THE ANTI-PROLIFERATIVE, ANTIOXIDATIVE AND ANTI-INFLAMMATORY PROPERTIES OF THE D2 FRACTION AND HPLC SEMI-PURIFIED SUB-FRACTIONS OF *DICEROCARYUM SENECIOIDES*

by

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DECLARATION

I declare that the dissertation hereby submitted to the University of Limpopo for the degree of Master of Science in Biochemistry has not been previously submitted by me for a degree at this or any other University, that it is my work in design and execution, and that all material contained herein has been duly acknowledged.

P.K Chokoe (Ms) — September 2011

Initials and Surname (Title) — Date

Signature: _____

DEDICATION

This work is dedicated to my son, Phuti, who has brought so much joy to his mommy's life; and to my late brother, Lekgau, whose encouragement I will forever cherish.

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ABBREVIATIONS

°C	Degrees Celsius
Apaf-1	Apoptosis protease activating factor-1
ATCC	American Type Culture Collection
Bax	Bcl-2-associated protein X
BH_4	Tetrahydrobiopteine
Bcl-2	B-cell leukemia-2
CGD	Chronic granulomatous disease
CO_2	Carbon dioxide
COX	Cyclooxygenase
Coxibs	COX-2 specific inhibitors
D1	*n*-Hexane fraction
D2	Dichloromethane fraction
D3	Water fraction
DCM	Dichloromethane
DCF	Dichlorofluorescein
DCFH	Dichlorofluorescin
DCFH-DA	Dichlorofluorescin diacetate
dH_2O	Distilled water
DMSO	Dimethylsulfoxide
DP	Dual plate
DPI	Diphenylene iodonium
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DNA	Deoxyribonucleic acid
EMW	Ethyl acetate: methanol: water
eNOS	Endothelial nitric oxide synthase
FAD	Flavin adenine dinucleotide (oxidized)
FADD	Fas-associated death domain
FBS	Fetal bovine albumin
Fe^{2+}	Ferrous ion
Fe^{3+}	Ferric ion
$FeCl_3$	Ferric chloride
FMN	Flavin mononucleotide

FRAP	Ferric reduction antioxidant potential
GDP	Guanosine diphosphate
GSNO	S-nitrosoglutathione
GTP	Guanosine triphosphate
H_2	Hydrogen
H_2O	Water
H_2O_2	Hydrogen peroxide
H_3PO_4	Phosphoric acid
HGE	Human granulocytic ehrlichiosis
HOCl	Hypochlorous acid
HPLC	High performance liquid chromatography
IDP	Iodonium diphenyl
IgG	Immunoglobulin G
INF-γ	Interferon gamma
iNOS	Inducible nitric oxide synthase
$K_3Fe(CN)_6$	Potassium ferricyanide
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
$NaNO_2$	Sodium nitrite
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
NSAID	Non-steroidal anti-inflammatory drug
$^1O_2$	Singlet oxygen
O_2	Molecular oxygen
O_2^-	Superoxide anion
$OH^\bullet$	Hydroxyl radical
$ONOO^-$	Peroxynitrite
PBS	Phosphate-buffered saline
PDE	Phosphodiesterases
PHOX	Phagocyte oxidase
PMA	Phorbol 12-myristate 13-acetate
PMN	Polymorphonuclear neutrophils

PSN	Penicillin, streptomycin and neomycin
RNA	Ribonucleic acid
RNOS	Reactive nitrogen and oxygen species
ROS	Reactive oxygen species
RPMI	Roswell Park Memorial Institute
RTCA	Real time cell analysis
SH	Sulfhydryl/ thiol
TCA	Trichloroacetic acid
TLC	Thin layer chromatography
TNF1	Tumour necrosis factor 1
Tris	Tris(hydroxymethyl)aminomethane
UV	Ultraviolet
Zn^{2+}	Zinc ion

ABSTRACT

Dicerocaryum senecioides is a crawling herb that is found growing mostly in sandy areas of southern and south-eastern Africa and its small, hairy leaves have been used over the years as food, shampoo, and for treatment of various ailments. In this study, the dichloromethane (D2) fraction was prepared from a crude methanol extract of *D. senecoides* leaves, and its effect on the proliferation of RAW 264.7 murine macrophages was investigated. Treatment of the macrophages with the extract resulted in a dose- and time-dependent decrease in cell viability as determined by the MTT assay and real time cell analysis. Cytotoxicity of the D2 fraction on the macrophages was demonstrated to be due to apoptosis by staining the cells with DAPI nucleic acid stain. Anti-inflammatory activity of D2 fraction on RAW cells was determined by evaluating intracellular ROS production by the DCFH-DA fluorescent assay. Cells treated with the D2 fraction and stimulated with PMA were found to have a lower fluorescence intensity compared to untreated, stimulated cells; thus mimicking the response observed in the resting cells. The percentage fluorescence in untreated, stimulated cells doubled, while no significant change was observed in the D2-treated cells. The effect of the D2 fraction on iNOS activity was also assessed. The fraction reduced the NO synthesised by iNOS in cells treated with the D2 fraction and stimulated with LPS dose-dependently. The D2 fraction was further fractionated by semi-preparative HPLC; and thin layer chromatography was used to analyse phytocompounds of the 96 HPLC sub-fractions as well as to screen these sub-fractions for anti-oxidative activity. Sub-fractions 1-7 and 33-39 showed an intensely pronounced DPPH-scavenging compound and this scavenging ability was confirmed by a quantitative DPPH assay that provided parallel results. The reducing potential of the sub-fractions was assessed by evaluating their Fe^{3+}-reducing ability through the FRAP assay. Sub-fractions 1-7 and 33-39 displayed remarkable reducing potential. Taken together with the DPPH-scavenging activity, these findings suggest that HPLC sub-fractions 1-7 and 33-39 possess a compound(s) with impressive antioxidant activity. These findings merit the D2 fraction as an extract that can be used to control chronic inflammation as it does not only inhibit free radical production, but also

scavenges excessive ROS and has the ability to induce apoptosis in the macrophages responsible for dysregulated production of the free radicals. The extract also has commendable chemoprotective and chemotherapeutic potential as it demonstrated pro-apoptotic activity along with prevention of excess free-radical production.