HEPATOCURATIVE AND ANTIOXIDANT EFFECT OF ETHYL-ACETATE AND N-BUTANOL FRACTIONS OF *Detarium microcarpum* STEM BARK IN CCl_4 INDUCED LIVER DAMAGE IN WISTAR RATS

BY

THERESA YEBO GANA

DEPARTMENT OF BIOCHEMISTRY
FACULTY OF SCIENCE
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA.

MAY, 2015

HEPATOCURATIVE AND ANTIOXIDANT EFFECT OF ETHYL-ACETATE AND N-BUTANOL FRACTIONS OF *Detarium microcarpum* STEM BARK IN CCl_4 INDUCED LIVER DAMAGE IN WISTAR RATS

BY

GANA THERESA YEBO B.Sc (ABU) 2010
M.sc/SCIE/5202/2011-2012

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

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN BIOCHEMISTRY.

DEPARTMENT OF BIOCHEMISTRY
FACULTY OF SCIENCE
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA.

MAY, 2015

DECLARATION

I declare that the work in this Project Thesis entitled Hepatocurative and Antioxidant Effect of Ethyl-acetate and N-butanol Fractions of *Detarium microcarpum* Stem Bark in CCl_4 Induced Liver Damage in Wistar Rats has been carried out by me in the Department of Biochemistry, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and the list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other Institution.

_____	_____	_____
Name of Student	Signature	Date

CERTIFICATION

This project thesis entitled HEPATOCURATIVE AND ANTIOXIDANT EFFECT OF ETHYL-ACETATE AND N-BUTANOL FRACTIONS OF *Detarium microcarpum* STEM BARK IN CCL_4 INDUCED LIVER DAMAGE IN WISTAR RATS by THERESA YEBO GANA, meets the regulations governing the award of Master of Sciences of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

Prof. D.A Ameh	_____	_____
Chairman, Supervisory Committee	Signature	Date
Dr. K.M Anigo	_____	_____
Member, Supervisory Committee	Signature	Date
Prof. I.A Umar	_____	_____
Head of Department	Signature	Date
Prof. A.Z Hassan	_____	_____
Dean, Postgraduate School	Signature	Date

ACKNOWLEDGEMENTS

My most sincere gratitude goes to God almighty for keeping me alive throughout my study period in this institution and also for the gift of knowledge, wisdom and understanding leading to the completion of this research work. In high regards, my heartfelt gratitude goes to my supervisors, Prof. D.A Ameh and Dr. K.M Anigo for their enthusiasm, their valuable contributions, and for creating sufficient time to supervise my research work to completion.

I sincerely thank the Head, Biochemistry Department, and the academic staff of Biochemistry Department for their invaluable contributions in my study. My gratitude to Mr U.S. Nndidi, Mr Aliyu Mohammed, Non-academic and Technical Staff of the Department of Biochemistry for their assistance.

Special thanks to Mr. Muazu Ahmed and Mr. Kabiru Musa of the department of Pharmacology, and to Mr. Bamidele of Anatomy Department. Their affection and practical support at a demanding time made it possible to undertake and complete this project.

I express my deep appreciation to my ever-loving and caring Parent, Dr. & Mrs Francis N. Gana. To my siblings; Dr. Godwin Gana, Pharm. Thomas Gana, Mrs Faith Yisa and and my Inlaw, Dr Amos Yisa and Mrs Cynthia Gana. My thanks also go to my nieces and cousins.

Finally, I appreciate all the contributions and assistance of my friends and colleagues; Rahinat Yakubu, Iyabo Bawalla, Habiba Kassim, Joy Ameloko, Ameh Joseph, Gbenga Amode, Jude Okpala, Binda Andongma and Isaac Eleojo. I am indebted and most grateful to them all.

DEDICATION

To

God Almighty

To

My Family

For their abundant support, understanding and love

ABSTRACT

The stem bark of *Detarium microcarpum* (Guill and Perr.) is used in traditional medicine for the treatment of liver disease in middle belt region of Nigeria. To substantiate this folkloric claim, ethyl-acetate and n-butanol fractions of *Detarium microcarpum* stem bark was investigated for its hepatocurative and antioxidant effect in CCl_4 induced liver damage in rats. Aqueous extraction was carried out on *Detarium microcarpum* stem bark and the crude extract was further fractionated sequentially using ethyl-acetate and n-butanol solvents. In the *in-vitro* studies, phytochemical screening of the crude extract showed the presence of phenolic, flavonoids, tannins, saponins, alkaloids and glycosides while total phenolic content assay, total flavonoid content assay, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Reducing power and H_2O_2 free radical scavenging activities were carried out on ethyl-acetate and n-butanol fractions. The total phenol content for n-butanol and ethyl acetate fractions were 2.97±0.31 and 11.54±0.20 mg/g Gallic acid equivalents while total flavonoid content were 234.42±0.71 and 45.76±2.59 mg/g quercetin equivalents. Ethyl acetate fraction showed the highest DPPH free radical scavenging activity with 65.31% inhibition while n-butanol showed the highest reducing power and H_2O_2 free radical scavenging activities with 65.31% and 52.55% which informed the choice of n-butanol fraction for further studies. In the *in-vivo* studies, the LD_{50} of n-butanol fraction of *Detarium microcarpum* stem bark was >5000 mg/kg body weight of rats. CCl_4 (1ml/kg body weight) as a 1:1(v/v) solution in olive oil was used to induce liver damage followed by subsequent treatment with n-butanol fraction of *Detarium microcarpum* stem bark at three different doses (100, 150 and 200 mg/kg bw/day) while silymarin (100 mg/kg bw/day) was used as standard drug for 28 days. The liver weight was significantly ($p<0.05$) increased in the negative control group when compared with the CCl_4 treated groups. There was significant

($p<0.05$) reduction in the serum activities of alanine aminotransaminase (ALT), aspartate aminotransaminase (AST), alkaline phosphatase (ALP), direct and indirect bilirubin for CCl_4 treated groups compared to the negative control group. Total protein (TP) and albumin (ALB) in the negative control group were reduced but not significantly ($p>0.05$) compared to the CCl_4 treated groups. In endogenous antioxidant activities, there was significant ($p<0.05$) reduction of malondialdehyde (MDA) in CCl_4 treated groups compared to the negative control group. A significant ($p<0.05$) increase was also observed in superoxide dismutase (SOD) and catalase (CAT) activities of CCl_4 treated groups compared to the negative control group. These results may suggest hepatocurative and antioxidant effects of *Detarium microcarpum* stem bark in CCl_4 induced liver damaged animals.

TABLE OF CONTENTS

CONTENT PAGE

Title Page……...…….……....i

Declaration……....ii

Certification…..…….....…iii

Acknowledgements…...……...……iv

Dedication…..……...……v

Abstract……...……....vi

Table of Contents……...……....viii

List of Tables……..……...…….xiv

List of Figures……..……...xv

List of Appendices……xvi

1.0 INTRODUCTION……...1

1.1 Preamble……...……1

1.2 Statement of Research Problem…….4

1.3 Justification……..5

1.4 Aim and Objectives…….5

1.4.1 Aim……5

1.4.2 Specific objectives…….....6

1.5 Null Hypothesis.……...……6

2.0 LITERATURE REVIEW……..7

2.1 *Detarium microcarpum*. Guill and Perr……....7

2.1.1 Classification of the plant…….....7

2.1.2 Description, distribution and habitat of *Detarium microcarpum*……7

2.1.3 General uses of *Detarium microcarpum* plant……8

2.1.4 Ethno-medicinal uses……..9

2.2 Phytochemical profile of *Detarium microcarpum* plant**……..11**

2.3 Pharmacological activities…..……13

2.3.1 Antidiabetic activity……..……..13

2.3.2 Antibacterial and antifungal activities……....13

2.3.3 Antiviral activity……14

2.3.4 Enzyme inhibition……..14

2.3.5 Antisnake venom activity……...14

2.4. The Liver…….**15**

2.4.1 Structure and functions...……..15

2.4.2 Liver cells…...……...17

2.4.3 Xenobiotics and liver metabolism……..…18

2.4.4 Mechanisms of hepatic injury..……19

2.5 Mode of action of liver toxicants……...20

2.5.1 Carbon tetrachloride (CCl_4) induced hepatotoxicity…..……..20

2.6 Liver injuries.……..…21

2.6.1 Cholestatic liver injury…..……22

2.6.2 Fatty liver (Steatosis)….……23

2.6.3 Cell death……..23

2.7 Biochemical alterations in hepatic damage……...26

2.7.1 Serum aminotransferase enzymes ……..26

2.7.2 Serum alkaline phosphatase……26

2.7.3 Serum total protein and albumin…….27

2.7.4 Serum bilirubin……27

2.8 Silymarin……...…….……28

3.0MATERIALS AND METHODS…….30

3.1 Materials……...30

3.1.1 Chemicals/reagents……...……...30

3.1.2 Plant sample collection and identification……..32

3.1.3 Experimental animals……..32

3.2 Methodology…….32

3.2.1 Preparation of plant sample……..…….……...32

3.2.2 Aqueous extract preparation…….32

3.2.3 Fractionation…….33

3.2.4 Qualititative phytochemical analysis…….33

3.2.5 Quantitative phytochemical analysis…….35

3.2.6 *In-vitro* antioxidant activity……..……36

3.2.7 Acute toxicity studies……37

3.2.8 Induction of liver damage…….……...……..38

3.2.9 Experimental design…….……38

3.2.10 Biochemical analysis……..……40

3.2.11 Determination of oxidative stress parameters…….……46

3.3 Statistical Analysis…….47

4.0 RESULT…….……49

4.1 Qualitative Screening of Phytochemicals……49

4.2 Total flavonoid / total phenolic content……..……..49

4.3 *In-vitro* Antioxidant Activity……..…..52

4.3.1 DPPH radical scavenging activity……...52

4.3.2 Reducing power assay…….54

4.3.3 Hydrogen peroxide (H_20_2) radical scavenging activity…….……..56

4.4 Lethal Dose Determination……..……59

4.5 Effect of n-butanol fraction on body weight / organ weight..……...61

4.4.1 Effect of n-butanol fraction on body weight……..61

4.4.2 Effect of n-butanol fraction on relative organ weight……..……..63

4.5 Biochemical Parameters…….……....65

4.5.1 Effect of n-butanol fraction on serum liver damage biomarkers/liver function parameters in CCl_4 induced liver damage in rats……65

4.5.2 Effect of n-butanol fraction on kidney function parameters of CCl_4 induced liver damage in rats…….69

4.6 Oxidative Stress Parameters……....71

4.6.1 Effect of n-butanol fraction on oxidative stress parameters in CCl_4 induced liver damage in rats……..……71

5.0 DISCUSSION……..….73

6.0 Summary, Conclusion and Recommendations……...81

6.1 SUMMARY**……81**

6.2 CONCLUSION……..82

6.3 RECOMMENDATIONS……..……...….82

REFERENCE……..……83

LIST OF TABLES

TABLE **TITLE** **PAGE**

Table 2.1: Some Phytochemical Constituents of *Detarium microcarpum*……...11

Table 4.1: Qualitative Analysis of Phytochemicals in the Aqueous Crude Extract of *Detarium microcarpum* Stem Bark…….....……..50

Table 4.2: Total Flavonoid and Phenolic Content in n-butanol and Ethyl-acetate Fractions of *Detarium microcarpum* Stem Bark…….....…….51

Table 4.3: 50% Inhibition Concentration (IC_{50}) of n-butanol and Ethyl-acetate Fractions of *Detarium microcarpum* Stem Bark against DPPH, Reducing power and H_2O_2 Radicals……58

Table 4.4: Lethal Dose Determination of n-butanol Fraction of *Detarium microcarpum* Stem Bark in Rats for 48 hr…….……..60

Table 4.5: Effect of n-butanol Stem Bark Fraction of *Detarium microcarpum* on Body Weight of CCl_4 Induced Liver Damage in Rats …….....62

Table 4.6: Effect of n-butanol Fraction of *Detarium microcarpum* Stem Bark on Relative Organ Weight (%) of CCl_4 Induced Liver Damage in Rats…..64

Table 4.7: Effect of n-butanol Fraction of *Detarium microcarpum* Stem Bark on Serum Liver Damage Biomarkers of Rats with CCl_4 Induced Liver Damage in Rats……67

Table 4.8: Effect of n-butanol Fraction of *Detarium microcarpum* Stem Bark on Liver Function Parameters of CCl_4 Induced Liver Damage in Rats…...68

Table 4.9: Effect of n-butanol Fraction of *Detarium microcarpum* Stem Bark on Kidney Function Parameters of CCl_4 Induced Liver Damage in Rats….70

Table 4.10: Effect of n-butanol Fraction of *Detarium microcarpum* Stem Bark on Oxidative Stress Parameters in CCl_4 Induced Liver Damage in Rats….72

LIST OF FIGURES

FIGURES **TITLE** **PAGE**

Figure 1: *Detarium microcarpum* Plant……...…….…….……12

Figure 2: Structure of the Liver…….…..…...……16

Figure 3: Experimental design…...…….…..…….……..31

Figure 4: DPPH Radical Scavenging Activity of Ascorbic Acid (AA), n-butanol Fraction (BF) and Ethyl-acetate Fraction (EF) of *Detarium microcarpum* Stem Bark at Different Concentrations…….…….**.....**53

Figure 5: Reducing Power of Ascorbic Acid (AA), n-butanol Fraction (BF) and Ethyl-acetate Fraction (EF) of *Detarium microcarpum* Stem Bark at Different Concentrations……..…….…….…….55

Figure 6: Hydrogen Peroxide Radical Scavenging Activity of Ascorbic Acid (AA), n-butnol Fraction (BF) and Ethyl-acetate Fraction (EF) of *Detarium microcarpum* Stem Bark at Different Concentration……..……...57

LIST OF APPENDICES

Appendice I: Standard curve for Quecertin……..……..…….98

Appendice II: Standard curve for Gallic acid…….……..…99

CHAPTER ONE

INTRODUCTION

1.1 Preamble

Herbal medicines are herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products (WHO, 2001). Notwithstanding the extent of significant advancement in modern medicine in recent decades, plants still make an important contribution to health care. Traditionally they are used worldwide for the prevention and treatment of disease. Herbal plants were prescribed even when their active compounds were unknown because of their effectiveness and relatively low cost (Bhawna and Kumar, 2010). This observation is particularly more relevant to people in the developing countries of the world where the majority of the populations are living in the rural areas.

The liver plays an important role in regulating various physiological processes. It is essential in the body for maintenance, performance and regulating homeostatic functions. It is involved with almost all the biochemical pathways for growth, fight against diseases, nutrient supply, energy provision and reproduction. In addition, it aids metabolism of carbohydrate, protein and fat, detoxification, secretion of bile and storage of vitamins (Ahsan *et al.,* 2009). Because of its central role in drug metabolism, it is the most vulnerable tissue for drug toxicity (Sunil *et al.,* 2012). The role played by the liver in the removal of substances from the portal circulation makes it susceptible to persistent attack by offending foreign compounds, culminating in liver dysfunction (Bodakhe and Ram, 2007). The liver secretes bile, prothrombin, fibrinogen, blood-clotting factors and heparin, a mucopolysaccharide sulfuric acid ester that prevents

blood from clotting within the circulatory system (Bhawna and Kumar, 2010). Toxic chemicals, xenobiotics, alcohol consumption, malnutrition, anaemia, medications, autoimmune disorders (Marina, 2006), viral infections (hepatitis A, B, C, D, etc.) and microbial infections (Sharma and Ahuja, 1997) are harmful and cause damage to the hepatocytes.

Reactive oxygen species (ROS) are continuously generated during metabolic processes to regulate a number of physiological functions essential to the body (Valko *et al*., 2007). These reactive oxygen species are prone to withdraw electrons from biological macromolecules such as proteins, lipids, nucleic acids in order to gain stability in the biological system. This disruption may be attributed to a number of factors such as the inability of the cells to produce sufficient amounts of antioxidants, nutritional deficiency of minerals or vitamins (Abd Ellah, 2010). When the production of ROS exceeds the capability of the body to detoxify these reactive intermediates, oxidative stress would develop (Mena *et al.,* 2009). Oxidative stress can be induced by variety of factors such as radiation or exposure to heavy metals and xenobiotics (e.g carbon tetrachloride). This may lead to drastic harm to the body such as membrane damage, mutations due to attenuation of DNA molecules, and disruption to various enzymatic activities in metabolism of the body (McGrath *et al*., 2001; Valko *et al*., 2006; Chanda and Dave, 2009).

Medicinal plants are important sources of antioxidants (Rice, 2004). Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells (Nunes *et al.,* 2012). Natural antioxidants either in the form of raw extracts or their chemical constituents are very effective in preventing the destructive processes caused by oxidative stress (Zengin *et al*., 2011). Recently interest in naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical

products, because they are multifaceted in their multitude and magnitude of activity and provide enormous scope in correcting imbalance (Djeridane *et al*., 2006; Wannes *et al.*, 2010). The beneficial medicinal effects of plant materials typically results from the combinations of secondary products present in the plant (Wink, 1999). Phytochemical constituents of medicinal plants (e.g. polyphenols, carotenoids, flavonoids, phenolics, vitamins C and E), act as antioxidants by preventing damages to cell membrane due to cellular oxidative processes that may result in diseases (Omoregie and Osagie, 2011). They are found in all parts of plants such as leaves, fruits, seeds, roots and bark (Mathew and Abraham, 2006).

Antioxidants are broadly divided into enzymic antioxidants and non enzymic antioxidants. Enzymic antioxidants include the superoxide dismutases, glutathione peroxidase and catalase (Klaunig and Kamendulis, 2004). Non-enzymic antioxidants, which include vitamin E, vitamin C, β-carotene, reduced glutathione, and coenzyme Q function to quench reactive oxygen species (Clarkson and Thompson, 2000). Antioxidants have various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging (Rao *et al.,* 2004). Many chemicals damage mitochondria, an intracellular organelle that produces energy, its dysfunction release excessive amount of oxidants which in turn damage hepatic cells. Activation of some enzymes in the cytochrome P_{450} system, such as CYP2E1, also leads to oxidative stress (Jaeschke *et al*., 2002).

Carbon tetrachloride (CCl_4) is a well known hepatotoxin used in diverse experimental models (Singh *et al.,* 2008). In addition to hepatic problems, it causes dysfunction of the kidneys, lungs, testis, brain, and blood by generating free radicals (Ozturk *et al*., 2003; Khan *et al.,* 2009). Carbon tetrachloride (CCl_4) is rapidly transformed to trichloromethyl

radical (CCl_3^*) and its derivative trichloromethyl peroxy radical (CCl_3OO^*), generated by cytochrome P_{450} of liver microsomes (Brent and Rumack, 1993). These free radicals react with membrane lipids leading to their peroxidation (Singh *et al.,* 2008). Membrane disintegration of hepatocytes with subsequent release of membrane associated enzymes and necrosis are some of the consequences of CCl_4 induced liver damage.

1.2 Statement of Research Problem

Hepatotoxicity is one of the very common ailments resulting into serious debilities ranging from severe metabolic disorders to even mortality (Anil *et al*., 2010). Liver injury due to chemicals or infectious agents may lead to progressive liver fibrosis and ultimately cirrhosis and liver failure (Anand, 1999). According to the report published by USFDA, more than 900 drugs, toxins, and herbs have been reported to cause liver injury, and drugs account for 20 – 40% of all instances of hepatic failure (Soni *et al.,* 2011).

Liver ailments represent a major global health problem. Chronic liver cirrhosis and drug induced liver injury is the ninth leading cause of deaths in western and developing countries (Baranisrinivasan *et al*., 2009; Saleem et *al*., 2010).

In Nigeria, as in other parts of sub-saharan Africa, the major causes of liver cirrhosis include infections particularly chronic hepatitis B virus (HBV) infection (Otu, 1987 and Cook, 1980) and hepatitis C virus infection (Bojuwoye, 1996). There is increasing evidence that free radicals and reactive oxygen species play a crucial role in various steps that initiate and regulate the progression of liver diseases independently of the original agent (Jemal *et al*., 2007).

1.3 Justification

Liver diseases remain one of the major threat to public health and a worldwide problem (Asha and Pushpangdan, 1998). The use of medicinal plants with high level of antioxidant constituents has been proposed as an effective therapeutic approach for hepatic damages (Govind, 2011).

Many antioxidants have been used to protect organs from the free radical challenges. Several researches are attempting to explore the possibility of using herbs containing antioxidants as organ curative agents. In view of severe undesirable side effects of synthetic agents, there is a growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatocurative activity.

Though the stem bark of *Detarium microcarpum* is used traditionally in Nigeria as a valuable remedy to treat liver diseases, no detailed pharmacological investigation have been done to that respect. This is why the present study is being undertaken to investigate it's hepatocurative and antioxidant activity.

1.4 AIM AND OBJECTIVES

1.4.1 Aim

The aim of this study is to investigate the hepatocurative and antioxidant effect of ethylacetate and n-butanol fractions of *Detarium microcarpum* stem bark in CCl_4-induced liver damage in rats.

1.4.2 Specific objectives

i. To identify and quantify levels of some phytochemicals present in the crude extract, ethyl-acetate and n-butanol fractions of *Detarium microcarpum* stem bark.
ii. To carry out *in-vitro* antioxidant activity (such as DPPH, reducing power and H_20_2 radical scavenging activity) on ethyl-acetate and n-butanol fractions of *Detarium microcarpum* stem bark and determine the lethal dose (LD_{50}) of the fraction with the highest activity.
iii. To determine the effects of the fraction with the highest activity on some biochemical parameters (such as ALT, AST, ALP, Bilirubin, Total protein, Albumin, Urea and Creatinine) in CCl_4 induced liver damage in rats.
iv. To determine the effect of the fraction with highest activity on some endogenous antioxidant enzyme levels and lipid peroxidation in the tissues of the animals induced with CCl_4.

1.5 Null Hypothesis

Ethyl-acetate and n-butanol fractions of *Detarium microcarpum* stem bark do not have any hepatocurative and antioxidant effect on CCl_4 induced liver damage in rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Detarium microcarpum.* Guill and Perr.

