

**BIOCHEMICAL EVALUATION OF *TULBAGHIA VIOLACEA* HARV. RHIZOMES
IN DIET INDUCED HYPERCHOLESTROLEMIC RATS**



University of Fort Hare
Together in Excellence

A THESIS

“Submitted in fulfilment of the requirements for the degree of

PHILOPHIAE DOCTOR (PhD BIOCHEMISTRY)”

**DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY,
FACULTY OF SCIENCE AND AGRICULTURE,
UNIVERSITY OF FORT HARE,**

By

OLORUNNISOLA, OLUBUKOLA SINBAD

SUPERVISOR: PROF AJ AFOLAYAN

CO-SUPERVISOR: PROF G BRADLEY

MAY, 2012

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DEDICATION

This thesis is dedicated to the Lord God Mighty Jesus Christ.

DECLARATION

I, the undersigned, declare that this dissertation submitted to the University of Fort Hare for the award of degree of Philosophiae Doctor (PhD Biochemistry) in the Faculty of Science and Agriculture, School of Biological and Environmental Science contains my independent work and has not been submitted for any degree at any other University in partial or entirely for the award of any degree. I further declare that all sources cited are indicated and acknowledged by means of comprehensive list of references.

Name: OLORUNNISOLA OLUBUKOLA SINBAD.....

Signature:.....

Date:

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TABLE OF CONTENTS

DEDICATION.....	iii
DECLARATION	iv
ACKNOWLEDGMENT	v
TABLE OF CONTENT	vi
LIST OF TABLES.....	xv
LIST OF PLATES.....	xv
LIST OF FIGURES.....	xvii
LIST OF ABBREVIATIONS.....	xxiv
ABSTRACT.....	xxvi
CHAPTER 1	
Introduction and Literature Review	
1.1 Introduction.....	1
1.2 Atherosclerosis	2
1.2.1 Prevalence of Atherosclerosis and Risk Factors	3
1.2.2 Classification of Atherosclerosis Lesions	4
1.2.3 Pathophysiology of Atherosclerosis	7
1.2.4 Early Fatty Streak Development	9
1.2.5 Early Fibroatheroma	9

1.2.6 Advanced Atheroma	10
1.2.7 Complex Lesion Development	10
1.2.8 Role of Oxidative Stress in Etiology of Atherosclerosis	11
1.2.9 ATHEROSCLEROSIS: DIAGNOSIS AND TREATMENT	12
1.2.9.1 Treatment of Atherosclerosis	12
1.3. Medicinal Plants and Disease Management	14
1.4. Aims and Objectives of the Study	18
1.4.1 Specific Objectives	18
1.4.1.1 Ethnobotanical Survey of Plants	18
1.4.1.2 <i>In vitro</i> and <i>in vivo</i> Antioxidant activities of Extract of <i>T. violacea</i>	18
1.4.1.3 <i>In vitro</i> and <i>in vivo</i> Toxicity Evaluation of Extract of <i>T. violacea</i>	18
1.4.1.4 Anti-hyperlipidemia and clinical significance of <i>T. violacea</i> Extract	18
1.4.1.5. <i>In vivo</i> Anti-atherosclerotic Properties of <i>Tulbaghia violancea</i> Rhizomes in South African Herbal Medicine	19
CHAPTER 2.....	20
Ethnobotanical Survey of Medicinal Plants used in the Management of Heart Diseases in the Nkonkobe Municipality South Africa.....	20
2.0 Introduction	21
2.1 Materials and Methods	22

