gratitude to the University of the Witwatersrand, the National Research Foundation and the National University of Science and Technology (NUST) for sponsorship that made this work possible. I am obliged to the Dean and staff at the Faculty of Medicine (NUST) for their continued support throughout the course of my study.

Last but not least I owe my sincere and earnest gratitude to my family and friends for the support, love and encouragement throughout the course of my study. My most sincere gratitude goes to my best friend Bright for the unwavering support and motivation in all my endeavours.

TABLE OF CONTENTS

DECLARATION	ii
DEDICATION	iii
ABSTRACT	iv
ACKNOWLEDGEMENTS	vi
LIST OF FIGURES	xiii
LIST OF TABLES	xiv
LIST OF ABBREVIATIONS	xv
CHAPTER 1-INTRODUCTION AND LITERATURE REVIEW	1
1.0. Background	2
1.1. <i>Ficus thonningii</i>	3
1.1.1. Botany and Taxonomy	3
1.2. Biochemistry	5
1.2.1. Nutritional value.....	5
1.2.2. Phytochemical composition	7
1.2.2.1. Flavonoids	7
1.2.2.2. Tannins	8
1.2.2.3. Alkaloids	9
1.2.2.4. Terpenoids	9
1.2.2.5. Essential oils.....	10
1.2.2.6. Other phytochemicals.....	10
1.3. Ethnomedicinal uses.....	11

1.3.1.	Leaves.....	11
1.3.2.	Stem Bark.....	12
1.3.3.	Roots.....	12
1.3.4.	Latex.....	13
1.3.5.	Uses of <i>F. thonningii</i> in combination with other plants	13
1.4.	Phytopharmacology.....	14
1.4.1.	Antimicrobial activity.....	14
1.4.1.1.	Antibacterial effects	14
1.4.1.2.	Antiprotozoal properties.....	17
1.4.1.3.	Antifungal effects	18
1.4.2.	Anthelmintic properties.....	19
1.4.3.	Antioxidant activity.....	19
1.4.4.	Analgesic effects	20
1.4.5.	Anti-inflammatory effects	21
1.4.6.	Cardioprotective effects	21
1.4.7.	Hypoglycemic effects.....	22
1.4.8.	Antidiarrheal effects	22
1.5.	The gastrointestinal tract	23
1.6.	Effect of phytochemicals on the GIT	25
1.7.	Nutrition and growth in neonates	27
1.8.	Justification of the present study	27
1.9.	Aims and objectives	29
CHAPTER 2-MATERIALS AND METHODS.....		31

2.1.	Plant collection and preparation	32
2.2.	Dry matter and moisture content determination	32
2.3.	Extraction	33
2.4.	Brine shrimp (<i>Artemia salina</i>) toxicity assay	33
2.4.1.	Hatching and preparation of nauplii	33
2.4.2.	Brine shrimp assay	34
2.5.	Phytochemical determination	35
2.5.1.	Terpenoids: Salkowski's test	35
2.5.2.	Alkaloids: Mayer's reagent test	35
2.5.3.	Saponins: Froth test	36
2.5.4.	Flavonoids: Shinoda's test	36
2.5.5.	Anthraquinones	36
2.5.6.	Phlobatannins	37
2.6.	Ethics clearance and study setting	37
2.7.	Housing and general care of the rat pups	37
2.8.	Study design	38
2.9.	Treatments	38
2.10.	Termination and tissue sample collection and storage	40
2.11.	Blood sample preparation	40
2.12.	Growth performance determination	41
2.12.1.	Body mass gain	41
2.12.2.	Visceral organ mass	41
2.12.3.	Linear growth	42
2.13.	Biochemical profiling for general health status of the rat pups	42

2.14.	Morphology and morphometry of abdominal viscera.....	43
2.15.	Determination of hepatic storage substrates.....	43
2.15.1.	Hepatic lipids.....	43
2.15.2.	Hepatic glycogen.....	44
2.16.	Statistical analysis	44
CHAPTER 3-RESULTS		45
3.1.	Plant extract yield and phytochemical screening	46
3.1.1.	Extract yield	46
3.1.2.	Phytochemical composition	46
3.2.	Brine shrimp acute toxicity assay.....	48
3.3.	Toxicity, mortality and morbidity	48
3.4.	The effect of <i>F. thonningii</i> extracts on growth of rat pups.....	51
3.4.1.	Body mass gain	51
3.4.2.	Linear growth	54
3.4.3.	Morphometry and histology of the GIT and viscera	56
3.5.	The effect of <i>F. thonningii</i> extracts on metabolic substrates	63
3.6.	The effects of <i>F. thonningii</i> extracts on the hepatic storage substrates of rat pups	65
3.7.	Clinical biochemical measures for general health status of suckling rats.....	67
3.8.	Effect of <i>F. thonningii</i> extracts on the packed cell volumes of rat pups.....	70
CHAPTER 4-DISCUSSION.....		72
4.1	Summary	73

4.2.	Phytochemical constituents of <i>F. thonningii</i>	73
4.3.	Brine shrimp toxicity assay	74
4.4.	Overall effect of <i>F. thonningii</i> extracts on growth of rat pups.....	75
4.5.	Effects of <i>F. thonningii</i> on GIT morphology and morphometry.....	76
4.6.	Effects on the morphology and morphometry and markers of function of other visceral organs	79
4.7.	Effects of <i>F. thonningii</i> extracts on metabolism	83
CHAPTER 5-CONCLUSIONS AND RECOMMENDATIONS		86
5.0.	Conclusions	87
5.1.	Limitations of the study.....	87
5.2.	Perspectives and recommendations for future studies	87
APPENDICES.....		89
APPENDIX 1: Animal Ethics Clearance		90
REFERENCES		91

LIST OF FIGURES

Figure 1.1: The leaves and fruit of the common wild fig, <i>F. rokko</i> , the Zimbabwean variant of <i>Ficus thonningii</i>	4
Figure 3.1: The toxicity effects of methanolic extracts of <i>Ficus thonningii</i> against brine shrimp (<i>Artemia salina</i>) larvae.....	49
Figure 3.2: The toxicity effects of aqueous extracts of <i>Ficus thonningii</i> against brine shrimp (<i>Artemia salina</i>) larvae	50
Figure 3.3: Induction and terminal masses of suckling rat pups after 7 days of treatment with <i>Ficus thonningii</i> extracts.....	53
Figure 3.4: Non-fasting glucose concentrations of suckling rats after 7 days of treatment with crude extracts of <i>Ficus thonningii</i>	64
Figure 3.5: Packed cell volumes (PCVs) of suckling rat pups after 7 days of treatment with crude <i>Ficus thonningii</i> extracts	71

LIST OF TABLES

Table 1.1: Antibacterial activity of <i>Ficus thonningii</i> extracts and common diseases associated with the susceptible bacteria.....	15-16
Table 3.1: Phytochemical constituents of crude leaf extracts of <i>Ficus thonningii</i>	47
Table 3.2: Effect of <i>Ficus thonningii</i> extracts on body mass gain of rat pups	52
Table 3.3: Effect of <i>Ficus thonningii</i> extracts on the linear growth of rat pups.....	55
Table 3.4: Gross morphometry of the GIT of suckling rats after 7 days of treatment with crude extracts of <i>Ficus thonningii</i>	57-58
Table 3.5: Visceral organ weights of suckling rats after 7 days of treatment with crude extracts of <i>Ficus thonningii</i>	59-60
Table 3.6: Effect of <i>Ficus thonningii</i> on the morphometry of the small intestine of suckling rats.....	61
Table 3.7: Effect of <i>Ficus thonningii</i> on the morphometry of the caecum of suckling rats.....	62
Table 3.8: Effects of <i>Ficus thonningii</i> extracts on hepatic storage substrates of rat pups..	66
Table 3.9: Clinical biochemical parameters of suckling rats after 7 days of treatment with crude extracts of <i>Ficus thonningii</i>	68-69

LIST OF ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alanine aminotransaminase
AMPK	Adenosine monophosphate activated protein kinase
ANOVA	Analysis of variance
BUN	Blood urea nitrogen
DM	Dry matter
GIT	Gastrointestinal tract
GLUT	Glucose transporter
MIC	Minimum inhibitory concentration
LC ₅₀	Median Lethal Concentration
PCV	Packed Cell Volume
SCFA	Short chain fatty acids
SGLT	Sodium-dependent glucose transporter
SD	Standard deviation
TG	Triglyceride
w/w	weight/ weight
b.w	body weight
p.o	per os

**CHAPTER 1-INTRODUCTION AND
LITERATURE REVIEW**

1.0. Background

Since prehistoric times, mankind has relied on plants as a source of food, shelter and medicine (Fowler, 2006). Before the invention of allopathic medicine, man used plant medicines on an empirical basis for curing ailments (Pal and Shukla, 2003). Today, apart from their commercial exploitation in manufacturing, cosmetic and pharmaceutical industries, plants are still extensively used in ethnomedical and ethnoveterinary practice. Plant-based medicines have also contributed significantly to the development of conventional drugs. A large proportion of medications used today are either derived or synthetically modeled from naturally-occurring plant molecules (Pal and Shukla, 2003). Examples include; aspirin (acetylsalicylic acid) from willow bark (*Salix ssp*), digoxin from foxglove (*Digitalis purpurea*), morphine from opium poppy (*Papaver somniferum*) and quinine from *Cinchona* bark (Benzie and Wachtel-Galor, 2011).

The prohibitive cost of conventional drugs and the limited availability of proper diagnostic facilities and services to both rural and urban communities in Africa have driven a continued dependence on traditional plant therapeutics. About 90 % of the African population still relies on plants and plant extracts as a source of primary health care (Benzie and Wachtel-Galor, 2011).

There is a general misconception that natural remedies are safer, healthier and have fewer side effects when compared to allopathic medicine. However, the lack of proper dosages, inconsistencies in extraction, lack of quantification and quality control of active and toxic principles in plant-based medicines, poses a high risk of toxicity to the patient (Iwu, 2002).

The pharmacological activity exhibited by plant medicines and their usefulness in disease management has led to a resurgence of interest in the identification and characterisation of their active compounds. Researchers have employed various scientific tests and clinical trials to validate the safety, effectiveness and possible toxicity of plant medicines.

The common wild fig, *Ficus thonningii*, is one of the many fruit-bearing trees that have traditionally been used for treating diseases in Africa and beyond. The therapeutic properties of this tree are of paramount importance to traditional medicine. It is extensively used as a remedy for the treatment and cure of many diseases which include diarrhoea, gonorrhoea, vomiting, diabetes mellitus, respiratory infections, urinary tract infections, fever and mental illnesses (Ajayi, 2008; Usman, *et al.*, 2009). *Ficus thonningii* is also widely used by nursing mothers to stimulate lactation (galactagogue) (Orwa *et al.*, 2009). Despite its widespread use in ethnomedicinal systems, the safety and efficacy of *Ficus thonningii* cannot be reliably guaranteed by the history and extent of its traditional use.

1.1. *Ficus thonningii*

1.1.1. Botany and Taxonomy

F. thonningii is a multistemmed, evergreen or briefly deciduous tree with a dense, rounded to spreading crown mainly distributed in upland forests of tropical and sub-tropical Africa (Agroforestry tree Database, 2011). The tree grows at altitudes of between 1 000–2 500 m and it grows best in light, deep and well drained soils (Hines and Eckman, 1993; Agroforestry tree Database, 2011). The leaves are alternate or whorled, mid-dark green and sub-glossy above whilst paler below (Palgrave, 2002) as shown in Figure 1.1.



Figure 1.1: The leaves and fruit of the common wild fig, *F. rokko*, the Zimbabwean variant of *Ficus thonningii*. Photograph reproduced with permission from Jean-Yves Rasplus©

(http://www.figweb.org/Ficus/Subgenus_Urostigma/Section_Galoglychia/Subsection_Chlamydodora/Ficus_rokko.htm)

They can be rounded or tapering, 4.5-12 cm long, hairless or finely hairy with a prominent midrib (Hyde and Wursten, 2011). The bark is usually smooth, pale to dark grey with vertical lenticels and the tree usually has aerial roots that hang from branches (Hyde and Wursten, 2011; Agroforestry tree Database, 2011). The fruits which are borne singly or in pairs are round, 10-20 mm in diameter, usually hairy and turn yellowish and rarely pink when ripe (Schmidt *et al.*, 2002). *F. thonningii* is a flowering tree that is pollinated by wasps which enjoy a symbiotic relationship and live in the syconium of its fruit

(Agroforestry tree Database, 2011). The tree can easily be propagated using seeds and cuttings (Danthu *et al.*, 2002).

Ficus thonningii has been described as a complex of taxa under which *Ficus rokko*, *Ficus rhodesiaca*, *Ficus burkei*, *Ficus petersii*, *Ficus persicifolia* and a number of other *Ficus* species are all lumped (Berg, 1990; Schmidt *et al.*, 2002; Burrows and Burrows, 2003). However, it is important to note that most of the trees grouped under the *F. thonningii* complex have the same name in the local African languages. The Shona name for *F. rokko*, *F. natalensis*, *F. burkei* and *F. rhodesiaca* is ‘Mutsamvi’ (Burrows and Burrows, 2003; Hyde and Wursten, 2011) and in Angola, ‘Mulemba’ is the local name given to *F. burkei*, *F. psiliopoga* and *F. petersii* (Burrows and Burrows, 2003). More importantly, the traditional medicinal uses and value to the local people appears to be the same for these closely related variants.

1.2. Biochemistry

1.2.1. Nutritional value

F. thonningii leaves are a good source of protein (Tegbe *et al.*, 2006). Protein content in *F. thonningii* leaves ranges between 18.7-20.5 g/100 g dry matter (DM) (Tegbe *et al.*, 2006). This makes them a useful dietary source of essential and non-essential amino acids. *F. thonningii* leaves therefore have the potential to be used to mitigate against protein deficiency diseases in children particularly in drought prone areas (Lockett *et al.*, 2000).

The leaves are also good sources of micronutrients. They have an ash content of up to 17.34% w/w (Jokthan *et al.*, 2003). *F. thonningii* also has high levels of calcium, 180.05 mg/100 g dry weight (Tegegne, 2008) as compared to that in bovine milk (118 mg/100 ml). Potassium levels range between 0.91 and 1.25 g/100 g dry weight and magnesium levels between 260 and 357.2 mg/100 g dry weight (Tegegne, 2008; Bamikole and Ikhatua, 2010).

F. thonningii leaves have also been shown to contain a high crude fibre content which reaches up to 19.41 % w/w (Tegbe *et al.*, 2006). Crude fibre is important, particularly in ruminant nutrition, for the production of volatile fatty acids which account for about 80 % of the metabolic energy requirements (Tagang *et al.*, 2010). Owing to its good nutrient profile, *F. thonningii* is used as forage for ruminants (Bamikole and Ikhatua, 2010) and rabbits (Jokthan *et al.*, 2003; Tegbe *et al.*, 2006). A comparative study done to evaluate the utilisation of *F. thonningii* and *Mangifera indica* (mango) leaves by rabbits showed that rabbits on *F. thonningii* leaves had a significantly higher average body mass compared to those on *Mangifera indica* leaves (Jokthan *et al.*, 2003).

African ethnic groups have reported the use of *F. thonningii* in cuisine. The evergreen leaves are cooked as a vegetable by the Igede and Fulani of Nigeria (Igoli *et al.*, 2002; Lockett *et al.*, 2000), and the Senegalese (Gueye and Diouf, 2007). The leaves are similarly used in Angola, Sudan, Benin and Ethiopia (Danthu *et al.*, 2002; Achigan-Dako and Ndanikou, 2009).

In addition to its widespread use as a food supplement and vegetable, *F. thonningii* is extensively used as medicine for treating disease, maintaining health and restoring tissue functioning and vitality. The broad pharmacological activity of *F. thonningii* can be seen from its application in the treatment of diverse infectious and non-infectious diseases. Its therapeutic potential can be credited to the presence of various phytochemicals that exhibit physiological effects.

1.2.2. Phytochemical composition

F. thonningii contains different classes of secondary metabolites. These non-nutritive compounds, commonly known as phytochemicals, are produced by the plant for protection against biotic and abiotic stresses. They are generally responsible for the protective and disease preventive properties of many plants (Cowan, 1999). A number of researchers have used qualitative methods for phytochemical screening of *F. thonningii*. The major drawback of using these methods is that they only confirm the presence or absence of various classes of phytochemicals but cannot identify and quantify specific compounds. A survey of literature reveals that the main classes of phytochemicals isolated from *F. thonningii* are: flavonoids, tannins, alkaloids, terpenoids and essential oils (Ndukwe *et al.*, 2007; Usman *et al.*, 2009; Ahur *et al.*, 2010)

1.2.2.1. Flavonoids

Flavonoids are polyphenolic compounds characterised by a 2 phenyl-benzo [α] pyrane or flavane nucleus which consists of two benzene rings linked through a heterocyclic pyrane

ring (Cushnie and Lamb, 2005). The presence of flavonoids in various parts (stem bark, leaves and roots) of *F. thonningii* has been reported by researchers (Ndukwe *et al.*, 2007; Usman *et al.*, 2009). Greenham *et al.*, (2007) reported the presence of flavone-C-glycosides in *F. thonningii* leaves. These were further identified as orientin, vitexin and isovitexin. *F. thonningii* contains stilbenes which include resveratrol, resveratrol glucosides and stilbene glucosides (Greenham *et al.*, 2007). Stilbenes are a special type of flavonoid produced by plants in response to pathogens and other abiotic stresses such as ultraviolet (UV) radiation (Chong *et al.*, 2009). Resveratrol and its glycosylated derivatives are derived from the phenylpropanoid pathway and have numerous implications in plant disease resistance and human health as they elicit biological and pharmacological activity (Vitrac *et al.*, 2004).

1.2.2.2. Tannins

Phytochemical screening using the ferric chloride test and the tannic acid test revealed the presence of tannins in methanolic, n-butanolic and aqueous extracts of *F. thonningii* leaves (Ndukwe *et al.*, 2007; Usman *et al.*, 2009). Tannin concentrations in *F. thonningii* leaves have been estimated to be about 90 mg/100 g DM (Bamikole *et al.*, 2004). Tannins are antinutritional phytochemicals that are associated with reduced feed intake, growth retardation and reduced nutrient absorption (Kim and Miller, 2005). They bind to dietary, digestive and structural proteins and they also reduce the absorption of iron in the GIT (Kim and Miller, 2005). Some animals secrete proline-rich tannin binding proteins as defense against the antinutritional effects of tannins (Kumar, 1992).

1.2.2.3. Alkaloids

Alkaloids are a large class of plant secondary metabolites comprised of low molecular weight, nitrogen-containing compounds. They have varied physiological effects and this has led to their use as pharmaceuticals, stimulants and narcotics (Ramada *et al.*, 2009). *F. thonningii* contains alkaloids (Ndukwe *et al.*, 2007, Ahur *et al.*, 2010) but reports of specific alkaloids isolated from *F. thonningii* are scanty in literature.

