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

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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CO-SUPERVISOR: PROF G BRADLEY

MAY, 2012
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: 

ACKNOWLEDGMENTS

I am grateful to the Lord God Jesus Christ for his protection, provision and guidance in the course of this programme.

I would like to express my sincere gratitude to my supervisor Prof AJ Afolayan. Thank you for your useful advice and comments not only about my work but also about life in general. The Lord God will reward you perfectly. I am also very grateful to my co-supervisor; Prof G Bradley for the interest, support and constructive criticisms he gave me during the course of this program.

I convey my deepest thanks and regards to my family especially my wife and children for their endurance, constant support and encouragement.

Avery special thanks to National Research Foundation of South Africa for their financial support.

I will like to thank every member of the phytomedicine research group most especially my friends Olufunmiso O. Olajuyigbe and Dr Ezekel Green for his love, endurance and encouragement.

My appreciation also goes to Miss Nolukholo Mketo for her love, support and contribution toward the success of this work.

Lastly, I will like to thank the traditional healers, Sangomas and herbalists who provided ethnobotanical information during our survey.
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Figure 26d: Photomicrograph of cross section of the aorta of atherogenic rats treated with atrovastin
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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,
O$_2^-$ Superoxide anions
$\text{H}_2\text{O}_2$ - Hydrogen peroxide

NO - Nitric oxide

TCFA - Thin-cap fibroatheroma

PLT - Platelet
GENERAL ABSTRACT
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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 (H$_2$O$_2$), 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) μg/ml. An IC$_{50}$ value of 12.59 μg/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 $\Gamma GT$ 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 dimethy 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 μg/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-cholesterolema 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.
INTELECTUAL 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.

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Supervisor signature              Student signature