2.2. Study Area	22
2.1 Methodology	22
2.2 Intellectual Property Agreement Statement	23
2.3 Compliance Statement.....	23
2.4 Results and Discussion	23
CHAPTER 3.....	27
<i>In vitro</i> and <i>in vivo</i> toxicity evaluation of methanolic extract of <i>Tulbaghia violacea</i> in Wistar Rats.....	27
3.0 Introduction	28
3.1 Material and Methods	29
3.2 Collection of Plant Materials	29
3.3 Preparation and Extraction of Plant Materials	30
3.4 Extraction of Essential Oil	30
3.5 Brine Shrimps Lethality Test	30
3.6 <i>In vivo</i> Toxicity Evaluation	31
3.6.1 Animals	31
3.6.2 Acute Oral Toxicity	32
3.6.3 Sub-chronic Toxicity	32
3.6.4 Hematological and Biochemical Analysis	33

3.6.5 Histological Studies	33
3.7 Statistical Analysis	33
3.8 Results and Discussion	34
3.8.1 Brine Shrimps Cytotoxicity Bioassay	34
3.8.2 Acute Toxicity	38
3.8.3 Sub-chronic Toxicity	38
3.8.4 Biochemical and Hematology parameters	40
3.8.4.1 Biochemical Parameters	42
3.8.4.2 Hematological parameters	47
3.9 Conclusion	50
CHAPTER 4	51
<i>In vitro and In vivo</i> Antioxidant Activities of Methanolic Extract of <i>Tulbaghia violacea</i>	
Rhizomes Harv.....	51
4.0 Introduction	52
4.1 Material and Methods	53
4.1.1 Chemicals	53
4.1.2 Collection of Plant Materials	53
4.1.2.1 Preparation and Extraction of Plant Materials	53
4.1.3 Extraction of Essential Oil	53

4.1.3.1GC-MS Analysis of the Oil	54
4.1.3.2 Identification of Chemical Component.....	54
4.1.4 Phytochemical Contents of <i>T. violacea</i> Rhizomes	54
4.1.4.1 Total Phenolic Determination	54
4.1.4.1 Total flavonoid Determination	55
4.1.4.2 Total Tannin Determination	55
4.1.5 <i>In vitro</i> Antioxidant Activity	56
4.1.5.1 DPPH Radical Scavenging Activity	56
4.1.5.2 Nitric Oxide Scavenging Activity	56
4.1.5.3 Lipid Peroxidation and Thiobarbituric Acid Reaction	57
4.1.5.4.1 Reducing Power of Oil Extract	58
4.1.5.4.2 Reducing Power of Fresh and Dried Extracts of <i>T. violacea</i>	58
4.1.5.5 H ₂ O ₂ Inhibitory Activity of Fresh and Dried Extracts of <i>T. violacea</i>	59
4.2 <i>In vivo</i> Antioxidant Evaluation of <i>T. violacea</i>	59
4.2.1 Animals	59
4.2.2 Experimental Design	60
4.2.3 Preparation of Liver Homogenate	60
4.3 Biochemical Estimation parameters	61
4.3.1 Total Protein and Albumin	61

4.3.2 Malonydialdehyde	61
4.3.3 Superoxide Dismutase	61
4.3. 4 Catalase Activity	62
4.3.5 Estimation of Glutathione Peroxidase Activity.....	62
4.3.6 Estimation of Reduced Glutathione.....	63
4.4 Statistical Analysis	63
4.5 Results and Discussion	63
4.5.1 GC.MS Analysis of the Essential Oil	63
4.5.2 Total phenolics, flavonoid, flavonol, tannin and proanthocyanidin Content.....	64
4.5.3 <i>In vitro</i> Scavenging Activities of extracts <i>T. violacea</i>	66
4.5.3.1 DPPH Radical Scavenging Activity of extracts of <i>T. violacea</i>	66
4.5.3.2 Nitric Oxide Scavenging Activity of extracts of <i>T. violacea</i>	69
4.5.3.3 Lipid Peroxidation Activity.....	71
4.5.3.4 Reducing Power of the Extract	73
4.5.3.5 Hydrogen Peroxide Radical Scavenging Activity.....	75
4.5.4 <i>In vivo</i> Antioxidant Activity.....	77
4.5.4.1 Effect of the Extracts on Lipid Peroxidation in Normal Rats	77
4.5.4.2 Effect of the Extracts on Serum Antioxidant enzymes in Rats fed Normal Diet.....	79