1.2.2.4. Terpenoids

F. thonningii leaves also contain triterpenoids. Triterpenoids are compounds with a carbon skeleton based on 6 isoprene units which are derived biosynthetically from squalene (Harbone, 2008). Triterpenoids produce several pharmacologically active groups such as steroids, saponins and cardiac glycosides (Ramada *et al.*, 2009). Triterpenes isolated in *F. thonningii* stem bark extracts include saponins and cardiac glycosides (Ndukwe *et al.*, 2007; Usman *et al.*, 2009). The concentration of saponins in *F. thonningii* leaves was shown to be as high as 300 mg/100 g DM (Bamikole *et al.*, 2004). Saponins have been associated with reduced feed intake, retarded growth and lysis of erythrocytes (Kumar, 1992).

The Moraceae family is a rich source of cardiac glycosides which are ubiquitous in the *Ficus* genus e.g. in *F. racemosa*, *F. religiosa* (Joseph and Raj, 2011; Poongothai *et al.*, 2011). Cardiac glycosides are inhibitors of Na⁺/K⁺-ATPase and have been used in the

treatment of cardiovascular conditions such as heart failure and atrial arrhythmias (Prassas and Diamandis, 2008).

1.2.2.5. Essential oils

F. thonningii leaves have also been shown to contain essential oils which are composed mainly of 6, 10, 14 trimethyl-2-pentadecanone (18.8%), phytol (14.7 %), acorenone (7.6 %) and β -gurjunene (6.3%) (Ogunwande *et al.*, 2008). Essential oils have antimicrobial effects hence can be useful in the treatment of infectious diseases. On the other hand, the antimicrobial activity of these essential oils can be detrimental to the balance of microbial populations in the GIT (Acamovic and Brooker, 2005).

1.2.2.6. Other phytochemicals

Other phytochemicals with antinutritional properties in *F. thonningii* leaves include phytate (130 mg/100 g DM) and oxalate (230 mg/100 g DM) (Bamikole *et al.*, 2004). Phytate and oxalate reduce the bioavailability of essential dietary minerals e.g. iron, zinc and calcium (Sandberg, 2002). *F. thonningii* leaves also contain other secondary metabolites such as lignins, lignans and active proteins such as ficin, which add to its pharmacological and biological activity (Ahur *et al.*, 2010). Lignins are components of insoluble dietary fibre and lignans are associated with reduced risk of cardiovascular disease (Peterson *et al.*, 2010).

1.3. Ethnomedicinal uses

Although all parts of *F. thonningii* are medicinally useful, people prefer to use the leaves and bark which exude latex because latex has traditionally been associated with potency (Ahur *et al.*, 2010). In traditional medicine, plant extracts are prepared as; infusions, decoctions or macerations. An infusion is prepared by pouring hot water on the plant material and steeping for a defined period of time (Handa, 2008). A decoction is prepared by pouring cold water on plant extracts, bringing to the boil and allowing the mixture to simmer for a defined period of time (Handa, 2008). Macerations are prepared by soaking plant material in cold water at room temperature (Handa, 2008).

1.3.1. Leaves

Decoction and/or macerations of fresh *F. thonningii* leaves, taken orally, have been used for the treatment of diarrhoea, gonorrhoea and diabetes mellitus (Njoronge and Kibunga, 2007). The leaves are also used for treating liver disorders and disease conditions associated with jaundice (Ahur *et al.*, 2010). In Angola, boiled *F. thonningii* leaves are used for treating wounds. The wounds are washed with leaf extracts and in cases of gingivitis, bleeding gums are rubbed with leaves (Bossard, 1996). Leaf extracts are also used for treating bronchitis and urinary tract infections (Cousins and Huffman, 2002). A decoction of the leaves is used in Mali for treating urinary schistosomiasis (Bah *et al.*, 2006). In Nigeria, a maceration of the leaves is used for treating stomach pains, gastritis, gastric ulcers and colitis (Nwude and Ibrahim, 1980). *F. thonningii* leaves are also used

for the treatment of bone movement disorders, ringworm, thrush, scabies and athlete's foot rot (Moshi *et al.*, 2009; Alawa *et al.*, 2002).

1.3.2. Stem Bark

Traditionally, the stem bark is pound and the infusion is used for treating influenza, sore throat, colds, arthritis, rheumatism and to relieve inflammation (Dalziel, 1937, Orwa *et al.*, 2009). In Tanzania the bark is also used to stimulate lactation (Minja, 1994). *F. thonningii* bark decoction is also used in Mali and Senegal, to treat respiratory diseases such as; pneumonia, bronchitis, emphysema (Prelude medicinal plants database, 2011). The bark of *F. thonningii* is used for treating diarrhoea, cysts, skin diseases and ulcers in Ethiopia (Teklehaymanot and Gidday, 2007; Prelude medicinal plant database, 2011). The bark decoction is also used for fertility enhancement and induction of the menstrual cycle (Watt and Breyer-Brandwijk, 1962; M Kangare-Minja, 1989) and in Southern Africa it is used as relief for constipation and bowel disorders (Gelfand *et al.*, 1985).

1.3.3. Roots

F. thonningii roots are used for the treatment of malaria, fever, hepatitis and dental pains (Prelude medicinal plant database, 2011). In Zimbabwe the roots have been reportedly used for preventing miscarriages and for stopping nose-bleeding (Gelfand *et al.*, 1985). Additionally, *F. thonningii* roots are used for relieving stomach pains, diarrhoea, pneumonia and chest pains (Njoronge and Kibunga, 2007; Teklehaymanot and Gidday,

2007). In East Africa *F. thonningii* roots are used for treating diseases believed to be caused by evil spirits hence the tree is believed to be sacred (Wondimu *et al.*, 2007).

1.3.4. Latex

F. thonningii exudes a white, sticky latex that turns pinkish with time (Arbonnier, 2004). This milky latex is used for treating fever, tooth decay and ringworm (Alawa *et al.*, 2002; Arbonnier, 2004). The latex is commonly used for treating cataract in the eye (Alawa *et al.*, 2002) and is also used as a vermifuge (Mali and Mehta, 2007).

1.3.5. Uses of *F. thonningii* in combination with other plants

The combination of different plants could be characterised by synergistic effects resulting in a higher drug efficacy than that expected from each plant if used individually (Evans *et al.*, 2002). In African ethnomedicine *F. thonningii* is combined with sour sop (*Anona muricata*), basil (*Ocimum gratissimum*) and bamboo (*Bambusa vulgaris*) to prepare a remedy for treating respiratory diseases (Diafouka, 1997). In Mali, *F. thonningii* leaves are combined with the leaves and fruits of the African Palmyra palm (*Borassus aethiopum*) and the mixture is used for massaging, bathing and fumigating those suffering from paralysis and polio (Malgras, 1992).

Researchers have scientifically and clinically validated some of the ethnomedical practitioners' claims about the use of crude *F. thonningii* extracts in disease management.

The pharmacological properties of the plant extracts have been elucidated using both *in vitro* and *in vivo* methods.

1.4. Phytopharmacology

1.4.1. Antimicrobial activity

In vitro antimicrobial studies have been used to validate the pharmacodynamic basis for the traditional use of *F. thonningii* in the treatment of infectious diseases. *F. thonningii* extracts have antibacterial, antiprotozoal and antifungal effects (Ndukwe *et al.*, 2007; Usmna *et al.*, 2009; Kone *et al.*, 2004; Moundipa *et al.*, 2005; Oyelana *et al.*, 2011).

1.4.1.1. Antibacterial effects

F. thonningii extracts have been shown to exhibit antibacterial activity against both Gram positive and Gram negative pathogenic bacteria. Table 1.1 below is a summary of the antibacterial activity of *F. thonningii* extracts and their minimum inhibitory concentrations (MIC). It is important to note that the diseases commonly caused by microorganisms susceptible to *F. thonningii* extracts, correspond with the ethnomedicinal claims of diseases treated with the plant's extracts in traditional medicine.

Table 1.1. Antibacterial activity of *Ficus thonningii* extracts and common diseases associated with the susceptible bacteria.

Plant part	Susceptible microorganism	MIC	Reference	Common diseases associated with microorganism
Ethanollic leaf extracts	<i>Streptococcus pyrogenes</i>			
	(Group A)	23 µg/ml	Kone <i>et al.</i> , 2004	Tonsilitis, septicaemia, rheumatic fever
	Beta- hemolytic	47 µg/ml	Kone <i>et al.</i> , 2004	
	<i>Enterococcus faecalis</i> (aminoside resistant)	94 µg/ml	Kone <i>et al.</i> , 2004	Urinary tract infections
n-butanolic stem - bark extracts	<i>Escherichia coli</i>	1.25 mg/ml	Usman <i>et al.</i> , 2009	Urinary tract infections, neonatal meningitis
Aqueous stem-bark extracts	<i>Staphylococcus aureus</i>	1.25 mg/ml	Usman <i>et al.</i> , 2009	Wound infections, boils, impetigo, pneumonia, endocarditis

Table1.1 continued

Aqueous stem-bark extracts	<i>Klebsiella spp</i>	1.25 mg/ml	Usman <i>et al.</i> , 2009	Urinary tract infections, pneumonia and wound infections
	<i>Pseudomonas aeruginosa</i>	2.5 mg/ml	Usman <i>et al.</i> , 2009	Pulmonary infections, urinary tract infections, gastroenteritis
Methanolic stem-bark extracts	<i>Bacillus subtilis</i>	12.5 mg/ml	Ndukwe <i>et al.</i> , 2007	Generally non-pathogenic
	<i>Salmonella typhi</i>	1.25 mg/ml	Ndukwe <i>et al.</i> , 2007	Enteric fever, gastroenteritis
	<i>Providencia stauti</i>	6 mg/ml	Ndukwe <i>et al.</i> , 2007	Nosocomial infections, urinary tract infections, respiratory tract infections and wounds

MIC= Minimum Inhibitory Concentration

The antibacterial effects of crude extracts of *F. thonningii* could be credited to the presence of phytochemicals. The terpenoids, flavonoids and tannins present in *F. thonningii* are probably responsible for its activity against some microorganisms. Terpenoids disrupt microbial cell membranes and hence give plants bactericidal effects (Cowan, 1999). Phytol, a diterpene found in *F. thonningii* has been shown to possess antibacterial effects. The mean inhibitory concentration of phytol (2 mg/ml) against *Mycobacterium tuberculosis* was shown to be comparable to that of ethambutol, a clinically useful drug (0.95-3.8 mg/ml) (Rajab *et al.*, 1998).

Flavonoids complex with extracellular and soluble proteins as well as with bacterial cell walls and hence exhibit bactericidal effects (Cowan, 1999). Tannins inactivate microbial adhesions, enzymes and cell envelope proteins and may complex with polysaccharides (Ya *et al.*, 1988, Cowan, 1999). Aromatic alkaloids intercalate with DNA and thus are potential antimicrobial agents (Raaman, 2006). These phytochemicals have been isolated from *F. thonningii* extracts (Ndukwe *et al.*, 2007; Usman *et al.*, 2009) and could contribute to its antibacterial effects.

1.4.1.2. Antiprotozoal properties

F. thonningii is used for treating malaria (Titanji *et al.*, 2008; Chinsebu and Hedimbi, 2010). Despite the claims that *F. thonningii* possesses antimalarial activity, Jansen *et al.*, (2010) reported no significant antiplasmodial activity against *Plasmodium falciparum* the protozoan parasite that causes malaria. However, amongst the compounds isolated from essential oils of *F. thonningii*, is the C-15 compound; 6, 10, 14 trimethyl-2-pentadecanone.

This is an aliphatic methyl ketone which possesses insect repellent properties (Innocent *et al.*, 2008). It is possible that *F. thonningii* just possesses good insect repellent properties and hence reduces the contact of the vector with humans, minimising incidence of malaria transmission (Innocent *et al.*, 2008; Jansen *et al.*, 2010).

F. thonningii was shown to exhibit amoebicidal activity against *Entamoeba histolytica*. Lethality concentrations were low (LC₅₀-48hr = 12.71 µg/ml) and comparable to the commercial drug metronidazole (LC₅₀-48 hr = 46.08 µg/ml) (Moundipa *et al.*, 2005). The antiprotozoal properties of *F. thonningii* could be a result of the activity of alkaloids, terpenes, stilbenes anthraquinones, flavonoids and essential oils (Schwikkard and Van Heerden, 2002).

1.4.1.3. Antifungal effects

The traditional use of *F. thonningii* in the treatment of athlete's foot rot suggests that it may have antifungal compounds. Oyelana *et al.*, (2011) showed that leaf extracts of *F. thonningii* (25 mg/ml) had antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Fusarium solani*, *Penicillium chrysogenum*, *Penicillium oxalicum* and *Rhizopus stolonifer*. The MIC of *F. thonningii* leaf extracts was comparable to that of the positive control, cefoxitin, a cephamycin antibiotic of the beta-lactam group reported active against the fungal isolates used in the study. The extracts showed a significant arrest of mycelia growth at higher concentrations (75 and 100 mg/ml).

Two antifungal chitinases (GLx Chi-C and Chi-B) were isolated in the latex *Ficus microcarpa* (synonym for *F. thonningii*) (Taira *et al.*, 2005). The chitinases exhibit strong antifungal activity (Taira *et al.*, 2005). They bind to fungal cell walls and hydrolyse the fungal cell wall component, chitin (Hou *et al.*, 1998).

1.4.2. Anthelmintic properties

The presence of the proteolytic compound ficin in *Ficus thonningii* latex justifies its use as an anthelmintic agent. Ficin is a cysteine endopeptidase that is found in the latex of many *Ficus* spp. It is known to digest living intestinal parasites (Etkin and Ross, 1982; Krief *et al.*, 2005). This supports the use of *F. thonningii* used as a vermifuge in ethnomedicine (Mali and Mehta, 2007).

1.4.3. Antioxidant activity

Flavonoids are good antioxidants which scavenge and reduce free radical formation (Grassi *et al.*, 2010). The C-glucosyl flavonoids (orientin, vitexin and isovitexin) isolated from *F. thonningii* possess antioxidant properties and free radical scavenging activity. They have been identified in medicinal plants with anti-inflammatory and antioxidant activity such as the pigeon pea (*Trollius ledebouri* Reichb), linseed (*Linum usitatissimum*) oil and in rooibos tea (*Aspalathus linearis*) (Joubert and Ferreira, 1996; Von Gadow *et al.*, 1997).

Owing to these antioxidant activities, *F. thonningii* leaf extracts have been shown to possess protective effects on the erythrocyte membrane from acetaminophen-induced membrane peroxidation (Ahur *et al.*, 2010). The antihaemolytic and haematinic potential is possibly due to its antagonistic activity against the depletion of glutathione and hence prevention of the generation of free radicals which result in oxidative stress (Ahur *et al.*, 2010).

In addition to flavonoid antioxidant activity, the stilbenes present in *F. thonningii* also exhibit antioxidant activity. Resveratrol and its methylated derivative, trans-3,3', 5,5-tetrahydroxy-4-methoxystilbene, possess antioxidative effects against oxidative stress induced by reactive nitrogen species and reactive oxygen species (Olas *et al.*, 2003; Olas *et al.*, 2008). Resveratrol and its derivatives have also been shown to reduce peroxynitrite which is one of the most potent reactive nitrogen species (Olas *et al.*, 2008). High levels of peroxynitrite are generated in inflammation-based disease conditions (Ischropoulos and Al Mehdi, 1995). The anti-inflammatory properties of *F. thonningii* are probably a result of the action of these stilbenes and flavonoids. There is also a likelihood of synergistic interactions between the flavonoids and stilbenes present in *F. thonningii* (Wu *et al.*, 2009).

1.4.4. Analgesic effects

F. thonningii has been reported to possess analgesic properties that are comparable to aspirin in both peripheral and central induced pain (Otimenyin, 2004). Using the acetic acid-induced writhing reflex model in mice, Otimenyin (2004) demonstrated that

methanolic extracts of *F. thonningii* (500 mg/kg) administered intraperitoneally had a percentage inhibition (79.7%) comparable to aspirin (80%). This shows that *F. thonningii* has analgesic effects that can be useful in the management of peripherally induced pain (Otimenyin, 2004). Otimenyin (2004) also reported the analgesic effects of the plant in central pain using the hot plate test method in mice.

1.4.5. Anti-inflammatory effects

The anti-inflammatory properties of *F. thonningii* have been validated using egg albumin and carageenan-induced oedema in rats (Otimenyin, 2004; Coker *et al.*, 2009). Phytol, the aliphatic diterpene found in *F. thonningii* has anti-inflammatory effects and it is a potential therapeutic agent for the treatment of rheumatoid arthritis and other chronic inflammatory diseases such as asthma (Ogunlesi *et al.*, 2009). Unsaturated phytol exhibits antioxidant activity and is believed to be the side group that gives tocotrienols their higher antioxidative effects as compared to tocopherols (Yu *et al.*, 2005).

1.4.6. Cardioprotective effects

Ficus thonningii possesses cardio-suppressant and hypotensive properties. In a study carried out to investigate the cardioprotective effects of ethanolic stem bark extracts of *F. thonningii*, the plant extracts were shown to exhibit negative chronotropic and inotropic effects on both electronically driven and spontaneously beating atrial muscle strips (Musabayane *et al.*, 2007). *F. thonningii* extracts (120 mg/kg b.w given p.o for 5 weeks)

also reduced mean arterial pressures of both normal and diabetic rats (Musabayane *et al.*, 2007).

The cardioprotective effects of *F. thonningii* could be credited to the presence of resveratrol. Resveratrol has been reported to prevent and slow the progression of various diseases which include cancer and cardiovascular diseases (Baur and Sinclair, 2006). It has also been shown to possess vasoprotective and reno-protective effects (Ramada *et al.*, 2009).

1.4.7. Hypoglycemic effects

Ethanollic extracts of *F. thonningii* have been shown to exhibit hypoglycaemic effects in rats (Bwititi and Musabayane, 1997). These hypoglycemic effects are proof of principles the ethnomedicinal use of the plant extracts in the treatment of diabetes mellitus. Oral glucose tolerance tests performed on diabetic and non diabetic rats treated with stem bark ethanollic extracts of *F. thonningii* showed a dose dependant hypoglycaemic effect comparable to that of metformin (Musabayane *et al.*, 2007).

1.4.8. Antidiarrheal effects

F. thonningii is extensively used for treating diarrhoea in both livestock and humans (Njoronge and Kibunga, 2007). The antidiarrhoeal properties of *F. thonningii* could probably be the result of anti-secretory, anti-inflammatory and antibacterial properties arising from the presence of tannins and astringent phenolics such as triterpenoids and

saponins (Tripathi, 1994). Tannins and tannic acid present in *F. thonningii* extracts are known to reduce secretion by denaturing proteins of the intestinal mucosa and forming protein tannates which make the mucosa more resistant to chemical alteration (Tripathi, 1994). Plants with anti-diarrhoeal properties also act by decreasing intestinal motility, stimulating water absorption and reducing electrolyte secretion (Njoronge and Bussman, 2006).

While the secondary metabolites produced by *F. thonningii* have been shown to exhibit beneficial pharmacological effects, there is paucity of information regarding their effects on their first point of contact with the body, the gastrointestinal tract.

1.5. The gastrointestinal tract

For systemic therapeutic purposes, the main route of administration of *F. thonningii* is oral. The gastrointestinal tract (GIT) is the first point of contact of the body with the plant's extracts. The GIT is the vital interface of the body with its external environment (Schneeman, 2002). The principal functions of the GIT include digestion, absorption, metabolism, immune defense and osmoregulation (Barltrop and Brueton, 1990). Its efficacy in carrying out these diverse functions heavily depends on its structural integrity.