2.1.1 Classification of the plant

Kingdom	-	Plantae
Class	-	Magnoliopsida
Order	-	Fabales
Family	-	Fabaceae
Genus	-	*Detarium*
Speces	-	*Detarium microcarpum.* Guill and Perr.
Phylum	-	Tracheophyta

2.1.2 Description, distribution and habitat of *Detarium microcarpum*

Detarium microcarpum is an African tree belonging to the Fabaceae family (legumes). Typically, it is found in high rainfall savannah areas, dry forests and fallow lands on sandy or iron rich hard soils as well as scattered trees on farms. It also occurs in dry savannah as a more stunted tree with smaller fruits (Vautier *et al*., 2007) reaching 10 m high and with a dense rounded crown; in wet areas it can grow up to 25 m tall. The leaves alternate with 3–4(–6) pairs of leaflets, short hairy when young, leaflets alternate to subopposite, ovate, oblong to elliptical, 7–11 cm × 3.5–5 cm, base rounded, apex usually emarginate, thickly leathery. Flowers are bisexual, regular, sessile, fragrant; sepals 4, elliptical, white or cream, densely pubescent outside; petals absent; stamens (8–) 10, free; ovary superior, sessile, 1-celled, style slender, stigma terminal, head-shaped. Fruit an ovoid or rounded, indehiscent drupe-like pod, 2.5–4.5 cm in diameter,

more or less flattened, glabrous, yellowish when ripe, with greenish mealy pulp, fibrous and sweet, 1-seeded. Seed orbicular, 15–20 mm × 6.5–8.5 mm. Seedling with epigeal germination. The root system is horizontal; bole usually straight, cylindrical, 30cm in diameter; bark scaling on older branches, grey, brown or reddish; crown irregular (Kouyate and van Damme, 2006).

D. microcarpum is classified as a major African medicinal plant. It is commonly known in English as sweet dattock, trees. *D. microcarpum* is known locally in Ghana as *Takyikyiriwa, Twutwiriwa*; in Senegal as *Kpagra, Kpayhga*; in Nigeria *Taura*, (Hausa): *Ofo,* (Igbo): *Ogbogbo*, (Yoruba): *Gungorochi,* (Nupe): *Konkehi,* (Fulfulde): *Galapo*, (Kanuri): *Agashidam,* Tiv (Irvine, 1961; Keay *et al.*, 1964; Dalziel, 1955). *D. microcarpum* is found in semi-arid sub-Saharan Africa from Senegal to Cameroon, extending east to the Sudan. It has an irregular distribution, but it can be locally very common. *Detarium microcarpum* is naturally distributed in the drier regions of West and Central Africa (Benin, Cameroon, Central African Republic, Chad, Gambia, Ghana, Guinea, Guinea Bissau, Côte d'Ivoire, Mali, Niger, Nigeria, Senegal, Sudan and Togo) Contu, (2012). Unlike the other species of its family, *D. microcarpum* grows in dry savannah, in humid forest (Kouyaté and Van Damme, 2006). It is most common in wooded savannahs or savannahs, semi-cleared dry forest areas and fallows, growing in sandy or hard soils with high iron content (Kouyaté and Lamien, 2011).

2.1.3 General uses of *Detarium microcarpum* plant

Fruits of plant bark and leaves are used not only for texture and flavour, but also for their chemical and nutritional properties (Abulude *et al.,* 2004). The seeds are used as frankincense and to make necklaces for women. The seeds and leaves are eaten as a condiment and vegetable (Kouyate and van Damme, 2006). The seed oil was reported to

have low biogenic and oxidative rancidity; a desired property in oils meant for consumption, industrial purposes and pharmaceutical applications (Okorie *et al*., 2010). The kernel of the seed is deep purple brown, and is more or less oily and edible. Nutritionally, the seed which is used as a traditional soup thickener contain lipids, carbohydrates, proteins, crude fibre and the essential elements: Na, K, Mg, Ca, S, P and Fe (Abreu and Relva, 2002; Abreu *et al*., 1998). Saponins, phytates and cyanides are reportedly present as anti-nutrients (Anhwange *et al*., 2004).

The defatted seed yield gum, which have been utilized as a bio-adhesive agent in the formulation of muco-adhesives and sustained release tablets (Okorie, 2004). The gum content of the seeds was reported to be high; Linoleic acid was the predominant fatty acid (Njoku *et al*, 1999). *D. microcarpum* produces timber which can serve as a mahogany substitute. Its hard dark brown wood provides very good quality timber, which is used in carpentry and construction (Vautier *et al.*, 2007). It is also used for good quality charcoal and fuel wood delivering 19 684 kJ kg-1 of calorific power (Kaboré, 2005). It is the most important commercial fuel wood species and is harvested preferentially from the state forests in Burkina Faso (Kaboré, 2005; Savadogo *et al.*, 2007). Its foliage are avoided by animals and the roots used in perfume (Vautier *et al.*, 2007).

2.1.4 Ethno-medicinal uses

Among the Ibos in the south eastern Nigeria, *Detarium microcarpum* is a revered plant, mythically believed to be chip of the primal trees that germinate and grow in God's own garden. They are the main object in traditional worship, symbolizing truth, honesty and integrity (Ejizu, 1986). Throughout West Africa the genus *Detarium* is believed to possess medico-magical powers. In African ethnomedicine, the bark, leaves and roots of

Detarium microcarpum are widely used throughout its distribution area because of their diuretic and astringent properties. They are prepared as infusions or decoctions to treat rheumatism, venereal diseases, urogenital infections, haemorrhoids, caries, biliousness, stomach-ache, intestinal worms and diarrhoea including dysentery. They are also used against malaria, leprosy and impotence. A decoction of the powdered bark is widely taken to alleviate pain, e.g. headache, sore throat, back pain and painful menstruation. The fresh bark or leaves are applied to wounds, to prevent and cure infections (Kouyate and van Damme, 2006). The leaves, stems, roots, barks, as well as the fruits have found tremendous usage in treatment of various ailments e.g. tuberculosis, meningitis, itching and diarrhea (Obun *et al.*, 2010).

In Burkina Faso, the fruit pulp of *D. microcarpum* is used to treat skin infection. In Mali the bark is used to treat measles, itching, hypertension, nocturia and tiredness, while the decoction of the leaves or roots is used for paralysis, meningitis, tiredness, cramps and difficult delivery (Kouyate, 2005). In Niger and Togo, the fruit preparation is used for dizziness, while in Benin a decoction of the leaves is used in treating convulsions and fainting. Apart from medicinal uses, the fruit of *D. microcarpum* is sweet and commonly eaten fresh, while the pulp is used in making cakes, as well as a substitute for sugar. The seeds are used as frankincense to ward off evil spirits (Akah *et al*., 2012).

In West Africa the roots are part of a medico-magical treatment for mental conditions, and for protection against bad spirits. In veterinary medicine the leaves and roots are used to treat diarrhoea in cattle in southern Mali, and in Benin to treat constipation. In Niger cattle are made to inhale the smoke of the leaves to treat fever (Kouyate and van Damme, 2006).

2.2. Phytochemical Profile of *Detarium microcarpum* Plant

Table 2.1: Some phytochemical constituents of *Detarium microcarpum*

Compound	Plant part	Reference
1. Clerodane diterpenes e.g. i) 3, 4- epoxyclerodan-13E-en-15-oic acid(1), ii) 5α, 8-α (2-oxokolavenic acid(2) iii)3,4-dihydroxyclerodan-13E-en15-oic acid(4) iv) 3, 4-dihydroclerodan-13z-en-15-oic acid(5) v) 2-oxokolavenic acid(3) Copalic acid(6)	Fruit pulp	Cavin *et al*., (2006).
2. Sitosterol, lupol, β-sitosterol, stigmasterol, campesterol	Bark extract	Abreu *et al.,* (1998)
3. γ-quinide (-)-bomesitol, D-pinitol, myoinositol, sucrose, D-glucose, D-fructose	Bark extract	Abreu and Relva, (2002)
4.Saponins, Proteins, Carbohydrates, Reducing sugar, Resins, Flavonoids, Glycosides, Terpenoids, Steroids, Fats and Oil	Root extract	Okolo *et al.,* (2012)



Figure 1: *Detarium microcarpum* plant: (www.westafricanplants.senckenberg.de/images/pictures/detarium_microcarpum_ms_2151_552_ff9925.jpg)

2.3 Pharmacological Activities

2.3.1 Antidiabetic activity

Methanol extract of *D. microcarpum* roots and its fraction significantly ($P<0.05$) reduced blood sugar level in alloxan-diabetic rats without producing hypoglycemia, an effect attributed to the flavonoids abundantly present in the extract, Okolo *et al.*, (2012).

2.3.2 Antibacterial and antifungal activities

The methanol crude alkaloids extracted from the bark of *D. microcarpum* plant was found to be very active against *E. coli, P.aeruginosa*, and *S. aureus,* these showed that alkaloids from this plant could be used as broad spectrum antibiotic against diseases caused by the test microbes (Garba *et al*., 2013). Similarly, ethanol extract of the bark of *D. microcarpum* was shown to exhibit antimicrobial action against some pathogenic organisms including *Pseudomonas aeruginosa, Klebsiella pneumonia, Citrobacter freunditis, Staphylococcus aureus, Streptococcus pyrogenes and Listeria monocytogenens* (Abreu *et al*., 1998). Kouyate and van Damme, (2006) reported some antibacterial action of *Detarium microcarpum* bark extract against some bacteria including *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

A study by Olugbuyiro and Akinbohun, (2012) demonstrated a good anti-mycobacterial property of the stem bark extract of *Detarium microcarpum* and projects it as promising natural product agents for generating anti-infective against *Mycobacterium tuberculosis.* Ebi and Afieroho, (2011) reported that using the aqueous methanol extract of the seeds of *D. microcarpum* a broad spectrum antimicrobial activity was observed against clinical isolates of *Staphylococcus, aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella. pneumonia, Salmonena. paratyphi and Candida albicans*. This activity was attributed to the presence of steroidal saponins and

flavonoids. Inhibition of the growth of the plant pathogenic fungus-*Cledosporium eucumerinum* by the pulp extract of *D. microcanpum* was reported by Cavin et al., (2006). All these studies point to the potential antimicrobial usefulness of *Detarium microcarpum.*

2.3.3 Antiviral activity

The flavones present in the methanol extract of *D. microcarpum* was shown by Mahmood et al., (1993) to strongly inhibit HIV-1 or HIV-2 virus. Olugbuyiro *et al.,* (2009) reported the antiviral activity of the fractions of the methanol stem bark extract of *D. microcarpum* using-7 Replicon assay. They demonstrated that the active fraction MTH-1700 selectively inhibited Hepatitis C-Virus (HCV). The extract was also shown to exhibit moderate antitumor activity against breast cancer (Abreu *et al.*, 1999). Also the bark extract of *D. microcarpum* showed significant molluscicidal activity against *Lymnaea natalensis* (Mahmood *et al.,* 1993).

2.3.4 Enzyme inhibition

The clerodane diterpenes isolated from the fruits of *D. microcarpum* (Cavin *et al.,* 2006) has been shown to inhibit the growth of the plant pathogenic *Cladosporium cucumerinum* and of the enzyme acetylcholinesterase (AChE). One of the compounds-5α,8α (2-oxokolavenic acid was ten times as potent as galanthamine, a clinically useful drug for Alzheimer's disease. Inhibition of AChE is currently the most efficient approach in managing the symptoms of Alzheimer's disease.

2.3.5 Antisnake venom activity

The leaves of *D. microcarpum* are commonly used in the northern part of Nigeria to treat snake bite. Studies by Iful, (2008) reported that the leaf extract of *D. microcarpum* potently reduced mortality in *Echis carinatus* (carpet viper) venom treated animals. The

study also revealed that the extract relaxed the rabbit isolated jejunum and contracted the rat phrenic nerve-diaphragm muscle.

2.4. The Liver

2.4.1 Structure and function

The liver is the largest organ in the body and serves many vital functions such as removal of damaged red blood cells from the blood in co-ordination with spleen, produces bile, clotting factors, stores vitamins, minerals, protein, fats and glucose from diet. The most important task of the liver is to filter toxic substances from the body, like alcohol, chemotherapeutic drugs, antibiotics and toxicants. If accumulation of toxins is faster than the liver metabolizing ability, hepatic damage may occur (Waugh *et al.*, 2001; Bigoniya *et al.*, 2009). The liver plays a key role in metabolism of nutrients and various xenobiotics, such as food additives, drugs or environmental pollutants. It is the largest gland in human body, harboring important processes associated with e.g. regulation of carbohydrate, lipid, amino acid and hormone metabolism, the synthesis and degradation of plasma proteins, the storage of vitamins and metals, the secretion of bile and finally with xenobiotics metabolism (Kmiec, 2001; Sherlock, and James, 2002; Baynes and Marek, 2005).

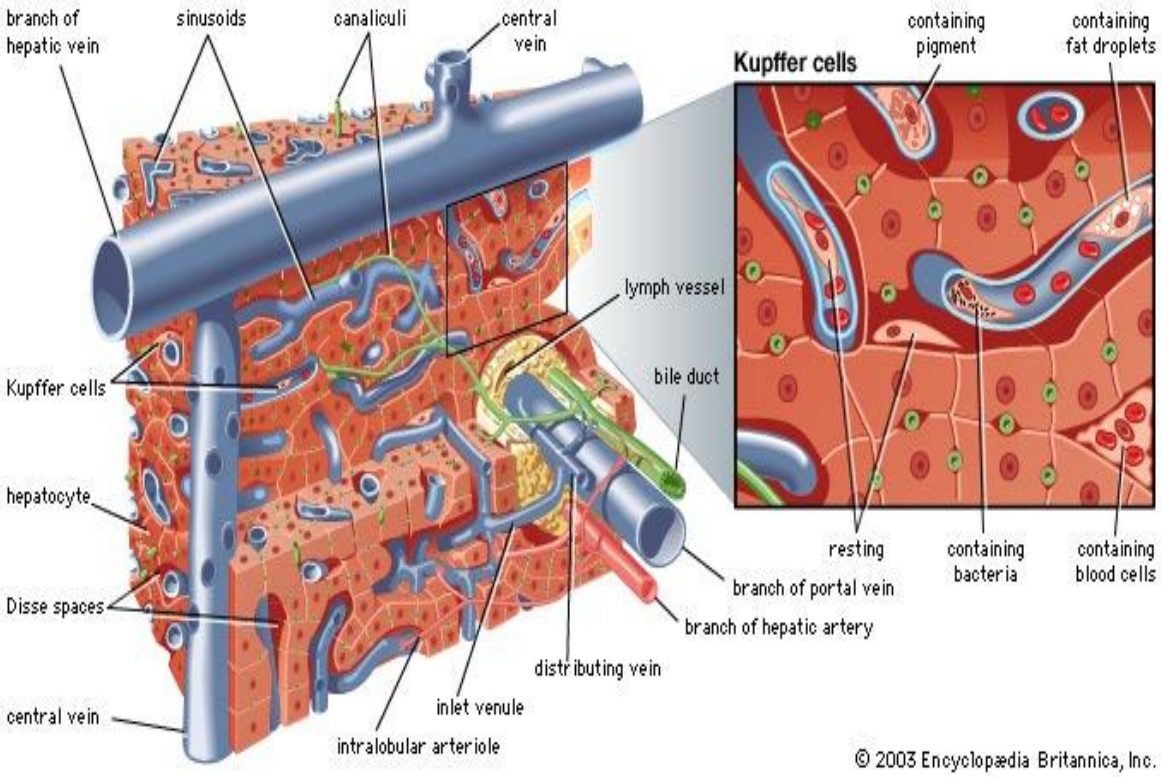


Figure 2: Structure of the liver: Detailed views of hepatic lobules with depicted compartments of vascular and biliary system and cell types found in liver. (scheme assumed according to Encyclopaedia Britannica, Inc.; www.britannica.com)

2.4.2 Liver cells

The principal cellular population found in the liver, are hepatocytes. Hepatocytes actively participate in the metabolism of proteins, lipids, metals and vitamins, as well as in the processes of excretion, detoxification and energy storage. Liver are interlaced by blood sinusoids, which represent a source of other cell populations essential for liver functions. Endothelial cells represent the major cell population found in sinusoids and they mediate communication between hepatocytes and inner space of sinusoids, as well as prevent pathogen infiltration into the liver parenchyma (Kmiec, 2001; Baynes and Marek, 2005).

Moreover, the wall of blood sinusoids is also lined with mononuclear phagocytes (Kupffer cells), hepatic stellate cells and pit cells. Kuppfer cells are liver macrophages activated by gut-derived bacterial endotoxins, which are characterized by high phagocytic, endocytic and secretory activities, important also for paracrine interactions between hepatocytes and hepatic stellate cells (HSCs). Lymphocytes are also distributed in liver tissue with yet another cell polulation, so-called pit cells, which have characteristic features of natural killer cells (NK) and thus exert cytotoxic activity against tumor cells (Kmiec, 2001). Furthermore, all cell populations located within sinusoids also contribute to exchange of metabolites between plasma and hepatocytes, degradation of undesirable particles, such as microbial agents or cellular debris, and regulation of blood flow. They also show spontaneous cytotoxicity targeted towards virus-modified hepatocytes, thus helping to maintain liver integrity and homeostasis (Kmiec, 2001; Sherlock, and James, 2002; Baynes and Marek, 2005).

2.4.3 Xenobiotics and liver metabolism

Liver plays a key role in xenobiotic metabolism facilitating excretion of chemicals from the body. However, detoxification processes may be also accompanied with increased toxicity of reactive metabolites. This is for example the case of activation of procarcinogens to carcinogens, where more reactive diols, quinones and/or epoxides are produced during the promutagen metabolism thus leading to generation of e.g. DNA adducts and/or oxygen species-related genotoxic stress (Zatloukalova, 2008). Activities of detoxification enzymes depend on many factors, such as age, gender, genetic factors/polymorphisms, or by previous exposure to various chemicals thus resulting in drug or toxic response individuality (Rodriguez-Antona and Ingelman-Sundberg, 2006).

The ability of humans to metabolize and clear drugs is a natural process that involves the same enzymatic pathways and transport systems that are utilized for normal metabolism of dietary constituents. The human body identifies almost all drugs as foreign substances (xenobiotic) and subjects them to various chemical processes to make them suitable for elimination. This involves chemical transformations to reduce fat solubility and to change biological activity. Although almost all tissues in the body have some ability to metabolise chemicals, smooth endoplasmic reticulum in the liver is the principal metabolic clearing house for both endogenous chemicals (example, fatty acids, and steroid hormones) and exogenous substances like drugs (Zatloukalova, 2008). Drugs, food additives or numerous environmental pollutants are xenobiotics daily ingested with food, inhaled or absorbed through skin, thus leading to exposure of various body organs to their toxic metabolites. Xenobiotics enter the cells by either passive or protein-assisted membrane transport and they are metabolised by 2 principal groups of enzymes (Nebert and Gonzalez, 1987; Nebert and Dalton, 2006): Phase 1 reaction, in which enzymes carry out oxidation, reduction, or hydrolytic reactions, is

thought to prepare a drug for phase 2 in which enzymes form a conjugate of the substrate (the phase 1 product). The cytochrome P_{450} enzymes catalyze phase 1 reactions. These processes tend to increase water solubility of the drug and can generate metabolites which are more chemically active and potentially toxic. Most of phase 2 reactions take place in the cytosol and involve conjugation with endogenous compounds via transferase enzymes. Chemically active products from phase 1 are made relatively inert and suitable for elimination by the phase 2 step (Smith *et al*., 1998; Werck-Reichhart and Feyereisen, 2000; Liston *et al.*, 2001).

2.4.4 Mechanism of hepatic injury

Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. Many chemicals damage mitochondria, an intracellular organelle that produces energy. Its dysfunction releases excessive amount of oxidants which, in turn, injure hepatic cells. Activation of some enzymes in the cytochrome P_{450} system such as CYP2E1 also leads to oxidative stress (Jaeschke *et al.*, 2002). Injury to hepatocyte and bile duct cells lead to accumulation of bile acid in the liver. This promotes further liver damage (Patel *et al.*, 1998). Non-parenchymal cells such as Kupffer cells, fat-storing stellate cells, and leukocytes (neutrophil and monocyte) also have roles in the mechanism. The liver is subject to toxic injury more often than the other organ. This is not surprising, since the portal vein blood that drains the absorptive surface of the intestine tract flows directly to liver. Thus the liver is exposed to all ingested substances that are absorbed into the portal blood (Cullen, 2005). Cell death is a process accompanying many physiological, biochemical and pathological situations in organisms.

2.5 Mode of Action of Liver Toxicant

2.5.1 Carbon tetrachloride (CCl_4) induced hepatotoxicity

In recent years, attention has been focused on the role of biotransformation of chemicals to highly reactive metabolites that initiate cellular toxicity. Many compounds, including clinically useful drugs, can cause cellular damage through metabolic activation of the chemicals to highly reactive compounds such as free radicals. CCl_4 has probably been studied more extensively both biochemically and pathologically than any other hepatotoxin (Cui *et al*., 2009; Kim *et al*., 2010). Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver disease (Johnson and Kroening, 1998). Its toxic effect on the liver has been extensively studied (Junnila *et al*., 2000; Amin and Mahmoud, 2009; Cui *et al.,* 2009; Kim *et al.,* 2010). In CCl_4 induced hepatotoxicity model, upon administration of CCl_4 to animals, it undergoes enzymatic activation, majorly by CYP2E1, into the trichloromethyl free radical ($CCl_3^{\cdot}$) within the membrane of the endoplasmic reticulum this causes cell death with accumulation of lipid peroxidation and intracellular calcium ions and triggers secondary damage from the inflammatory process (Medina and Moreno-Otero, 2005). This is followed by chloromethylation, saturation, peroxidation and progressive destruction of the unsaturated fatty acid of the endoplasmic recticulum membrane phospholipids. These processes are known as lipid peroxidation, leading to functional and structural disruption of hepatocytes (Tatiya *et al*., 2012).