4.5.4.3 Effect of extract of <i>T. violacea</i> on lipid peroxidation and antioxidant Enzymes in Diet Induced Hypercholesterolemia rats.....	81
4.6 Conclusion	87
CHAPTER 5.....	88
Anti-hyperlipidemia of <i>T.violacea</i> Extract.....	88
5.0 Introduction.....	89
5.1 Material and Methods.....	90
5.1.1 Collection of Plant Materials.....	90
5.1.2 Plant Material and Preparation.....	90
5.2 Animals.....	90
5.3 Cholesterol Supplemented diet.....	90
5.4 Experimental Design.....	91
5.5 Determination of Biochemical Parameters	91
5.5.1 Assessment of Lipid Profile and Biochemical Parameters.....	91
5.6 Statistical Analysis.....	92
5.7 Results and Discussion.....	92
5.7.1 Effect of rhizome of <i>T. violacea</i> on Serum Lipid profile in Rats on normal diet	92
5.7.2 Effect of <i>T. violacea</i> on Serum Lipid Profile of hypercholesterolemic Rats	94
5.7.3 Effect of extract of <i>T. violacea</i> on changes in Body Weight of Rats	98

5.7.4 Effect of Extract on Biochemical Parameters.....	99
5.8 Conclusion.....	102
CHAPTER 6.....	103
<i>In vivo</i> Anti-atherogenic Properties of RTV in Diet Induce Atherogenic Rats.....	103
6.0 Introduction.....	104
6.1 Materials and Methods.....	105
6.2 Plant Collection and Extract Preparation.....	105
6.2.1Preparation and Extraction of Plant Materials was as described in section 3.3.....	105
6.3 Animals.....	105
6.4 Experiment Design.....	106
6.5 Sample Collection and Preparation.....	107
6.6 Hematological Analysis.....	107
6.7 Biochemical Assay.....	107
6.7.1 Determination of catalase activity (CAT).....	108
6.7.2 Determination of superoxide dismutase activity.....	108
6.7.3 Malondialdehyde.....	109
6.8 Histopathological Examination of Liver and Aorta.....	109
6.9 Statistical Analysis.....	109
6.10 Results and Discussion.....	110

6.11 Conclusion.....	120
CHAPTER 7.....	121
7.0 GENERAL DISCUSSION	122
7.1 Toxicity Evaluation.....	123
7.2 Antioxidant Activity Evaluation.....	124
7.3 Antilipidemia and Antiatherosclerotic and Activities.....	125
7.4 Possible Mechanisms.....	126
7.5 Conclusion.....	127
7.6 Recommendation.....	128
References.....	129

LIST OF TABLES

Table 1a: The classification of atherosclerotic lesions according to the American Heart Association.....	5
Table 1b: Classification of autopsy-verified atherosclerotic lesions	6
Table 2: The plants used for the treatment of cardiovascular diseases in Nkonkobe Municipality of South Africa and their predisposing factors	25
Table 3: Body weights of male and female rats after 28 days of oral administration of methanolic extracts of <i>T. violacea</i> rhizomes.....	40
Table 4: Relative organ weight of rats after 28 days treatment with methanolic rhizome extracts of <i>T. violacea</i>	40
Table 5: Effect of daily administration of rhizomes of <i>T. violacea</i> extracts for 28 days on biochemical profiles of the control and treated rats in the sub-chronic toxicity study.....	45
Table 6: Haematological parameters of rats treated with the <i>T. violacea</i> extract for 28 days.	49
Table 7: GC-MS analyses of <i>T. violacea</i> rhizome essential oil	64
Table 8: Polyphenol content of methanolic extracts of fresh and dried rhizomes of <i>T. violacea</i>	65
Table 9: Effective inhibitory concentration for nitric oxide and lipid peroxidation of oil extract of rhizomes of <i>T. violacea</i>	69
Table 10: Effective inhibitory concentration for DPPH, hydrogen peroxide radical scavenging activity and lipid peroxidation.....	70