Structurally, the GIT is made up of four specialised layers of tissues: the mucosa, the submucosa, the muscularis and the serosa. The outermost layer of the GIT is the serosa and it is composed of an epithelial layer and an underlying connective tissue (Walthall *et al.*, 2005). The muscularis is mainly smooth muscle comprising of the inner circular layer

and the outer longitudinal layer innervated by a specialised nerve plexus (Myenteric plexus). The submucosa is mainly composed of connective tissue supplied with blood vessels, lymphoid tissue and a specialised nerve plexus (Meissner's plexus) (Buddington *et al.*, 2003). The mucosa is the innermost layer of the GIT and it is in direct contact with the external environment. The structure, integrity and maturational state of the mucosa greatly influence the systemic bioavailability of nutrient and non-nutrient compounds (Buddington *et al.*, 2003) and hence have a direct impact on growth and development.

The mucosa plays an important role in the release of gastric juices, enzymes, hormones and other regulatory proteins that are critical in the growth and function of the GIT. Pepsin, gastrin, somatostatin, ghrelin, obestatin, cholecystokinin, leptin are amongst some of the active substances produced by the GIT mucosal cells (Chen *et al.*, 2006).

In the small intestine, the mucosal surface is folded into villi and microvilli to increase the surface area available for absorption. Each villus is made up of columnar epithelial cells (enterocytes) which originate from the crypt and mature into absorptive cells as they migrate up the villus (Barltrop and Brueton, 1990). Neonatal enterocytes are highly permeable and have the ability to take up macromolecules intact by non-selective pinocytosis (Pacha, 2000). The permeability of the neonatal GIT is a physiological adaptation for the uptake of immunoglobulins and growth factors from the mother's milk. In rats, normal intestinal closure which is stimulated by glucocorticoids, will only occur 3 weeks after birth (Pacha, 2000; Sangild, 2006). Therefore, interaction of nutrients and xenobiotics with the neonatal rat GIT is expected to significantly differ from that of the adult rat.

1.6. Effect of phytochemicals on the GIT

The exposure of the GIT to foreign compounds can have both beneficial and detrimental physiological effects. Some phytochemical compounds have the ability to induce the release of various tropic and trophic regulatory molecules and hence elicit proliferative effects on the mucosal cells of the GIT. An example is the inductive effect of plant based protease inhibitors on the release of gastrin by the gastric G cells and cholecystokinin by the duodenal I cells (Buddington *et al.*, 2003). Gastrin augments the release of gastric acid in the stomach and also enhances the proliferation and maturation of parietal cells (Jain and Samuelson, 2006). Cholecystokinin on the other hand, increases pancreatic secretions and has an effect on satiety (Beglinger, 2002). Phytochemicals can also mimic regulatory peptides, bind to specific receptors and activate or amplify signaling pathways. Examples are phytoestrogens which have been reported to influence the absorption of nutrients by enterocytes (Buddington *et al.*, 2003).

While some phytochemicals have beneficial effects, some adversely affect the structural integrity of the GIT and reduce both its digestive and absorptive capacity. Polyphenolics e.g. tannins have an astringent and purgative effect that supports their use in the treatment of diarrhoea but they are also known to exhibit a puckering effect and hence reduce the surface area of the epithelial cells of the intestinal mucosa (Acamovic and Brooker, 2005; Bajec and Pickering, 2008).

Some phytochemicals are inhibitors of digestive proteins. Saponins, polysaccharides and tannins are well known inhibitors of proteases (Buddington *et al.*, 2003). Herbal medicines can also reduce carrier-mediated transport of nutrients from the lumen into enterocytes. Research suggests that the decrease in transport of glucose caused by polyphenols is most likely to be the result of non-specific binding to carrier proteins such as sodium dependent glucose transporter 1 (SGLT-1) and glucose transporter 2 (GLUT 2) (Kwon *et al.*, 2007; Hanhineva *et al.*, 2010). Tannins bind and precipitate both surface and luminal proteins and hence reduce digestive efficiency of the GIT (Shahidi and Naczki, 1992).

Phytochemicals also affect the microbial population of the GIT. Polyphenolic compounds, alkaloids and flavones are common antimicrobial agents naturally produced by plants to fight off bacterial and fungal infections (Cowan, 1999; Buddington *et al.*, 2003). While the use of the compounds is advantageous in the reduction of pathogenic microorganisms, the same compounds can have unfavourable effects on the delicate balance of the natural flora of microorganisms that are resident in the GIT. Intestinal microbes have critical roles in digestion which include; fermentation and production of essential nutrients such as amino acids (arginine, cysteine and glutamine), synthesis of vitamins (B and K complex) and production of short chain fatty acids (SCFA) (Cencic and Chingwaru, 2010). SCFA are a good energy source, which can provide 10-30 % of basal metabolic requirements to the growing animal (Cencic and Chingwaru, 2010). The disruption of the microbial load by phytochemicals leads to reduced metabolism of essential and non essential elements consequently resulting in subnormal growth and development. The GIT microflora is also involved with the production of bile acids and anticarcinogenic compounds (O'Sullivan *et al.*, 2005). These microorganisms further act as a barrier against pathogens by preventing

mucosal adherence and by producing bacteriocins. They also have key roles in the development of both the humoral and cell mediated immune function of the neonate (Bauer *et al.*, 2006; Cencic and Chingwaru, 2010).

1.7. Nutrition and growth in neonates

Growth is defined as the increase in size (hypertrophy) and/or number (hyperplasia) of cells (Malina *et al.*, 2004). The growth and development of an organism is determined by the complex interaction of genetic, environmental and nutritional factors. Although hormones, growth factors and other regulatory proteins have key roles in regulating growth, cellular growth is ultimately controlled by the intracellular availability of nutrients (Russell and Rhoads, 2008). The nutritional state of the neonatal period influences cell proliferation and consequently determines the size of the organs and the organism (Winick and Noble, 1966). Nutritional insults during the suckling period can have irreversible effects on the growth of cells and their critical organisation into functional organs (Sefcikova and Mozes, 2002). The interaction of phytochemicals with the GIT mucosal surface, hydrolytic enzymes, secretory bodies, nutritional elements and normal gut flora in neonates could have inhibitory and irreversible effects on both body mass gain and linear growth.

1.8. Justification of the present study

In Africa, the common wild fig (*Ficus thonningii*) is extensively used as an alternative and a complement to conventional drugs. As reviewed earlier, *F. thonningii* has a broad range

of pharmacological effects that arise from the presence of physiologically active phytochemicals such as flavonoids, terpenoids, tannins and alkaloids. Furthermore, *F. thonningii* has a remarkable nutritional profile and it is a common household functional food. Due to its high nutritional value and therapeutic potential, mothers may be tempted to use *F. thonningii* for their babies particularly in drought prone areas where food security is threatened. Nursing mothers could expose their suckling infants to bioactive compounds from *F. thonningii* by giving them either directly as a medicinal supplement or indirectly via breast milk. The bioactive compounds found in *F. thonningii* extracts, may affect the proliferation and differentiation of intestinal epithelial cells, resulting in irreversible structural and functional changes in the neonatal GIT.

Researchers have mainly focused on the physiological effects of *F. thonningii* in adult rats but the neonatal rat is a better model to assess the effects of *F. thonningii* in babies owing to its sensitive, rapidly growing and relatively more permeable GIT. The permeability of the neonatal GIT allows the passage of various phytochemicals through to the portal circulation and hence allows the assessment of the effects of the plant's extracts on the liver (Linderoth *et al.*, 2005). Exclusively fed on milk, the neonatal rat can be used to accurately evaluate the effects of *F. thonningii* on the GIT, independent of other dietary factors.

Despite its wide spread use both as food and as medicine, there is currently no research on the effects of *F. thonningii* on the growth and clinical biochemistry of neonates. The effects of this plant on the morphology and morphometry of the GIT of neonates have not been thoroughly researched. In view of this existing research gap, this study sought to fill

the void of knowledge that surrounded the subject. The objective of this study was to determine the effects of orally administered aqueous and alcoholic leaf extracts of *F. thonningii* on: growth performance, GIT and accessory organ morphology and morphometry, and clinical biochemistry of neonates using suckling Sprague Dawley rat pups as an animal model for the human baby.

1.9. Aims and objectives

The main objective of this study was to determine the effect of aqueous and alcoholic extracts of *F. thonningii* leaves on growth performance, GIT morphology and clinical biochemistry of suckling Sprague Dawley rats.

The specific objectives of the study were to:

1. Identify the major classes of phytochemicals in *F. thonningii* extracts that could be responsible for its physiological effects.
2. Determine the acute toxicity of *F. thonningii* by evaluating the effects of aqueous and methanolic extracts on Brine shrimp (*Artemia salina*) larvae.
3. Determine the effects of orally administered aqueous and methanolic extracts of *F. thonningii* leaves on:
 - a) Growth performance of suckling Sprague Dawley rat pups by measuring:
 - i. body mass gain,

- ii. femur and tibia growth and density as a measure of linear growth.
- b) The morphology and morphometry of the GIT and accessory organs by determining:
 - i. lengths and/or masses of visceral organs,
 - ii. histology of the caecum, liver and proximal segments of the small intestine,
 - iii. the height of the villi and the depths of the crypts.
- c. Metabolic substrates by measuring:
 - i. circulating glucose, triglycerides, cholesterol,
 - ii. hepatic glycogen and hepatic lipid content of the rats.
- d. The general health profile of the rats by measuring:
 - i. plasma levels of markers of liver function; alanine transaminase (ALT), alkaline phosphatase (ALP) and circulating levels of total bilirubin,
 - ii. plasma protein levels (albumin, globulin and total protein),
 - iii. blood urea nitrogen (BUN), creatinine, phosphates and calcium,
 - iv. packed cell volume.

CHAPTER 2-MATERIALS AND METHODS

2.1. Plant collection and preparation

Fresh *Ficus thonningii* leaves were sourced from Gweru, in the Midlands Province of Zimbabwe, wrapped in paper and transported overnight to the University of the Witwatersrand, Johannesburg. Gweru (19.75 °S and 29.9 °E) (Hove, 2000), has an altitude of 1430 m above sea level and an average annual rainfall of 643 mm (Moyo, 2006). Identification, authentication and verification of the species was done by botanists and nature conservationists; John and Sandra Burrows in Johannesburg, South Africa. The leaves were thoroughly cleaned of all foreign material and dried in an oven (Salvis®, Salvis Lab, Schweiz, Switzerland) at 40 °C, for 24 hours (Okwari and Ofemi, 2011). The crisp dry leaves were ground to powder using a Waring blender (Waring ®, Lasec SA Company, USA).

2.2. Dry matter and moisture content determination

The dry matter content of *Ficus thonningii* leaves was determined by weighing a representative sample of fresh leaves before drying them in an oven (Salvis®, Salvis Lab, Schweiz, Switzerland) until a constant mass was obtained. The formula:

$$\text{Moisture content (\%)} = \frac{\text{Fresh mass} - \text{Dry mass}}{\text{Fresh mass}} \times 100$$

was used to calculate the moisture content. Dry matter was obtained from calculating the difference between the fresh (wet) mass and the moisture content.

2.3. Extraction

Crude methanolic and aqueous extracts of *F. thonningii* were made by mixing 80 g of the dry leaf powder with 1L of 80 % methanol (Merck Chemicals, Johannesburg, South Africa) and 1L of distilled water respectively (Okwari and Ofemi, 2011). The mixtures were incubated for 24 hours at 25°C. After extraction, the mixtures were filtered using Whatman Grade No.1 filter paper (Whatman Ltd, England, UK) and the residue was discarded. The filtrate was concentrated using a rotor evaporator (Labocon (Pty) Ltd, Krugersdorp, Transavaal, South Africa) before drying in an oven (Salvis®, Salvis Lab, Schweiz, Switzerland) at 40 °C. The resultant residue was recovered and weighed to determine extract yield (Obrefuna *et al.*, 1992; Aniagu *et al.*, 2008).

2.4. Brine shrimp (*Artemia salina*) toxicity assay

The brine shrimp lethality assay was carried out as described by Vanhaecke *et al.*, (1981) and Wanyoike *et al.*, (2004).

2.4.1. Hatching and preparation of nauplii

Brine shrimp eggs (cysts) were hatched by incubating them in 2.9 % saline solution for 24 hours, at room temperature. The incubation chamber was continuously aerated by an infusion pump (Havard Apparatus, MRC, Millis, USA) with a small tube extending to the bottom. After 24 hours, the larvae (nauplii) were harvested by attracting them with light

and collecting them with a pipette. The live nauplii were transferred into small beakers containing 2.9 % saline solution.

2.4.2. Brine shrimp assay

Fifty milligrams of methanolic *F. thonningii* extracts were dissolved in 5 ml of dimethylsulfoxide (DMSO) and made up to 100 ml with 2.9 % saline solution. The methanolic solution was serially diluted to achieve the following concentrations: 5000, 500, 50, 5, 0.5 and 0.05 µg/ml. The first aqueous doses used (up to 5 mg/ml) were non toxic so the stock concentration of aqueous extracts was increased to 200 mg/ml. This was then diluted to achieve the following concentrations: 200, 100, 50, 10, 5 and 2.5 mg/ml. The extract solutions were transferred to a 24-well plate. Two milliliters of each solution were added to each well, in triplicate, in decreasing concentrations. Saline solution (2.9 %) was used as the negative control. Potassium dichromate a chemical toxicant with cytotoxic properties was used as positive control at 3000, 300, 30 and 3 µg/ ml. Ten nauplii were transferred into each well using a 50 µL pipette and the plates were incubated for 24 hours at room temperature. The numbers of dead nauplii in each well were then counted using an ophthalmic surgical microscope (Leica M620, Japan). The nauplii were considered dead if no movement of appendages were observed within 10 seconds. The percentage mortality was calculated using the following formula:

$$\text{Mortality (\%)} = \frac{\text{Number of dead nauplii}}{\text{Initial number of live nauplii}} \times 100$$

A graph relating % mortality and the decimal logarithm of concentration of extracts was plotted using non linear regression. The concentration required to kill 50 % of the nauplii

within 24 hours (median lethal concentration/ LC₅₀-24 hr) was calculated from the three replicates and averaged for each treatment. Results were presented as Mean LC₅₀ ± SEM.

2.5. Phytochemical determination

Preliminary phytochemical screening tests were carried out to determine the major classes of phytochemical classes present in both the aqueous and methanolic *F. thonningii* leaf extracts. The phytochemicals were classified based on a qualitative colour reaction with specific reagents as originally described by Harbone, (1973); Sowofora, (1993) and Trease and Evans, (2002).

2.5.1. Terpenoids: Salkowski's test

To test for terpenoids, 2 ml of chloroform were added to 0.5 g of plant extracts and 3 ml of concentrated sulphuric acid resulting in the formation of two layers. A reddish brown colour development at the interface of these layers showed a positive confirmation of the presence of terpenoids, or specifically plant steroids (Ayoola *et al.*, 2008).

2.5.2. Alkaloids: Mayer's reagent test

Plant extracts (200 mg) were boiled in 20 ml of 1 % sulphuric acid and 2 ml of 50 % ethanol were added. Twenty 20 ml of ammoniacal chloroform added. The phases were separated using dilute sulphuric acid and 5 drops of Mayer's reagent added. The formation of a cream precipitate indicated the presence of alkaloids (Siddiqui *et al.*, 2009).

2.5.3. Saponins: Froth test

A millilitre of each plant extract filtrate was shaken in 10 ml of distilled water for 30 seconds. Persistent frothing indicated the presence of saponins (Sowofora, 1993).

2.5.4. Flavonoids: Shinoda's test

Plant extracts (200 mg) were dissolved in ethanol and chips of magnesium ribbon were added. A millilitre of concentrated hydrochloric acid was then added and colour development observed. A pink or red colour showed the presence of flavonoids (Trease and Evans, 2002).

In another method, 5 ml of 10 % ammonium hydroxide was added to 2 ml of the plant extract filtrate. After thorough mixing, 1 ml of concentrated sulphuric acid was added. A yellow colour showed the presence of flavonoids (Siddiqui *et al.*, 2009).

2.5.5. Anthraquinones

Half a gram of extracts was boiled in 10 ml 2M sulphuric acid, filtered and shaken with 5 ml chloroform to form two layers. The organic layer was pipetted into a new tube and 1 ml of 10 % ammonium hydroxide was added before colour changes were observed (Ayoola *et al.*, 2008).

2.5.6. Phlobatannins

Plant extracts (0.5 g) were boiled in 1 % hydrochloric acid and a red precipitate showed the presence of phlobatannins (Jana and Shekhawat, 2010).

2.6. Ethics clearance and study setting

The Animal Ethics Screening Committee of the University of the Witwatersrand approved the study (Ethics number - 2011/22/2A) (Appendix 1). All animal studies were carried out in the multipurpose animal unit of the Central Animal Services at the University of the Witwatersrand. Laboratory analyses were carried out in the School of Physiology, at the University of the Witwatersrand.

2.7. Housing and general care of the rat pups

Five Sprague Dawley rat dams, each with 8 ± 1 , six-day old pups were obtained from the Central Animal Services. Each dam and its respective litter were housed in perspex cages lined with wood shavings. Temperatures were maintained at 22 ± 2 °C. Twelve hour light and dark cycles (lights on at 07:00 a.m) were followed. Adequate ventilation was provided at all times. The pups were individually identified by marking with colour-coded, non-toxic permanent marker ink on their tails. During the course of the study, the pups were allowed to nurse freely with their dams. The dams were provided with standard rat chow (Epol, South Africa) and drinking water *ad libitum*.

2.8. Study design

The 40, six-day old, suckling pups were randomly allocated to five treatment groups each consisting of 8 pups of either sex. Pups in each litter were split into different groups to avoid maternal effect bias.

2.9. Treatments

The six-day old rat pups were weighed at induction and they were given a day to familiarize with the environment before receiving treatments. The pups were weighed daily before receiving the following treatments at a volume of 5 ml/kg body weight:

- i. Group 1 - Distilled water
- ii. Group 2 - Low aqueous dose (50 mg aqueous extract/kg body weight)
- iii. Group 3 - High aqueous dose (500 mg/kg body weight)
- iv. Group 4 - Low methanolic dose (50 mg alcoholic extract/kg body weight)
- v. Group 5- High methanolic dose (500 mg/kg body weight)

The plant extract solutions were suspended in distilled water and administered once daily by orogastric gavage using an orogastric gavage tube, for 7 days. The dams did not receive any treatments but they were weighed as part of a routine health monitoring regime. The doses used are within the range 50-1000 mg/kg b.w used by other researchers (Aniagu *et al.*, 2008; Musabayane *et al.*, 2007; Coker *et al.*, 2009) and below the LD₅₀ of *F. thoningii*

(> 3000 mg/kg b.w) (Aniagu *et al.*, 2008). All pups were monitored for any behavioral changes (isolation from other pups and reduced activity), clinical signs of toxicity (respiratory distress, colour of the mucus membrane, uncoordinated movements and staining of the perineum) throughout the course of the experiments.