Liver toxicant CCl_4 causes lipid peroxidative degradation of biomembrane, which is one of the principal cause of hepatotoxicity (Cotran *et al*., 1994). CCl4 induces oxidative stress in many settings **(**Kalava and Menon, 2012) In liver CCl_4 is biotransformed by cytochrome P_{450} to produce its active metabolite trichloromethyl free radical (CCl_3*)

(Kaplowitz *et al.,* 1986), which binds to the macromolecule and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxide which inturn gives toxic aldehyde that causes damage to liver. Secondary mechanisms link carbon tetrachloride metabolism that could promote the generation of toxic products arising directly from carbon tetrachloride metabolism or from peroxidative degeneration of membrane lipids. The possible involvement of toxic intermediates radical species such as trichloromethyl (CCl_3*), trichloromethylperoxy ($OOCCl_3$*) and chlorine (Cl*) free radicals as well as phosgene and aldehydic products of lipid peroxidation (Bigoniya *et al*., 2009).

This radical can bind to cellular molecules (nucleic acid, protein and lipid) impairing crucial cellular processes such as lipid metabolism, with the potential outcome of fatty degeneration (steatosis). This radical can also react with oxygen to form a highly reactive specie, trichloromethylperoxy radical (CCl_3OO*). CCl_3OO* initiates the chain reaction of lipid peroxidation which attacks and destroys polyunsaturated fatty acids, in particular those associated with phospholipids. This affects the permeability of mitochondrial, endoplasmic reticulum and plasma membranes resulting in the loss of cellular calcium sequestration and homeostasis, which can contribute heavily to subsequent cell damage. Carbon tetrachloride significantly increases serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphate (ALP) and total bilirubin whereas decreasing total protein, albumin and total cholesterol (Bigoniya *et al*., 2009).

2.5.2 Liver injuries

Liver disorders are mainly caused by toxic chemicals, such as antibiotics chemotherapeutic agents, peroxidised oil, aflatoxin, CCl_4, chlorinated hydrocarbons etc,

most of the lipid peroxidation and by generation of reactive oxidative intermediates in liver (Harsh, 2005). Also, excess consumption of alcohol, infections and autoimmune/disorders are causes of liver disorder. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver. Enhanced lipid peroxidation produced during the liver microsomal metabolism of ethanol may result in hepatitis and cirrhosis (Ross and Wilson, 2005; Tortora and Grabowski, 2003).

2.5.3 Cholestatic liver injury

This form of liver dysfunction is defined physiologically as a decrease in the volume of bile formed or an obstruction of bile flow or an impaired secretion of specific solutes into bile. Cholestasis is characterized biochemically by elevated serum levels of compounds normally concentrated in bile, particularly bile salts and bilirubin. When biliary excretion of the yellowish bilirubin pigment is impaired, this pigment accumulates in skin and eyes, producing jaundice, and spills into urine, which becomes bright yellow or dark brown. This is because drug-induced jaundice reflects a more generalized liver dysfunction, it is considered a more serious warning sign in clinical trials than mild elevation of liver enzymes (Zimmerman, 1999).

The mechanism underlying cholestatic liver injury has been linked to an impairment of bile salt transport by inhibition or down regulation of ATP-dependent bile salt transporters and also to alterations of actin resulting in disruption of the cytoskeleton and an impaired transport of bile along the canalicular system and into the bile ducts (Cullen, 2005). Interaction with bile acid transporters is a mechanism that has been observed under administration of several drugs and toxicants leading to toxic liver injury and is believed to be a central event in the development of cholestatic liver injury (Lewis, 2000).

2.6.2 Fatty liver (Steatosis)

Fatty liver (steatosis) is defined biochemically as an appreciable increase in the hepatic lipid (mainly triglyceride) content in the hepatocytes, which is less than five percent (5%) by weight in normal human liver. At the same time there is a decrease in plasma lipids and lipoproteins. The term hepatic steatosis also refers to an intracellular accumulation of lipid droplets in the cytoplasm. Steatosis is of two types. Primary steatosis is often observed in patients displaying symptoms of the metabolic syndrome including obesity, diabetes, hypertriglycerinaemia and insulin resistance. Secondary hepatic steatosis is extrinsically induced by alcohol, several drugs, copper accumulation in Wilson's disease and other factors (Pessayre *et al.,* 2001).

Histologically, in standard paraffin-embedded and solvent-extracted sections, hepatocytes containing excess fat appear to have multiple round empty vacuoles that displace the nucleus to the periphery of the cell. Hepatic steatosis may also be accompanied by hepatocellular necrosis, inflammation and fibrosis, in which case it is termed as 'non-alcoholic steatohepatitis' (NASH). The morphology of steatosis has been linked to primary mitochondrial failure and has been implicated for microvesicular steatosis, non-alcoholic steatohepatitis (NASH) and cytolytic hepatitis (Fromenty and Passayre, 1995). Acute exposure to many hepatotoxicants, e.g., carbon tetrachloride, and drugs can induce steatosis (Zimmerman, 1999).

2.6.3 Cell death

Based on morphology, liver can be destroyed or damaged by two different modes, oncotic necrosis (necrosis) or apoptosis.

2.6.3.1 Necrosis

Necrosis is a form of cell injury that results in the premature death of cells in living tissue (Proskuryakov *et al*., 2003). Some of the types of necrosis include: coagulative necrosis, fat necrosis and fibrinoid necrosis. Necrosis may be caused by factors external to the cell or tissue, such as infection, toxins, mechanical trauma (physical damage to the body that causes cellular breakdown) any damage to blood vessels (which may disrupt the blood supply to that area); and ischemia that result in the unregulated digestion of cell components (Raffray and Gerald, 1997: Proskuryakov *et al.*, 2003). Under extreme conditions tissues and cells die through an unregulated process of destruction of membranes and cytosol (Nazarian *et al.,* 2009). Cells that die due to necrosis do not follow the apoptotic signal transduction pathway but rather various receptors are activated that result in the loss of cell membrane integrity and an uncontrolled release of products of cell death into the intracellular space (Proskuryakov *et al.,* 2003).

2.6.3.2 Apoptosis

Apoptosis is a controlled form of cell death that serves as a regulation point for biological processes and can be thought of as the counterpoint of cell division by mitosis. This selective mechanism is particularly active during development and senescence. Although apoptosis is a normal physiological process, it can also be induced by a number of exogenous factors, such as xenobiotic chemicals, oxidative stress, anoxia and radiation. Apoptosis is characterized by cell shrinkage, chromatin condensation, nuclear fragmentation, formation of apoptotic bodies, and generally a lack of inflammation, (Iful, 2008).

Apoptosis is always a single cell event with the main purpose of removing cells no longer needed during development or eliminating aging cells during regular tissue turnover. Under these conditions, apoptotic bodies are phagocytosed by Kupffer cells or taken up by neighbouring hepatocytes. In the absence of cell contents release, the remnants of apoptotic cells disappear without causing an inflammatory response. Because of effective regeneration, apoptotic cell death during normal tissue turnover or even a moderately elevated rate of apoptosis is of limited pathophysiological relevance in the liver. However, if the rate of apoptosis is substantially increased, the apoptotic process cannot be completed. In this case, cells undergo secondary necrosis with breakdown of membrane potential, cell swelling, and cell contents release (Ogasawara *et al.,* 1993).

2.6.3.3 Cirrhosis / Fibrosis

Fibrosis is a progressive disease that occurs in response to chronic injury and is characterized by the accumulation of excessive amounts of fibrous tissue, specifically fibril forming collagens type I and III, and a decrease in normal plasma membrane collagen type IV. Formation of so-called pseudo-septa and regeneration nodules leads to a diversion of the blood flow and impairs supply with nutrients and oxygen. Furthermore, toxic liver fibrosis is accompanied by both bile ductular proliferation and inflammation under various conditions (Muller *et al.,* 1996). Fibrosis can develop around central veins and portal tracts or within the space of Disse. The excessive extracellular matrix protein deposition and the loss of sinusoidal endothelial cell fenestrae and of hepatocyte microvilli limit exchange of nutrients and waste material between hepatocyte and sinusoidal blood (Iful, 2008). With continuing collagen deposition, the architecture of the liver is disrupted by interconnecting fibrous scars. When the fibrous scars subdivide the remaining liver mass into nodules of regenerating

hepatocytes, fibrosis has progressed to cirrhosis and the liver has limited residual capacity to perform its essential functions. The primary cause of hepatic fibrosis/cirrhosis in humans worldwide is viral hepatitis. However, biliary obstruction and in particular alcoholic and non-alcoholic steatohepatitis are of growing importance for the development of hepatic fibrosis (Bataller and Brenner, 2005). In addition, fibrosis can be induced by heavy metal overload (Gutierrez-Ruiz and Gomez-Quiroz, 2007).

2.7 Biochemical Alterations in Hepatic Damage

2.7.1 Serum aminotransferase enzymes

Serum concentrations of aspartate aminotransferase (AST) or glutamate oxaloacetate transaminase (SGOT) and alanine aminotransferase (ALT) or glutamate pyruvate transaminase (SGPT) are the most commonly used biochemical markers of hepatocellular necrosis (Friedman *et al.*, 1996). These serum activities presumably increase as a result of cellular membrane damage and leakage (Kaplan, 1993). Serum levels of SGOT and SGPT are increased on damage to the tissues producing them. Serum aminotransferase activities are increased in all types of hepatic injury. Thus serum estimation of SGPT which is fairly specific for liver tissue is of greater value in liver cell injury, whereas SGOT level may rise in acute necrosis or ischemia of other organs such as the myocardium, besides liver cell injury.

2.7.2 Serum alkaline phosphatase

Serum alkaline phosphatase is produced by many tissues especially bone, liver, intestine, placenta and is excreted in the bile. Serum alkaline phosphatase increases to some extent in most types of liver injury. The highest concentrations are observed with cholestatic injuries (Friedman *et al.*, 1996). Alkaline phosphatase (ALP) catalyse the

hydrolysis of phosphate esters, and is found in biliary epithelium and the bile canalicular region of hepatocytes. Its function is not well established, but is thought to be involved in metabolite transport across cell membranes. Elevation of the level of ALP can suggest intrahepatic, extrahepatic biliary obstruction, or infiltrative diseases of the liver (Field *et al*., 2008).

2.7.3 Serum total protein and albumin

One of the most important liver functions is protein synthesis. Albumin is a major part of the total protein (TP) made specifically by the liver. Liver damage causes disruption and disassociation of polyribosomes on endoplasmic reticulum and thereby reducing the biosynthesis of protein. The TP levels including Alb levels will be depressed in hepatotoxic conditions due to defective protein biosynthesis in liver. Restoring the normal levels of TP including Alb is an important parameter for liver recovery (Navarro and Senior, 2006).

2.7.4 Serum bilirubin

Bilirubin is the breakdown product of normal haem -a part of haemoglobin in red blood cells-catabolism of aged erythrocytes. Bilirubin, loosely bound to albumin in plasma to form a soluble species taken up from the Disse spaces of liver sinusoids into hepatocytes, where it is esterified at its propionyl sites with glucuronic acid under the catalytic activity of uridinediphosphoglucuronate 1A1 transferase enzymes. Esterified bilirubin is excreted into bile as water-soluble bilirubin diglucuronide. Serum concentration of bilirubin is a marker of the liver's ability to take up bilirubin from the plasma into the hepatocyte, conjugate it with glucuronic acid, and excrete bilirubin glucuronides into bile. Elevated level of serum conjugated bilirubin implies regurgitation of bilirubin glucuronides from hepatocytes back into plasma, usually

because of intrahepatic or extrahepatic obstruction to bile outflow and cholestasis. The liver has substantial reserve capacity, and normal serum bilirubin levels can be maintained until there is enough injury to reduce the liver's capacity to clear bilirubin from plasma. Serum concentration of bilirubin is very specific for potentially serious liver damage, and is an important indicator of the loss of liver function (Field *et al.,* 2008). Reduction in the level of serum bilirubin is a strong indication of restoring normal liver function.

2.8 Silymarin

Silymarin is a standardized plant extract obtained from the seeds of milk thistle (*Silybum marianum)* silymarin, a mixture of flavonoid complexes, is the active component of milk thistle plant that protects liver and kidney cells from toxic effects of drugs, including chemotherapy (Post-White *et al.*, 2007). It is composed of a mixture of four isomeric flavonolignans: silibinin, isosilibinin, silydianin and silychristin. It contains about 60% polyphenol silibin and is used as a hepatoprotective agent (Boigk *et al.,* 1997). Silymarin is widely used for protection against various liver diseases in Europe and around the world.

In addition to its free radical scavenging properties, silymarin increases antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, and inhibits lipid peroxidation (Zhao *et al.*, 2000). It is reported to offer protection against various chemical hepatotoxins such as CCl_4, and alcoholic liver (Crocenzi and Roma, 2006). Silymarin act as an antioxidant by scavenging preoxidant free radicals and by increasing the intracellular concentration of glutathione (GSH). It also exhibits a regulatory action of cellular membrane permeability and increase its stability against xenobiotics injury, increasing the synthesis of ribosomal RNA by stimulating DNA polymerase-I, exerting

a steroid like regulatory action on DNA transcription and stimulation of protein synthesis and regeneration of liver cells (Dehmlow *et al*., 1996; Saller *et al*., 2007).

Silymarin efficacy is not limited to the treatment of toxic and metabolic liver damage; it is also effective in acute, chronic hepatitis and in inhibiting fibrotic activity (Saller *et al.,* 2007). It acts as inhibitor of the transformation of stellate hepatocytes into myofibroblasts, this process is responsible for the deposition of collagen fibres leading to cirrhosis (Fraschini *et al*., 2002).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals/reagents

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Bilirubin, Albumin, Total protein, Urea and Creatinine assay kits were purchased from Reckon Diagnostics Pvt. Ltd. (Gujarat, India). Catalase, Malondialdehyde and Superoxide dismutase kits were purchased from Northwest Life Science Specialties LLC (Vancouver, Washington). All other chemicals and reagents used in this study were of analytical grade.

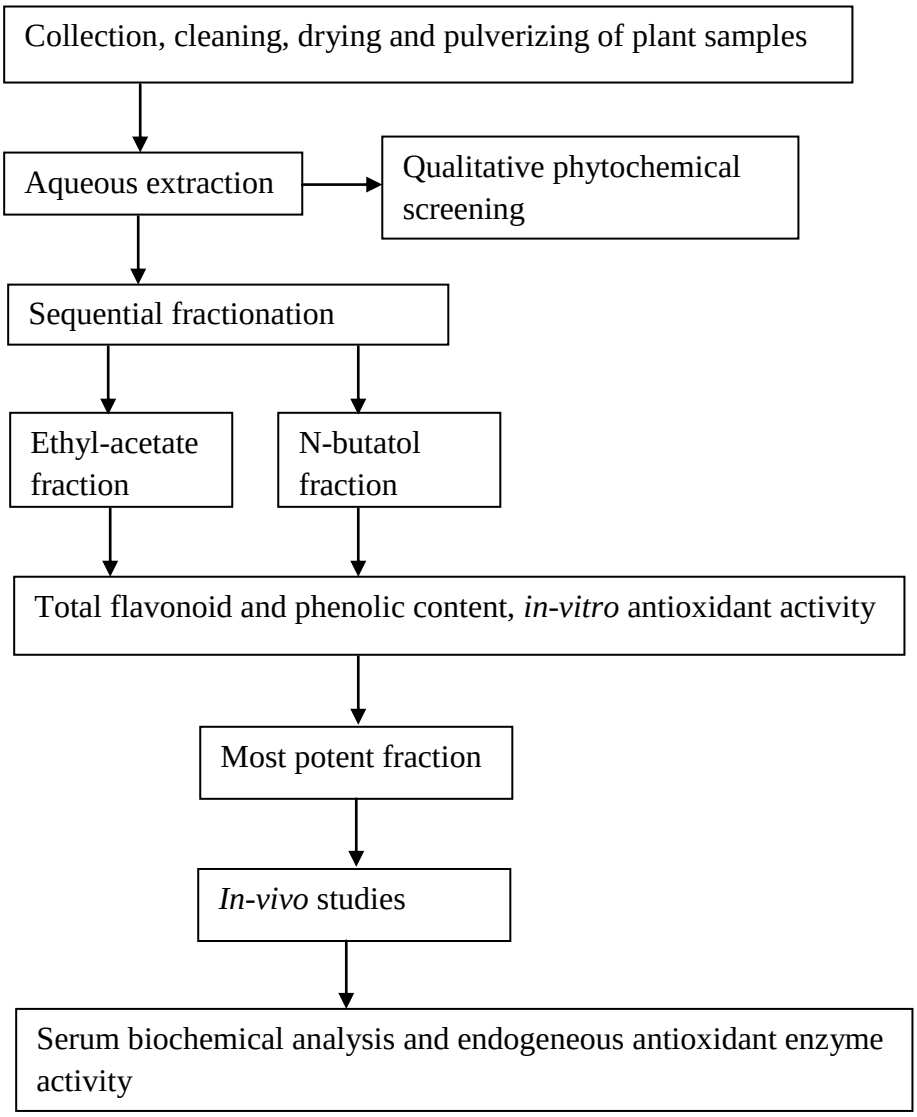


Figure 3: Experimental design

3.1.2 Plant sample collection and identification

The stem bark of *Detarium microcarpum* was collected in February, 2013 from Minna, Niger State and authenticated at the Herbarium unit in the Department of Biological Sciences, Ahmadu Bello University, Zaria where the voucher number V/N 3105 was obtained.

3.1.3 Experimental animals

A total of 48 healthy Wistar rats weighing between 100-170g were obtained and kept in well aerated laboratory cages in the animal house, Department of Pharmacology, ABU, Zaria. They were allowed to adjust to laboratory environment for a period of 2 weeks before the commencement of the experiment. The animals were fed with Growers mash from the vital feeds company and water was provided *ad libitum* during the stabilization period. The animals were divided into treatment groups and the control groups.

3.2 Methodology

3.2.1 Preparation of plant sample

The collected plant samples were rinsed in clean water and air dried at room temperature. The dried plant samples were pulverized into powder using laboratory mill, the powder obtained was used to prepare the extracts.

3.2.2 Aqueous extract preparation

To 1000 grams of powdered stem bark, 2.5litres portion of distilled water was added and stored at room temperature for 48 hours and shaken at intervals. At the end of the extraction, the crude extract was filtered using muslin cloth and Whatmann filter paper No.1. The aqueous extract obtained was evaporated to dryness at 45□C using water bath.

The powdered extract was stored in an air tight sample bottle in a refrigerator until required for analysis.

3.2.3 Fractionation of crude extract

The crude extract of *D.microcarpum* stem bark was subjected to liquid-liquid partition separation to separate the extract into different fractions. Reconstituted extract (200 ml) was placed in a separatory funnel and 200 ml of ethylacetate and n-butanol solvents were added sequentially as a 1:1 (v/v) solution and rocked vigorously (Abbot and Andrews, 1970). The sample was left standing for 30 min for each solvent on the separatory funnel until a fine separation line appeared clearly indicating the supernatant from the sediment before it was eluted sequentially. The process was repeated thrice in order to get adequate quantity for each fraction. The ethylacetate, n-butanol as well as the aqueous residue fractions were evaporated to dryness in a water bath to afford the fractions (grams) respectively. The various fractions obtained were kept in sealed container at 4□C in a refrigerator until use.

3.2.4 Qualitative phytochemical analysis

Chemical tests for the screening and identification of bioactive chemical constituents in the powdered stem bark of *Detarium microcarpum* under study were carried out in extracts using the standard procedures as described by Sofowara, (1993), Trease and Evans, (1989) and Harborne, (1973).

3.2.4.1 Test for tannins

Grounded stem bark (0.5g) of *Detarium microcarpum* was boiled in 20ml of distilled water in a test tube and filtered. 0.1% ferric chloride ($FeCl_3$) was added to the filtered samples and observed for brownish green or a blue black colouration which showed the presence of tannins.

3.2.4.2 Test for saponins

Grounded stem bark (2g) of *Detarium microcarpum* was boiled together with 20ml of distilled water in a water bath and filtered. Filtered sample (10ml) was mixed with 5ml of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and for the formation of emulsion which indicated the presence of saponins.

3.2.4.3 Test for flavonoids

A few drop of 1% NH_3 solution is added to the grounded stem bark of *Detarium microcarpum* in a test tube. A yellow coloration showed the presence of flavonoid compound.

3.2.4.4 Test for glycosides

Grounded stem bark (5ml) of *Detarium microcarpum* was mixed with 2ml of glacial CH_3CO_2H containing 1 drop of $FeCl_3$. The above mixture was carefully added to 1ml of concentrated H_2SO_4 so that the concentrated H_2SO_4 was underneath the mixture. A brown ring appearance indicated the presence of the cardiac glycoside constituent.

3.2.4.5 Test for alkaloids

The grounded stem bark of *Detarium microcarpum* (0.5g) was added to 5ml of dilute sulphuric acid H_2SO_4 (1%) on a steam bath. The solution was filtered, and the filtrate was treated with a few drops of Dragendorff's reagent. Reddish brown turbidity or precipitate indicated the presence of alkaloids.

3.2.4.6 Test for phenolic compounds

The grounded stem bark of *Detarium microcarpum* (500 mg) was dissolved in 5 ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

3.2.5 Quantitative phytochemical analysis

3.2.5.1 Total flavonoid content

The total flavonoid content was measured by the aluminum chloride colorimetric assay (Olajire and Azeez, 2011). 1 ml each of n-butanol and ethylacetate fractions (10 mg/ml) and standard solution of Quercetin (2-10mg/ml) were added to 4 ml of distilled H_2O. To the above mixture, 0.3ml of 5% $NaNO_2$ was added. After 5 min, 0.3ml of 10% $AlCl_3$ was added. After 6 min, 2 ml of 1M NaOH was added and the total volume made up to 10 ml with distilled H_2O. The solution was mixed well and the absorbance was measured at 510 nm against a blank (1ml of sample was replaced by 1ml of distilled water). The total flavonoids content of the fractions was expressed as mg of Quercetin equivalent (QE) per gram dry mass (mg QE/g dw). All samples were analysed in triplicates.