Table 11: Effect of crude extract of rhizomes of <i>T. violacea</i> on thiobarbituric acids (TBARS) and reduced glutathione (GSH) in tissues of rats maintaining on high-cholesterol diet	83
Table 12: Effect of methanolic extracts of <i>T. violacea</i> on lipids profile of rats fed a normal diet.....	93
Table 13: Effect of methanolic extracts of <i>T. violacea</i> rhizomes on serum lipid profile and glucose in diet induced hypercholesterolemic rats.....	95
Table 14: Effect of extracts of <i>T. violacea</i> rhizomes on serum levels of biochemical parameters in diet induced hypercholesterolemic rats.....	100
Table 15: Food intake and percentage change in weight of experimental animals.....	111
Table 16: Effect of methanolic extract of <i>T. violacea</i> on serum lipid profile of diet induced atherosclerotic rats	113
Table 17: Effect of methanolic extract of <i>T. violacea</i> on serum biochemical parameters of diet induced atherosclerotic rats	116
Table 18: Effect of <i>T. violacea</i> extract on markers of endothelium dysfunction in diet induced atherosclerotic rats	119

LIST OF PLATES

Plate 1a: <i>Tulbaghia violacea</i> Harv. (TV) A: <i>Tulbaghia violacea</i> in its natural habitat in University of Fort Hare Environ (Alice).....	18
Figure 1b: The rhizomes of <i>Tulbaghia violacea</i>	18

LIST OF FIGURES

Figure 1: The sequence of events in the development of atherosclerosis	8
Figure 2: Determination of IC ₅₀ of fresh methanolic extract of rhizome (FMRTV) of <i>T. violacea</i> against brime shrimps nauplii.....	35
Figure 3: Determination of IC ₅₀ of dried methanolic extract of rhizome (DMRTV) of <i>T. violacea</i> against brime shrimps nauplii.....	36
Figure 4: Determination of IC ₅₀ of essential oil of rhizome of <i>T. violacea</i> against brime shrimps nauplii.....	37
Figure 5: Percentage weight increase of the control and treated female rats in the subchronic toxicity study.....	41
Figure 6: Percentage weight increase of the control and treated male rats in the subchronic toxicity study.....	41
Figure 7a “Photomicrograph of cross section of the liver of normal Rats”	46
Figure 7b: “Photomicrograph of cross section of the liver of Rats treated with 500 mg/kg/ body weight”	46
Figure 8: DPPH Scavenging effects of oil extracts from rhizomes of <i>T. violacea</i> , vitamin C and BHT	68
Figure 9: DPPH Scavenging effects of fresh and dried extracts from rhizomes of <i>T. violacea</i> and vitamin C.....	69
Figure 10: lipid peroxidation Scavenging effects of oil extracts from rhizomes of <i>T. violacea</i> , vitamin C and BHT	71