2.10. Termination and tissue sample collection and storage

Blood for glucose and triglyceride determination was collected before euthanasia by the tail-prick method. Circulating glucose were measured using a glucometer (Accu-Chek Active Roche Diagnostics, Germany) and the triglycerides using a portable triglyceride meter (Acutrend, Roche Diagnostics). Pups were then euthanased by intra-peritoneal injection of sodium pentobarbitone (100 mg/kg b.w) (Euthanase®, Centaur labs, Johannesburg, South Africa). The thorax was opened and 1 ml of blood was collected by cardiac puncture using a 21G needle, into lithium-heparin tubes. The tubes were gently inverted for 30 seconds immediately after collection to thoroughly mix the blood with the anticoagulant. The pups were dissected to remove the abdominal visceral organs. The luminal contents of the stomach, large intestine and caecum were gently squeezed out. Gross morphometric measurements of the stomach, caecum, large intestine and the small intestine were recorded. Additionally, the liver, pancreas, spleen and the kidneys were weighed using a balance (Precisa 310M, Precisa Instruments, Switzerland). Sections of the small intestine, caecum, spleen, and liver were collected and preserved in 10 % phosphate-buffered formalin for histology. Liver samples were stored in a freezer (Bosch, Johannesburg, South Africa) at -20 °C for the determination of hepatic metabolic substrates.

2.11. Blood sample preparation

Packed cell volumes (PCV) were determined by spinning whole blood at 12800 rpm for 2 minutes in a microhaematocrit centrifuge (IDEXX StatSpin® VT Centrifuge, Netherlands).

Plasma samples were prepared by spinning the blood collected in the lithium-heparin tubes in a centrifuge (SorvalIIRT®6000B, DuPont Instruments, New York, USA) at 8000 rpm, for 10 min, at 22 °C. After centrifugation, plasma was transferred into microtubes (Eppendorf, Hamburg Germany) plain tubes and stored in a freezer (Bosch, Johannesburg, South Africa) at -20 °C for biochemical profiling.

2.12. Growth performance determination

2.12.1. Body mass gain

The pups were weighed daily to determine the effects of the extracts on body mass gain. The difference between the induction mass and the termination mass was taken as the total gain in body mass for the period of treatment. The average daily mass gain was calculated by dividing the total body mass gain by the number of days the pups received treatment.

2.12.2. Visceral organ mass

The visceral organs collected at termination were weighed and relative organ masses were calculated using the formula:

$$\text{Relative organ mass} = \frac{\text{Absolute organ mass}}{\text{Termination mass}} \times 100$$

2.12.3. Linear growth

The lengths of the tibia and femur were measured to determine linear growth. To achieve this, the long bones were dissected out and cleaned of all flesh. The lengths were then measured using a ruler and recorded in millimeters. Femur length was determined by measuring the distance between the proximal-most point of the femur head and the distal-most point of the femur. Tibia length was determined by measuring the distance between the tibia head closest to femur and the medial malleolus. The bones were then dried to a constant weight in an oven (Salvis®, Salvis Lab, Schweiz, Switzerland) at 45° C and their dry masses measured using a balance (Precisa 310M, Precisa Instruments, Switzerland).

2.13. Biochemical profiling for general health status of the rat pups

Twelve biochemical parameters were analysed to assess the effect of *F. thoningii* crude extracts on the general health status of the rat pups. Plasma concentrations of alkaline phosphatase (ALP), alanine amino transaminase (ALT), total bilirubin, total protein, amylase, globulin, albumin, creatinine, urea, cholesterol, phosphate and calcium were determined using a calibrated colorimetric chemistry analyser (IDEXX VetTest® Chemistry Analyser, Netherlands). All assays were carried out according to the manufacturer's instructions. In summary, plasma samples were thawed and 150 µL pipetted into the chemistry analyser. Ten microlitres of plasma sample were automatically

loaded onto each pre-inserted disk and analysed for 6 minutes before results were displayed.

2.14. Morphology and morphometry of abdominal viscera

Preserved sections of the small intestine, caecum, and liver were routinely processed in paraffin-embedded blocks. These were sectioned into: 5 μm liver sections and 8 μm intestine and caecum sections. The sections were stained using haematoxylin-eosin stain (Otimenyin *et al.*, 2004) after which they were examined under a light microscope. Villi height, crypt depth and thickness of the serosa and mucosal layers of the intestinal segments and caecum were measured using an eye-piece mounted micrometer (Reichert $\text{\textcircled{R}}$, Austria). To screen for possible hepatocellular injury, microscopic examination of hepatocellular necrosis, inflammation, sinusoidal obstruction, dilation and /or congestion was carried out (Ramachandran and Kakar, 2008).

2.15. Determination of hepatic storage substrates

2.15.1. Hepatic lipids

Liver lipids were determined as described by Bligh and Dyer (1959). In summary, homogenised liver samples were steeped overnight in a mixture of chloroform: methanol (2:1). The extracts were filtered, mixed with normal saline solution (0.09 %) and allowed to separate. The bottom phase was recovered, dried under vacuum and the lipids were recovered by resuspending them in chloroform. The amount of lipids in each sample was

determined by redrying in predried, preweighed glass vials. Lipid weight for each sample was expressed as a percentage of the original weight of the sample.

2.15.2. Hepatic glycogen

Liver glycogen was determined using the indirect hydrolysis method (Passonneau and Lauderdale, 1974). In summary, 0.1 g of liver tissue was homogenised in 1 ml of 0.03 M HCl. Glycogen was hydrolysed by adding 1 ml of 1 M HCl and incubating in a boiling water bath for 2 hrs. Homogenates were then neutralised by adding 1 ml of 1M NaOH. Glucose concentrations were measured using a glucose oxidase based colorimetric reaction on a glucometer (Accu-Chek Active Roche Diagnostics, Germany).

2.16. Statistical analysis

Statistical analysis was carried out using Graph Pad Prism version 5.0 (Graph Pad Software Inc. California, USA). Data was expressed as Mean \pm SD and statistically analysed using One way Analysis of Variance (ANOVA). Differences between means were tested for statistical significance ($p < 0.05$) using the Bonferroni post hoc test for significant data.

CHAPTER 3-RESULTS

3.1. Plant extract yield and phytochemical screening

3.1.1. Extract yield

The extract yield from dry material was 4.24 % for aqueous extracts and 3.8 % for methanolic extracts. The moisture content of *F. thonningii* leaves was 67%.

3.1.2. Phytochemical composition

Table 3.1 shows the results obtained following the screening of *Ficus thonningii* leaf extracts for the presence of phytochemicals. Detectable levels of flavonoids, saponins, phlobatannins and anthraquinones were observed in both aqueous and methanolic extracts while terpenoids and alkaloids were detected in methanolic extracts only. Aqueous extracts contained relatively higher levels of saponins than aqueous extracts.

Table 3.1: Phytochemical constituents of crude leaf extracts of *F. thonningii*

Phytoconstituent	Methanolic extract	Aqueous extract
Terpenoids/ steroids	++	-
Alkaloids	+	-
Saponins	+	+++
Flavonoids	++	+
Phlobatannins	+	+
Anthraquinones	+	+

KEY: (+++) = very high, (++) = high, (+) = low, (-) = absent

3.2. Brine shrimp acute toxicity assay

Percentage mortalities of brine shrimp (*Artemia salina*) larvae in *F. thoningii* extracts are as shown in Figure 3.1 and 3.2. The median lethality concentration (LC_{50-24 hr}) for methanolic extracts of *F. thoningii* was $31.62 \pm 2.8 \mu\text{g/ml}$ while the LC_{50-24 hr} of aqueous extracts was $119.3 \pm 1.10 \text{ mg/ml}$. Potassium dichromate was used as a positive control and its LC_{50-24 hr} was $0.3 \mu\text{g/ml}$.

3.3. Toxicity, mortality and morbidity

The rat pups showed no behavioral changes or obvious signs of distress or clinical signs of toxicity. There were no mortalities resulting from the administration of *F. thoningii* extracts.

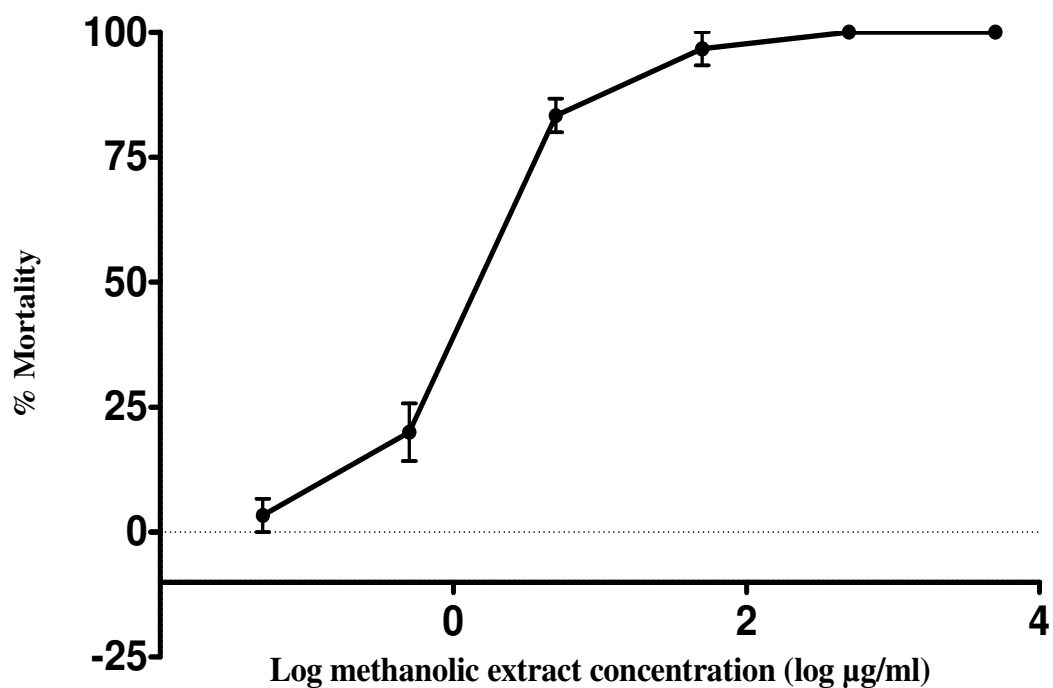


Figure 3.1: The toxicity effects of methanolic extracts of *Ficus thonningii* against brine shrimp (*Artemia salina*) larvae. Each point represents the mean mortality and error bars indicate the standard error of the mean (SEM).

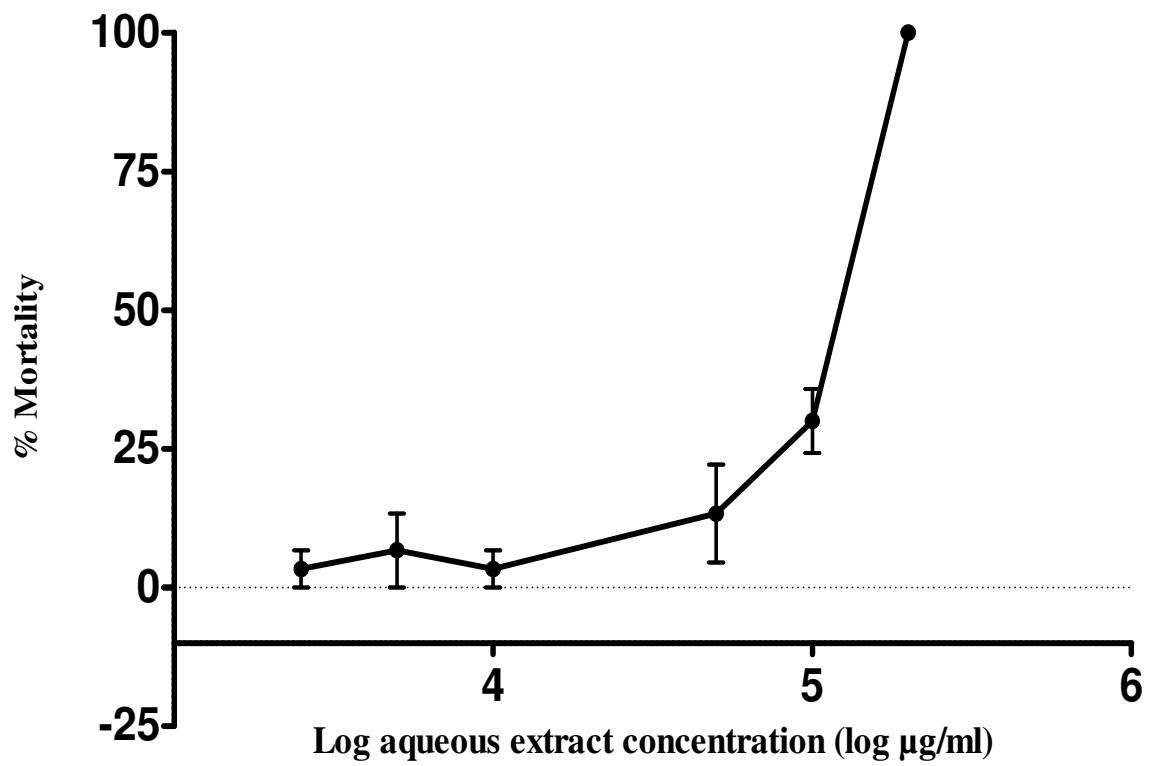


Figure 3.2: The toxicity effects of aqueous extracts of *Ficus thonningii* against brine shrimp (*Artemia salina*) larvae. Each point represents the mean mortality and error bars indicate the standard error of the mean (SEM).

3.4. The effect of *F. thonningii* extracts on growth of rat pups

3.4.1. Body mass gain

Table 3.2 shows the effects of *F. thonningii* extracts on the body mass gain of rat pups. There were no significant differences between the induction masses of all rat pups ($p = 0.9903$) (Table 3.2). Following treatment with aqueous and methanolic extracts for 7 days, all rat pups showed a significant increase in body mass ($p < 0.001$) (Figure 3.3) but no significant differences were observed between the body masses of the rat pups in the treatment groups at the termination of treatment ($p = 0.9678$) (Table 3.2).

Table 3.2. Effect of *Ficus thonningii* extracts on body mass gain of rat pups

	Treatments				
	Control	Low aqueous	High aqueous	Low methanolic	High methanolic
Body mass gain (g)	17.26 ± 2.71	17.68 ± 3.30	16.55 ± 3.65	16.25 ± 3.60	14.62 ± 3.51
Average daily gain (g/day)	2.20 ± 0.33	2.21 ± 0.41	2.07 ± 0.46	2.09 ± 0.45	1.83 ± 0.44

Data is expressed as mean ± SD (n = 8).

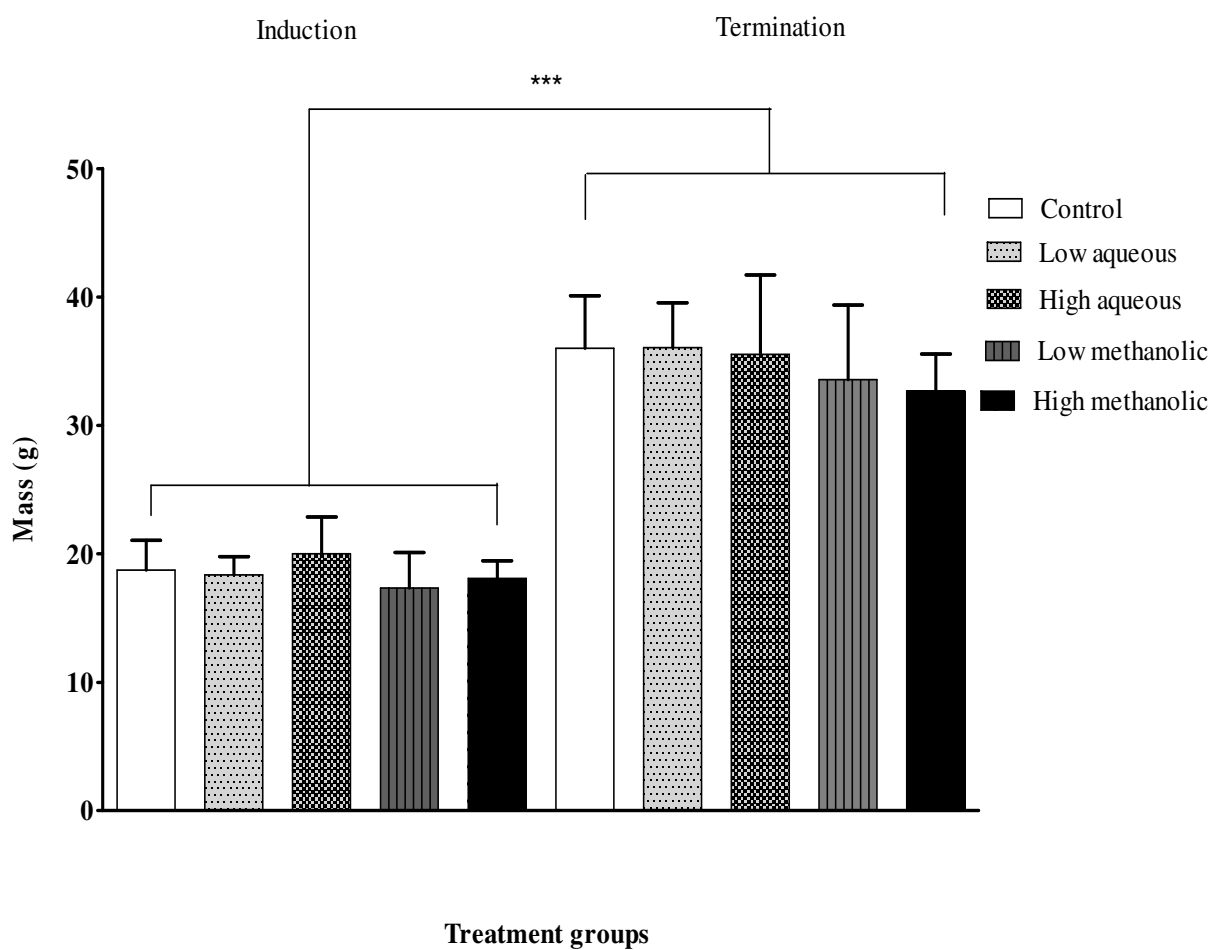


Figure 3.3: Induction and terminal masses of suckling rat pups after 7 days of treatment with *Ficus thonningii* extracts. The low doses were administered at 50 mg/kg b.w and high doses at 500 mg/kg b.w. Data is presented as means and error bars indicate SD (n=8). *** = p < 0.001.

3.4.2. Linear growth

Table 3.3 shows the lengths and masses of the tibia and femur of the rat pups after treatment with *F. thoningii* extracts. The lengths of the tibia and femur of treated groups did not differ significantly from the control. Similarly, there were no significant differences observed between the masses of the tibia and femur of treated animals in comparison with the control. The densities of both the tibia and femur of treated pups were not significantly different from the control group (Table 3.3).

