UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

In Vitro Medicinal Properties of Novel Compounds from *Croton steenkampianus*

By
Adeboye Mutiu Adelekan

Submitted in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHIAE: PLANT SCIENCE

Department of Plant Science

Faculty of Natural and Agricultural Sciences
University of Pretoria

Promoter: Prof JJM Meyer

2009

© University of Pretoria

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

I declare that the thesis/dissertation, which I hereby submit for the degree PHD Plant Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE:

Date: 26/01/2009

ACKNOWLEDGEMENTS

Glory be to God almighty for His grace and mercy over me throughout the study.

I also wish to thank the following people for the role they have played in the course of the research:

Prof JJM Meyer for the assistance, guidance and support given to me.

Prof AI Louw for his contribution.

The National Research Foundation and University of Pretoria for their financial support.

Dr Ahmed Hussein for his help in the isolation and identification of the compounds and for arranging antiplasmodial bioassay.

Dr Adrian Basson for help with the cytotoxicity testing.

Dr Benedict Bapela for assistance with the TB assay.

Dr Emmanuel Tshikalange for help with anti-HIV assay.

Prof P Smith (University of Cape Town), Bruce Tshilamulele and Luis David (Smithsonian Tropical Research Institute, Panama) for help with the malaria bioassays.

Eric Palmer, Department of Chemistry, University of Pretoria for the assistance with the NMR.

Prof Peet van Roogen for assistance with x-ray crystallography.

My wife Tsholofelo D Adelekan for her support and encouragement.

All my friends and well-wishers.

SUMMARY

In Vitro Medicinal Properties of Novel Compounds from *Croton steenkampianus*

by

Adeboye Mutiu Adelekan
Promoter: Prof JJM Meyer
Department of Plant Science
Doctor of Philosophiae

The effect of infectious diseases on the population in the developing countries is of utmost concern. Malaria, tuberculosis (TB) and human immunodeficiency virus (HIV) are the three major infectious disease threats. They account for approximately half of the mortality caused by infectious diseases, which is almost half of the mortality in the developing countries. With no vaccine likely in the foreseeable future, drugs remain the best means of controlling infectious diseases. In the industrialized nations at the present time, some 50% of all prescribed drugs are derived or synthesized from natural products (animals, marine species, plants and micro-organisms). It has been estimated that plants are the most important source of medicine for more than 80% of the world's population. As previous work on the leaves of *Croton steenkampianus* gave promising results and revealed that it still contained bioactive compounds that could be isolated, it was chosen for further work.

The bioassay guided fractionation of the ethanol crude extract using silica and Sephadex column chromatography resulted in the isolation of six compounds: three flavoniods (quercetin, tamarixetin and eriodictyol), one new indane (**1**) (2,6-dimethyl-1-oxo-4 indanecarboxylic acid) and two new diterpenes (steenkrotin A (**2**) and steenkrotin B (**3**)) with novel skeletons. The structure of the compounds was determined using NMR, IR, UV, MS and X-ray crystallography.

Ethanol crude extract, quercetin, steenkrotin A, steenkrotin B and the indane were tested against four strains of *Plasmodium falciparum* (D6, D10, Dd2 and W2). Quercetin showed good antiplasmodial activity against the D10 and Dd2 strains. The antiplasmodial activity of steenkrotin A and crude extract were moderate. The antimalarial activity of steenkrotin A in particular is promising, as it showed more activity against resistant strains. The indane, and steekrotin B were not active against the strains of *P. falciparum* used (IC_{50} > 10 µg/m). The IC_{50} of the compounds improved when they were combined with chloroquine. However, the IC_{50} of chloroquine was still the lowest. The compounds showed moderate bioactivity against *Bacillus cereus* and *Escherichia coli*. The three new compounds (**1**, **2** and **3**) tested against *Mycobacterium* (H37Rv) were not active (IC_{50} > 10 µg/ml). The indane (**1)** showed anti-HIV activity at 50 µg/ml against reverse transcriptase. The antioxidant activity of the compounds tested ranged from weak to excellent (>280.00 µg/ml for compound **1** and **2** to 0.05 µg/ml for quercetin).

The cytotoxicity of the compounds and extract were determined against Vero cells lines. Their IC_{50} values ranged from 34.0 to 305.9 µg/ml, which is higher and better than that of chloroquine. The IC_{50} values obtained are: chloroquine (25.0), quercetin (33.6), steenkrotin A (35.0), ethanol extract (45.0), tamarixetin (53.8), indane (248.2) and steenkrotin B (305.9).

CONTENTS

Chapter 6: Cytotoxicity of the isolated compounds

Chapter 7: General discussion and conclusion

Appendix

LIST OF FIGURES

Chapter 1

Chapter 2

Chapter 4

Chapter 5

Chapter 6

LISTS OF TABLES

LIST OF ABBREVIATIONS

^{13}C-NMR: Carbon nuclear magnetic resonance

1H-NMR: Proton nuclear magnetic resonance

AIDS: Acquired immune deficiency syndrome

APAD: 3-Acetylpyrimidine adenine dinucleotide

COSY: Correlated spectroscopy

DEPT: Distortionless enhancement by polarization transfer

DHFR: Dihydrofolate reductase

DHODase: Dihydroorotate dehydrogenase

DHPS: Dihydropteroate synthase

DMSO: Dimethylsufoxide

DPP: Dimethylallyl pyrophosphate

EDTA: Ethylenediaminotetra-acetic acid

FPIX: Ferriprotoporphyrin IX

HEPES: N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid

HIV: Human immunodeficiency virus

HMBC: Heteronuclear multiple bond correlation

HMQC: Heteronuclear multiple quantum correlation

HSQC: Heteronuclear singlequantum coherence

IPP: Isopentenyl pyrophosphate

IR: Infrared

LD_{50}: 50% Lethal dose

MS: Mass spectroscopy

MTCT: Mother-to-child transmission

MTT: 3-[4, 5-Dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide

NBT: Nitroblue tetrazolium

NMR: Nuclear magnetic resonance

NOESY: Nuclear overhauser effect spectroscopy

NSP: National strategic plan

PBS: Phosphate buffer saline

PEP: Post-exposure prophylaxis

PF: Potentiating factor

SP: Sulphadoxine-pyrimethamine

STD: Sexual transmitted disease

STI: Sexual transmitted infection

TLC: Thin layer chromatography

TMS: Tetramethylsilane

TRIS: N-tris (hydroxymethyl) aminomethane

UNAIDS: Joint United Nations programme on HIV/AIDS

UNGASS: United Nations general assembly session on HIV/AIDS

UNICEF: United Nations children's fund

USAID: United States agency for international development

UV: Ultraviolet

WHO: World health organization