Figure 11: lipid peroxidation Scavenging ability of methanolic extracts of fresh and dried rhizomes of <i>T. violacea</i> (RTV)	72
Figure 12: Reducing power of methanolic extract of fresh and dried rhizome of <i>T. violacea</i>	74
Figure 13: Ferric ion reducing power of oil extracts from rhizomes of <i>T. violacea</i> , vitamin C and BHT.....	75
Figure 14: Hydrogen peroxide Scavenging ability of methanolic extracts of fresh and dried rhizomes of <i>T. violacea</i> (RTV).....	77
Figure 15: Malondialdehyde (MDA) levels in plasma of rats 28 days after administration....	78
Figure 16: Superoxide dismutase (SOD) levels in the plasma of rats 28 days after administration	80
Figure 17: Glutathione peroxidase (GPX) levels in the plasma of rats 28 days after administration	80
Figure 18: Catalase (CAT) levels in the plasma of rats 28 days after administration.....	81
Figure 19: Effects of methanolic extract of rhizomes of <i>T. violacea</i> on antioxidant enzymes from aorta homogenate of rats fed a high cholesterol diet	84
Figure 20: Effects of methanolic extract of rhizomes of <i>T. violacea</i> treatment on antioxidant enzymes from liver homogenate of rats feed a high cholesterol diet.....	85
Figure 21: Effects of methanolic extract of rhizomes of <i>T. violacea</i> treatment on antioxidant enzymes from heart homogenate of rats feed a high cholesterol diet.....	86
Figure 22a: Photomicrograph of liver section from a rat treated with high cholesterol diet ..	96
Figure 22b: Photomicrograph of liver section from a normal rat	96

Figure 22c: Photomicrograph of liver section of rat fed with high cholesterol diet and RTV	96
Figure 22d: Photomicrograph of liver section of rat fed with high cholesterol diet and Atorvastatin.....	97
Figure 23: The growth response of rats fed with normal diet, high cholesterol diet (HCD) and HCD plus RTV extract.....	99
Figure 24: Effect of RTV extract on activities of tissue antioxidant enzymes in aorta of diet induced atherogenic rats.....	115
Figure 25: Effect of RTV extract on activities of tissue antioxidant enzymes in liver of diet induced atherogenic rats.....	115
Figure 26a: Photomicrograph of cross section of the aorta of Normal.	117
Figure 26b: Photomicrograph of cross section of the aorta of atherogenic rats.....	117
Figure 26c: Photomicrograph of cross section of the aorta of atherogenic rats treated with RTV extracts.....	118
Figure 26d: Photomicrograph of cross section of the aorta of atherogenic rats treated with atrovastin.....	118

LIST OF PAPERS PUBLISHED FROM THIS THESIS

Paper I

Olorunnisola OS, Bradley G, Afolayan AJ (2011). Ethnobotanical information on plants used for the management of cardiovascular diseases in Nkonkobe Municipality, South Africa. *Journal of Medicinal Plants Research*. 5(17), 4256-4260.

Paper II

Olorunnisola OS, Bradley G, Afolayan AJ (2011). Antioxidant properties and cytotoxicity evaluation of methanolic extract of dried and fresh rhizomes of *Tulbaghia violacea*. *African Journal of Pharmacy and Pharmacology*. Vol. 5(22): 2490-2497.

Paper III

Olorunnisola OS, Bradley G, Afolayan AJ (2012). Chemical composition, antioxidant activity and toxicity evaluation of essential oil of *Tulbaghia violacea* Harv. *Journal of Medicinal Plants Research*. 6 (12), pp. 2340-2347,

Paper IV

Olorunnisola OS, Bradley G, Afolayan AJ (2012). Effect of methanolic extract of *T. violacea* rhizomes on Antioxidant enzymes and lipid profile in normal rats. *African Journal of Pharmacy and Pharmacology*. 6(14): 1026 – 1030.

Paper V

Olorunnisola OS, Bradley G, Afolayan AJ (2012). Protective Effect of Extract of *T. violacea* Rhizome against Hypercholesterolemia Induced Oxidative Stress in Wistar Rat. *Molecules* 17, 6033-6045.

Paper VI

Olorunnisola OS, Bradley G, Afolayan AJ (2012). Acute and sub-chronic toxicity studies of methanolic extract of rhizome of *Tulbaghia violacea*. Harv. in Rats. African Journal of Biotechnology Vol. 11(83), pp. 14934-14940,

Paper VII

Olorunnisola OS, Bradley G, Afolayan AJ (2012). Antilipidemia effect of crude methanolic extract of rhizomes of *Tulbaghia violacea* in diet induces hypercholesterolemia rats. African Journal of Biotechnology. 11(70): 13498-13505.