Table 3.3: Effect of *Ficus thonningii* extracts on the linear growth of rat pups

	Treatments				
	Control	Low aqueous	High aqueous	Low methanolic	High methanolic
Tibia					
Mass (mg)	38.50 ± 7.41	37.25 ± 6.36	36.25 ± 9.68	32.88 ± 5.94	32.63 ± 4.63
Length (mm)	15.88 ± 0.88	15.94 ± 0.56	16.00 ± 0.96	15.81 ± 1.10	15.94 ± 1.21
Density (mg/mm)	2.54 ± 0.31	2.34 ± 0.40	2.25 ± 0.51	2.04 ± 0.41	2.04 ± 0.21
Femur					
Mass (mg)	40.88 ± 5.08	41.13 ± 7.26	37.38 ± 8.05	36.00 ± 5.40	33.88 ± 3.00
Length (mm)	12.88 ± 0.35	13.00 ± 0.53	13.38 ± 0.70	13.25 ± 0.53	13.25 ± 0.60
Density (mg/mm)	3.18 ± 0.40	3.16 ± 0.50	2.79 ± 0.50	2.71 ± 0.36	2.56 ± 0.30

Data expressed as mean ± SD (n=8).

3.4.3. Morphometry and histology of the GIT and viscera

Table 3.4 shows data obtained following gross morphometric measurements of the GIT of suckling rats after 7 days of treatment with *F. thonningii* extracts. The relative masses of the stomach were significantly higher in rats treated with the high dose (500 mg/kg b.w) of *F. thonningii* methanolic extracts in comparison to the control ($p = 0.0007$). The low methanolic dose exhibited no significant increase in relative masses of the stomach. There was an increase in the relative masses of the caeca across all treatment groups but this increase was not statistically significant ($p = 0.0817$). However, histological examination of the caeca showed a significant dose dependent increase in the thickness of mucosal layers of the aqueous and methanolic treatment groups ($p = 0.0001$) (Table 3.7). The muscularis and serosa of the caecum were not significantly different across all treatments.

There were no significant differences observed between the relative masses of the small intestine across all treatment groups ($p = 0.2155$). Microscopic morphometric measurements showed a non significant increase in the villi height ($p = 0.2431$) and crypt depth ($p = 0.6069$) of treated groups in comparison to the control (Table 3.6).

The relative masses of the large intestine, liver, pancreata, spleen and kidneys of treated groups were not significantly different from the control ($p > 0.05$) (Tables 3.4 and 3.5).

Table 3.4: Gross morphometry of the GIT of suckling rats after 7 days of treatment with crude extracts of *Ficus thonningii*

Organ		Treatment				
		Control	Low aqueous	High aqueous	Low methanolic	High methanolic
S.I	(g)	1.40 ± 0.42	1.42 ± 0.25	1.63± 0.28	1.45± 0.43	1.60 ± 0.32
S.I	(%)	3.65 ± 1.18	4.01 ± 1.02	4.73 ± 1.31	4.51 ± 1.40	4.97 ± 1.22
S.I	(mm)	577. 22 ± 54.26	588.50 ± 52.58	603. 75 ± 33.67	582.22 ± 74.67	616.88 ± 38.72
L.I	(g)	0.16 ± 0.06	0.15 ± 0.03	0.16 ± 0.04	0.15 ± 0.04	0.16 ± 0.02
L.I	(%)	0.44 ± 0.21	0.43 ± 0.11	0.45 ± 0.10	0.47 ± 0.13	0.49 ± 0.07

Table 3.4 (continued)

L.I	(mm)	78.89 ± 3.92	76.00 ± 6.97	78.25 ± 8.00	73.33 ± 11.73	73.13 ± 2.59
Caecum	(g)	0.15 ± 0.04	0.16 ± 0.03	0.22 ± 0.05	0.19 ± 0.07	0.19 ± 0.04
Caecum	(%)	0.42 ± 0.14	0.45 ± 0.12	0.64 ± 0.16	0.58 ± 0.28	0.59 ± 0.14
Stomach	(g)	0.23 ± 0.04	0.23 ± 0.02	0.23 ± 0.03	0.24 ± 0.04	0.25 ± 0.02
Stomach	(%)	0.63 ± 0.09	0.63 ± 0.06	0.64 ± 0.06	0.71 ± 0.06	0.77 ± 0.08**

KEY: S.I= Small intestine; L.I = Large intestine. Absolute values are expressed in grammes (g) and millimeters (mm) and relative masses as a percentage (%) of the body mass at the time of termination. ** = p< 0.01. Data is expressed as mean ± SD (n=8).

Table 3.5: Visceral organ weights of suckling rats after 7 days of treatment with crude extracts of *Ficus thonningii*

		Treatment				
Organ		Control	Low aqueous	High aqueous	Low methanolic	High methanolic
Liver	(g)	1.25 ± 0.20	1.25 ± 0.12	1.25 ± 0.17	1.27 ± 0.24	1.23 ± 0.13
	(%)	3.48 ± 0.76	3.49 ± 0.53	3.58 ± 0.61	3.88 ± 0.72	3.79 ± 0.49
Spleen	(g)	0.22 ± 0.05	0.24 ± 0.05	0.22 ± 0.05	0.21 ± 0.06	0.19 ± 0.03
	(%)	0.63 ± 0.08	0.66 ± 0.12	0.62 ± 0.10	0.61 ± 0.10	0.59 ± 0.10

Table 3.5 (continued)

Pancreas	(g)	0.10 ± 0.02	0.11 ± 0.04	0.12 ± 0.05	0.09 ± 0.03	0.10 ± 0.03
	(%)	0.27 ± 0.05	0.29 ± 0.09	0.34 ± 0.11	0.27 ± 0.05	0.31 ± 0.13
Kidneys	(g)	0.42 ± 0.05	0.46 ± 0.06	0.43 ± 0.08	0.42 ± 0.08	0.41 ± 0.04
	(%)	1.17 ± 0.10	1.27 ± 0.12	1.22 ± 0.11	1.25 ± 0.13	1.26 ± 0.09

KEY: Absolute masses are expressed in grammes (g) and relative masses as a percentage (%) of the terminal body mass. Data is expressed as mean \pm SD (n=8).

Table 3.6: Effect of *Ficus thonningii* extracts on the morphometry of the small intestine of suckling rats

	Treatment				
	Control	Low aqueous	High aqueous	Low methanolic	High methanolic
Villus height (µm)	50.42 ± 5.78	58.24 ± 10.75	59.95 ± 7.58	60.25 ± 9.77	60.32 ± 4.77
Crypt depth (µm)	7.66 ± 1.80	8.41 ± 1.85	9.30 ± 1.26	8.82 ± 2.44	8.82 ± 1.37
Muscle (µm)	3.31 ± 0.27	3.38 ± 0.63	3.6 ± 0.68	3.58 ± 0.43	2.77 ± 0.25
Serosa (µm)	1.00 ± 0.00	1.10 ± 0.22	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
VH/CD ratio	6.78 ± 1.25	7.02 ± 0.79	6.51 ± 0.89	6.96 ± 1.05	7.00 ± 1.39

KEY: VH= villus height, CD = crypt depth. Data is expressed as mean ± SD (n=8).

Table 3.7: Effect of *Ficus thonningii* on the morphometry of the caecum of suckling rats

	Treatment				
	Control	Low aqueous	High aqueous	Low methanolic	High methanolic
Mucosal layer (μm)	11.20 \pm 0.84	14.80 \pm 0.8**	16.20 \pm 1.30***	12.75 \pm 2.22	16.00 \pm 0.82***
Muscularis + serosa (μm)	3.00 \pm 0.71	3.80 \pm 0.84	3.80 \pm 0.84	3.25 \pm 0.50	3.25 \pm 0.5
Total thickness (μm)	14.4 \pm 0.55	18.6 \pm 1.34**	20.00 \pm 2.12***	16.00 \pm 2.58	19.25 \pm 0.96**

** = $p < 0.01$, *** = $p < 0.0001$. Data is expressed as mean \pm SD (n=5). The low aqueous, high aqueous and high methanolic groups had significantly higher mucosal layer thickness and total thicknesses when compared to the control and the low methanolic groups.

3.5. The effect of *F. thonningii* extracts on metabolic substrates

Figure 3.4 shows the concentrations of non fasting blood glucose in suckling rat pups following treatment with *F. thonningii* extracts. The high methanolic group had a significantly lower mean non fasting blood glucose concentration compared to the control group and the aqueous groups ($p = 0.0008$). The low methanolic group was not significantly different from the high methanolic group. The circulating non fasting levels of triglycerides were high in all groups including the control. Triglycerides values fell above the limit detectable by a TG meter (6.8 mmol/L) across all groups.

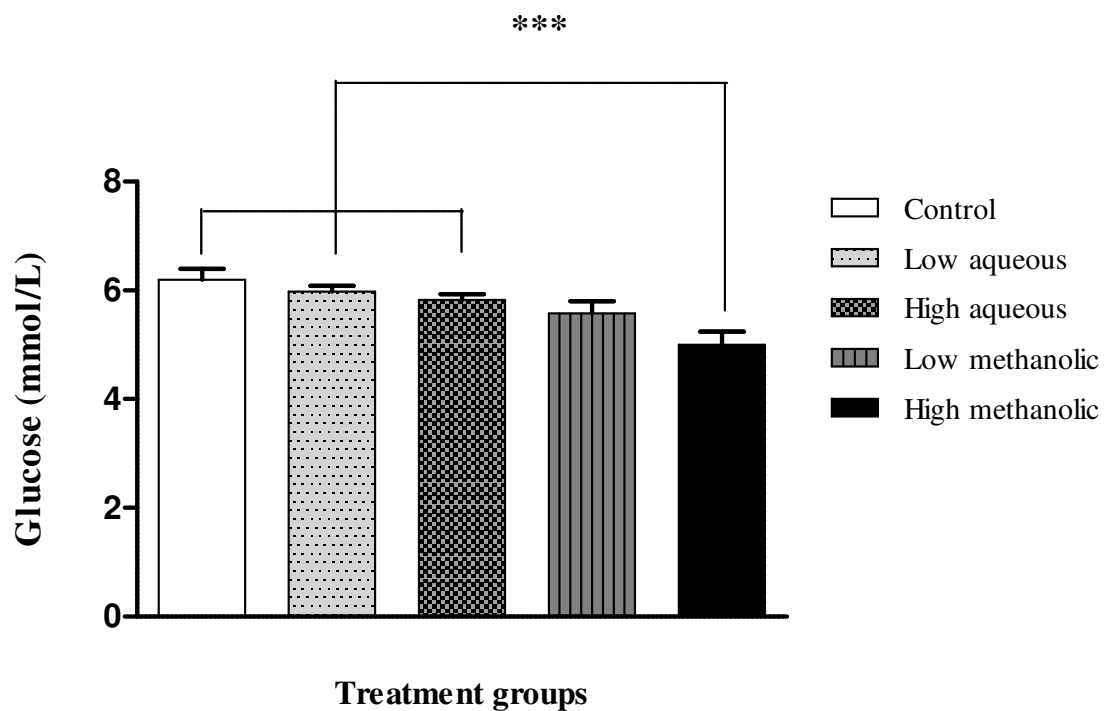


Figure 3.4: Non fasting blood glucose concentrations of suckling rats after 7 days of treatment with crude extracts of *Ficus thonningii*. The low doses were administered at 50 mg/kg b.w and high doses at 500 mg/kg b.w. Data is presented as means and error bars indicate SD (n=8).*** = p < 0.0001.

3.6. The effects of *F. thonningii* extracts on the hepatic storage substrates of rat pups

The effects of *F. thonningii* extracts on liver lipids and glycogen levels are shown in Table 3.8. There were no significant differences between the levels of lipids in treated groups in comparison to the control ($p = 0.05$). Glucose concentrations (from the hydrolysis of glycogen) were not significantly different across all treatments ($p = 0.7762$).

Table 3.8: Effects of *F. thonningii* extracts on hepatic storage substrates of rat pups

	Treatment				
	Control	Low aqueous	High aqueous	Low methanolic	High methanolic
Lipids (%)	4.89 ± 1.00	4.29 ± 0.97	5.89 ± 1.47	5.98 ± 0.97	5.78 ± 1.07
*Glycogen (mmol/L)	3.85 ± 0.74	4.31 ± 0.57	4.03 ± 0.86	3.68 ± 1.06	3.96 ± 1.5

Data is presented as Mean ± SD (n = 8). *Glycogen expressed as glucose equivalents.

3.7. Clinical biochemical measures for general health status of suckling rats

The clinical biochemical parameters of suckling rats treated with aqueous and methanolic extracts of *F. thonningii* are shown in Table 3.9. All groups including the control group had high levels of alkaline phosphatase (ALP), falling above the normal reference value (370 U/L) (Espandiari *et al.*, 2008). However there were no significant differences between the ALP values of the treated groups in comparison to the control. There were no significant differences observed in the plasma concentrations of phosphates, calcium, creatinine, albumin, globulin and total proteins across all treatment groups. Although there was a general decrease in the plasma levels of urea, total bilirubin and alanine amino transaminase (ALT) of the treated groups in comparison with the control, the decrease was insignificant ($p > 0.05$). Cholesterol levels were within normal ranges but slightly on the high side across all treatment groups including the control. There were no differences between the cholesterol levels of treated groups in comparison to the control ($p = 0.9854$).

Table 3.9: Clinical biochemical parameters of suckling rats after 7 days of treatment with crude extracts of *Ficus thonningii*

Plasma biochemical parameter	Treatment					Reference values
	Control	Low aqueous	High aqueous	Low methanolic	High methanolic	
Cholesterol (mmol/L)	4.44 ± 0.35	4.44 ± 0.46	4.31 ± 0.54	4.44 ± 0.70	4.37 ± 0.72	3.8-4.6*
Urea (mmol/L)	7.26 ± 2.09	5.90 ± 1.10	5.81 ± 1.90	7.50 ± 1.8	5.98 ± 1.77	6.6-8.39*
Creatinine (µmol/L)	31.75 ± 6.60	48.00 ± 54.99	31.38 ± 8.20	48.8 ± 48.36	33.63 ± 4.00	34.00-57.00
Phosphate (mmol/L)	3.14 ± 0.36	3.32 ± 0.52	3.05 ± 0.25	3.22 ± 0.24	2.93 ± 0.25	1.87-3.61
Calcium (mmol/L)	2.88 ± 0.34	2.91 ± 0.19	2.80 ± 0.13	2.99 ± 0.22	2.75 ± 0.17	2.92-3.08*
Total protein (g/L)	42.63 ± 2.72	44.35 ± 2.49	42.00 ± 2.45	44.25 ± 5.70	43.38 ± 6.25	41.3-44.7*

Table 3.9 continued

Globulin (g/L)	20.12 ± 1.89	20.75 ± 2.82	19.00 ± 1.69	17.25 ± 3.92	18.25 ± 2.43	15.6-18.4*
Albumin (g/L)	22.50 ± 1.60	23.50 ± 1.69	22.75 ± 1.67	27.13 ± 6.79	25.38 ± 6.95	25.4-26.6*
ALT (U/L)	31.00 ± 13.04	27.00 ± 11.55	25.63 ± 9.58	31.50 ± 18.28	28.38 ± 7.65	37-69 [#]
ALP (U/L)	410.38 ± 41.44	385.75 ± 86.75	371.00 ± 84.71	414.88 ± 77.29	363.50 ± 85.86	290-370 [#]
Amylase (U/L)	1117.88 ± 136.67	1061.63 ± 128.18	1092.75 ± 128.14	1140.75 ± 289.32	1064.50 ± 195.05	326.00-2246
Total bilirubin (µmol/L)	10.38 ± 3.82	10.38 ± 3.89	8.25 ± 3.45	6.5 ± 2.93	8.25 ± 2.87	2.00-12.00

ALP = alkaline phosphate, ALT = alanine transaminase. * Papworth and Clubb, 1995: # Espandiari *et al.*, 2008. All other reference values are as supplied by IDEXX Laboratories, Netherlands. Data is presented as Mean ± SD (n=8).

3.8. Effect of *F. thoningii* extracts on the packed cell volumes of rat pups

Figure 3.5 shows the comparison between PCVs of the treatment groups and the control.

The group receiving the high aqueous dose of *F. thoningii* extracts showed a significant decrease in the packed cell volume in comparison to the control ($p=0.048$). All other values from the different groups were not significantly different from the control.

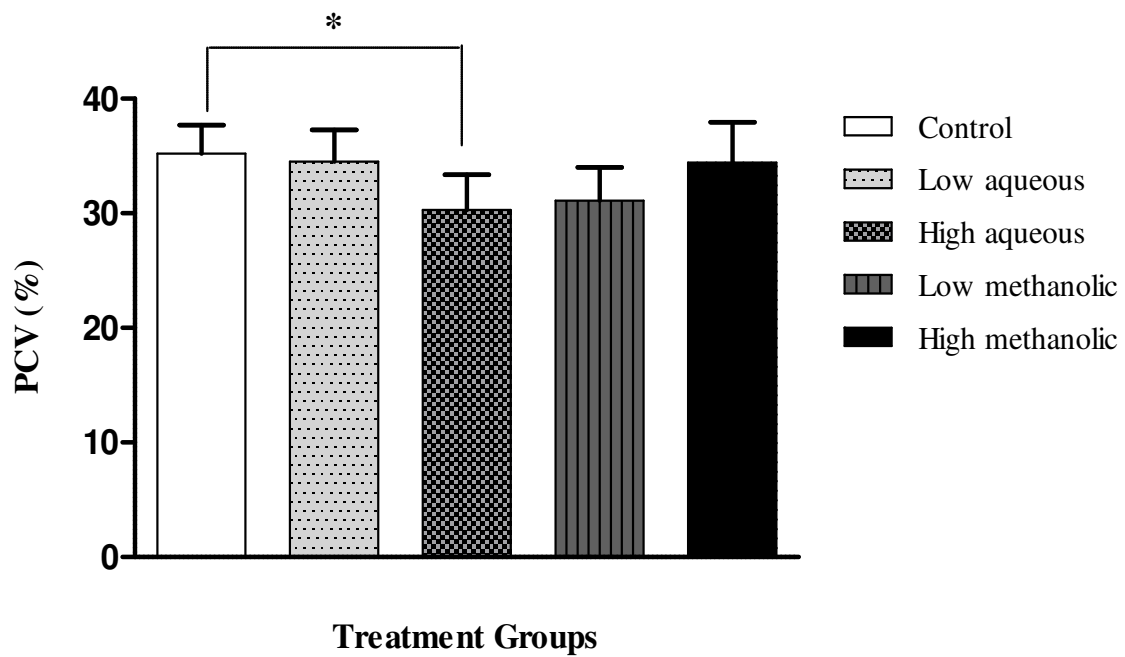


Figure 3.5: Packed cell volumes (PCVs) of suckling rat pups after 7 days of treatment with crude *Ficus thonningii* extracts. The low doses were administered at 50 mg/kg b.w and high doses at 500 mg/kg b.w. Data is presented as means and SD (n= 8). * = p <0.05.

CHAPTER 4-DISCUSSION

4.1 Summary

The aim of this study was to investigate the effects of *Ficus thonningii* extracts on growth, morphology, morphometry of the GIT and visceral organs and the clinical biochemistry of suckling rats. Methanolic extracts of *F. thonningii* were cytotoxic to Brine shrimp larvae but aqueous extracts were non toxic. Results obtained from the animal study revealed that short term, oral administration of *F. thonningii* extracts may not promote precocious maturation of the neonatal rat intestine but had significant trophic effects on the stomach and the mucosal layers of the caecum. Treatment of neonatal rats with *F. thonningii* extracts may not result in disruption in the normal function of the liver and the kidneys. The results also confirmed that methanolic extracts of *F. thonningii* possess glucose-lowering effects. The physiological effects exhibited by *F. thonningii* are a result of the presence of various biologically active secondary metabolites. Phytochemical analysis of *F. thonningii* extracts confirmed the presence of alkaloids, flavonoids, terpenoids, tannins and saponins. Methanolic extracts of *F. thonningii* exhibited toxic effects in *Artemia salina* larvae but at the doses used in this study, there was no evidence of toxicity in the neonatal rats.