3.2.5.1 Total phenolic assay

The total phenolic contents of n-butanol and ethylacetate fractions were determined by using Folin-Ciocalteu method (Chun *et al.,* 2003). Extract or standard solution (1ml) of gallic acid (2-10mg/l) was added to 9 ml of distilled water. Folin Ciocalteau reagent (1ml) was added to the mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added and the solution was diluted to volume with distilled H_2O and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank (distilled H_2O) was measured at a wave length of 750 nm. Total polyphenol contents were reported as mg Gallic Acid Equivalent (GAE)/g of extract. All samples were analysed in triplicates.

3.2.6 *In-vitro* antioxidant activity

3.2.6.1 DPPH radical scavenging activity assay

Procedure: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay used by Chan *et al.* (2007) was adopted with slight modification. Different dilutions of the n-butanol and ethylacetate fractions (0.2-1.0 mg/ml) were prepared. DPPH solution was also prepared by dissolving 6.0 mg of DPPH in 100 ml methanol. Then, 1 ml of extract from each dilution was added into the test tube containing 2 ml of DPPH solution. Control was prepared by adding 1 ml of methanol to 2 ml of DPPH solution. Ascorbic acid at various concentrations (0.2-1.0mg/ml) was used as standard. The mixture was shaken vigorously and was left to stand in the dark for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. A blank was prepared without adding extract. IC_{50} (Inhibitory concentration to scavenge 50% free radicals) was determined. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. All samples were analysed in triplicates. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. The scavenging activity of each fraction on DPPH radical was calculated using the following equation:

Scavenging activity (%) = (1– absorbance of test/absorbance of control) × 100

IC_{50} was calculated from equation of line obtained by plotting a graph of % inhibition against sample concentration (mg/ml).

3.2.6.2 Reducing ability

Procedure: The reductive potential of samples was determined according to the method of (Oyaizu, 1986). 1 ml of different concentrations (0.2-1.0 mg/ml) of the n-butanol and ethylacetate fractions were mixed with potassium ferricyanide (2.5 ml, 1% w/v) and 2.5 ml of phosphate buffer (pH 6.6). The mixture was incubated at 50°C for 20

min. 2.5 ml TCA (10% w/v) was added to it and centrifuged at 3000 rpm for 10 min. Supernatant (2.5ml) was taken and 2.5 ml water and 0.5 ml ferric chloride (0.1%w/v) were added to it. The absorbance was measured at 700 nm. Increased absorbance reading indicated increased reducing power. A blank was prepared without adding extract. Ascorbic acid at various concentrations (0.2-1.0mg/ml) was used as standard. All samples were analysed in triplicates.

Percentage (%) of reduction power = [1-(1-As/Ac)] x 100

Ac= absorbance of standard at maximum concentration tested

As= absorbance of sample

3.2.6.3 Scavenging of Hydrogen peroxide

Procedure: Hydrogen peroxide scavenging activity was carried out by the method of Gulcin *et al.*, (2005). A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (pH 7.4). Various concentrations (0.2-1.0mg/ml) of n-butanol, ethylacetae fractions and ascorbic acid were prepared. Extract or standard (1ml) in were added to 2 ml of hydrogen peroxide solution in phosphate buffer. The absorbance was measured at 230nm after 10 minutes against a blank solution that contained extract or standard without hydrogen peroxide. All samples were analysed in triplicates. IC_{50} values were calculated.

H_2O_2 scavenging activity (%) = $(A_0 – A_1) / A_0 \times 100$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

3.2.7 Acute toxicity studies

Acute toxicity for n-butanol fraction was determined by the method described by the Lorke, (1983) to select a suitable dose for the studies of the effects of the fraction. In the first stage, rats were divided into 3 groups of 3 rats each and administered by gavage at

doses 10mg, 100mg and 1000mg/kg to each animal per group. They were observed for 24hrs for signs of toxicity, including death. In the second phase, rats were divided into 3 groups of one rat each and treated with the fraction based on the findings in the first phase. The LD_{50} was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose, i.e., the geometric mean of the consecutive doses with 0 and 100% survival rates were recorded.

3.2.8 Induction of liver damage

Liver damage was induced by the administration of carbon tetrachloride (CCl_4). Rats were injected intraperitoneally with a single dose of CCl_4 (148 mg/kg body weight) as a 1:1 (v/v) solution in olive oil and were fasted for 36 h before the administration of n-butanol fraction (Manoj and Aqued, 2003). This was done once a week for a period of four weeks. The administration of n- butanol fraction was done daily by oral intubation for the period of 28 days.

3.2.9 Experimental design

3.2.9.1 Animal grouping

The rats were randomized into 6 groups, with six animals per group. The extracts were reconstituted in distilled water, and administered orally on daily basis by gastric intubation for 28 days.

Group 1(NC): Normal control were fed normal chow and water *ad libitum.*

Group 2 (negative control): Animals were given CCl_4 in olive oil only

Group 3 (CCl_4+100): Animals were given CCl_4 in olive oil + 100mg/kg fraction.

Group 4 (CCl_4+150): Animals were given CCl_4 in olive oil + 150mg/kg fraction.

Group 5(CCl_4+200): Animals were given CCl_4 in olive oil + 200mg/kg fraction

Group 6(CCl_4+Std): Animals were given CCl_4 in olive oil and silymarin (100mg/kg).

3.2.9.2 Collection and preparation of sera sample

At the end of the 28 days, the rats were weighed and sacrificed by decapitation using chloroform anaesthesia. Blood samples were collected from the head wound in plain bottles (for biochemical parameters). The blood samples collected in plain tubes were allowed to clot and the serum separated by centrifugation using Labofuge 300 centrifuge (Heraeus) at 3000 rpm for 10 minutes and the supernatant (serum) collected was subjected to biochemical analysis.

3.2.9.3 Relative organ weight

Different organs namely the liver, lungs, kidneys, spleen and heart were carefully dissected out and weighed in grams. The organ weight ratio of each animal was calculated. The relative weight was calculated as g/100 g body weight.

$$\text{Relative Organ Weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

3.2.9.4 Collection and preparation of the liver

Immediately after the blood was collected, the liver was quickly dissected out, 1g was weighed and crushed using mortar and pestle and homogenized in 10ml of 50Mm Sodium phosphate buffer (pH 7.4). This was centrifuged at 4000rpm for 10 minutes then, the supernatant was collected using pasteur pipette for endogenous antioxidant activity assay.

3.2.10 Biochemical analysis

3.2.10.1 Determination of aspartate amino-transferase (AST) activity

Serum AST activity was determined by the method described by Reitman and Frankel, (1957).

Assay principle: In this reaction L-Aspartate and α-Ketoglutarate react in the presence of AST in the sample to yield oxaloacetate and L-glutamate. The oxaloacetate is reduced by malate dehydrogenase (MDH) to yield L-malate with the oxidation of NADH to NAD. The reaction is monitored by measurement of the decrease in absorbance of NADH at 340nm. The rate of deduction in absorbance is proportional to AST activity in the sample.

L-aspartate + α-ketoglutatrate $\xleftrightarrow{AST}$ L-glutamate + oxaloacetate

Oxaloacetate + NADH + H^+ $\xleftrightarrow{MDH}$ L-malate + NAD^+

Procedure: Serum sample (0.05ml) and 1.0ml of substrate solution were mixed and absorbance was read at 1minute and thereafter at 30, 60, 90, 120secs at 340nm against the reference blank in which the sample used was distilled water. The mean change in absorbance per minute (▲A/min) was determined and result calculated. Activity was expressed as IU/L.

Calculation

Serum AST activity = (IU/L) = ▲A/min. x F.

Where F = 3376 (based on the millimolar extinction coefficient of NADH at 340nm).

3.2.12.2 Determination of alanine amino-transferase (ALT) activity

Serum ALT activity was determined by the method described by Reitman and Frankel, (1957).

Assay principle: Alanine aminotransferase (ALT) catalyzes the transamination of L-alanine to α-ketoglutarate, forming L-glutamate and pyruvate. The pyruvate formed is reduced to lactate by lactate dehydrogenase (LDH) with simultaneous oxidation of reduced NADH to NAD^+. The change in absorbance is directly proportional to the ALT activity measured at 340nm.

L-alanine + α-ketoglutatrate $\xleftrightarrow{ALT}$ L-glutamate + Pyruvate

Pyruvate + NADH + H^+ $\xleftrightarrow{LDH}$ Lactate + NAD^+

Procedure: Serum sample (0.05ml) and 1.0ml of substrate solution were mixed and absorbance was read at 1minute and thereafter at 30, 60, 90, 120 secs at 340nm. The mean change in absorbance per minute (▲A/min) was determined and result calculated. Activities were expressed as IU/L.

Calculation

Serum ALT activity = (IU/L) = ▲A/min. x F.

Where F = 3376 (based on the millimolar extinction coefficient of NADH at 340nm).

3.2.12.3 Alkaline phosphate (ALP)

Activity of alkaline phosphatase (ALP) was determined by the method described by King and Armstrong, (1980).

Assay principle: Alkaline Phosphatase in a sample hydrolyses para-nitrophenyl phosphate into paranitrophenol and phosphate, in the presence of magnesium ions. The

rate of increase in absorbance of the reaction mixture at 405nm due to liberation of paranitrophenol is proportional to the alkaline phosphatase activity.

p-Nitrophenyl Phosphate + $H_2O \xrightarrow{\text{Alkaline Phosphatase}}$ p-Nitrophenol + Posphate

Procedure: Buffered substrate (1.0ml) was added to 0.02ml of the serum sample. It was mixed well and absorbance was measured at 30, 60, 90, 120 seconds at 405nm against the reference blank (distilled water). The mean change in absorbance per minute was determined and the test results calculated.

Calculation

Serum ALP activity = (IU/L) = ▲A/min. x F

Where F = 2713 (calculated on the basis of molar extinction coefficient for p-nitrophenol and ratio of total assay volume to the sample volume).

3.2.12.4 Estimation of serum bilirubin

Serum bilirubin concentration was determined by the methods of Jendrassik and Grof, (1938).

Assay principle: Bilirubin is estimated by reacting it with diazotised sulfanilic acid obtained from sodium nitrite and sulfanilic acid solutions. Bilirubin when reacted with diazotised sulfanilic acid forms a pink colored Azo-compound. The unconjugated or free bilirubin takes longer time to react and requires caffeine as accelerator.

Procedure for Total bilirubin: Working reagent (0.1ml) was added to 0.05ml of serum sample and then mixed well. Solution 3-bilirubin (0.1ml) was added to the test sample and mixed. This was incubated for 5 minutes at room temperature and the absorbance of the test sample was read against sample blank at 546nm.

Calculation

Serum total bilirubin (mg/dl) = (Absorbance of sample-Absorbance of blank) x F (23)

Procedure for Direct bilirubin: To 1ml of normal saline, 0.05ml of serum sample was added and mixed well. 0.1ml of working reagent was added to the test sample and mixed. This was incubated for 3 minutes at room temperature and the absorbance of the test sample was read against sample blank at 546nm.

Calculation

Serum Direct bilirubin (mg/dl) = (Absorbance of sample-Absorbance of blank) x F (13)

3.2.12.5 Estimation of serum albumin

Estimation of Serum Albumin by Bromocresol Green (BCG) Method (Doumas *et al*., 1997)

Assay principle: In an acidic medium, albumin binds with bromocresol green causing a shift in the absorption spectra of the yellow BCG dye. The blue green colour formed is directly proportional to the albumin present when measured at 630 nm.

Albumin + BCG $\longrightarrow$ BCG-Albumin complex

Procedure: Albumin reagent (2.5ml) was pipetted into blank, standard and test tubes respectively. Serum sample (0.01ml) was added to the test tube while 0.01ml of standard reagent was added to standard tube. The tubes were shaken for proper mixing and allowed to stand at room temperature (not less than 25^0C) for 10minutes. The absorbance of the test and standard tubes were read after 10minutes against reagent blank at 630nm.

Calculation

$$\text{Albumin Concentration (g/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of standard}$$

3.2.12.6 Estimation of total protein

Estimation of Total protein by Biuret Method (Lowry *et al*., 1951).

Assay principle: In an alkaline medium, protein reacts with the copper in the Biuret reagent causing an increase in absorbance. The increase in the absorbance, at 540nm (530-570nm or with Green/Yellow filter) due to formation of the coloured complex, is directly proportional to the concentration of protein.

Procedure: Reagent (2.5ml) was added to 0.05ml serum sample and mixed well. It was then incubated at room temperature for 10minutes. The absorbance was read at 540nm against reagent blank.

Calculation

$$\text{Total Protein Concentration (g/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of standard}$$

3.2.12.7 Estimation of serum creatinine concentration

Serum Creatinine was determined according to the method of Bartels *et al*. (1972).

Assay principle: Creatinine present in the serum reacts with alkaline picrate to form a colored complex. The rate of formation of colored complex is directly proportional to creatinine concentration. This rate of reaction (intensity of color produced) is measured at 510 nm and is compared with that of the standard.

$$\text{Creatinine} + \text{Picric Acid} \xrightarrow{\text{alkaline medium}} \text{Creatinine picrate (coloured complex)}$$

Procedure: Creatinine working reagent (1.0ml) was pipetted in standard and test tubes respectively. Serum sample (0.1ml) was added to the test tube while 0.1ml of standard reagent was added to the standard tube. The tubes were shaken well for proper mixing and aspirated. The absorbance of standard (ST, ST1) and Test (TS, TS1) were recorded at 20secs and 80secs at 510nm, against distilled water.

Calculation

$$\text{Creatinine concentration (mg/dl)} = \frac{TS2 - TS1}{ST2 - ST1} \times \text{Concentration of standard}$$

TS1 = Test 1

TS2 = Test 2

ST1 = Standard 1

ST2 = Standard 2

3.2.12.8 Estimation of serum urea concentration

The serum urea concentration was determined by modified Berthelot's reaction method of Faweett and Scout, (1960).

Assay principle: Urease breaks down urea in to ammonia and carbon dioxide. In alkaline medium, ammonia reacts with hypochlorite and salicylate to form dicarboxyindophenol, a coloured compound. The reaction is catalysed by sodium nitroprusside. The intensity of colour produced is measured at 578nm (570-620nm).

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

Procedure: The standard reagent (1ml) was pipetted into a standard tube. Serum sample (0.01ml) was pipetted into a test tube and 0.01ml of distilled water was pipetted into blank tube. Urea working reagent E (1.0ml) was added into the blank, standard and test tubes respectively. The tubes were shaken to mix well and then incubated for 10minutes at room temperature (25-30^{0}C) for 5minutes at 37^{0}C. Working color reagent-C (1.0ml) was added to the blank, standard and test tubes respectively and mixed well then incubated for 10minutes at room temperature (25-30^{0}C) for 5 minutes at 37^{0}C. The absorbance of the test and standard were read against blank at 578nm.

Calculation

$$\text{Urea concentration (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of standard}$$

3.2.13 Determination of oxidative stress parameters

3.2.13.1 Catalase activity

Catalase activity was determined by the method described by Aebi, (1984).

Assay principle: Catalase scavenges hydrogen peroxide converting it to water and molecular oxygen. The activity of catalase in the sample will be determined by following the rate of decrease in absorbance at 240nm.

$$2H_20_2 \xrightarrow{\text{Catalase}} 2H_20 + O_2$$

Procedure: Tissue homogenate 10µL was added to 2.8ml of 50mM potassium phosphate buffer (pH of 7.0) in 3ml cuvette. The reaction was initiated by adding 0.1ml of fresh 30mM $2H_20_2$ and the decomposition rate of $2H_20_2$ was measured at 240nm for 5mins on a spectrophotometer. A molar extinction coefficient of $0.041mM^{-1}$ cm^{-1} was used to calculate the catalase activity.

3.2.13.2 Superoxide dismutase (SOD) activity

Superoxide Dismutase activity was measured using the method described by Martin *et al*. (1987).

Assay principle: Auto oxidation of haematoxylin (with increase of absorbance at 560nm) is inhibited by SOD activity at the assay pH 7.8; the percentage of the inhibition is linearly proportional to the amount of SOD present within a specific range. SOD activity in the sample is determined by measuring the amount of haematin at 560nm.

Heamatoxylin $\xrightarrow{\text{Auto-oxidation}}$ H_20 + Heamatin (active form of heamatoxylin)

Procedure: To 920µL of phosphate buffer (0.05M, pH7.8) was added 40µL of sample. A reagent test was also prepared by replacing the sample with 40µL of sample dilution buffer (0:85% NaCl). The mixture was incubated for 2 minutes at 25^0C before the addition of 40µL of haematoxylin. Following the addition of 40µL of haematoxylin, absorbance of the sample test and reagent test were read at 560nm immediately and after 5minutes against the sample blank which is distilled water.

3.2.13.3 Lipid peroxidation by measuring the malondialdehyde (MDA) level

Thiobarbituric acid reactive substance (TBARS) in the tissue was estimated using the method of Fraga *et al.* (1988).

Assay principle: The formation of malondialdehyde is the basis for the well known TBA method used for evaluating the extent of lipid peroxidation. A low pH of 2-3 and high temperature (100^0C), malondialdehyde (MDA) binds thiobarbituric acid (TBA) to form a pink complex which absorbs maximally at 532nm.

Procedure: To 0.5ml of tissue homogenate, 0.5ml saline and 1.0ml of 10% TCA were added. 0.25ml of 0.1M TBA was added to the mixture. The mixture was incubated for 1hour at 95^0C, cooled and centrifuged at 3000rpm for 20min. the absorbance of the pink colour produced was read at 535nm.

3.3 Statistical Analysis

The results are presented as mean ± standard deviation (SD). Within and between groups, comparisons were performed by the analysis of variance (ANOVA) (using SPSS 20.0 for Windows Computer Software Package). Significant differences were

compared by Duncan's new Multiple Range test; a probability level of less than 5% ($P< 0.05$) was considered significant Duncan, (1955).

CHAPTER 4

RESULT

4.1 Qualitative Screening of Phytochemicals

Phytochemical screening of the aqueous crude extract of *Detarium microcarpum* stem bark showed the presence of alkaloids, saponins, flavonoids, phenols, glycosides and tannins Tables (4.1).

4.2 Total Flavonoid / Total Phenolic Content

The aqueous extract of *Detarium microcarpum* stem bark was fractionated with ethyl-acetate and n-butanol solvent and the result of total flavonoids and phenolic content of ethylacetate and n-butanol fractions are presented in Table (4.2). The amount of total flavanoid was determined with the Quercetin reagent as a standard compound and the total flavanoid were expressed as mg/g Quercetin equivalent using the standard curve equation: $y = 0.012x + 0.001$, $R^2 = 0.962$, Where y is absorbance at 510 nm and x is total flavonoid content in the ethyl acetate and n-butanol fractions expressed in mg/g. Figure 3 shows the content of total flavonoid that were measured in terms of Quercetin equivalent. The total flavonoid varied from 45.76±2.59 in ethyl acetate fraction to 234.42±0.71 in n-butanol fraction shown Table 4.2. The total phenolic content was determined using Gallic acid as a standard compound and the result expressed as mg/g gallic acid equivalent using the standard curve equation: $y = 0.100x + 0.185$, $R^2 = 0.999$, Where y is absorbance at 750 nm and x is total phenolic content in the ethyl acetate and n-butanol fractions expressed in mg/g. Figure 4 shows the content of total phenols that were measured in terms of Gallic acid equivalent. The total phenol varied from 2.97±0.31 in n-butanol fraction to 11.54±0.20 in ethyl acetate fraction.

Table 4.1: Qualitative analysis of phytochemicals in the aqueous crude extract of *Detarium microcarpum* stem bark.

Phytochemicals	***D. microcarpum* stem bark**
Alkaloids	+
Saponins	+
Flavonoids	+
Phenols	+
Glycosides	+
Tannins	+

+ =Present

Table 4.2: Total flavonoid and Phenolic content in n-butanol and ethylacetate fractions of *Detarium microcarpum* stem bark.

Parameters	TFC(mg/g)*	TPC(mg/g)**
BF	234.42±0.71	2.97±0.31
EF	45.76±2.59	11.54±0.20

Values are means ± SD of three (3) determinations, * mg quercetin/g fraction, ** mg gallic acid/g fraction. TFC: total flavonoid content, TPC: total phenolic content, BF: butanol fraction, EF: ethyl acetate fraction

4.3 *In-vitro* Antioxidant Activity

4.3.1 DPPH radical scavenging activity

The aqueous extract of *Detarium microcarpum* stem bark was fractionated with ethyl-acetate and n-butanol solvents. Fig 5 shows % inhibition of DPPH radical scavenging activities of ascorbic acid, ethyl acetate and n-butanol fractions at different concentrations. The radical scavenging activity increased with increasing concentrations, ascorbic acid having: 29.86%, 38.93%, 61.07%, 65.90% and 71.67%, ethyl acetate fraction having: 27.15%, 32.39%, 48.41%, 56.48% and 65.31%, n-butanol fraction having: 22.14%, 34.51%, 42.34%, 57.66% and 63.55% scavenging activities for 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml fraction, respectively. DPPH radical scavenging activity of ethyl acetate fraction was considerably stronger than n-butanol fraction when compared to the standard (ascorbic acid). The lower IC_{50} value reflects to higher antioxidant activity of bark fraction. The IC_{50} value for ascorbic acid was found to be 0.58 mg/ml, ethyl acetate fraction 0.68mg/ml and n-butanol fraction 0.71mg/ml Table 4.3.