Paper VIII

Olorunnisola OS, Bradley G, Afolayan AJ (2012). Protective effect of *Tulbaghia violacea* Harv. on aortic pathology, tissue antioxidant enzymes and liver damage in diet -induced atherosclerosis rats. . Internation Journal. Molecular. Science. 13, 12747-12760

LIST OF ABBREVIATIONS

TC, Total cholesterol; LDL, low-density lipoprotein;

TG, Triglycerides

HDL, high density lipoprotein;

TBARS, Thiobarbituric acid reactive substances;

SOD- Superoxide dismutase;

GPx - Glutathione peroxidase;

RBCs- Red blood cells;

WBCs, White blood cells;

Hb, Hemoglobin;

NO – Nitric oxide;

HCD - High cholesterol diet

ECM - Extracellular matrix.

EC- Endothelial cells

SMC- Smooth muscle cells

RTV- Rhizomes of *Tulbaghia violacea*.

ABTS- 2, 2'- azinobis [3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt.

DPPH- 1, 1-diphenyl-2- picrylhydrazyl,

O₂^{•-} Superoxide anions

H₂O₂- Hydrogen peroxide

NO - Nitric oxide

TCFA - Thin-cap fibroatheroma

PLT - Platelet

GENERAL ABSTRACT

GENERAL ABSTRACT

Discovery of cheap, nontoxic and readily available antiatherosclerotic drugs is an extraordinary challenge in this modern world. Atherosclerosis and cardiovascular diseases have been predicted to be the leading cause of death by the year 2030. Hence, this thesis was designed to search for plant (s) with anti-atherogenic properties, investigate its possible side effects and extrapolate its likely mechanism(s) of action.

An ethnobotanical survey was employed in identification of locally important plants used for the management and treatment of cardiovascular diseases and its predisposing factors in Nkonkobe Municipality, Eastern Cape in South Africa. Information on the names of plants, their parts used and methods of preparation was collected through a questionnaire which was administered to herbalists, traditional healers and rural dwellers. The most frequently used plant (Rhizomes of *Tulbaghia violacea* Harv.) was investigated for toxicity using brine shrimp lethality (*in vitro*) and *in vivo* toxicity test (acute and subchronic) on rats to determine safety dosage. The *in vitro* antioxidant and free radical scavenging activity of the plant was investigated using models such as 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide anions, hydrogen peroxide (H₂O₂), nitric oxide (NO), 2,2'-azinobis [3-ethylbenzothiazoline-6-sulfonic acid] diammonium salt (ABTS), lipid peroxidation inhibition and the ferric reducing agent. Phytochemical content and the effect of oral administration of fresh methanolic extract rhizomes of *Tulbaghia violacea* (250, 500 mg/kg. bwt/day) on Lipid peroxidation (TBARS), serum and tissue antioxidant enzymes in normal, hypercholesterolemic and diet induced atherogenic rats were also assessed. More so, the potential of the extract (250 and 500 mg/kg. bwt) to protect against atherogenic diet (4% cholesterol 1% cholic acid and 0.5% thiouracil) induced fatty streaks formation, dyslipidemia, oxidative stress and endothelial dysfunction was also investigated.

Ethnobotanical study revealed that 19 plant species are used for the treatment of heart related diseases in the Municipality. 53 % of the plants mentioned were used for the management of chest pain, 47 % for high blood pressure, 42 % for heart disease, 16 % for stroke and 11 % for the treatment of hypercholesterolemia. *Tulbaghia violacea* was repeatedly mentioned as the plant species used for the treatment of high blood pressure and predisposing factors in the study area.