4.2. Phytochemical constituents of *F. thonningii*

Preliminary, qualitative phytochemical screening of *F. thonningii* leaves revealed the presence of alkaloids, tannins, anthraquinones, terpenoids (steroids), flavonoids and saponins. Previous studies on the phytochemistry of *F. thonningii* have also reported the presence of these phytochemicals (Ndukwe *et al.*, 2007; Usman *et al.*, 2009; Ahur *et al.*,

2010). These phytochemicals could be responsible for the broad pharmacological activity of *F. thonningii* that supports its widespread use in African traditional medicine. Although the methods used in this study were qualitative, the intensity of colour development in the determination of terpenoids, flavonoids and alkaloids was higher in methanolic extracts as compared to aqueous extracts. These observations suggest that there were higher concentrations of these phytochemicals in methanolic extracts than there were in the aqueous extracts. This is probably because methanol has a higher polarity index than water hence will extract a higher concentration of phenolic compounds (Cowan, 1999). Additionally, most plant secondary metabolites are organic in nature and hence partition well in organic solvents. This justifies the use of palm wine as a solvent in ethnomedicinal systems of West Africa (Akinsulire *et al.*, 2007). On the other hand, the degree of frothing observed when the aqueous extracts were tested for saponins was higher than in methanolic extracts. This suggests that there were higher saponin levels in aqueous extracts as compared to methanolic extracts. Saponins possess hydrophilic glycoside moieties which increases their solubility in water as compared to methanol (Cowan, 1999).

4.3. Brine shrimp toxicity assay

The scientific validation of potential acute and chronic toxic effects of plant medicines is crucial in light of their widespread use and the common misconception that “green medicine” is always safe. The brine shrimp lethality test was used to determine the toxicity of *F. thonningii* extracts. Plant extracts with LC₅₀-24 hr values greater than 1 mg/ml are considered to be non toxic (Latha *et al.*, 2010). The low LC₅₀-24 hr of methanolic extracts

(31.62 µg/ml) shows that the extracts are highly toxic to brine shrimp larvae, while aqueous extracts were non-toxic (LC₅₀-24 hr = 119.3 mg/ml). These results also suggest that there are higher concentrations of bioactive compounds in methanolic extracts in comparison with aqueous extracts. However, although the methanolic extracts of *F. thonningii* were toxic to brine shrimp larvae, they did not exhibit any visible toxic effects in the rat pups. *F. thonningii* is generally considered non toxic when administered orally at low doses (Aniagu *et al.*, 2008; Ahur *et al.*, 2010). The median lethal dose (LD₅₀) of *F. thonningii* aqueous extracts administered orally in adult Wistar rats was reported to be above 3000 mg/kg body weight (Aniagu *et al.*, 2008). However, rats showed 100% mortality after intraperitoneal administration of 600 mg/kg body weight of the same extracts and the LD₅₀ intraperitoneally was reported to be 584 mg/kg b.w (Aniagu *et al.*, 2008). This implies that some of the toxic principles in *F. thonningii* are metabolised in their passage through the GIT and the liver. Clinical trials of *F. thonningii* extracts in humans have to be done before conclusions on its safety can be drawn.

4.4. Overall effect of *F. thonningii* extracts on growth of rat pups

Oral administration of *F. thonningii* extracts for 7 days did not result in any significant changes in increase in body mass in all treatment groups compared to the control despite the presence of antinutritional factors. *F. thonningii* leaves when used as fodder have been shown to increase the body weight in rabbits owing to the high levels of crude protein (Jokthan *et al.*, 2003). The protein content of the extracts was not measured but it is likely that the growth-promoting effects of crude extracts could have been lower due to loss of protein during the extraction process. Proteins are amphoteric in nature and therefore

require specific pH conditions to solubilise. The concentrations of proteins in extracts could have therefore been lower than the original concentration from the *F. thoningii* leaves. As a result, leaf extracts of *F. thoningii* might not exhibit as much growth promoting effects as the fodder.

The lengths and densities of anti-gravity bones (tibia and femur) can be used as a reliable index of growth (Eshet *et al.*, 2004). Unlike body mass which is usually influenced by several factors such as food intake and the hydration status of the animal, linear growth measurements can be used as a more accurate determination of growth patterns (Hills and Parizkova, 2002). The tibial and femoral lengths and densities of treated animals in this study were not significantly different from the control group. This shows that *F. thoningii* extracts did not have significant effects on the linear growth of the suckling pups.

4.5. Effects of *F. thoningii* on GIT morphology and morphometry

Morphological assessment of the developing gut is a useful tool in evaluating the effects of dietary changes on the physiology of neonates (Barltrop and Brueton, 1990). Rats have an altricial mode of GIT development which is characterised by a relatively immature gut at birth which undergoes major structural and functional changes postnatally (Linderoth *et al.*, 2005). Dietary changes during the suckling period are often followed by changes in mass, surface area, digestive and absorptive capacity of the neonatal GIT (Sangild, 2006).

Phytochemicals increase proliferation of the enterocytes and growth of the gut mucosa (Pusztai *et al.*, 1999) and hence they can induce precocious maturation of the neonatal

GIT. Lectins from the red kidney bean (*Phaseolus vulgaris*), administered orally for 3 days, were shown to induce precocious structural and functional maturation of the GIT in suckling rats (Linderoth *et al.*, 2005). In the present study, the administration of *F. thonningii* extracts for 7 days led to a significant increase in the stomach mass and mucosal layer thicknesses of caeca of the suckling rat pups. The increase in stomach weights can be an indication that *F. thonningii* extracts contain phytochemicals that mimic the action of and/or increase the release of gastrin and other regulatory peptides which increase the proliferation of parietal cells and hence exhibit trophic effects on the gut mucosa (Jain and Samuelson 2006). Unfortunately, histology was not performed on the stomach to ascertain whether the increase in mass was due to hypertrophy or hyperplasia or both. The higher activity of the high methanolic dose when compared to the low methanolic dose suggests a dose-related effect. Higher doses could have higher concentrations of phytochemicals hence more profound physiological effects.

Although the increase in relative caecum weights of the pups was not statistically significant ($p=0.0817$), histological analysis showed that *F. thonningii* extracts significantly increased the thickness of the caecal mucosa and submucosal layers. This could be the result of the presence of soluble, non-digestible, fermentable polysaccharides in the extracts. Non-digestible polysaccharides (also known as prebiotics) increase proliferation of the mucosal cells of the caecum and colon (Steed and Macfarlane, 2009). They do so by stimulating the activity and growth of intestinal saccharolytic microbes which ferment non-starch polysaccharides to short chain fatty acids (SCFAs) (Steed and Macfarlane, 2009). SCFAs especially butyrate, have trophic effects on the caecal mucosa and are likely to be responsible for the architectural changes observed in the caecal walls

of rats in this study (Blottiere *et al.*, 2003; Lobo *et al.*, 2007). An increase in caecal weight and the production of SCFAs could be useful in ruminants which depend on SCFAs for up to 80 % of their metabolic energy requirements (Tagang *et al.*, 2010). Previous studies with neonatal rats fed African potato extracts (*Hypoxis hemerocallidea*) (Erlwanger and Cooper, 2008) and *Aloe vera* extracts (Beya, 2008) showed an increase in caecal mucosal layers and caecal weights.

If present, the prebiotic compounds in *F. thoningii* extracts may have also increased the production of lactic acid by lactic acid-producing bacteria. Lactic acid is a known luminal trophic factor which increases the mitotic index of caecal crypt cells (Ichikawa and Sakata, 1997). Future studies could look at the effects of *F. thoningii* on the SCFA concentrations in colonic and caecal contents to evaluate the usefulness of *F. thoningii* as a prebiotic.

There was no significant difference in the relative masses of the small intestines from pups on the different treatments. Though not statistically significant, the increase in villi height and crypt depths of treated animals could be of biological significance as it shows the potential trophic effects that *F. thoningii* extracts could exhibit if administered for a longer duration or at higher doses. The phytochemicals in *F. thoningii* did not appear to have any adverse effects on the integrity of the sensitive neonatal GIT. Histomorphological analysis showed that there was no mucosal damage, villous atrophy or any apparent pathology in the intestine of treated rats.

4.6. Effects on the morphology and morphometry and markers of function of other visceral organs

The liver is the major organ involved with the metabolism and biotransformation of xenobiotics in the body (Rajesh *et al.*, 2009). Contact with toxic principles may disrupt the integrity of hepatic parenchymal cells. A rise in the serum levels of alkaline phosphatase (ALP) is usually indicative of obstructive changes in the hepato-biliary system (Thapa and Walia, 2009). Hepatic cell damage can also be detected by tracking circulating levels of the cytosolic enzyme alanine transaminase (ALT) which is released into the blood following changes in membrane permeability and necrosis. Unlike ALP, ALT is more specific to the liver and hence is a more reliable index of liver damage (Rajesh *et al.*, 2009). Circulating levels of both enzymes; ALT and ALP, were used as markers of liver damage in this study. The results obtained show that at the doses used, *F. thonningii* extracts did not seem cause any parenchymal cell lesions, necrosis or drug induced-hepatitis. It was notable that ALP levels were significantly elevated in all of the pups. ALP is also associated with osteoblastic activity and hence its levels are elevated in rapidly growing animals (Alhassan *et al.*, 2009). Rats have been reported to have relatively higher circulating concentrations of ALP compared to other mammals and neonatal rats have high concentrations due to milk feeding which induces the production of the isozyme (Koyama *et al.*, 1987). This explains the elevated (>370 U/L) ALP concentrations observed in all rat pups including the controls. An isoform of ALP is also found in the intestinal mucosa, predominantly in duodenal epithelial cells where it regulates bicarbonate secretion, limits bacterial transepithelial passage and detoxifies lipopolysaccharides (Dziedziejko *et al.*, 2005; Lalles, 2010). However, this study did not measure the specific isoforms of ALP.

Serum albumin, total bilirubin and total protein concentrations can be used as clinical tools in assessing the hepato-synthetic function (Thapa and Walia, 2007). Albumin is the main plasma protein produced in the liver and plasma concentrations of albumin reflect the extent of the functioning liver cell mass (Alhassan *et al.*, 2009). Decreased plasma albumin concentrations are indicative of liver injury and a decreased rate of its synthesis (Olatunji *et al.*, 2005) or increased loss e.g. protein losing enteropathy and nephropathy (Umar and Dibousse, 2010; Littman *et al.*, 2000).

Raised concentrations of bilirubin in the blood are indicative of the failure of the liver to excrete bilirubin and/or the blockage of bile ducts. The concentrations of these biochemical markers of liver function in the blood were unaltered by administration of *F. thonningii* extracts at the doses used, confirming that *F. thonningii* was not hepatotoxic in the short term. Histopathological analysis of *F. thonningii*-treated rat livers showed no changes in hepatic lobular architecture and no signs of inflammation. However, the veins and sinusoids were slightly dilated in all treatment groups suggesting possible hepatic congestion (Jones *et al.*, 1997).

The exposure of the kidney to toxins, drugs and other xenobiotics leads to tubule damage and necrosis consequently disrupting normal renal physiology (Bovee, 1986; Markowitz *et al.*, 2003). The glomerular filtration rate (GFR) is the gold standard for measuring kidney function (Stevens and Levey, 2009). Due to limitations in the study design, timed collections of urine for the determination of GFR were difficult to achieve. As a result, serum concentrations of: creatinine and urea, inorganic phosphates, calcium and albumin

were used as surrogate markers of kidney function. Serum creatinine concentrations depend exclusively on the GFR therefore can be used as a more reliable clinical estimation of GFR (Stevens and Levey, 2009). Increased levels of plasma creatinine and urea could indicate compromised tubular function and/or impaired glomerular filtration (Car *et al.*, 2006). These markers have limitations and cannot be compared to the use of GFR and fractional excretion of sodium (FENa) in assessing nephrotoxicity. Despite their limitations, serum concentrations of creatinine and urea are still useful as first line markers of kidney function (Bovee, 1986; Stevens and Levey, 2009). Based on the use of these markers, the results obtained in this study showed that short term administration of crude extracts of *F. thonningii* exhibited no nephrotoxic effects or alterations in renal physiology. These findings are in line with the observations of other researchers. Musabayane *et al.*, (2007) observed no cytotoxic effects in bovine and porcine kidney cell lines after a 72-hr exposure to ethanolic extracts of *F. thonningii*. There were no observed changes in renal fluid flow and electrolytes excretion rates of adult Wistar rats following treatment with stem bark ethanolic extracts of *F. thonningii* (Musabayane *et al.*, 2007). Extrapolation of these observations to humans is difficult as differences are likely to exist in the physiological and metabolic responses to the doses given.

A delicate balance exists between the production and destruction of blood cells. Some phytochemicals e.g. saponins have cytotoxic effects and disrupt this existing equilibrium (Makkar *et al.*, 2007). Coker *et al.*, (2009) reported that *F. thonningii* extracts had no deleterious effects on the haematonic value, whilst ethyl acetate leaf extracts of *F. thonningii* were reported to improve red blood cell counts in acetaminophen-treated rats (Ahur *et al.*, 2010). Contrary to these reports, the results of this study showed a significant

reduction in the packed cell volumes of rats treated with high aqueous doses of *F. thonningii*. High levels of saponins were detected in the aqueous extracts. Saponins haemolyse red blood cells by interacting with membrane components such as proteins, phospholipids and cholesterol (Makkar *et al.*, 2007). However, although the PCVs were reduced in the high aqueous group, there was no significant rise in the circulating bilirubin concentrations to confirm destruction of red blood cells. Reduced levels of haemoglobin resulting from reduced bioavailability of iron can also lead to a reduction in the haematocrit. *F. thonningii* has high levels of iron but the availability of iron can be reduced by the presence of antinutritional factors such as tannins, phytate and other polyphenolic phytochemicals (Sandberg, 2002). Salivary glands in rats secrete proline-rich proteins as defense against tannins. The presence of tannins and phytate in the gut might have reduced the bioavailability of iron. Hepatic iron stores in the neonate are depleted during the suckling period (Smythe and Miller, 1928; Keen *et al.*, 1981) and the neonate relies on dietary supply. Reduction in the uptake and bioavailability of iron at this stage is likely to lead to a reduction in erythropoiesis and consequently microcytic anaemia. A complete haematological analysis including the determination of red blood cell counts, mean corpuscular volumes and mean corpuscular haemoglobin could be carried out to determine the effects of *F. thonningii* extracts on the haematopoietic system. However, these could not be done due to the small volume of blood collected from the pups as a result of their small blood volume.

4.7. Effects of *F. thonningii* extracts on metabolism

Ficus thonningii has been shown to possess hypoglycemic effects (Bwititi and Musabayane, 1997; Musabayane *et al.*, 2007) and this supports its ethnomedicinal use in the treatment and management of diabetes mellitus. In this study, the high methanolic dose (500 mg/kg b.w) significantly reduced glucose concentrations in comparison with the control. Although glucose concentrations were significantly lowered in this group, they remained within the normal reference range (2.78-7.5 mmol/L) for young healthy rats. This shows that the extracts did not elicit undesirable hypoglycemic effects.

There are a number of possible mechanisms underlying the glucose lowering effects of *F. thonningii* extracts. At intestinal level, the polyphenolic compounds in *Ficus thonningii* (e.g. flavonoids, tannins, proanthocyanins) could attenuate the uptake of glucose by inhibiting the activity of glucose transport proteins (Kwon *et al.*, 2007). Polyphenolic compounds have been shown to inhibit glucose uptake through the Na⁺-dependent SGLT-1 and GLUT 2, hence they reduce both the apical and basolateral transport of glucose in the enterocytes (Kwon *et al.*, 2007; Hanhineva *et al.*, 2010).

The phytoestrogen resveratrol (trans-3, 5, 4'-trihydroxystilbene), previously shown to be present in *F. thonningii* leaf extracts (Greenham *et al.*, 2007), could have also contributed to its glucose-regulating effects. Resveratrol exhibits hypoglycaemic effects by enhancing glucose uptake by muscle cells and by activating hepatic AMPK (Shimizu *et al.*, 2000; Deng *et al.*, 2008; Minikawa *et al.*, 2011).

The presence of phytol in *F. thonningii* could be another contributory factor to its glucose-handling effects. Phytanic acid, a compound produced from the metabolism of phytol is also reported to possess hypoglycaemic and hypolipidaemic effects as shown by Heim *et al.*, (2002). In rats, phytanic acid increases the expression of glucose transporters and glucokinase and hence increases glucose uptake in the hepatocytes (Heim *et al.*, 2002).

F. thonningii extracts may also contain other glucose-regulating phytochemicals which either have a direct effect on insulin production/secretion, mimic insulin or could have an effect on gene expression and signaling pathways in glucose metabolism. However, insulin concentrations were not measured in this study.

The introduction of milk feeding at birth is usually associated with an increase in the biosynthesis of triglycerides (TGs) in the neonatal rat liver (Jamdar *et al.*, 1978). A concomitant rise in serum TG levels occurs to a peak at day 9 after birth and TG levels remain high until the day of weaning (Jamdar *et al.*, 1978). This could be a plausible explanation for the unusually high levels of lipids in both the treatment groups and the controls.

Most triglyceride values fell above 6.8 mmol/L (the upper limit of the TG meter used) (Accutrend, Roche Diagnostics). Instruments with a higher detection limit should be employed for measuring circulating TGs when using rat neonatal models. Regardless of this fact, *F. thonningii* seems to decrease blood glucose levels without promoting the

biosynthesis of triglycerides and cholesterol and this is beneficial in the prevention of cardiovascular diseases.

CHAPTER 5-CONCLUSIONS AND RECOMMENDATIONS

5.0. Conclusions

The results obtained in this study are of considerable importance as they introduce the first *in vivo* evidence that *F. thonningii* might exhibit trophic, maturational and developmental changes on the neonatal GIT. The antinutritional factors present in *F. thonningii* extracts did not exhibit any adverse effects on the GIT and visceral organ morphometry and function. Results also showed that methanolic extracts of *F. thonningii* possess glucose-lowering effects. However, the mechanisms underlying these effects should be further explored.

5.1. Limitations of the study

The study's main focus was on morphometric changes and structural changes of the neonatal rat GIT and so the study did not investigate effects of *F. thonningii* extracts on GIT function. Perfused gut loops, immunohistochemistry and cell cultures should be used in future studies to shed more light on the absorptive, transport, immune defense and metabolic function of the intestinal tissue. The study design could also be modified to facilitate timed-collection of urine samples for a more accurate determination of GFR and fractional excretion indices.

5.2. Perspectives and recommendations for future studies

Increasing the duration of exposure of the neonates to the plant extracts would be advantageous in order to evaluate the functional significance of any observed changes.

However, the effects of dietary manipulations in neonatal rats are more accurately determined before the pups open their eyes. After eye opening (day 14 or 15), changes in the GIT structure cannot be exclusively attributed to the experimental intervention as the rats start to forage on the other particulate material in their cages.