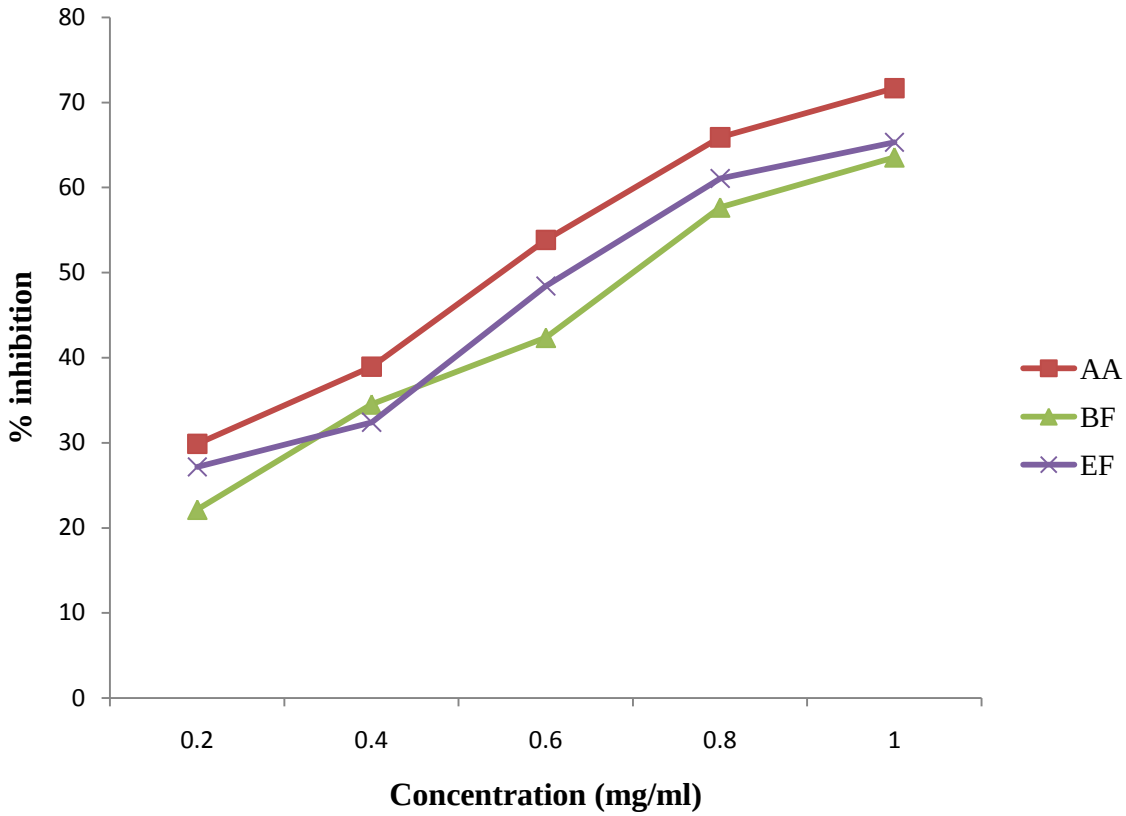


Figure 5: DPPH radical scavenging activity of ascorbic acid (AA), n-butanol fraction (BF) and ethyl acetate fraction (EF) of *Detarium microcarpum* stem bark at different concentrations.

4.3.2 Reducing ability assay

Fig 6 shows % reducing ability of ascorbic acid, ethyl acetate and n-butanol fractions at different concentrations. The % reducing ability increased with increasing concentrations, and at a concentration of 1.0mg/ml, the reducing ability of ascorbic acid, n-butanol and ethyl acetate fractions was 71.40%, 65.31% and 56.24% respectively. The Reducing ability of n-butanol was considerably stronger than that of ethyl acetate fraction when compared to the standard (ascorbic acid). The lower IC_{50} value reflects a higher antioxidant activity of bark fraction. The IC_{50} value for ascorbic acid was found to be 0.23 mg/ml, n-butanol fraction 0.30mg/ml and ethyl acetate fraction 0.53mg/ml Table 4.3.

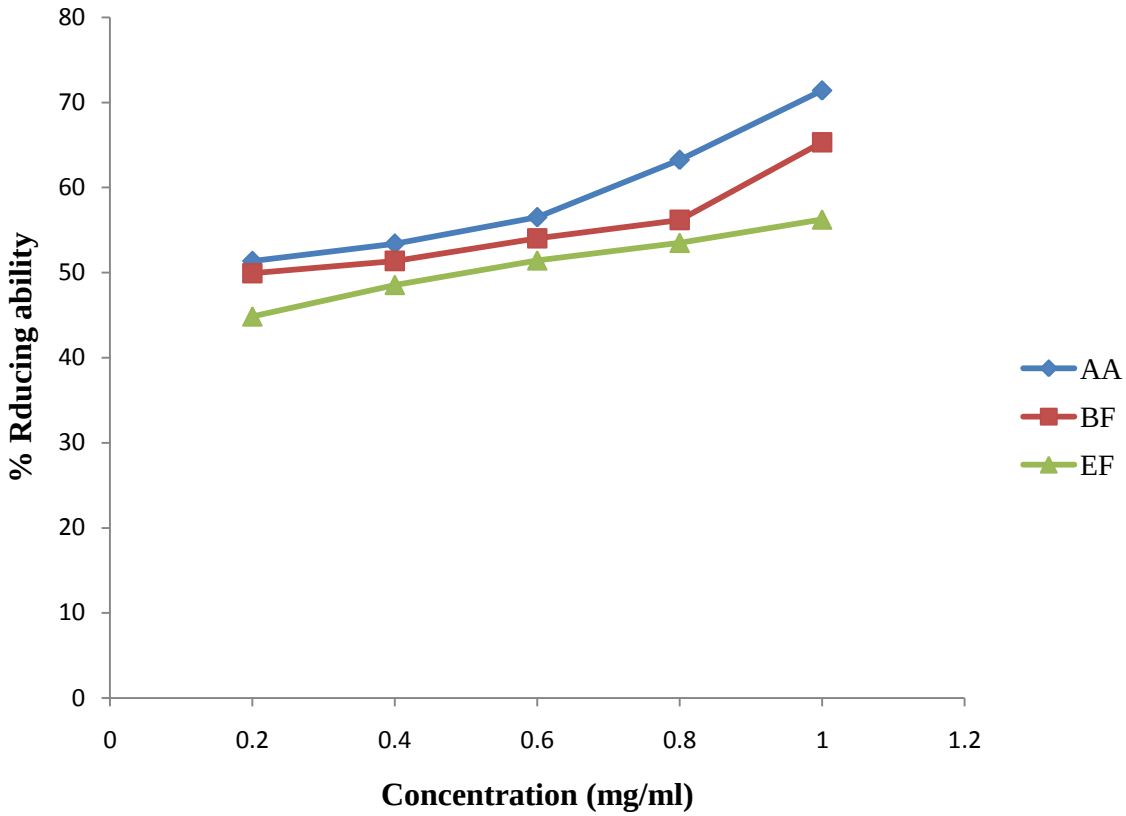


Figure 6: Reducing ability of ascorbic acid (AA), n-butanol fraction (BF) and ethyl acetate fraction (EF) of *Detarium microcarpum* stem bark at different concentrations.

4.3.3 Hydrogen peroxide (H_20_2) radical scavenging activity

Fig 7 shows % inhibition of hydrogen peroxide (H_20_2) scavenging activity of ascorbic acid, ethyl acetate and n-butanol fractions at different concentrations. The % inhibition of hydrogen peroxide (H_20_2) increased in a dose dependent manner and at a concentration of 1.0mg/ml % inhibition of hydrogen peroxide (H_20_2) of ascorbic acid, n-butanol and ethyl acetate fractions was 56.46%, 52.55% and 43.87% respectively. The % inhibition of hydrogen peroxide (H_20_2) of n-butanol was considerably stronger than that of ethyl acetate fraction when compared to the standard (ascorbic acid). The lower IC_{50} value reflects to a higher antioxidant activity of bark fraction. The IC_{50} value for ascorbic acid was found to be 0.081 mg/ml, n-butanol fraction 0.098mg/ml and ethyl acetate fraction 0.113mg/ml Table 4.3.

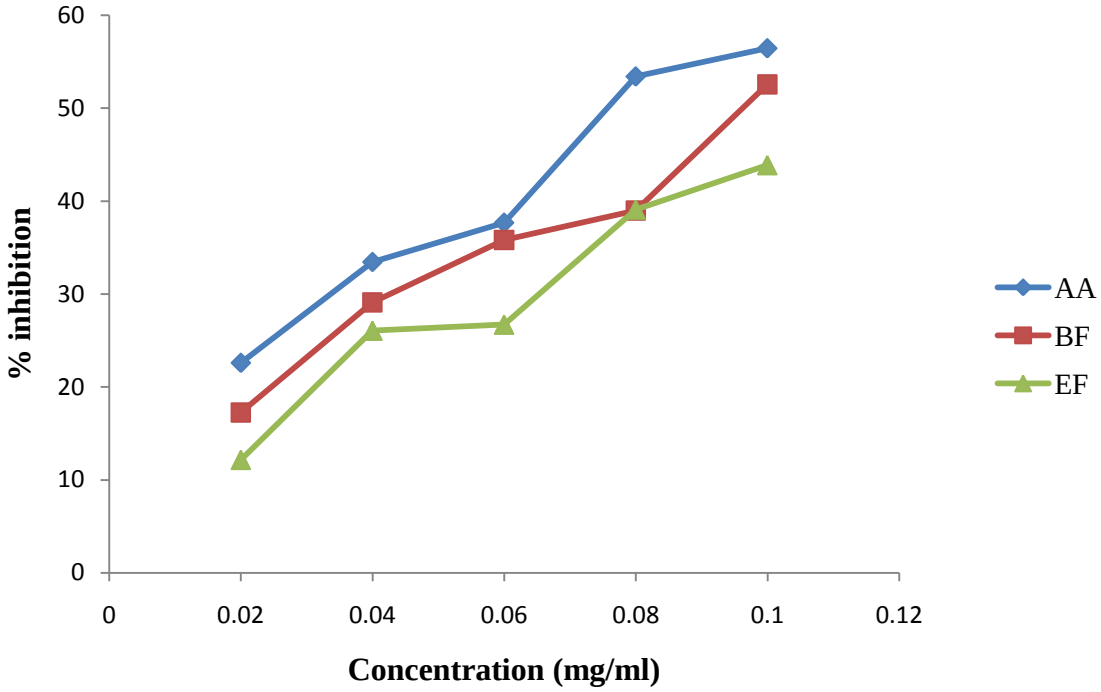


Figure 7: Hydrogen peroxide radical scavenging activity of ascorbic acid (AA), n-butanol fraction (BF) and ethyl acetate fraction (EF) of *Detarium microcarpum* stem bark at different concentrations.

Table 4.3: 50% inhibition concentration (IC_{50}) of n-butanol and ethyl acetate fractions of *Detarium microcarpum* stem bark against DPPH, Reducing power and H_2O_2 radicals.

Antioxidant radicals	50% Inhibition concentration (IC_{50})		
	BF (mg/ml)	EF (mg/ml)	AA (mg/ml)
DPPH Radical	0.71	0.68	0.58
Reducing power	0.3	0.53	0.23
H_2O_2 Radical	0.098	0.113	0.081

BF: butanol fraction, EF: ethyl acetate fraction, AA: Ascorbic acid

4.4 Lethal Dose Determination

Table 4.4 shows the lethal dose determination of n-butanol stem bark extracts of *D. microcarpum* in albino rats for 48 hours. There was no mortality within 48 hours after oral administration of 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight of the fraction to the rats in both the first and second phases respectively. Therefore, the LD_{50} value was estimated to be > 5000mg/kg

Table 4.4: Lethal dose determination of n-butanol fraction of *Detarium microcarpum* stem bark in rats for 48 hrs.

Group	Dose (mg/kg)	Number of animals	Motality	
			24hrs	48hrs
Phase 1	10	3	0	0
	100	3	0	0
	1000	3	0	0
Phase 2	1600	1	0	0
	2900	1	0	0
	5000	1	0	0

4.5 Effect of n-butanol Fraction on Body Weight / Organ Weight

4.5.1 Effect of n-butanol fraction on body weight

Table 4.5 shows the body weight change of CCl_4 treated rats with n-butanol fraction of *Detarium microcarpum* stem bark for a period of 28 days. The results showed a significant ($P<0.05$) decrease in the body weight of negative control group (from 163.75±6.55g to 145.50±14.06g) compared with normal control which had a progressive increase in body weight (from 115.00±13.08g to 142.00±19.52g). However no significant ($P>0.05$) change was observed in all CCl_4 treated groups compared to the negative control group.

Table 4.5: Effect of n-butanol fraction of *D. microcarpum* stem bark on body weight of CCl_4 induced liver damage in rats

Groups (n=5)	Mean Initial Body weight (g)	Mean Final Body weight (g)	Change in Body weight (g)
NC	115.00±13.08[a]	142.00±19.52[b]	27.00±15.89
NGC	163.75±6.55[a]	145.50±14.06[b]	19.25.±13.30
CCl_4+100	146.33±15.87[a]	153.33±24.97[a]	13.00±10.73
CCl_4+150	132.40±9.86[a]	132.60±10.92[a]	2.20±1.92
CCl_4+ 200	120.33±15.87[a]	120.00±30.95[a]	17.00±12.43
CCl_4+ Std	119.40±18.53[a]	127.40±14.40[a]	8.00±9.92

Values expressed as mean ± Standard deviation, Values with different superscripts along the row differ significantly (*P<0.05*), Std: Standard, NC: Normal Control Rats, NGC: Negative control, CCl_4+100: CCl_4 induced liver Rats+100mg/kg n-butanol fraction, CCl_4+150: CCl_4 induced liver Rats+150mg/kg n-butanol fraction, CCl_4+200: CCl_4 induced liver Rats+200mg/kg n-butanol fraction, CCl_4+Std: CCl_4 induced liver Rats +100mg silymarin.

4.5.2 Effect of n-butanol fraction on relative organ weight

Table 4.6 shows the relative organ weight (%) for liver, lung, kidney, spleen and heart. There was a significant ($P<0.05$) increase in the weight of the liver (4.8± 0.29) of negative control group compared with the normal control group (3.4±0.35). Treatment with n-butanol fraction and silymarin significantly ($P<0.05$) reduced the weight of the liver (100, 150, 200mgkg n-butanol fraction) of all CCl_4 treated groups (3.8±0.22, 3.9±0.46 and 3.9±0.25), silymarin (3.6±0.36) compared with the negative control group. There was no significant ($P>0.05$) change in the weight of Lungs, Kidney, Spleen and Heart of negative control group and CCl_4 treated group compared to normal control group.

Table 4.6: Effect of n-butanol fraction of *Detarium microcarpum* stem bark on relative organ weight (%) of CCl_4 induced liver damage in rats.

Groups (n=5)	Liver	Lung	Kidney	Spleen	Heart
NC	3.4 ± 0.35^{a}	0.9 ± 0.20^{a}	0.7 ± 0.06^{a}	0.6 ± 0.14^{ab}	0.3 ± 0.03^{ab}
NGC	4.8 ± 0.29^{d}	1.0 ± 0.19^{ab}	0.7 ± 0.04^{ab}	0.6 ± 0.10^{ab}	0.3 ± 0.02^{a}
CCl_4+100	3.8 ± 0.22^{abc}	1.0 ± 0.13^{ab}	0.7 ± 0.04^{ab}	0.5 ± 0.12^{a}	0.3 ± 0.01^{a}
CCl_4+150	3.9 ± 0.46^{bc}	0.9 ± 0.15^{a}	0.7 ± 0.07^{ab}	0.7 ± 0.11^{b}	0.4 ± 0.01^{b}
CCl_4+ 200	3.9 ± 0.25^{bc}	1.2 ± 0.36^{b}	0.8 ± 0.04^{b}	0.6 ± 0.08^{ab}	0.4 ± 0.05^{b}
CCl_4+ Std	3.6 ± 0.36^{abc}	1.2 ± 0.11^{ab}	0.8 ± 0.07^{b}	0.6 ± 0.19^{ab}	0.4 ± 0.04^{ab}

Values expressed as mean ± Standard deviation, Values with different superscripts along the row differ significantly (*P<0.05*), Std: Standard, NC: Normal Control Rats, NGC: Negative control, CCl_4+100: CCl_4 induced liver Rats+100mg/kg n-butanol fraction, CCl_4+150: CCl_4 induced liver Rats+150mg/kg n-butanol fraction, CCl_4+200: CCl_4 induced liver Rats+200mg/kg n-butanol fraction, CCl_4+Std: CCl_4 induced liver Rats +100mg silymarin.

4.5 BIOCHEMICAL PARAMETERS

4.5.1 Effect of n-butanol fraction on serum liver damage biomarkers/liver function parameters in CCl_4 induced liver damage in rats.

4.5.1.1 Serum liver damage biomarkers

The effect of n-butanol fraction of *Detarium microcarpum* stem bark on serum liver damage biomarkers of CCl_4 induced liver damage in rats is presented in Table 4.7. There was a significant ($P<0.05$) increase in serum ALT (84.00±7.31), AST (52.60±5.68) and ALP (77.60± 9.81) in negative control group compared to the normal control group having (35.80±5.45, 20.60±1.95 and 48.60±3.65) respectively. A significantly ($P<0.05$) reduced serum ALT (53.60 ±8.41, 37.80± 8.64 and 52.40±5.18), AST (30.80±4.15, 24.20± 5.89 and 26.80±4.09) and ALP (68.00 ±7.81, 56.80±8.50 and 59.80±1.79) levels were observed (in 100, 150 and 200mg/kg n-butanol fraction) of CCl_4 treated groups and silymarin (100mg/kg) treated group ALT (39.60±6.77), AST (25.00±3.16) and ALP (50.00±6.9) compared to negative control group.

4.5.1.2 Liver function parameters

The effect of n-butanol fraction of *Detarium microcarpum* stem bark on serum concentrations of total protein (TP), albumin (ALB), Direct Bilirubin (DB) and Indirect Bilirubin (IB) of CCl_4 induced liver damage in rats is shown in Table 4.8. There was a significant ($P<0.05$) increase in the level of DB (9.52±1.18) and IB (8.36±1.84) in negative control group compared to DB (4.64±0.67) and IB (5.62±1.24) in normal control group. A significant ($P<0.05$) reduction in DB (in 100,150 and 200mg/kg n-butanol fraction) having values of 5.42±0.94, 4.66±1.97, 6.46±1.80 and IB in 100,150 and 200mg/kg n-butanol fraction) having values of 5.90±1.12, 5.32±1.84 and 4.90±1.15 in CCl_4 treated groups and silymarin (100mg/kg) treated group for DB (5.72±0.27) and IB (5.88±2.10) compared with negative control group.

There was no significant (P>0.05) change in the level of TP for negative control group when compared with normal control. However there was a significant (P<*0.05*) increase in the level of TP (in 100mg/kg n-butanol fraction) CCl_4 treated group (69.80±4.60) when compared to negative control group.

There was a significant (P<*0.05*) decrease in the level of ALB in negative control group (33.00±2.94) compared with normal control group (39.20±1.64) whereas, CCl_4 treated groups had no significant (P>0.05) change compared to negative control group.

Table 4.7: Effect of n-butanol fraction of *Detarium microcarpum* stem bark on serum liver damage biomarkers of rats with CCl_4 induced liver damage.

Groups (n=5)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
NC	35.80±5.45[a]	20.60±1.95[a]	48.60±3.65[a]
NGC	84.00±7.31[c]	52.60±5.68[d]	77.60± 9.81[d]
CCl_4+100	53.60 ±8.41[b]	30.80±4.15[c]	68.00 ±7.81[c]
CCl_4+150	37.80± 8.64[a]	24.20± 5.89[ab]	56.80±8.50[ab]
CCl_4+ 200	52.40±5.18[b]	26.80±4.09[bc]	59.80±1.79[bc]
CCl_4+ Std	39.60±6.77[a]	25.00±3.16[ab]	50.00±6.9[a]

Values expressed as mean ± Standard deviation, Values with different superscripts down the column differ significantly ($P<0.05$), std: Standard, ALP: Alkaline Phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, NC: Normal Control Rats, NGC: Negative control group, CCl_4+100: CCl_4 induced liver Rats+100mg/kg n-butanol fraction, CCl_4+150: CCl_4 induced liver Rats+150mg/kg n-butanol fraction, CCl_4+200: CCl_4 induced liver Rats+200mg/kg n-butanol fraction, CCl_4+Std: CCl_4 induced liver Rats +100mg silymarin.

Table 4.8: Effect of n-butanol fraction of *Detarium microcarpum* bark on serum liver function parameters of rats with CCl_4 induced liver damage

Groups (n=5)	DB (mg/dl)	IB (mg/dl)	TP(g/dl)	ALB(g/dl)
NC	4.64± 0.67[a]	5.62±1.24[a]	63.00±3.08[a]	39.20±1.64[b]
NGC	9.52±1.18[c]	8.36±1.84 [b]	62.25±1.71[a]	33.00±2.94[a]
CCl_4+100	5.42±0.94[ab]	5.90±1.12[a]	69.80±4.60[b]	38.40±5.41[ab]
CCl_4+150	4.66±1.97[a]	5.32±1.84[a]	66.40±3.65[ab]	37.20±5.89[ab]
CCl_4+ 200	6.46±1.80[b]	4.90±1.15[a]	66.60±2.30[ab]	35.20±2.86[a]
CCl_4+ Std	5.72±0.27[ab]	5.88±2.10[a]	64.80±2.59[a]	36.00±3.81[ab]

Values expressed as mean ± Standard deviation, Values with different superscripts down the column differ significantly ($P<0.05$), std: Standard, DB: Direct Bilirubin, IB: Indirect Bilirubin, TP:Total Protein, ALB: Albumin., NC: Normal Control Rats, NGC: Negative control group, CCl_4+100: CCl_4 induced liver Rats+100mg/kg n-butanol fraction, CCl_4+150: CCl_4 induced liver Rats+150mg/kg n-butanol fraction, CCl_4+200: CCl_4 induced liver Rats+200mg/kg n-butanol fraction, CCl_4+Std: CCl_4 induced liver Rats +100mg silymarin.

4.5.2 Effect of n-butanol fraction of *Detarium microcarpum* stem bark on kidney function parameters of CCl_4 induced liver damage in rats.

Serum urea and creatinine concentrations after treatment of CCl_4 induced liver damage with n-butanol fraction of *Detarium microcarpum* stem bark is shown in table 4.9. The result showed a significant ($P<0.05$) increase in the levels of serum urea in negative control group (4.36±0.39) compared to the normal control (3.28± 0.60) and a significant ($P<0.05$) reduction in serum urea (in 150 and 200mg/kg n-butanol fraction) of CCl_4 treated groups (3.52±0.41 and 3.40±0.56) compared to the negative control group.

There was no significant ($P>0.05$) difference in creatinine concentrations of all the groups.

Table 4.9: Effect of n-butanol fraction of *Detarium microcarpum* stem bark on Kidney Function Parameters of CCl_4 induced liver damage in rats.