The brine shrimp cytotoxicity test revealed that fresh, dried methanolic extracts and essential oil of the *T. violacea* exhibited a high degree of cytotoxic activity with IC_{50} values of 18.18 (fresh) and 19.24 (dried) $\mu\text{g/ml}$. An IC_{50} value of 12. 59 $\mu\text{g/ml}$ was obtained for the essential oil of the plant. The low cytotoxicity values obtained, suggested that rhizome of *T. violacea* may serve as a potential source of antimicrobial and anticancer agents.

In vivo acute study of single oral administration of 5g/kg dose does not produce mortality or significant behavioral changes during 14 days observation. In the sub-chronic study, the extract (250, 500 mg/kg/bwt/ day) administered for a period of 28 days showed no mortality or morbidity. The weekly body and organ weight of the rats showed no significant differences between the control and the rats treated with the extract. The extract at all doses does not show any effect on of biomarkers of liver or renal damage. However, a significant decrease in the activity of γGT was observed in the extract treated groups. Hematological evaluation revealed that oral administration of fresh methanolic extracts of rhizomes of *T. violacea* does not cause anaemia or leucocytosis in the animals. Furthermore, histopathology results of the internal organs revealed no detectable inflammation. These results demonstrated that the rhizome extract of *T. violacea* was potentially safe for consumption orally even in chronic concentration.

In vitro antioxidant evaluation showed that the essential oil, fresh and dried methanolic extracts exhibited potent antioxidant activities in a concentration dependent manner. Phytochemical investigation reveals that the fresh and the dry extract of RTV are rich in flavonoid, flavonol, phenols, tannin and proanthocyanidin, while the essential oil contained dimethyl disulfide, dimethyl trisulfide, (methyl methylthio) methyl, 2,4-dithiapentane (11.35 %) and (methylthio) acetic acid, 2- (methylthio) ethanol, 3-(methylthio) - and propanenitrile (7.20 %). The fresh extract had higher radicals scavenging activity than the essential oil or dried extract, with 50 % inhibition of DPPH, hydrogen peroxide and lipid peroxidation at a concentration of 35.0 ± 0.12 , 19.3 ± 0.11 and 17.9 ± 0.15 $\mu\text{g/ml}$ respectively. Oral administration of methanolic extract of RTV in 125, 250 and 500 mg/kg to female Wistar rats significantly inhibited reduction of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT). The extracts also inhibited ($p < 0.05$) lipid peroxidation in normal, high cholesterol and diet induced atherosclerosis fed rats in a dose dependant manner.

Also the extract (250 and 500 mg/kg/bwt/day) caused a significant ($p < 0.05$) improvement in body weight of treated animals compared with untreated hypercholesterolemia control rats. The extracts also protected significantly ($p < 0.05$) against atherogenic diet induced liver damage or fatty streaks formation in the aorta as revealed by histological examination. The anti-cholesterolemia and anti-atherosclerotic activities of the extract compared favorably well with standard drugs Gemfibrozil and Atorvastatin respectively.

Conclusively, rhizomes of *T. violacea* possess significant anti-atherogenic activity and its mechanism of action(s) may be due to its antioxidant and anti-hypercholesterolemia properties. The results of this study also suggested that rhizome of *T. violacea* is relatively safe for human consumption and it may be used as an alternative to garlic.

INTELLECTUAL PROPERTY AND AGREEMENT STATEMENT

All the elderly and the traditional healers who contributed to this ethnobotanical survey were adequately financially rewarded, and given verbal assurances that the results of this research shall not be used for commercial purposes but to serve as information to the community and the entire Eastern Cape.

ETHICAL COMMITTEE APPROVAL

The study involving the use of animals in this project was carried out following the approval of the Ethical Committee on Animal Use and Care of the University of Fort Hare.

COMPLIANCE STATEMENT

No part of this study in any form has been or will be commercialized; instead the entire thesis is meant to be used as a tool for information dissemination on the medicinal plants used for the treatment of cardiovascular diseases in Eastern Cape Province of South Africa.

Supervisor signature

Student signature

CHAPTER 1

LITERATURE REVIEW