The reduction of PCVs observed at high doses of aqueous extracts should be complemented with a complete haematological analysis before conclusions can be drawn. Erythrocyte counts, haemoglobin levels, mean corpuscular volumes and leukocyte counts could be useful in determining the effects of the plant's extracts on haematological parameters.

APPENDICES

APPENDIX 1: Animal Ethics Clearance

REFERENCES

- Acamovic, T. & Brooker, J.D. 2005. Biochemistry of plant secondary metabolites and their effects in animals. *Proceedings of the Nutrition Society* **64**, 403-412.
- Achigan-Dako, E. and Ndanikou, S. 2009. Diversity of traditional vegetables and local taxonomy. In: *Traditional vegetables in Benin, diversity, distribution, ecology, agronomy and utilization*. Achigan-Dako, E., Pasquini, M., Assogba-Komlani, F., Ndanikou, S., Dansi, A. and Ambrose-Oji, B. 2009 (eds). IFS- Darwin Initiative, Benin, pp. 22.
- Agroforestry Tree Database. 2011.
(<http://www.worldagroforestrycentre.org/sea/Products/AFDbases/AF/asp/speciesInfo.asp?SpID=866>) accessed 03/07/11.
- Ahur, V.M., Madubunyi, I., Adenkola, A.Y. & Udem, S.C. 2010. The effect of acetyl acetate extract of *Ficus thonningii* (Blume) leaves on erythrocyte osmotic fragility and haematological parameters in acetaminophen-treated rats. *Comparative Clinical Pathology* **10**, 1107-1111.
- Ajayi, A.O. 2008. Antimicrobial nature and use of some medicinal plants in Nigeria. *African Journal of Biotechnology* **7**, 595-599.
- Akinsulire, O.R., Aibim, I.E., Adenipekun, T., Adelowotan, T. & Odugbemi, T. 2007. *In vitro* antimicrobial activity of crude extracts from plants *Bryophyllum pinnatum* and *Kalanchoe crenata*. *African Journal of Traditional, Complementary and Alternative Medicines* **4**, 338-344.
- Alawa, J.P., Jokthan, G.E. & Akut, K. 2002. Ethnoveterinary medical practice for ruminants in the sub-humid zone of the northern Nigeria. *Preventive Veterinary Medicine* **54**, 79-90.
- Alhassan, A.J., Sule, M.S, Aliyu, S.A. & Aliyu, M.D. 2009. Ideal hepatotoxicity model in rats using carbon tetrachloride (CCl₄). *Bayero Journal of Pure and Applied Sciences* **2**, 185-187.
- Aniagu, S.O., Nwinyi, F.C., Akumka, D.D., Agbain, E.O., Dzarma, S., Ajoku, P., Adelusola, K.A., Ibe, J., Inyang, U.S. & Gamaniel, K.S. 2008. Short-term toxicity

studies of *Ficus thonningii* Blume Moraceae leaf extracts in rats. *International Journal of Food Science and Technology* **43**, 456-464.

Arbonier, M.A. 2004. *Trees, Shrubs and Lianas of West African Dry Zones*. 2nd edition, Margraf, Netherlands, pp. 412.

Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Ezennia, E.C. & Atangbayila, T.O. 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. *Tropical Journal of Pharmaceutical Research* **7**, 1019-1024.

Bah, S., Diallo, D., Dembele, B. & Paulsen, S. 2006. Ethnopharmacological survey of plants used for the treatment of schistosomiasis in Niono district, Mali. *Journal of Ethnopharmacology* **105**, 387-399.

Bajec, M.R. & Pickering, G.J. 2008. Astringency: Mechanisms and perception. *Critical Reviews in Food Science and Nutrition* **48**, 1-18.

Baltrop, D. & Brueton, M, J. 1990. The gastrointestinal tract and short term toxicity tests. In: *Short Term Toxicity Tests for Non-genotoxic Effects* (Bourdeoui, P. eds), John Wiley and Sons, pp 99.

Bamikole, M.A., Ikhatua, U.J., Arigbede, O.M. Babayemi, O.J. & Etela, I. 2004. An evaluation of the acceptability as forage of some nutritive and antinutritive components and of the dry matter degradation profiles of five species of *Ficus*. *Tropical Animal Health and Production* **36**, 157-167.

Bamikole, M.A. & Ikhatua, U.J. 2010. Nutritional evaluation of *Ficus thonningii*-*Panicum maximum* mixtures in West African dwarf goats. *Nutrition and Food Science* **40**, 280-288.

Bauer, E., Williams, B.A., Smidt, H., Vestegen, M.W.A. & Mosenthin, R. 2006. Influence of the gastrointestinal microbiota on development of the immune system in the young animals. *Current Issues in Intestinal Microbiology* **7**, 35-52.

- Baur, J.A. & Sinclair, D.A. 2006. Therapeutic potential of resveratrol: The *in vivo* evidence. *Nature Reviews: Drug Discovery* **5**, 493-506.
- Beglinger, C. 2002. Overview: cholecystokinin and eating. *Current Opinion in Investigational Drugs* **3**, 587-588.
- Benzie, I.F.F. and Watchtel-Galor, S. 2011. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. CRC Press, pp. 2.
- Berg, C.C. 1990. Annotated checklist of the *Ficus* species of the African floristic region with special reference and a key taxa of Southern Africa. *Kirkia* **13**, 268-269.
- Beya, W. 2008. The effect of crude aqueous and alcohol extracts of *Aloe vera* on the GIT and accessory organs of suckling rats. Masters thesis, University of the Witwatersrand, Johannesburg, South Africa.
- Bligh, E.G. & Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* **37**, 911-917.
- Blottiere, H.M., Buecher, B., Galmiche, J.P. & Cherbut, C. 2003. Molecular analysis of the effect of short chain fatty acids on intestinal cell proliferation. *Proceedings of the Nutrition Society* **62**, 101-106.
- Bossard, E. 1996. La médecine traditionnelle au centre et à l'ouest de l'Angola. Ministerio da ciência e da tecnologia. Instituto de investigação científica tropical. Lisboa, pp 49.
- Bovee, K.C. 1986. Renal function and Laboratory Evaluation. *Toxicologic Pathology* **14**, 26-36.
- Buddington, R., Kimura, Y. & Nagata, Y. 2003. Phytochemicals and gastrointestinal health. In : *Phytochemical functional foods* (Johnson, I & Williamson, G. eds.), Woodhead publishing Ltd, Cambridge, England, pp. 161.
- Burrows, J. & Burrows, S. 2003. *Figs of Southern and South-Central Africa*, Umdaus Press. Hatfield, South Africa, pp 62-100.

- Bwititi, P. & Musabayane, C.T. 1997. The effect of plant extracts on plasma glucose in rats. *Aota Medica et Biologica (Japan)* **45**, 167-169.
- Car, B.D., Eng, V.M., Everds, N.E. & Bounos, D.I. 2006. Clinical pathology of the rat. In: *The Laboratory Rat 2nd edition* (Suckow, M.A., Wasbroth, S.H. & Franklin, C.L eds). Elsevier academic press, USA, pp. 138.
- Cencic, A. & Chingwaru, W. 2010. The role of functional foods, nutraceuticals and food supplements in intestinal health. *Nutrients* **2**, 611-625.
- Chen, D., Aihara, T., Zhao, C.M., Hakanson, R. & Okabe, S. 2006. Differentiation of the gastric mucosa I. The role of histamine in control of function and integrity of oxyntic mucosa: understanding gastric physiology through disruption of targeted genes. *American Journal of Gastrointestinal and Liver Physiology* **291**, 539-544.
- Chinsebu, K.C. & Hedimbi, M. 2010. An ethnobotanical survey of plants used to manage HIV/AIDS opportunistic infections in Katima, Caprivi region, Namibia. *Journal of Ethnobiology and Ethnomedicine* **6**, 25.
- Chong, J., Poutaraud, A. & Hugueney, P. 2009. Metabolism and roles of stilbenes in plants. *Plant Science* **177**, 143-155.
- Coker, M.E., Emikpe, B.O., Adeniyi, B.A. & Budale, B.A. 2009. The inflammatory potential, haematological and histological changes induced in rats due to the administration of methanolic extracts of *Ficus thonningii* leaves. *African Journal of Pharmacy and Pharmacology* **3**, 273-276.
- Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clinical Microbiology Review* **12**, 564-582.
- Cousins, D. & Huffman, M.A. 2002. Medicinal properties in the diets of gorillas: an ethno-pharmacological evaluation. *African Study Monographs* **23**, 65-89.
- Cushnie, T.P.T. & Lamb, A.J. 2005. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents* **26**, 343-356.
- Dalziel, J.M. 1937. *The useful plants of west tropical Africa*. The Crown Agents for the colonies, Westminster, London, pp. 612.

- Danthu, P., Solovier, P., Gaye, A., Sarr, A., Seck, M. & Thomas, I. 2002. Vegetative propagation of some West African species by cuttings. *Agroforestry Systems* **55**, 57-63.
- Deng, J.Y., Hsreh, P.S., Huang, J.P., Lu, L, S. & Hung, L.M. 2008. Activation of estrogen receptor is crucial for resveratrol-stimulating muscular glucose uptake via both insulin dependent and independent pathways. *Diabetes* **57**, 1814-1823.
- Diafouka, A.J.P. 1997. Analyse des usages des plantes medicinales dans 4 regions de Congo Brazzaville. These de doctorat, Universite libre de Bruxelles, Faculte des Sciences, Laboratoire de Botanique Systematique et de Phytosociologie, pp. 431.
- Dziedziejko, V., Safranow, K., Zylka, D.S. Mokrzyńska, A.M., Millo, B, Machoy, Z. & Chlubek, D. 2005. Comparison of the rat and human alkaline phosphatase isoenzymes and isoforms using HPLC and electrophoresis. *Biochemica et Biophysica Acta* **1752**, 26-33.
- Erlwanger, K.H. & Cooper, R.G. 2008. The effects of orally administered crude alcohol and aqueous extracts of African potato (*Hypoxis hernerocallidea*) corm on the morphometry of viscera of suckling rats. *Food and Chemical Toxicology* **46**, 136-139.
- Eshet, R., Maor, G., Ari, B.T, Eliezer, M.B., Gat-Yablonski, G. and Phillip, M. 2004. The aromatase inhibitor letrozole increases epiphyseal growth plate height and tibial length in periparturient male mice. *Journal of Endocrinology* **182**, 165-172.
- Espandiari, P., Zhang, J., Schnackenberg, L.K., Miller, T.J., Knapton, A. Herman, E.H., Beger, R.D. and Hanig, J.P. 2008. Age related differences in susceptibility to toxic effects of valproic acid in rats. *Journal of Applied Toxicology* **28**, 628-637.
- Etkin, N.L. & Ross, P.J. 1982. Food as medicine and medicine as food. An adoptive framework for the utilization among the Hausa of Northern Nigeria. *Social Science & Medicine* **16**, 1559-1573.
- Evans, W.C., Trease, G.E. & Evans, D., 2002. *Trease and Evans Pharmacognosy*. 15th edition, Edinburgh, Saunders, pp. 249; 454.

- Fowler, M.W. 2006. Plants, medicines and man. *Journal of the Science of Food and Agriculture* **86**, 1797-1804.
- Gelfand, M., Mavi, S., Drummond, R.B. & Ndemera, B. 1985. *The Traditional Medicinal Practitioner. His principles of practice and pharmacopoeia*. Mambo Press, Gweru, Zimbabwe, pp. 411.
- Grassi, D., Desideri, D. & Ferri, C. 2010. Flavonoids antioxidants against atherosclerosis. *Nutrients* **2**, 889-902.
- Greenham, J.R., Grayer, R.J, Harbone, J.B. & Reynolds, V. 2007. Intra- and Interspecific variations in vacuolar flavonoids among *Ficus* species from the Budongo forest, Uganda. *Biochemical Systematics and Ecology* **35**, 81-90.
- Gueye, M. & Diouf, M. 2007. Traditional leafy vegetables in Senegal. Diversity and medicinal uses. *African Journal of Traditional, Complimentary Alternative Medicines* **4**, 469-475.
- Hanhineva, K., Torronen, R., Bondia-Pons, I., Pekkinen, J., Kolehmainen, M., Mykkanen, H. & Poutanen, K. 2010. Impact of dietary polyphenols on carbohydrate metabolism. *International Journal of Molecular Sciences* **11**, 1365-1402.
- Handa, S.S. 2008. An overview of the extraction techniques for medicinal and aromatic plants. In: *Extraction Techniques for Medicinal and Aromatic Plants*. (Handa, S.S., Khanuja, S.P.S., Longo, G. & Rakesh, D.D. eds). ICS-UNIDO, pp. 25-27.
- Harbone, J.B. 1973. *Phytochemical methods*. Chapman Hall, London, United Kingdom, pp. 49-188.
- Harbone, J.B. 2008. *Phytochemical methods: a guide to modern techniques of plant analysis* 3rd edn, Chapman and Hall.
(<http://book.google.co.za/books?id=2yvqeRtE8CwC&lpg=P>) accessed 03/07/11.
- Heim, M., Johnson, J., Boess, F., Bendik, I., Weber, P., Hunziker, W. & Fluhmann, B. 2002. Phytanic acid, a natural peroxisome proliferator activated receptor (PPAR) agonist, regulates glucose metabolism in rat primary hepatocytes. *The FASEB Journal* **17**, 718-720.

Hines, D.A. & Eckman, K. 1993. *Indigenous multipurpose trees of Tanzania, Uses and economic benefits for people*. FAO Corporate document repository. (<http://www.fao.org/docrep/x5327e/x27e00.htm>) accessed 24/08/2011.

Hills, P.A. and Parizcova, J. 2002. Assessment of growth in adolescent athletes. In: *Nutritional Assessment of Athletes* (Drinskell, J.A. and Wolinsky, I. eds). CRC Press, Florida, USA, pp. 115-134.

Hou, W.C., Chen, Y.C. & Lin, Y.H. 1998. Chitinase activity of sweet potato (*Ipomoea batatas* [L]. Lam var. Tainong 57). *Botanical Bulletin of Academia Sinica* **39**, 93-97.

Hove, T. 2000. A method for predicting long term average performance of photovoltaic systems. *Renewable energy* **21**, 207-227.

Hyde, M.A. & Wursten, B. 2011. *Flora of Zimbabwe*. (<http://www.zimbabweflora.co.zw/speciesdata/utilities/utility/species-search-binomial.php>) accessed 24/08/2011.

Ichikawa, H. & Sakata, T. 1997. Effects of L-lactic acid, short chain fatty acids and pH in cecal infusate on morphometric and cell kinetic parameters of rat cecum. *Digestive Diseases and Sciences* **42**, 1598-1610.

Igoli, J.O., Tor-Anyin, T.A., Usman, S.S., Oluma, H.O.A. & Igoli N.P. 2002. Folk medicines of the Benue valley of Nigeria. In: *Recent progress in Medicinal plants, Vol 7, Ethnomedicine and Pharmacognosy* (Singh, V.K, Govil, J.N., Hashim, S. Sing G. eds.) Raleigh, NC, Science Technology Publishers, pp. 327-338.

Innocent, E., Gikonyo, N.K. & Nkunya, M.H.H. 2008. Repellence property of long chain aliphatic methyl ketones against *Anopheles gambiae* s.s. *Tanzania Journal of Health Research* **10**, 50-54.

Ischiropoulos, H. & Almeidi, A.B. 1995. Peroxynitrite mediated oxidative protein modifications. *FEBS Letters* **364**, 279-282.

Iwu, M.M. 2002. Introduction therapeutic agents from ethnomedicine. In: *Ethnomedicine and Drug Discovery* (Iwu, M.M. & Wotton, J.C. eds) Elsevier Science BV. Amsterdam , The Netherlands, pp. 7-9.

- Jain, R. N. & L. C. Samuelson (2006). Differentiation of the Gastric Mucosa II. Role of gastrin in gastric epithelial cell proliferation and maturation. *American Journal of Physiology - Gastrointestinal and Liver Physiology* **291**, 762-765.
- Jamdar, S. C., Moon, M., Bow, S. & Fallon, H. J. 1978. Hepatic lipid metabolism. Age related changes in triglyceride metabolism. *Journal of Lipid Research* **19**, 763-770.
- Jana, S. and Shekawat, G.S. 2010. Phytochemical analysis and antibacterial screening of *in vivo* and *in vitro* extracts of Indian medicinal herb: *Anethum graveoleus*. *Plants* **4**, 206-212.
- Jansen, O., Angenot, L., Tits, M., Nicolas, J.P., De Mol, P., Nikiema, J-B. & Frederich, M. 2010. Evaluation of 13 selected medicinal plants from Burkina Faso for their antiplasmodial properties. *Journal of Ethnopharmacology* **13**, 143-150.
- Jokthan, G.E., Afikwu, E.V. & Olugbemi. 2003. The utilization of fig (*Ficus thonningii*) and mango (*Mangifera indica*) leaves by rabbits. *Pakistan Journal of Nutrition* **2**, 264-266.
- Jones, T.C., Hunt, D.R. & King, N.W. 1997. *Veterinary Pathology*. 6th edition. John Wiley and Sons, pp. 171.
- Joseph, B. & Raj, J.S. 2011. A comparative study on various properties of five medicinally important plants. *International Journal of Pharmacology* **7**, 206-211.
- Joubert, E. & Ferreira, D. 1996. Antioxidants of rooibos tea a possible explanation for its health promoting properties?. *The South African Journal of Food Science and Nutrition* **8**, 79-83.
- Keen, C.L., Lonnerdal, B., Clegg, M. and Hurley, L.S. 1981. Developmental changes in composition of rat milk: trace elements, minerals, protein, carbohydrates and fat. *Journal of Nutrition* **111**, 226-230.
- Kim, H-S. and Miller, D.D. 2005. Proline-rich proteins moderate the inhibitory effect of tea on iron absorption in rats. *Journal of Nutrition* **135**, 532-537.
- Kone, W.M., Kamanzi K.A., Terreaux, C., Hostettmann, K., Traore, D. & Dosso, M. 2004. Traditional medicine in Cote-d'Ivoire: Screening of 50 medicinal plants for antibacterial activity. *Journal of Ethnopharmacology* **93**, 43-49.