Groups (n=5)	Urea (mg/dl)	Creatinine (mg/dl)
NC	3.28 ± 0.60^{a}	63.00 ± 2.94^{ab}
NGC	4.36 ± 0.39^{c}	57.00 ± 2.00^{a}
CCl_4+100	4.06 ± 0.26^{bc}	61.00 ± 6.95^{ab}
CCl_4+150	3.52 ± 0.41^{ab}	63.50 ± 3.11^{ab}
CCl_4+ 200	3.40 ± 0.56^{a}	60.75 ± 6.34^{ab}
CCl_4+ Std	3.32 ± 0.36^{a}	61.00 ± 8.91^{ab}

Values expressed as mean ± Standard deviation, Values with different superscripts down the column differ significantly ($P<0.05$), std: Standard, NC: Normal Control Rats, NGC: Negative control group, CCl_4+100: CCl_4 induced liver Rats+100mg/kg n-butanol fraction, CCl_4+150: CCl_4 induced liver Rats+150mg/kg n-butanol fraction, CCl_4+200: CCl_4 induced liver Rats+200mg/kg n-butanol fraction, CCl_4+Std: CCl_4 induced liver Rats +100mg silymarin.

4.6 Oxidative Stress Parameters

4.6.1 Effect of n-butanol fraction of *Detarium microcarpum* stem bark on oxidative stress parameters of CCl_4 induced liver damage in rats.

The effect of n-butanol fraction of *Detarium microcarpum* stem bark on some oxidative stress parameters of CCl_4 induced liver damage in rats is presented in Table 4.10. There was a significant ($P<0.05$) increase in the level of malonyldialdehyde (MDA) in negative control group (2.16±0.17) compared to the normal control group (1.12±0.18) and a significant ($P<0.05$) reduction in MDA levels (in 100,150 and 200mg/kg n-butanol) of CCl_4 treated groups (1.52±0.30, 1.40±0.20, and 1.50±0.29) and silymarin (100mg/kg) treated group (1.28±0.13) respectively, compared to negative control group.

The result also revealed a significant ($P<0.05$) reduction in superoxide dismutase (SOD) activity in negative control group (1.60±0.16) compared to the normal control group (2.50± 0.22) and a significantly ($P<0.05$) increased SOD activity (in 100, 150 and 200mg/kg n-butanol fraction) having 1.98±0.15, 1.92±0.19, 2.24±0.18 and silymarin (100mg/kg) treated group having (2.30±0.46) values respectively.

Catalase (CAT) activities were significantly ($P<0.05$) reduced in negative control groups (54.80±2.78) compared to normal control group (72.80±3.27) and a significantly ($P<0.05$) increased CAT activity (in 100,150 and 200mg/kg n-butanol fraction) in CCl_4 treated group (64.00 ±1.58, 68.20±3.35 and 60.80±6.53) and silymarin (100mg/kg) treated group (68.00±3.39) respectively compared to negative control group.

Table 4.10: Effect of n-butanol fraction of *Detarium microcarpum* stem bark on oxidative stress parameters in CCl_4 induced liver damage in rats.

Groups (n=5)	MDA (µM)	SOD (U/ml)	CAT (U/ml)
NC	1.12 ± 0.18^{a}	2.50 ± 0.22^{d}	72.80 ± 3.27^{d}
NGC	2.16 ± 0.17^{c}	1.60 ± 0.16^{a}	54.80 ± 2.78^{a}
CCl_4+100	1.52 ± 0.30^{b}	1.98 ± 0.15^{bc}	64.00 ± 1.58^{bc}
CCl_4+150	1.40 ± 0.20^{ab}	2.24 ± 0.18^{bcd}	68.20 ± 3.35^{cd}
CCl_4+ 200	1.50 ± 0.29^{b}	1.92 ± 0.19^{b}	60.80 ± 6.53^{b}
CCl_4+ Std	1.28 ± 0.13^{ab}	2.30 ± 0.46^{cd}	68.00 ± 3.39^{cd}

Values expressed as mean ± Standard deviation, Values with different superscripts down the column differ significantly (*$P<0.05$*), std: Standard, MDA: Malonyldialdehyde, SOD: Superoxide dismutase, CAT: Catalase, NC: Normal Control Rats, NGC: Negative control group, CCl_4+100: CCl_4 induced liver Rats+100mg/kg n-butanol fraction, CCl_4+150: CCl_4 induced liver Rats+150mg/kg n-butanol fraction, CCl_4+200: CCl_4 induced liver Rats+200mg/kg n-butanol fraction, CCl_4+Std: CCl_4 induced liver Rats +100mg silymarin.

CHAPTER FIVE

DISCUSSION

The medicinal value of plants lies in some chemical substances that have a definite physiological action on the human body. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. The presence of phenols, flavonoids, alkaloids, saponins, glycosides and tannins were earlier reported in aqueous stem extract of *Taraxacum officinale* (Mir *et al.,* 2013).

Flavonoids contribute in different extents to the beneficial health effects due to their antioxidant potential and the modulation of multiple cellular pathways that are crucial in the pathogenesis of diseases (Williams *et al.,* 2004). The biological activities of the flavonoids are related to their antioxidant activity by various mechanisms, e.g. by scavenging or quenching free radicals, by chelating metal ions, or by inhibiting enzymatic systems responsible for the generation of free radicals (Mojzisova and Kuchta, 2001). The result from this study showed that the n-butanol fraction had significantly higher flavonoid content than the ethyl acetate fraction, Khanum et al. (2015) reported n-butanol fraction to have highest total flavonoid contents compared to other fractions.

Phenolic compounds are plant secondary metabolites, which play an important role in disease resistance (Zayachkivska *et al.,* 2005). Phenolic compounds could be a major determinant of antioxidant potentials of food items (Parr and Bolwell, 2000) and could therefore be a natural source of antioxidants. From this study, ethyl-acetate fraction had a significantly higher total phenolic content than n-butanol fraction. This is in agreement

with the report of Shah et al. (2014) that ethyl-acetate fraction had maximum quantity of total phenolic content compared to other fractions.

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical that can readily undergo reduction by an antioxidant. Because of the ease and convenience of this reaction it now has widespread use in the free radical-scavenging activity assessment (Battu and Kumar, 2012). From the results in table 4.3, both ethyl-acetate and n-butanol fractions scavenged DPPH radical in a concentration dependent manner and ethyl acetate fraction showed the highest DPPH free radical scavenging activity. The lower the IC_{50} values, the higher the scavenging potentials of the fractions. This finding is in agreement with an earlier Riaz et al. (2012) report that ethyl acetate fraction exhibited the highest percent inhibition of the DPPH radicals as compared to other fractions. This study has shown that ethyl acetate and n-butanol fractions may have proton-donating ability and could serve as free radical inhibitors.

A substance may act as an antioxidant due to its ability to reduce rective oxygen specie (ROS) by donating hydrogen atom. Besides hydrogen donation, another important mechanism by which polyphenols scavenge ROS is the electron transfer. Antioxidants with electron-donating abilities reduce ferricyanide to ferrocyanide; ferrocyanide is then quantified as Perl's Prussian Blue at 700nm (Singh and Rajini, 2004; Li *et al.* 2009). However the reducing power of ethyl-acetate fraction was weaker than that of n-butanol fraction which exhibited a stronger reducing power. Borar et al. (2011) reported that n-butanol fraction had a more prominent reducing capability than ethyl-acetate fraction. The reducing property of n-butanol fraction implies that it is capable of donating hydrogen atom in a dose dependent manner (Mazumder *et al.* 2006).

Hydrogen peroxide (H_2O_2) itself is not very reactive, but it can sometimes be toxic to the cell because it may give rise to hydroxyl radical in the cells. Thus, removal of H_2O_2 is very important for protection of food systems (Patel *et al.,* 2010). Hydrogen peroxide scavenging activities of n-butanol fraction was stronger than that of ethyl-acetate fraction. This result is in agreement with the report of Jan et al. (2013) that n-butanol fraction of *Monotheca buxifolia* fruit had higher H_2O_2 radical scavenging activity than ethyl-acetate fraction.

The oral minimum lethal dose (LD_{50}) of the n-butanol fraction of aqueous stem bark extract of *Detarium microcarpum* estimated as > 5000 suggests that the extract may have low toxicity. It was earlier established that any substance with LD_{50} estimate greater than 2000 mg/kg body weight by oral route may be considered of low toxicity and safe in humans (ASTME, 1987; Bruce, 1987). Earlier study by Palani et al., (2009), showed no mortality when a limit dose of 2000 mg/kg body weight of an extract was administered to animals orally and indicated that the extract has low acute toxicity.

A significant ($P<0.05$) decrease in body weight and significant ($P<0.05$) increase in liver weight was observed in the toxin control rats than those of the normal control group. The most widely used criteria for the toxic action of a drug in animals are reduction in body organ weights/body weight. It has been reported that increase or decrease in either absolute or relative weight of an organ after administering a chemical or drug is an induction of the toxic effect of that chemical (Orisakwe *et al*, 2003). The increase in liver weight on induction with CCl_4 was ameliorated on administering the n-butanol fraction of *D. microcarpum* and silymarin.

Hepatotoxicity was successfully induced by the administration of CCl_4 which was evident by changes in biochemical parameters and antioxidant enzymes in the liver. In this present study, there were high levels of serum liver damage biomarkers in the

experimental animals as a result of CCl_4 induction. AST and ALT are the most sensitive biomarkers used in the diagnosis of liver diseases (Pari and Kumar, 2002). During hepatic damage, cellular enzyme such as ALT, AST and ALP normally located in the cytosol leaks into the serum resulting in the elevation of their serum concentration and the increase of the liver weight and volume. In line with the above, the significant ($P<0.05$) elevation in the levels of these marker enzyme in toxin control rats when compared to the normal control, implies that damage to the liver had occurred. However, the treated groups showed a significant ($P<0.05$) reduction in the level of these enzymes when compared with the toxin control group, this is an indication of the curative potential of the fraction on CCl_4-induced hepatic damage which is in agreement with the earlier observation on curative effects of *E. massavensis* by Fahmy and Hamdi, (2011).

Bilirubin is excreted by the liver; as such injury to hepatocyte and bile duct cells or interference with the normal liver functions affects its rate of conjugation or excretion leading to accumulation of the bile acid inside the liver, which promotes further liver damage. Thus a high level of bilirubin is used as an index for liver function and bile excretion status (Usha *et al.,* 2008). The present results showed a significant ($P<0.05$) increase in the levels of total and conjugated bilirubin in toxin control group. These levels are however reduced significantly ($P<0.05$) by treatment, Thus, suggesting the enhancement of liver functions by the n-butanol fraction. This result is in line with the report on effect of *Spathodeacampanulata* by Ansah et al., (2013). The possible mechanism of action maybe associated with inhibition of CYP2E1 activity or scavenging of free radicals responsible for CCl_4 toxicity (Sunil *et al.,* 2012).

The liver is a major organ of protein synthesis and any disease in the liver can cause damage of hepatocytes with changes in protein and free amino acid metabolism leading

to decreased synthesis and increased wasting via catabolism (Yousef *et al.*, 2006; Wallace, 2007 and El-Shafey *et al.*, 2011). It is well established that CCl_4 administration causes a significant decrease in serum total protein and albumin levels (Ogeturk *et al*., 2004). Hepatic damage affects the synthetic functioning and decreases albumin production by the liver. In the present study, the non significant difference in the levels of albumin and total protein is in line with earlier investigation on *Cochlospermum tinctorium* which reported a non significant ($P>0.05$) reduction effect on protein level in CCl_4 induced hepatotoxicity which suggested that probably because the liver damage was acute and the reserve capacity of the liver coupled with the relative long half-life of these proteins did compensate for the damage (Etuk *et al*., 2009).

The kidneys are responsible for the elimination of unmodified drugs and metabolites. In addition, these organs are also capable to realize diffused biotransformation reactions. Earlier studies demonstrate that nephrotoxicity induced by chemical agents are one of the consequences of the accumulation of certain metabolites in kidneys (Sener *et al.*, 2003). The presence of increased level of urea in serum is a possible indicator of hepatic and/or kidney injuries induced through CCl_4 treatment (Ogeturk *et al.*, 2005). This result is in line with Khan and Siddique, (2012) who reported that elevation in plasma urea level can be attributed to the damage of nephron structural integrity. The serum creatinine levels of all the groups were not significantly ($p>0.05$) different from the normal control. According to Bhattacharya et al. (2005), the serum creatinine level does not rise until at least half of the kidney nephrons are damaged or destroyed.

Antioxidants play very important roles in cutaneous tissue repair as they significantly prevent tissue damage that stimulates wound healing process. The human body frequently produces reactive oxygen species (ROS) which are beneficial in small amounts. However, large amounts of these ROS are produced during increased

oxidative stress encountered in the body due to either environmental hazard, or impairment in the body metabolism due to varying disease conditions including drugs or having insufficient amount of dietary antioxidants. This situation may be dangerous and has to be curbed by exogenous supply of antioxidants as a choice of therapy or preventive measure (Tosun *et al.*, 2009). Recently, it has been reported that antioxidant properties of flavonoides from several plant extracts posses stimulatory action and exert a stimulatory action on transcription and gene expression of certain antioxidative enzymes (Sreelatha *et al*., 2009).

Catalase (CAT) is an enzymatic antioxidant widely distributed in all tissues and the highest activity is found in the red cells and liver. It is a haemoprotein containing four haeme groups, that catalyses the decomposition of H_2O_2 to water and O_2 and thus, protects the cell from oxidative damage by H_2O_2 and OH^- (Gupta, 2004). The significant ($P<0.05$) reduction in the activity of CAT activity in CAT level observed in toxin control group when compared with the normal control group revealed that exposure of the animals to CCl_4 resulted in depletion of antioxidant activities. In consonance with this present result, Szymonik-Lesuik et al. (2003) reported that CCl_4 intoxication leads to changes in antioxidant enzymes and reactive intermediates involved in the bio-activation of CCl_4 that may bind to those enzymes to prevent their activation. Administration of n-butanol fraction of *D. microcarpum* stem bark and silymarin enhanced the activity of CAT in CCl_4 treated groups, the enhancement may be to prevent the accumulation of excessive free radicals and protect liver from CCl_4 intoxication. Similar observation was reported by Khan et al. (2012). Balamurugan et al. (2009) reported that the liver cells innate ability to arouse and maintain defense against oxidant by secreting more antioxidants to overpower CCl_4. The n-butanol fraction may

have overpowered CCl_4 onslaught by suppressing the formation of ROS and protecting the antioxidant machinery.

Malondialdehyde (MDA), a stable metabolite of the free radical mediated lipid peroxidation cascade, is widely used as marker of lipid peroxidation. A significant ($P<0.05$) increase in MDA level was observed in toxin control group when compared with the normal control group. The increased MDA level suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals (Szymonik *et al.,* 2003; Liu *et al*., 2009; Kim *et al.,* 2010). Enhanced lipid peroxidation (LPO) is a measure of membrane damage as well as alteration in structure and function of cellular membranes (Halliwell *et al.,* 1995). However, CCl_4 induced elevation of MDA levels were lowered significantly ($P<0.05$) by treatment with n-butanol fraction of *D. microcarpum* stem bark when compared with toxin control group which may be due to the free radical scavenging activity of the fraction. This result is in agreement with the findings of Teselkin et al. (2000), who demonstrated that antioxidants prevent CCl_4 toxicity, particularly hepatotoxicity, by inhibiting lipid peroxidation.

Superoxide dismutase (SOD), a metallo-protein is the most sensitive enzyme index in liver injury and one of the most important enzyme in the enzymatic antioxidant defense system. It scavengers the superoxide anion to form hydrogen peroxide and oxygen, hence diminishing the toxic effect caused by this radical (Olaleye *et al*., 2010)**.** It plays an important role in antioxidative mechanisms, this enzyme acts when there is an overproduction of free radicals due to administration of CCl_4 (Srivastava and Shivanandappa, 2010). CCl_4 not only initiates lipid peroxidation but also reduces tissue SOD activities, and this depletion may result from oxidative modification of these proteins (Augustyniak *et al*., 2005). The result of the present study showed that the

activity of antioxidant enzyme SOD level was significantly ($P<0.05$) decreased in toxin control rats compared to normal control, this decrease maybe attributed to the exhaustion of these antioxidant factors in a trial to scavenge excessive production of ROS caused by the toxic effect of CCl_4 (Burk *et al.,* 2008). After administration of n-butanol fraction of *D. microcarpum* stem bark, there was a significant ($P<0.05$) increase in SOD activity in CCl_4 treated groups compared to toxin control group which suggests a diminished CCl_4 induced oxidative damage. This result is in line with earlier report by Khan et al. (2012).

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

1) The result of the preliminary phytochemical screening of the aqueous crude extract of *Detarium microcarpum* stem bark revealed the presence of alkaloids, saponins, flavonoids, phenols, glycosides and tannins.

2) The quantitative phytochemical analysis of ethyl-acetate and n-butanol fractions of *Detarium microcarpum* stem bark showed that the n-butanol fraction had significantly higher total flavonoid content while the ethyl acetate fraction had the highest total phenolic content.

3) The ethyl acetate fraction of *Detarium microcarpum* stem bark had the highest % inhibition for DPPH free radical scavenging activity while n-butanol fraction had the highest reducing ability and % inhibition for H_2O_2 free radical scavenging activity, which informed the choice of n-butanol fraction for the *in-vivo* studies.

4) The results of the biochemical study showed that administration of moderate dose of 150mg/kg n-butanol fraction was most effective ($P<0.05$) in lowering the serum liver enzymes level compared to the low dose of 100 and high dose of 200mg/kg thus, proving the curative effect of the extract at a moderate dose.

5) The effect of n-butanol fraction of *Detarium microcarpum* stem bark, significantly ($P<0.05$) reduced MDA and significantly ($P<0.05$) increased SOD and CAT in all the treated groups compared to the negative control group.

6.2 CONCLUSION

The results from this present study showed that fractions from *Detarium microcarpum* stem bark, contains phytochemicals of therapeutic interest with respect to antioxidant activities and it also reveals the curative effect of n-butanol fraction at a moderate dose of 150mg/kg body weight in effectively reducing the liver enzymes levels in CCl_4-induced liver damaged rats. This could be an indication that n-butanol fraction has the potential to attenuate oxidative stress via its antioxidant properties, hence, may be used as preventive agent against deleterious consequences of oxidative stress.

6.3 RECOMMENDATIONS

1. Further investigations are needed especially bioactivity-guided fractionation, isolation and identification of the constituents of *D. microcarpum* stem bark responsible for the observed pharmacological activities.

2. Further investigations are needed to elucidate the exact mechanism(s) of action of *D. microcarpum* stem bark in CCl_4 induced liver damage.

REFERENCE

Abbot, D. and Andrews, R.S. (1970). *An Introduction to chromatography* 2nd ed. Longman press, London. pp. 72-78.

Abd Ellah, M. (2010). Involvement of free radicals in animal diseases. *Comparative Clinical Pathology*. 19(6):615–619.

Abreu, P. and Relva, A. (2002). Carbohydrates from *Detarium microcarpum* bark extract. *Carbohydrate Research*. 337: 1663-1666.

Abreu, P.M, Martins, E.S, Kayser, O, Bindseil, K.U, Siems, K, Seemann, A and Frevert J. (1999). Antimicrobial, antitumor and antileischmania screening of medicinal plants from Guinea-Bissau. *Phytomedicine* 6 (3):187-195.

Abreu, P.M, Rosa, V.S, Araujo, E, Canda, A.B. Kayser, O, Bindseil, K.U, Siems, K and Seemann, A, (1998). Phytochemical analysis and antimicrobial evolution of *Detarium microcarpum* bark extracts. *Pharmaceutical and Pharmacology Letters* 8, 107-108.

Abulude, F.O., Onibon, V.O. and Oluwatoba, F. (2004). "Nutritional and nutritional compositions of some tree barks". *Nigerian Journal of Basic and Applied Sciences*, 13: 43-49.

Aebi, H. (1984). "Catalase in Vitro" *Methods in Enzymology* 105, 121-126.

Ahsan, M.R, Islam, K.M and Bulbul, I.J. (2009). Hepatoprotective activity of Methanol Extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. *European Journal of Scientific Research*. 37(2): 302-310.

Akah, P.A, Nworu, C.S, Mbaoji, F.N, Nwabunike, I.A and Onyeto, C.A. (2012). Genus *Detarium*: Ethnomedicinal, phytochemical and pharmacological profile. *Phytopharmacology*, 3(2) 367-375

Alexander, R.R. and J.M. Grifiths, (1993). Haemoglobin Determination by the Cyanomethaemoglobin Method. In: Alexander, R.R. and J.M. Grifiths (Eds.), *Basic Biochemical Methods.* John Wiley and Sons, New York, pp: 188-189.

Amin, A and Mahmoud-Ghoneim, D. (2009). *Zizyphus spina-christi* protects against carbon tetrachloride-induced liver fibrosis in rats. *Food and Chemical Toxicology*., 47(8): 2111-2119.

Anand, B. (1999). Cirrhosis of liver. *West L Med*.171:110-115.

Anhwange, B.A, Ajibola, V.O and Oniye, S. (2004). Chemical studies of the seeds of *Moringa oleifera* (*Lam*) and *Detarium microcarpum* (*Guill* and *Sperr*). *Journal of Biological Science.* 4(6): 711-715.

Anil, K.V, Kumar, K.V, Satish, R., Rama, T. Anil, K, Babul, D. and Samhitha, J. (2010). Hepatoprotective Effect of *Flemingia Strobilifera* R.Br. on Paracetamol induced Hepatotoxicity in Rats. *International Journal of PharmTech Research.* 2(3), 1924-1931.

Ansah, C, Dadzeasah, P.E and Asiamah, E. (2013). Aqueous stem bark extract of *Spathodeacampanulata* (p. Beauv) Modulates carbon tetrachloride Induced hepatic damage in rats. *American Journal of Pharmacology and Toxicology* 8 (1): 39-50.

Asha, V.V. and Pushpangadan, P. (1998). Preliminary evaluation of the anti-hepatotoxic activity of *Phyllanthus kozhikodianus*, *Phyllanthus maderspatensis* and *Solanum indicum*. *Fitoterapia* 59: 255-259.

ASTME, (1987). *Standard Method for Estimating Acute Oral Toxicity in Rats, American Society for Testing and Materials*, Philadelphia, 1163.

Augustyniak, A, Wazkilwicz E and Skrzydlewaka, E. (2005). Preventive action of green tea from changes in the liver antioxidant abilities of different aged rats intoxicated with ethanol. *Nutrition,* 21: 925-932.

Balamurugan, M, Parthasarathi, K, Cooper, E.L and Ranganathan, L.S (2009). Anti-inflammatory and anti-pyretic activities of earthworm extract-*Lampito mauritii* (Kinberg). *Journal of Ethnopharmacology* 121(2): 330-332.

Baranisrinivasan, P, Elumalai, E. K, Sivakumar, C, Therasa, S. V and David, E. (2009). Hepatoprotective effect of *Enicostemma littorale* blume and *Eclipta alba* during ethanol induced oxidative stress in albino rats. *International Journal of Pharmacology*. 5(4) 268–272.

Bartels, H. Bohnzer, M. and Heierli, C. (1972). Serum creatinine determination without protein precipitation. *Clinical chemistry Acta*, 37: 193-197.

Bataller, R and Brenner, D.A. (2005). Liver fibrosis. *Journal of Clinical Investigation.* 115:209–218.

Battu, G.R and Kumar, B.M. (2012). *In-vitro* antioxidant activity of leaf extract of *Aerva lanata* Linn. *International Journal of Pharmaceutical Science*, 2(4):74-78

Baynes, J.W.D and Marek H. (2005). *Medical Biochemistry*. Elsevier Mosby. pp 411-421

Bhattacharya, H, Lun, L and Gomez, R. (2005): Biochemical effects to toxicity of CCl_4 on rosy barbs (*Puntius conchonius*). *Our Nature* 3:20–25.

Bhawna, S and Kumar, S.U (2010). Hepatoprotective activtity of some indigenous plants. *International Journal of PharmTech Research*. 2(1), 568-572

Bigoniya, P, Singh, C. S and Shukla, A. (2009). A Comprehensive Review of Different Liver Toxicants Used in Experimental Pharmacology. *International Journal of Pharmaceutical Sciences and Drug Research*; 1(3): 124-135

Bodakhe, S.H and Ram, A. (2007). Hepatoprotective Properties of *Bauhinia variegata* Bark Extract. *Yakugaku Zasshi*., 127: 1503-1507.

Boham BA and Kocipai-Abyazan R. (1974).“Flavonoids and condensed tannins from leaves of Hawaiian Vaccinium vaticulatum and V. calycinium” *Pacific Science.* 48: 458-463.

Boigk, G, Stroedter, L, Herbst, H, Waldschmidt, J, Riecken, E. O and Schuppan, D. (1997). Silymarin retards collagen accumulation in early and advanced biliary fibrosis secondary to complete bile duct obliteration in rats. *Hepatology* 26(3)**:** 643-649.

Bojuwoye B.J (1996). Chronic diseases in Nigeria: An overview. *Dokita*. 23(1):1-4.

Borar, S, Punia, P and Kalia, A.N. (2011). Anitoxidant potential of n-butanol fraction from extract of *Jaminum mesnyi* Hance leaves. *Indian Journal of Experimental Biology*. 49: 39-43.

Brent, J and Rumack, B. (1993). Role of free radicals in toxic hepatic injury. II. Are free radicals the cause of toxin-induced liver injury? *Journal of Toxicology:Clinical Toxicology*. 31: 173-96.

Bruce, RD (1987). A confirmatory study of Up-and-Down Method of Acute Oral Toxicity Testing. *Fundamental and Applied Toxicology*. 8, 97 – 100.

Burk, R.F, Lane, J.M and Patal K. (2008). Relationship of oxygen and gluthathione in protection against CCl_4 induced hepatic microsomal lipid peroxidation and covalent binding in the rat. *Journal of clinical investigation*, 74; 1996-2001.

Cavin, A.L, Hay, A.E, Marston, A, Stoeckli-Evans, H, Scopelliti, R, Diallo, D and Hostettman, K (2006). Bioactive Diterpenes from the Fruits of *Detarium microcarpum. Journal of Natural Products*. 69(5): 768-773.

Chan, E.W.C, Lim, Y.Y and Omar, M. (2007). Antioxidant and antibacterial activity of leaves of Etlingera species (Zingiberaceae) in Peninsular Malaysia. *Food Chemistry*, 104 (4): 1586-1593.

Chanda, S. and Dave, R. (2009). “*In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: an overview,” *African Journal of Microbiology Research,* 3(13) 981–996.

Chun, O.K, Kim, D.O. and Lee, CY. (2003). Superoxide radical scavenging activity of the major polyphenols in fresh plums. *Journal of Agriculture and Food Chemistry* 51: 8067-8072.

Clarkson, P.M and Thompson, H.S. (2000) Antioxidants: what role do they play in physical activity and health? *American Journal of Clinical Nutrition* 72 66. (S): 637-46

Contu S. (2012). Detarium microcarpum. IUCN Red List of Threatened Species. (2).

Cook, G.C. (1980). *Liver diseases in Tropical Gastroenterology*. Oxford University Press, New York Delhi, pp. 95 – 117.

Cotran, R.S, Kumar, V and Robbins, S.L. (1994). Genetic Disorders. In: Cotran RS, Kumar V, Robbins SL. *Pathologic Basis of Disease*. Edn 5, W B Saunders Co, Philadelphia, pp143.

Crocenzi, F.A and Roma, M.G. (2006). Silymarin as a new hepatoprotective agent in experimental cholestasis: new possibilities for an ancient medication. *Current Medicinal Chemistry* 13(9)**:** 1055-1074.

Cui, C.P, Wei, P, Liu, Y, Zhang, D.J, Wang, L.S and Wu, C.T (2009). The protective role of Hepatopoietin Cn on liver injury induced by carbon tetrachloride in rats. *Hepatology Research*., 39(2): 200-206

Cullen, J.H. (2005). Mechanistic classification of liver injury. *Toxicologic Pathology* 33: 6-8.

Dalziel, J.M. 1955. *The Useful Plants of West Africa*. Crown for Oversea Government and Admin. 4 Mill Bank London, pp: 186-188.

Dehmlow, C.; Erhard, J. and de Groot, H. (1996). Inhibition of Kupffer cell functions as an explanation for the hepatoprotective properties of silibinin. *Hepatology*, 23(4) 749-754.

Djeridane, A, Yousfi, M, Nadjemi, B, Boutassouna, D, Stocker, P and Vidal, N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry.* 97:654–660.

Doumas, B.T, Watson, W and Homer G. (1997). Albumin standards and the measurement of serum albumin with bromcresol green. *Clinica Chimica Acta*. 258(1) : 21-30.

Duncan, D.B. (1955). Multiple range and multiple F-test. *Biometrics,* 11, 1–42.

Ebi, G.C and Afieroho, O.E. (2011). Phytochemical and antimicrobial studies on *Detarium microrcarpum* Guill and Perr (Caesalpiniaceae) seeds coats. *African Journal of Biotechnology* 11(3), 457-462.

Ejizu, C.I. (1986). Ofo: *Igbo ritual symbols*. Fourth Dimension publishers, Enugu, Nigeria pp 190.

El- Shafey, A.A.M, Seliem, M.M.E, El-Mahrouky, F, Gabr, W.M, and Kandil R.A. (2011). Some physiological and biochemical effects of Oshar extract and abamectin biocide on male albino rats. *Journal of American Science.* **7***(12)*: 254-261

El-Olemy, M.M, Al-Muhtadi, F.J and Afifi, A.F.A. (1994). *Experimental phytochemistry*: A laboratory manual. King Saud University Press, Saudi Arabia, 21-27.

Encyclopaedia Britannica, (2003) Inc.; www.britannica.com

Etuk, E.U, Agaie, B.M, Ladan, M.J and Garba, I. (2009). The modulatory effect of *Cochlospermum tinctorium* a rich aqueous root extract on liver damage induced by carbon tetrachloride in rats. *African Journal of Pharmacy and Pharmacology*. 3(4). 151-157.

Famhy, S.R and Hamdi, S.A.H. (2011). Curative effect of the egyptian marine *Erugosquilla massavensis* extract on carbon tetrachloride-induced oxidative stress in rat liver and erythrocytes. *European Review for Medical and Pharmacological Sciences*. 15: 303-312.

Fawcett, J.K and Scott, J.E. (1960): A rapid and precise method for the determination of urea. *Journal of Clinical Pathology*. 13:156-159.

Field, K. M, Dow, C and Michael, M. (2008). Liver function in oncology: biochemistry and beyond. *The Lancet Oncology*. 9 (11) 1092–1101.

Fraga, C. G., Leibovitz, B. E. and Tappel, A. L. (1988). Lipid peroxidation measured as thiobarbituric-reactive substances in tissue slices: characterization and comparison with homogenates and microsomes. *Free Radical Biology and Medicine*. 4:155-161.

Fraschini, F. Demartini, G. and Esposti, D. (2002). Pharmacology of silymarin. *Clinical Drug Investigation*, 22(1) 51–65.

Friedman, LS, Martin, P, Munoz, S.J. (1996). Liver Function Tests and the Objective Evaluation of the Patient with Liver disease. In: Zakin D, Boyer TD. *Hepatology*: A Textbook of Liver Disease. Edn 3, W B Saunders Co, Philadelphia, pp 791-833.

Fromenty, B. and Pessayre, D. (1995). Inhibition of mitochondrial beta-oxidation as a mechanism of hepatotoxicity. *Pharmacology and Therapeutics* 67:101–154.

Garba, S, Andrew, K and Gayus, J. (2013). Antibacterial activities of total alkaloids extracted from some medicinal plants. *Topclass Journal of Herbal Medicine*. 2(9) 217-222.

Govind, P. (2011). Medicinal plants against liver diseases. *Internatioal Journal of Pharmaceutical Research*. 2:115–121.

Gülçin, I. Alici H.A. and Cesur, M, (2005). Determination of *in-vitro* antioxidant radical scavenging activities of propofol. *Chemical Pharmarceutical Bulletin.* 53:281–285.

Gupta S.K (2004). *Drug screening methods*. First ed. New Delhi: jaypee Brothers, medical Publishers; pp 463-64.

Gutierrez-Ruiz, M.C, Gomez-Quiroz, L.E. (2007). Liver fibrosis: searching for cell model answers. *Liver International* 27:434–439.

Halliwell, B. (1995). How to characterize an antioxidant: an update. *Biochemical Society Symposia*. 61: 73-101.

Harborne, J.B (1973). Phytochemical Methods: A Guide to modern Techniques of Plant analysis. Chap,amA. And Hall London, pp 279.

Harsh, M. (2005). *Textbook of Pathology*. Edn 5, Jaypee Brothers: Medical Publishers (P) Ltd., New Delhi, pp 22-24 and 608-668.

Iful, S. (2008). Studies on the antivenorm activities of the aqueous extracts of *Paullinia pinnata* and *Detarium microcarpum*. Ph.D. Thesu University of Jos, Nigeria.

Iniaghe, O.M., Malomo, S.O and Adebayo, J.O. (2008). *Journal of Medicinal Plants Research.* 2 (10) 301-305.

Irvine, F.R. (1961). *The Woody Plants of Ghana with Special References to Their Uses*. Oxford University Press London. pp 863.

Jaeschke, H, Gores, G.J, Cederbaum, A.I, Hinson, J.A, Pessayre, D and Lemasters, J.J. (2002). Mechanisms of hepatotoxicity. *Toxicological Science*; 65: 166-176.

Jan, S, Khan, M.R, Rashid, U and Bokhari, J. (2013). Assessment of Antioxidant Potential, Total Phenolics and Flavonoids of Different Solvent Fractions of *Monotheca Buxifolia* Fruit. *Public Health Research Perspective.* 4(5): 246–254.

Jemal, A, Siegel R and Ward, E. (2007). Cancer statistics. *Cancer Journal for Clinicians*. 57:43–66.

Jendrassik, L. and Grof, P. (1938). Colorimetric Method of Determination of bilirubin. *Biochemische Zeitschrift*. 297:81-82

Jensen, J.E, Stainberg, S.E, Freese, P and Marino, E. (2004). Liver function tests. *Journal of Digestive Disorder*. 6: 1-3.

Johnson, D.E. and Kroening, C. (1998). Mechanism of carbon tetrachloride toxicity in cultured rat hepatocytes. *Pharmacology and Toxicology*., 83: 231-239.

Junnila, M, Rakho, A, Sukura, A and Lindberg, L.A. (2000). Reduction of carbon tetrachloride induced hepatotoxic effects by oral administration of betaine in male hans-wistar rats: a morphometric histological study. *Veterinary Pathology*. 37: 231-238

Kaboré, C. (2005*). Aménagement des forêts au Sahel–Point sur vingt années de pratiques au Burkina Faso. Ouagadougou, Burkina Faso. Ministère de l'Environnement et de l'Eau.*

Kalava, S.V. and Menon, S.G. (2012). Protective efficacy of the extract of *volvariella volvacea* (bulliard ex fries) singer. against carbon tetrachloride induced hepatic injury. *International Journal of Pharmaceutical Sciences and Research*, Vol. 3(8): 2849-2856.

Kaplan, M.M. (1993). Laboratory tests. In: Schiff L, Schiff ER. *Diseases of the Liver*. Edn 7, J B Lippincott, Philadelphia, pp 108-144.

Kaplowitz, N.T.Y, Simon, F.R and Stolz, A. (1986). Drug induced hepatotoxicity. *Annals of Internal Medicine.* 104: 826-839.

Keay, R.W.J, Onochie C.F.A, and Standfield, D.P (1964). *Nigeria Trees* vol. 1, Dept. of Forest Research, Ibadan Nigeria.

Khan, M.R and Siddique, F. (2012). Antioxidant effects of *Citharexylum spinosum* in CCl_4 induced nephrotoxicity in rat. *Experimental and Toxicologic Pathology*. 64:349-55.

Khan R.A, Khan M.R and Sahreen S. (2012). CCl_4–induced hepatotoxicity: Protective effect of rutin on p53, CYP2E1 and the antioxidantive status in rats. *BMC Complementary and Alternative Medicine*, 12:178.

Khan, M.R., Rizvi, W., Khan, G.N., Khan, R.A and Shaheen, S. (2009): Carbon tetrachloride-induced nephrotoxicity in rats: protective role of *Digera muricata*. *Journal of Ethnopharmacology*. 122: 91-99.

Khanum, R, Mazhar, F and Jahangir, M. (2015). Antioxidant evaluations of polar and non-polar fractions of *Cajanus cajan* seeds. *Journal of Medical Plants and Research*. 9(6) 193-198.

Kim, H.Y, Kim, J.K, Choi, J.H, Jung, J.Y, Oh, W.Y, Kim, D.C and Lee, S.M (2010). Hepatoprotective effect of pinoresinol on carbon tetrachloride-induced hepatic damage in mice. *Journal of Pharmacology and Science*., 112(1):105-112.

King, E.J and Amstrong, A.R, (1980). Method of King and Amstrong In; Varley, H, Gowenlock, AH, Bell, M. *Practical Clinical Biochemisty*, 5th Edn., William Heinmann Medical Books Ltd, London. Pp 897.

Klaunig, J.E, Kamendulis, L.M (2004). The role of oxidative stress in carcinogenesis. *Annual Review of Pharmacology and Toxicology.* 44:239-267.

Kmiec, Z. (2001) Cooperation of liver cells in health and disease. *Advances in Anatomy, Embryology and Cell Biology*, 161, III-XIII, 1-151.

Kouyaté, A.M and Lamien, N. (2011). "*Detarium microcarpum*, sweet detar," Conservation and Sustainable Use of Genetic Resources of Priority Food Tree Species in sub-Saharan Africa. *Biodiversity International* 4: 4.

Kouyaté A.M and van Damme P. (2006). "Medicinal plants/Plantes médicinales: Detarium microcarpum Guill. & Perr." *Prota* 11 (1).

Kouyate, AM (2005). *Aspects ethnobotaniques et etude de la variability morphologique, biochimique et phenologique de Detarium microcapum.* Guill and perr. Au Mali. PhD thesis faculty of Agriculture and Applied Biological Sciences. University of Gent. Gent. Belguim pp. 207.

Lewis, J.H. (2000). Drug-induced liver disease. *Medical Clinics North America* 84:1275–1311.

Li ,X, Wu, X and Huang, L. (2009). Correlation between antioxidant activities and phenolic contents of Radix *Angelicae sinensis* (Danggui). *Mol*ecule 14, 5349-5361.

Lim, H.K, Kim, H.S, Choi, H.S, Oh, S, Jang, C.G and Choi, J. (2000). Effects of acetylbergenin against D-galactosamine induced hepatotoxicity in rats. *Pharmacological Research* 42:471.

Liston, H.L, Markowitz, J.S and Devane, C.L. (2001). Drug glucuronidation in clinical psychopharmacology. *Journal of Clinical Psychopharmacology* 21(5)**:** 500-515.

Liu, J, Tan, H, Sun, Y, Zhou, S, Cao, J and Wang F (2009). The preventive effects of heparin-superoxide dismutase on carbon tetrachloride-induced acute liver failure and hepatic fibrosis in mice. *Molecular and Cellular Biochemistry*., 327(1-2): 219-228.

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of Toxicology*. 54, 275

Lowry, O.H, Rosebrough, N.J, Farr, A.L and Randall, R.J. (1951). Protein measurement with the Folinphenol reagent. *Journal of Biology and Chemistry.*, *193*, 265–267.

Mahmood, N, Pizza, C, Aquino, P, de Tommasi, N, Piacente, S, Colman, S, Buma, A and Itay, A.J. (1993). Inhibition of HIV infection by flavonoids. *Antiviral Research* 22, 189-199.

Manoj, B. and Aqued, K. (2003). Protective effect of *Law sonalba* L. against CCl_4-induced hepatic damage in albino rats. *Indian Journal of Experimental Biology.* 4:85-87.

Marina, N. (2006). Hepatotoxicity of antiretrovirals: incidence, mechanisms and management. *Journal of Hepatology*. 44 (1) S132–S139.

Martin, J.P, Dailey, M and Sugarman, E. (1987). Negative and positive assays of superoxide dismutase based on hematoxylin autooxidation. *Archives of Biochemistry and Biophysics* 255 329–336.

Mathew, S and Abraham, T. (2006). *In-vitro* antioxidant activity and scavenging effects of *Cinnamomum verum* leaf extract assayed by different methodologies. *Food and Chemical Toxicology* 44: 198-206.

Mazumder, U.K, Gupta, M, Chakraborty, G and Monda, S.K. (2006). *In-vitro* antioxidant activity of *Diospyros mamabarica* Kostel bark. *Indian Journal of Experimemtal Biology*. (44) 39-44.

McGrath, L.T, McGleenon, B.M, Brennan, S. McColl, D, McIlroy, S. and Passmor, A.P (2001). "Increased oxidative stress in Alzheimer's disease as assessed with 4-hydroxynonenal but not malondialdehyde," *Quarterly Journal of Medicine*. 94(9) 485–490.

Medina, J and Moreno-Otero, R. (2005). Pathophysiological basis for antioxidant therapy in chronic liver disease. *Drugs*, 65(17):2445-2461.

Mena, S. Ortega, A and Estrela, J.M. (2009). "Oxidative stress in environmental-induced carcinogenesis," *Mutation Research*, 674(1-2) 36–44.

Mir, M.A, Sawhney, S.S and Jassal, M.M.S (2013). Qualitative and quantitative analysis of phytochemicals of Taraxacum officinale. *Wudpecker Journal of Pharmacy and Pharmocology.* 2(1) 001 – 005.

Mojzisova, G and Kuchta, M. (2001). Dietary flavonoids and risk of coronary heart disease. *Physiological Research*. 50: 529-535.

Muller, D, Enderle, G.J, Low, O, Dietze, E and Krell, H. (1996). Bile ductular proliferation and altered leukotriene elimination in thioacetamide-induced fibrosis of rat liver. *Journal of Hepatology* 25:547–553.

Navarro, V.J and Senior, J.R. (2006). Drug-related hepatotoxicity. *New England Journal of Medicine*. 354:731-9.

Nazarian, R. M., Van Cott, E. M., Zembowicz, A and Duncan, L.M. (2009). Warfarin-induced skin necrosis. *Journal of the American Academy of Dermatology* 61, 325-332.

Nebert, D.W and Dalton, T.P (2006). The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. *Nature Reviews Cancer*, 6, 947 60.

Nebert, D.W and Gonzalez, F.J. (1987). P450 genes: structure, evolution, and regulation. *Annual Review of Biochemistry*, 56, 945-93.

Njoku, O.U, Obioma, U and Frank, E.U (1999). Investigation on some nutritional and toxicological properties of *Afzelia africana* and *Deterium microcarpum* seed oil. *Bolle Chimic Farmaceutico* 138, 165-168.

Nunes, P.X, Silva, S.F, Guedes, R.J and Almeida S. (2012). Biological oxidations and antioxidant activity of natural products, Phytochemicals as nutraceuticals - Global Approaches to Their Role in Nutrition and Health. Croatia: Tech publishers. pp 1-20.

Obdoni, B.O and Ochuko, P.O. (2001). "Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria" *Global Journal of Pure and Applied Science*. 8: 203-208

Obun, C.O. Yahaya, S.M, Kibon, A.A. Olafadehan, O.A. and Alison, S.D. (2010). Evaluation of *Detarium microcarpum* pulp meal as food ingredient in rabbits diets. *Electronic Journal of Environmental, Agriculture and Food Chemistry,* 9 (2) 308 – 314.

Ogasawara, J, Watanabe-Fukunaga, R and Adachi, M**.** (1993). Lethal effect of the anti-Fas antibody in mice. *Nature* 364:806–809.

Ogeturk, M, Kus, I, Colakoglu, N, Zararsiz, I, Ilhan, N and Sarsilmaz, M. (2005): Caffeic acid phenethyl ester protects kidneys against carbon tetrachloride toxicity in rats. *Journal of Ethnopharmacology.* 97,273-280.