- Koyama, I., Arai, K., Sakagishi, Y., Ikeziwa, H. & Komoda, T. 1987. Blood appearance of rat alkaline phosphatase originating from the duodenum *in vitro*. *Journal of Chromatography* **420**, 275-286.
- Kumar, R. 1992. Antinutritional factors, the potential risks of toxicity and methods to alleviate them. *Journal of Animal Sciences Supplement* **2**, 11.
- Krief, S., Hladik, C.M. & Haxaire, C. 2005. Ethnomedicinal and bioactive properties of plants ingested by wild chimpanzees in Uganda. *Journal of Ethnopharmacology* **101**, 1-15.
- Kwon, O., Eck, P., Chen, S., Corpe, C.P., Lee J, H., Kruhlak, M. & Levine, M. 2007. Inhibition of the intestinal glucose transporter GLUT 2 by flavonoids. *The FASEB Journal* **21**, 366-377.
- Lalles, J.P. 2010. Intestinal alkaline phosphatase: multiple biological roles in maintenance of intestinal homeostasis and modulation by diet. *Nutrition review* **68**, 323-332.
- Linderoth, A., Biernat, M., Prykhodko, O., Kornolovska, I., Pusztai, A., Pierzynowski, S.G. & Bjorn, W.R. 2005. Induced growth and maturation of the gastrointestinal tract after *Phaseolus vulgaris* lectin exposure in suckling rats. *Journal of Pediatric Gastroenterology and Nutrition* **41**, 195-203.
- Littman, M.P., Dambach, D.M., Vaden, S.L. & Giger, U. 2000. Familial protein-losing enteropathy and protein-losing nephropathy in soft coated Wheaten Terriers: 222 cases (1983-1997). *Journal of Veterinary Internal Medicine* **14**, 68-80.
- Lobo, A.R., Colli, C., Alvares, E.P. & Fillisetti, T.M.C. 2007. Effects of fructans-containing yacon (*Smallanthus sonchifolius* Poepp & Endl) flour on caecum mucosal morphometry, calcium and magnesium balance and bone calcium retention in growing rats. *British Journal of Nutrition* **97**, 776-785.
- Locket, C.T., Carlvett, C.C. & Grivetti, L.E. 2000. Food-related behaviours during drought: A Study of rural Fulani, North Eastern Nigeria. *International Journal of Food Science and Nutrition* **51**, 91-107.

Makkar, H.P.S., Siddhuraju, P. & Becker, K. 2007. Plant secondary metabolites. In: *Methods in Molecular Biology* (Walker, J.M eds). Humanapress, New Jersey, USA, pp. 171.

Malgras, D. 1992. Arbes et arbustes guerisseurs des savanes maliennes. Editions Karthala, 22-24, Boulevard Arago, 75013, Paris, pp 480, In: prelude medicinal plants database, 2011.

Mali, R.G. & Mehta, A.A. 2007. A review on anthemintic plants. *Natural Product Radiance* **7**, 466-475.

Malina, R.M., Bouchard, C. & Bar-Or, O. 2004. Growth, Maturation and Physical Activity 2nd edition. Human Kinetics, Champaign, USA, pp. 4.

Markowitz, G.S., Fine, P.L., Stack, J.I., Kunis, C.L., Radhakrishnan, J., Palecki, W., Park, J., Nasr, S.H., Hoh, S., Siegel, D.S. & D'Agati, D.V. 2003. Toxic acute tubular necrosis following treatment with Zoledronate (Zometa). *Kidney International* **64**, 281-289.

Minja, M.M.J. 1994. Medicinal plants used in promotion of animal health in Tanzania. *Animal Diseases Research Insit. Presses universitaires de Namur, Dar Es Salaam*, pp. 335-364.

Minikawa, M., Kawawo, A., Miura, Y. & Yagasaki, K. 2011. Hypoglycemic effect of resveratrol in type 2 diabetic model db/db mice and its actions in cultural L6 myotubes and RIN-5F pancreatic β -cells. *Journal of Clinical Biochemistry and Nutrition* **48**, 237-244.

Mkangare-Minja, M.M.J. 1989. Collection of Tanzanian medicinal plants for studies of biological activity. In: *Proceedings of the 7th Tanzania Veterinary Association Scientific Conferences. Tanzania Veterinary Association* **7**, 67-78.

Moshi, M.J., Otieno, D.F., Mbabazi P.K. & Weisheit, A. 2009. The ethnomedicine of the Haya people of Bugabo ward, Kagera Region, Northwestern Tanzania, *Journal of Ethnobiology and Ethnomedicine* **5**, 24.

- Moundipa, P.F., Flore, K.G.M., Bilong, C.F.B. & Bruchhaus, I. 2005. *In vitro* amoebacidal activity of some medicinal plants of the Bamun region (Cameroon). *African Journal of Traditional, Complementary and Alternative Medicine* **2**, 113-121.
- Moyo, D.Z. 2006. An abattoir study of prevalence and seasonal fluctuations of gastrointestinal nematodes of cattle in the Midlands Province, Zimbabwe. *Research Journal of Animal and Veterinary Sciences* **1**, 27-40.
- Musabayane, C.T., Gondwe, M., Kadyamaapa, D.R., Chuturgoon, A.A. & Ojewole, J.A.O. 2007. Effects of *F. thonningii* (Blume) Moraceae stem bark ethanolic extract on blood glucose, cardiovascular and kidney cell lines of the proximal (LLC-PK1) and distal tubules (MDBK). *Renal Failure* **29**, 389-397.
- Ndukwe, I.G., Bello, A.I., Habila, J.D. & John, C. 2007. Phytochemical and antimicrobial screening of the crude petroleum spirit and methanol extracts of the stem bark, leaves and roots of *Ficus thonningii* (Blume). *African Journal of Biotechnology* **16**, 2645-2649.
- Njoronge, G.N. & Bussmann, R.W. 2006. Herbal usage and informant concensus in ethnoveterinary management of cattle diseases among Kikuyus (Central Kenya). *Journal of Ethnopharmacology* **108**, 332-339
- Njoronge, G.N. & Kibunga J.W. 2007. Herbal medicine acceptance, sources and utilisation for diarrhoea management in a cosmopolitan urban area (Thika, Kenya). *African Journal of Ecology* **45**, 65-70.
- Nwude, N. & Ibrahim, M.A. 1980. Plants used in traditional veterinary medical practice in Nigeria. *Journal of Veterinary Pharmacology and Therapeutics* **3**, 271-273.
- Obrefuna, P.C.M., Sofola, O.A. & Ebeigbe, A.B. 1992. Dietary salt loading attenuates endothelium-dependant relaxation to histamine but not to acetylcholine in the rat's aorta. *Experimental Physiology* **76**, 135-138.
- Ogunlesi, M., Okiei, W., Ofor, E. & Osibote, E.A. 2009. Analysis of the essential oils from the dried leaves of *Euphorbia hirta* Linn (Euphorbiaceae), a potential medication for asthma. *African Journal of Biotechnology* **8**, 7042-7050.

Ogunwade, I.A., Sonibare, M.A., Thangi, T.D., Dung, N.X., Soladaye, M. O. & Monohunfolu, O.O. 2008. Comparative analysis of the oils of three *Ficus* species from Nigeria. *Journal of Essential Oil Research* **20**

Okwari, O.O. & Ofem, O.E. 2011. Aqueous extracts of *Dombeya Buttneri* improves blood parameters in rats. *International Journal of Current Research* **2**, 86-91.

Olas, B., Wachowiz, B., Stochmal, A. & Oleszek, W. 2003. Inhibition of oxidative stress in blood platelets by different phenolics from *Yucca schidigera* Roetzl. *Bark nutrition* **19**, 633-40.

Olas, B., Wachowicz, B., Nowak, P., Stochmal, A., Oleszek, W., Glowacki. & Bald, E. 2008. Comparative studies of the antioxidant effects of a naturally occurring resveratrol analogue trans 3,3',5,5-tetrahydroxy-4-methoxystilbene and resveratrol- against oxidation and nitration of biomolecules in blood platelets. *Cell Biology and Toxicology* **24**, 331-340.

Olatunji, L.A., Okwusidi, J.I., Olatunji, V.A., Oguntonye, S. & Soladoye, A.O. 2005. Protective effects of *Allium sativum* on carbon tetrachloride-induced hepatotoxicity in rats. *Pharmacognosy Magazine* **1**, 155-158.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. & Anthony, S. 2009. Agroforestry Tree Database: A tree reference and selection guide version 4.0. (<http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp> accessed on 12/6/11).

O'Sullivan, G.C., Kelly, P., O'Halloran, S., Collins, C., Collins, J.K., Dune, C. & Shanahan, F. 2005. Probiotics: An emerging therapy. *Current Pharmaceutical Design* **11**, 3-10.

Otimenyin, S.O., Uguru, M.O. & Atang, B.L. 2004. Anti-inflammatory and analgesic activities of *Ficus thonningii* and *Pseudocedrela kotschyi* extracts. *Nigerian Journal of Pharmaceutical Research* **3**, 82-85.

Oyelana, O.A., Durugbo, E.U., Olukanni, O.D., Ayodele, E.A., Aikulola, Z.O. & Adewole, A.I. 2011. Antimicrobial activity of *Ficus* leaf Extracts on some fungal and bacterial pathogens of *Dioscorea rotundata* from Southwest Nigeria. *Journal of Biological Sciences* **11**, 359-366.

- Pacha, J. 2000. Development of intestinal transport function in mammals. *Physiological Reviews* **80**, 1634-1656.
- Pal, S.K.P. & Shukla, Y. 2003. Herbal medicine: Current status and the future. *Asian Pacific Journal of Cancer Prevention* **4**, 281-288.
- Palgrave, K.C. 2002. *Trees of Southern Africa*. C.Struik, Cape Town, South Africa, pp. 110.
- Papworth, T.A. and Clubb, S.K. 1995. Clinical pathology in the neonatal rat. *Comparative Haematology International* **5**, 237-250.
- Passonneau, J.V. & Lauderdale, V.R. 1974. A comparison of three methods of glycogen measurement in tissue. *Analytical Biochemistry* **60**, 405-412.
- Peterson, J., Dwyer, J. Adlercreutz, H., Scalbert, A., Jacques, P. and McCullough, M. 2010. Dietary lignans: physiology and potential for cardiovascular disease risk reduction. *Nutrition Reviews* **68**, 571-603.
- Poongothai, A., Sreena, K.P., Sreejith, K., Uthiralingam, M. & Annapoorani, S. 2011. Preliminary phytochemical screening of *Ficus racemosa* Linn bark. *International Journal of Pharma and Bio-sciences* **2**, 431-434.
- Prassas, I. & Diamandis, E.P. 2008. Novel therapeutic applications of cardiac glycosides. *Nature Reviews: Drug Discovery* **7**, 926-935.
- Prelude medicinal plants database. 2011.
(http://www.metafro.be/prelude/view_plant?pi=06040 accessed 27/04/11.)
- Pusztai, A., Ewen, S.W.B., Grant, G. & Bardocz, S. 1999. Effect of lectins on digestion of food and body metabolism. *GOST-98 Effects of antinutrients on the nutritional value of legume diets* **1**, 22-28.
- Raaman, N. 2006. *Phytochemical Techniques*. New India Publishing, India, pp. 228.
- Rajab, M.S., Cantrell, C.L., Franzblau, S.G. & Fischer, N.H. 1998. Antimycobacterial activity of (E)-phytol and derivatives: a preliminary structure activity study. *Planta Medica* **64**, 2-4.

- Rajesh, S.V., Raj Kapoor, B., Kumar, R.S. & Raju, A.K. 2009. Effect of *Clausena Dentata* (Willd). M Roem against paracetamol induced hepatotoxicity in rats. *Pakistan Journal of Pharmaceutical Sciences* **22**, 90-93.
- Ramachandran, R. and Kakar, S. 2008. Histological patterns in drug-induced liver diseases. *Journal of Clinical Pathology* **62**, 481-492.
- Ramada, K.G., Doss, S. & Mathura, M. 2009. The chemical diversity of bioactive molecules and therapeutic potential of medicinal plants. In: *Herbal Drugs Ethnomedicine to Modern Medicine* (Ramawat, K.G., eds.), Springer-Verlag Berlin Heidelberg, pp. 7-31.
- Russell, W.E. & Rhoads, J.M. 2008. Nutrition and the regulation of growth. In: *Nutrition in pediatrics: basic science, clinical applications* 4th edition (Duggan, C., Watkins, J.B. & Walker, W.A). BC Decker. Ontario, USA, pp. 224.
- Sandberg, A-S. 2002. Bioavailability of minerals in legumes. *British Journal of Nutrition* **88**, 281-285.
- Sangild, P. T. 2006. Transitions in the life of the gut at birth. In: *Digestive Physiology of Pigs; Proceedings of the 8th Symposium* (Lindberg, J.E. and Ogle, B eds.), pp. 1695-1711.
- Sasidharan, S., Jinxuan, O., Latha, L.Y. and Amutha, S. 2011. *In vivo* toxicity study of *Ganoderma boninense*. *African Journal of Pharmacy and Pharmacology* **16**, 1819-1823.
- Schmidt, E., Lutter, M. & McClelland, W. 2002. *Trees and Shrubs of Mpumalanga and Kruger National Park*. Jacana, South Africa, pp. 80.
- Schneeman, B.O. 2002. Gastrointestinal physiology and functions. *British Journal of Nutrition* **2**, 159-163.
- Schwikkard, S. & Van Heerden, F.R. 2002. Antimalarial activity of plant metabolites. *Natural Products Reports* **19**, 675-692.

- Sefcikova, Z. & Mozes, S. 2002. Effect of early nutritional experience on the feeding behaviour of adult female rats. *Veternarni Medicina* **47**, 315-322.
- Shahidi, F. & Naczk, M. 1992. An overview of the phenolics of canola and rapeseed: chemical, sensory and nutritional significance. *Journal of the American Oil Chemists' Society* **69**, 917-924.
- Shimizu, M., Kobayashi, Y., Suzuki, M., Satsu, H. & Miyamoto, Y. 2000. Regulation of intestinal glucose transport by tea catechins. *Biofactors* **13**, 61-65.
- Siddiqui, S., Verma, A., Rather, A.A., Jabeen, F. & Meghvansi, M.K. 2009. Preliminary phytochemical analysis of some important medicinal and aromatic plants. *Advances in Biological Sciences* **3**, 188-195.
- Smythe, C.V. and Miller, R.C. 1929. The iron content of the albino rat at different stages of the life cycle. *Journal of Nutrition* **1**, 209-216.
- Sowofora, A. 1993. *Medicinal plants and Traditional Medicine in Africa*. Spectrum Books, Ibadan, Nigeria, pp. 55-71.
- Steed, H. & Macfarlane, S. 2009. Mechanisms of prebiotics – impact on health. In: *Prebiotics and Prebiotics Science and Technology* Volume 1 (Charalampopoulos, D. & Rastall, R.A. eds.). Springer, New York, USA , pp. 136-139.
- Stevens, L.A. and Levey, A.S. 2009. Measured GFR as a confirmatory test for estimated GFR. *Journal of the American Society of Nephrology* **20**, 2305-2313.
- Tagang, A., Ishaku, P.K and Abdullah, A. 2010. Volatile fatty acids: production in ruminants and the role of monocarboxylate transporters. A review. *African Journal of Biotechnology* **9**, 6229-6232.
- Taira, T., Ohdomari, A., Nakama, N., Shinioj, M. & Ishihara, M. 2005. Characterization of antifungal activity in Gazyumaru (*Ficus microcarpa*) latex chitinases: both the chitin binding and the antifungal activities of class 1 chitinase are reinforced with increasing ionic strength. *Bioscience, Biotechnology and Biochemistry* **69**, 811-818.

- Tegbe, T.S.B., Adeyinka, I.A., Baye, K.D. & Alawa, J.P. 2006. Evaluation of feeding graded levels of dried and milled *Ficus thonningii* leaves on growth performance, carcass characteristics and organ of weaner rabbits. *Pakistan Journal of Nutrition* **5**, 548-550.
- Tegegne, E.D., 2008. *Importance of Ficus thonningii Blume in Soil Fertility Improvement and Animal Nutrition in Gondar Zuria, Ethiopia*. Masters thesis, University of Natural Resources and Applied Life Science, Vienna.
- Teklehaymanot, T. & Giday, M. 2007. Ethnobotanical study of medicinal plants used by people in Zegie Peninsula, North Western Ethiopia. *Journal of Ethnobiology and Ethnomedicine* **3**, 12.
- Thapa, B.R. & Walia, A. 2007. Liver function tests and their interpretation. *Indian Journal of Pediatrics* **74**, 663-671.
- Titanji, V.P.K., Zofou, D. & Ngemenya, M.N. 2008. The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian folk medicine. *African Journal of Traditional Complimentary and Alternative Medicines* **5**, 302-321.
- Trease, G.E. and Evans, W.C. 2002. *Pharmacognosy* 15th edition. Saunders, London, United Kingdom. pp. 42-44, 221-229, 246-249, 304-306.
- Tripathi, K. D. 1994. *Essentials of Medical Pharmacology*. Jaypee Brothers Medical Publishers (P), New Dehli, pp. 775.
- Umar, S.B. & Dibousse, J.K. 2010. Protein-losing enteropathy: case illustrations and clinical review. *American Journal of Gastroenterology* **105**, 43-49.
- Usman, H., Abdulrahman, F.I. & Usman, A. 2009. Qualitative phytochemical screening and *in vitro* antimicrobial effects of methanol stem bark extract of *Ficus thonningii* (Moraceae). *African Journal of Traditional Complimentary and Alternative Medicines* **6**, 289-295.
- Vanhaecke, P., Persoone, G., Claus, C. and Sorgeloos, P. 1981. Proposal for a short-term toxicity with *Artemia nauplii*. *Ecotoxicology and Environmental Safety* **5**, 382-387.

- Vitrac, X., Kussa, S., Decendit, A., Deffieux, G. & Menllon, J.M. 2004. Grapevine polyphenols and their biological effects. In: *Biotechnology of medicinal plants, vitalizers and therapeutics* (Ramawat, K.G. eds.), pp. 33-75.
- Von Gadow, A., Joubert, E. & Hansmann, C.F. 1997. Comparison of the antioxidant activities of rooibos tea (*Aspalathus linearis*) with green, oolong and black tea. *Food Chemistry* **60**, 73-77.
- Walthall, K., Cappon, G.D., Hurtt, M.E. & Zoetis, T. 2005. Postnatal development of the gastrointestinal system: a species comparison. *Birth Defects Research Part B: Developmental and Reproductive Toxicology* **74**, 132-156.
- Wanyoike, G.N., Chhabra, S.C., Langat-Thoruwa, C.C. and Omar, S.A. 2004. Brine shrimp toxicity and antiplasmodial activity of five Kenyan medicinal plants. *Journal of Ethnopharmacology* **90**, 129-133.
- Watt, J.M. & Breyer-Branddwijk, G.M. 1962. *The medicinal and poisonous plants of Southern and Eastern Africa*, 2nd ed. E. and S Livingstone Ltd, Edinburgh and London, pp. 773.
- Winick, M. & Noble, A. 1966. A cellular response in rat during malnutrition at various ages. *Journal of Nutrition* **89**, 300-306.
- Wondimu, T., Arsi, A. & Ensemu, K. 2007. Ethnobotanical study of medicinal plants around 'Dheerera' town, Arsi Zone, Ethiopia. *Journal Ethnopharmacology* **112**, 152-161.
- Wu, N., Fu, K., Fu, Y.J., Zu, Y.G., Chang, F.R., Chen, Y.H., Liu, X.L, Kong, Y., Liu, W. & Gu, C.B. 2009. Antioxidant activities of extracts and main components of Pigeon pea (*Cajanus cajan* (L) Millsp) leaves. *Molecules* **14**, 1032-1043.
- Ya, C., Gaffney, S.H., Lilley, T.H., Haslam, E. 1988. Carbohydrate-polyphenol complexation. In: *Chemistry and Significance of Condensed Tannins* (Hemingway, R.W and Karchesy eds). Plenum press, New York, USA, pp. 553.
- Yu, F.L., Gapor, A.B.S. & Bender, W.S. 2005. Evidence for the protective effect of the polyunsaturated phytol side chain in tocotrienols on 17 β -estradiol epoxidation. *Cancer Detection and Prevention* **29**, 383-388.