Ogeturk, M, Kus, I, Kavakli, A, Zararsiz, I, Ilhan, N and Sarsilmaz, M. (2004). Effect of melatonin on carbontetrachloride induced changes in rat serum. *Journal of Physiology and Biochemistry.* 60 (3) 205–210.

Okolo, C.E, Akah, P.A and Uzodinma, S.U. (2012). Antidiabetic activity of root extract *of Detarium microcorpum.* (Fabiacase) Quill & Perr. *Phytopharmacology* 3 (1), 12-18.

Okorie, O, Okonkwo, T.J.N, Nwachukwu, N. and Okeke I. (2010). Potentials of *Detarium microcarpum* (guill and sperr) seed oil as a matrix for the formulation of haloperidol injection. 5(1).

Okorie, O. (2004). Some *Physicochemical and in vitro Bio-adhesive Properties of Defatted Detarium microcarpum* Guill and Sperr (Family:Caesalpinaeceae) Gum. Ph. D. Thesis (Unpublished), Department of Pharmaceutical Technology and Industrial Pharmacy, University of Nigeria, Nsukka, Enugu State, Nigeria.

Olajire, A. A and Azeez, L (2011). Total antioxidant activity, phenolic, flavonoid and ascorbic acid contents of Nigerian vegetables. *African Journal of Food Science and Technology*. 2(2) 022-029.

Olaleye, M, Afolabi, C, Adebayo, A, Ogunboye, A and Akindahunsi A, (2010). Antioxidant activity and hepatoprotective property of leaf extracts of *Boerhaavia diffusa Linn* against acetaminophen-induced liver damage in rats, *Food and Chemical Toxicology*. 48: 2200-2205

Olugbuyiro, J.A.O and Akinbohun, O.F (2012). *In-vitro* activity of *Bryophyllum pinnatum* and *Detarium microcarpum* plants against *Mycobacterium tuberculosis* and other bacteria. *Natural Products Research Bulletin* 1: 012-018.

Olugbuyiro, J.A.O, Moody, J.O and Hamann, M.T. (2012). *In-vitro* activity of *Bryophyllum pinnatum* and *Detarium microcarpum* plants against *Mycobacterium tuberculosis* and other bacteria. *Natural Products Research Bulletin* 1: 012-018.

Olugbuyiro J.A.O, Moody, J.O and Hamana, M.T. (2009). Inhibitory activity of *Detarium microcarpum* extract against hepatitis C virus. *African Journal of Biomedical Research* 12(2), 149- 151.

Omoregie, E.S. and Osagie, A.U. (2011). Effect of *Jatropha tanjorensis* leaves supplement on the activities of some antioxidant enzymes, vitamins and lipid peroxidation in rats. *Journal of Food Biochemistry,* 35(2): 409-424.

Orisakwe, O.E, Obi, E, Udemezue, O.O and Orish, V.N. (2003) Effect of halofantrine on testicular architecture and testosterone level in guinea pigs. *European Bulletin of Drug Research.,* 11(4) 105-109.

Otu, A.A (1987). Hepatocellular carcinoma, hepatic cirrhosis and hepatitis B virus infection in Nigeria. *Cancer*. 60(10): 2581 - 2585

Oyaizu, M., (1986). Studies on products of browning reaction: Antioxidantive activity of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*, 44: 307-315.

Ozturk, F, Ucar, M, Ozturk, I.C, Vardi, N and Batcioglu, K. (2003). Carbon tetrachloride-induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. *Urology*, 62(2): 353-356.

Palani, S, Raja, S, Venkadesan, D, Karthi, S, Sakthivel, K and Senthil, K.B. (2009). Antioxidant activity and hepatoprotective potential of *Terminalia pallid*. *Archives of Applied Science and Research*. 1 (1) 18–28.

Pari, L and Kumar, N.A. (2002). Hepatoprotective activity of *Moringa oleifera* on antitubercular drug-induced liver damage in rats. *Journal of Medical Foods.* 5: 171-177.

Parr, S. and Bolwell, G (2000). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of Science of Food and Agriculture*., 80: 985-1012.

Patel, A, Patel, A, Patel, A, Patel, N.M. (2010). Estimation of Flavonoid, Polyphenolic Content and *in-vitro* Antioxidant Capacity of leaves of *Tephrosia purpurea* Linn. (Leguminosae). *International Journal of Pharmaceutical Science and Research*. 1(1) 66-77.

Patel, T, Roberts, L.R, Jones, B.A and Gores, G.J. (1998). Dysregulation of apoptosis as a mechanism of liver disease: an overview. *Seminars in Liver Disease* 18(2)**:** 105-114.

Pessayre, D, Berson, A, Fromenty, B and Mansouri, A. (2001). Mitochondria in steatohepatitis. *Seminar in Liver Disease.* 21:57–69.

Post-White, J, Ladas, E.J and Kelly, K.M. (2007). Advances in the use of milk thistle (*Silybum marianum*). *Integrative Cancer Therapies* 6(2)**:** 104-109.

Proskuryakov, S.Y, Konoplyannikov, A.G and Gabai, V.L. (2003). Necrosis: a specific form of programmed cell death? *Experimental Cell Research* 283, 1-16.

Raffray, M and Gerald M.C. (1997). Apoptosis and necrosis in toxicology: A continuum or distinct modes of cell death? *Pharmacology & Therapeutics* 75, 153-177.

Rao, C.V, Ojha, S.K, Radhakrishnan, K, Govindrajan, R, Rastogi, S, Mehrotra, S and Pushpangadan, P. (2004). Antiulcer activity of *Utleria saclicifolia* rhizome extract. *Journal of Ethnopharmacology*. 91: 243-249.

Reitman, S and Frankel A. (1957). Colorimetric method for determination of serum glutamate oxaloaectate and glutamic pyruvate transaminase. *American Journal of Clinical Pathology*. 28: 56-58.

Riaz, T, Abbasi, M.A, Aziz, U.R Shahzadi, T, Ajaib, M and Khan K.M. (2012). Phytochemical screening, free r*adical scavenging, antioxidant* activity and phenolic content of *Dodonaea viscose* Jacq. *Journal of Serberia Chemical Society* 77 (4) 423–435

Rice, E. (2004). Flavonoids and isoflavones: absorption, metabolism and bioactivity. *Free Radical Biology and Medicine*. 36: 827-828.

Rodriguez-Antona, C and Ingelman-Sundberg, M. (2006). Cytochrome P_{450} pharmacogenetics and cancer. *Oncogene* 25, 1679-91.

Ross, A.W and Wilson, K.J.W (2005). *Anatomy and Physiology in Health and illness*, Ninth Edition, published by Elsevier Ltd., 307-310, 333-336.

Saleem, T, Chetty, C, Ramkanth, S, Rajan, V, Kumar, K and Gauthaman, K. (2010). Hepatoprotective Herbs–A Review. *International Journal of Research in Pharmaceutical Science*. 1:1-5.

Saller, R. Melzer, J. Reichling, J. Brignoli, R. and Meier, R. (2007). An updated systematic review of the pharmacology of silymarin. *Forschende Komplementmedizin.* 14(2) pp. 70- 80.

Samir, S. (2001). Hepatotprotective Natural Products. *Journal of Advance Pharmaceutica Research and Bioscience*. 2(5), 110-111

Savadogo, P.., Tigabu, M.., Sawadogo, L and Odén, P.C. (2007). Woody species composition, structure and diversity of vegetation patches of a Sudanian savanna in Burkina Faso. *Bois et Forêts des Tropiques* 294(4): 5–20.

Şener, G, Şehirli, A.Ö and Ayanoğlu-Dülger, G. (2003). Protective effects of melatonin, vitamin E, and N-acetylcysteine against acetaminophen toxicity in mice: a comparative study. *Journal of Pineal Research.* (35) 61-8

Shah, N.A, Khan, M.R, Sattar, S, Ahmad, B and Mirza, B. (2014). HPLC-DAD analysis, antioxidant potential andanti-urease activity of A*sparagus gracilis* collected from District Islamabad. *BMC Complementary and Alternative Medicine,* 14:347.

Sharma, M.P and Ahuja, V. (1997). Amoebic liver abscess: Clinicians perspective. *Bombay Hospital Journal* 39, 615-619.

Sherlock, S.D. and James, D. (2002) *Diseases of the liver and biliary system*. Blackwell Publishing. pp 26-34.

Singh, N, Kamath, V, Narasimhamurthy, K and Rajini, PS. (2008). Protective effects of potato peel extract against carbon tetrachloride-induced liver injury in rats. *Journal of Environmental Toxicology and Pharmacology*. 6:242–246.

Singh, N and Rajini, P.S. (2004). Free radical scavenging activity of an aqueous extract of potato peel. *Food Chem*istry 85, 611-616.

Smith, G, Stubbins, M.J, Harries, L.W and Wolf, C.R. (1998). Molecular genetics of the human cytochrome P_{450} monooxygenase superfamily. *Xenobiotica* 28: 1129-1165.

Sofowora, A (1993). Medicinal Plants and Traditional Medicine in Africa. Chichester John Wiley and sons New York, pp. 97-145.

Soni, M, Mohanty, P.K and Jaliwala, Y.A. (2011). Hepatoprotective activity of fruits of *Prunusdomestica*. *International Journal of Pharmaand Bioscience*; 2(2): 439-453.

Sreelatha, S, Padma, R and Umadevi, M. (2009). "Protective Effects of *Coriandrum sativum* Extracts on Carbontetrachloride induced Hepatotocity in Rats," *Food and Chemical Toxicology*. (47) 702-708.

Srivastava, A and T. Shivanandappa, (2010). "Hepatoprotective Effect of the Root Extract of *Decalepishamiltonii* against Carbon Tetrachloride-Induced Oxidative Stress in Rats," *Food Chemistry*, 118 (2) 411-417.

Sunil, M, Kunduri, R.D, Sashi, B.B and Jyotirmoyee J. (2012). Protective and curative effect of poly herbal formulation containing indigenous medicinal plants against various hepatotoxic agents in rats. *Asian Pacific Journal of Tropical Biomedicine* (2012) S1377 – S1381.

Szymonik-Lesiuk, S, Czechowska, G, Stryjecka-Zimmer, M, Słomka, M, Madro, A, Celiński, K and Wielosz M (2003). Catalase, superoxide dismutase, and glutathione peroxidase activities in various rat tissues after carbon tetrachloride intoxication. *Journal of Hepatobiliary Pancreatic Surgery*., 10(4): 309-315.

Tatiya, A.U, Surana, S.J, Sutar, M.Pand Gamit, N.H. (2012). Hepatoprotective effect of poly herbal formulation against various hepatotoxic agents in rats. *Pharmacognosy Research* 4:50-56.

Teselkin, Y.O, Babenkova, I.V, Kolhir, V.K, Baginskaya, A.I, Tjukavkina, N.A, Kolesnik, Y.A and Eichholz, A.A. (2000). Dihydroquercetin as a means of antioxidative defence in rats with tetrachloromethane hepatitis. *Phytotherapy Research.* 14 (3): 160.

Tortora, G.J and Grabowski, S.R (2003). *Principle of Anatomy and Physiology.* (10), 875- 879.

Tosun, M, Ercisli, S, Sengul, M, Oezr, H, Polat, T and Ozturk, E. (2009). Antioxidant properties and total phenolic content of *salvia* species from Turkey. *Biological Research.* 42, 175-181.

Trease, G.E, Evans, and W.C. (1989). A Textbook of Pharmacognosy. 13th edn. Bailliere Tindall Ltd.: London. pp 60-75.

Ujah, O. F, Ujah, I. R, Johnson, J.T and Oka, V.O. (2013). Effect of ethanolic leaf extract of *Moringa olifera* leaf on haematological and biochemical parameters of wistar rats. *Journal of Natural Product and Plant Resources*. 3 (2):10-14.

Usha, K., Mary, K.G and Hemalatha, P. (2008). Hepatoprotective effect of *Hygrophila spinosa* and *Cassia occidentalis* on carbon tetrachloride induced liver damage in experimental rats. *Indian Journal of Clinical Biochemistry*, 22(2): 132-135.

Valko, M. Rhodes, C.J, Moncol, J, Izakovic, M and Mazur M. (2006). "Free radicals, metals and antioxidants in oxidative stress-induced cancer," *Chemico-Biological Interactions*, 160 (1) 1–40,

Valko, M. Leibfritz, D. Moncol, J. Cronin, M.T.D, Mazur, M. and Telser, J. (2007). "Free radicals and antioxidants in normal physiological functions and human disease," *International Journal of Biochemistry and Cell Biology*, 39 (1) 44–84.

Van-Burden, T and Robinson, W. (1981). Formation of complexes between protein and Tannin acid. *Journal of Agriculture and Food Chemistry*. 1: 77.

Vautier, H, Sanon, M and Moctar, S, (2007). SEED LEAFLET No. 122. Forest & Landscape Denmark. Hørsholm Kongevej 11. DK-2970 Hørsholm. (www.SL.ku.dk). Accessed August 26, 2010, at 15:37.

Vinson, J.A and Zhang, J. (2005). Black and Green tea equally inhibit diabetic cataracts in a Streptozotocin-induced rat model of Diabetes. *Journal of Agriculture and Food Chemistry.* 53, 3710 – 371.

Wallace, A.H. (2007). *Principles and Methods of Toxicology*. Healthcare USA, Inc., New York, 5th ed., pp. 369-453.

Wannes, W.A, Mhamdi, B, Sriti, J, Jemia, M.B, Ouchikh, O, Hamdaoui, G, Kchouk, M.E and Marzouk, B. (2010). Antioxidant activities of the essential oil and methanol extracts from myrtle (*Myrtus communis* var. italica L.) leaf, stem and flower. *Food and Chemistry Toxicology*, 48:1362–1370.

Waugh, A, Grant, A, Ross, A.W and Wilson, K.J.W (2001). *Anatomy and Physiology in Health and Illness*. Edn 9, Churchill Livingstone, Spain, pp 307-311.

Werck-Reichhart, D and Feyereisen, R. (2000). Cytochromes P450: a success story. *Genome Biology*. 1(6): 3003.1-3003.9

WHO (2001). *Legal Status of Traditional Medicine and Complementary/ Alternative medicine*: A world wide review. WHO Publishing 1, Geneva.

Williams, R.J, Spencer J.P and Rice-Evans, C. (2004). Flavonoids: Antioxidants or signalling molecules? *Free Radical Biology and Medicine*., 36: 838-849.

Wink, M. (1999). *Introduction to Biochemistry, role and biotechnology of secondary products.* In: M Wink, Ed, and Biochemistry of secondary product Metabolism. CRC Press, Boca Ratom, FL;PP. 1-16

www.westafricanplants.senckenberg.de/images/pictures/detarium_microcarpum_ms_2151_552_ff9925.jpg. Accessed May 18, 2015, at 6:30.

Yousef, M.I, Awad, I.T and Mohamed, E.H. (2006). Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. *Toxicology*, 227*(3)*:240-247.

Zatloukalova, J. (2008). *Deregulation of cell proliferation and apoptosis by xenobiotics and cytostatics* (Unpublished P.hD thesis). Masarykova Univerzita, Czech Republic.

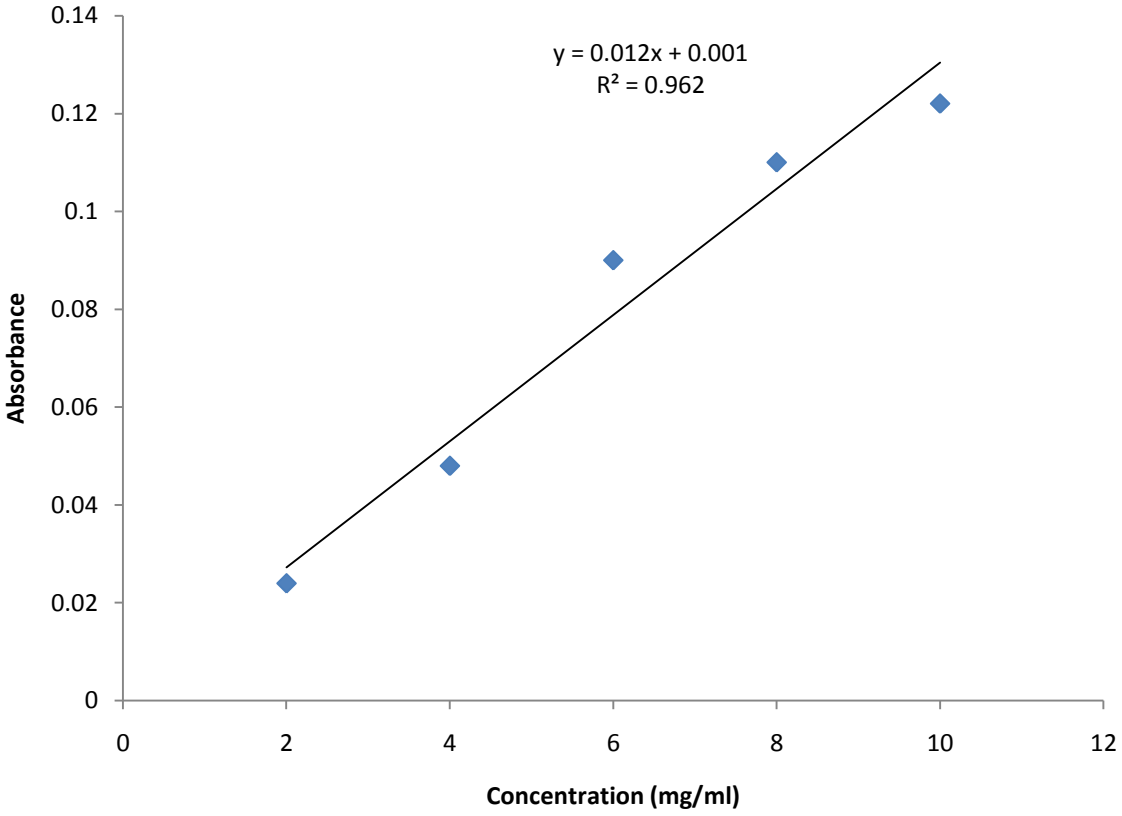
Zayachkivska, O.S, Konturek, S.G, Drozdowicz, D, Konturek, P.C, Brzozowski, T and Ghegotsky, M.R. (2005). Gastroprotective effects of flavonoids in plant extracts. *Journal of Physiology and Pharmacology.*, 56: 219-231.

Zengin, G, Cakmak, Y.S, Guler, G.O and Aktumsek, A. (2011). Antioxidant properties of methanolic extract and fatty acid composition of *Centaurea urvillei* DC. subsp. hayekiana Wagenitz. *Records of Natural Products* , 5:123–132.

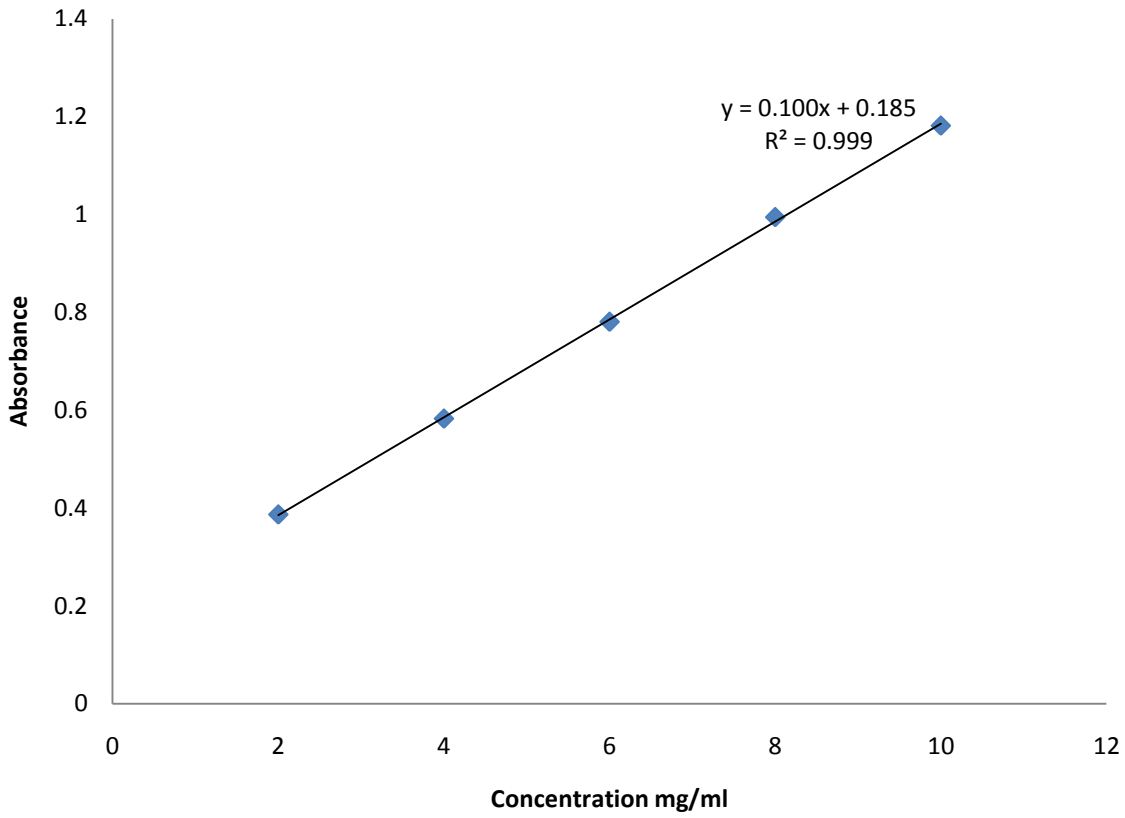
Zhao, J. Lahiri-Chatterjee, M, Sharma, Y and Agarwal, R. (2000). Inhibitory effect of a flavonoid antioxidant silymarin on benzoyl peroxide-induced tumor promotion, oxidative stress and inflammatory responses in SENCAR mouse skin. *Carcinogenesis* 21(4)**:** 811-816.

Zimmerman, H.J. (1999). *Hepatotoxicity*: The Adverse Effect of Drugs and other Chemicals on the Liver. 2nd Ed. Philadelphia: Lippincott, Williams & Wilkins. pp 427-456.

APPENDICES



Appendix I: Standard curve for Qucertin



Appendix II: Standard curve for Gallic acid.