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Antituberculosis activity of flavonoids from *Galenia africana* L. var. *africana*

BY

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Submitted in partial fulfilment of the requirements for the degree

DOCTOR OF PHILOSOPHIAE: PLANT SCIENCE

in the Faculty of Natural & Agricultural Science
University of Pretoria
Pretoria
(June 2009)

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DECLARATION

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DEDICATION

This thesis is dedicated to my late grandfather Abner F. Mativandlela and my family for being a positive motivating force in my life and supporting me through the trials and tribulations of life.

ABSTRACT

The recent increase in the incidence of tuberculosis (TB) with the emergence of multidrug-resistant (MDR) cases has lead to the search for new TB-drugs. *Mycobacterium tuberculosis* is a complex, resilient organism, and it is important to note that new drugs are required which can reduce TB's six month treatment time and can be effective against drug-resistant strains of mycobacteria. Plants contain numerous biological active compounds, many of which have been shown to have antimicrobial activity. The search for biologically active extracts based on traditional use of plants is relevant due to the appearance of microbial resistance to many antibiotics and the occurrence of fatal opportunistic infections. Ethanol extracts of seven selected ethnobotanically South African medicinal plants (*Artemisia afra, Dodonaea angustifolia, Drosera capensis, Galenia africana, Prunus africana, Syzygium cordatum* and *Ziziphus mucronata*) were investigated for their antimycobacterial activity against two *Mycobacterium* species.

The minimum inhibitory concentration (MIC) of ethanol extracts of *A. afra, Dodonaea angustifolia, Drosera capensis* and *G. africana* ranged from 0.781 to 6.25 mg/mL against a non-pathogenic strain of mycobacteria, '*M. smegmatis*'. *G. africana* showed the best activity, exhibiting an MIC of 0.781 mg/mL and a minimum bactericidal concentration (MBC) of 1.563 mg/mL against *M. smegmatis*. A drug sensitive strain of *M. tuberculosis* was found to be susceptible to the ethanol extracts of *Dodonaea angustifolia* and *G. africana*. (MICs 5.0 and 1.2 mg/mL respectively) when using the rapid radiometric-BACTEC method. The phytochemical analysis of *G. africana* led to the isolation and identification of three known compounds namely; (2*S*)-5,7,2'-trihydroxyflavanone, (*E*)-3,2',4'-trihydroxychalcone (not reported from natural sources) and (*E*)-2',4'-dihydroxychalcone. A novel chalcone '(*E*)-3,2',4'-trihydroxy-3'-methoxychalcone' was also isolated from the ethanol extract of *G. africana*. Isolation of (2*S*)-5,7,2'-trihydroxyflavanone, (*E*)-3,2',4'-trihydroxychalcone and *E*)-3,2',4'-trihydroxy-3'-methoxychalcone was reported for the first time from this plant. The MIC of novel compound against *M. tuberculosis* was found to be 50.0 µg/mL whereas (2*S*)-5,7,2'-trihydroxyflavanone and (*E*)-3,2',4'-trihydroxychalcone exhibited an MIC of 100.0 µg/mL.

During synergistic studies using (2*S*)-5,7,2'-trihydroxyflavanone and (*E*)-2',4'-dihydroxychalcone with the antituberculosis drug INH, it was found that the MICs of INH and the compounds were reduced sixteen and eight-fold respectively, resulting in a Fractional Inhibitory Concentration (FIC) of 0.1250 and 0.1875 respectively. The synergistic effect of two isolated compounds (2*S*)-5,7,2'-trihydroxyflavanone and (*E*)-2',4'-dihydroxychalcone) in *in vitro* studies also showed synergistic action, reducing their original MICs four-fold resulting in a FIC of 0.5. Since (2*S*)-5,7,2'-trihydroxyflavanone and (*E*)-2',4'-dihydroxychalcone from *G. africana* showed synergistic activity with INH, it is speculated that the compounds might have similar mechanism as that of INH. However, mechanistic studies on these compounds should be done in order to get an indication of the 'flavonoids and chalcones' interferences on mycolic acid synthesis, membrane synthesis and enzyme inhibition. Our investigation on the NADPH oxidase activity of (2*S*)-5,7,2'-trihydroxyflavanone with Mtr, found that this compound failed to exhibit any NADPH oxidase activity at 800 µM concentrations. Mtr is evidently not the target for the antimycobacterial activity of (2*S*)-5,7,2'-trihydroxyflavanone.

Fifty percent inhibitory concentration of the ethanol extract of *G. africana*, and the two purified compounds, (2*S*)-5,7,2'-trihydroxyflavanone and (*E*)-2',4'-dihydroxychalcone were found to be 120.0; 110.3 and 80.2 µg/mL respectively against the U937 cells. The MIC of the ethanol extract of *G. africana* in U937 macrophages infected with *M. tuberculosis* was found to be 50.0 µg/mL indicating the extract's intracellular antimycobacterial activity in real physiological conditions. The two purified compounds also showed good intracellular antimycobacterial activity. The MICs of (2*S*)-5,7,2'-trihydroxyflavanone and (*E*)-2',4'-dihydroxychalcone were found to be 100 and 50 µg/mL respectively. This study indicated that the intracellular activity of the ethanol extract is significant in macrophages. The activity might be due to *M. tuberculosis* being unable to avoid macrophage killing and its survival during phagocytosis, (including inhibition of phagosome-lysosome fusion, inhibition of the acidification of phagosomes, resistance to killing by reactive oxygen intermediates and modification of the lipid composition of the mycobacterial cell membrane, thereby altering its capacity to interact with immune or inflammatory cells).

It can be concluded that the traditional use of *G. africana* for TB has been scientifically validated to some extent. Isolated compounds and the ethanol extract of the plant warrant further investigation for their potential as antimycobacterial agents. Since synergistic activity of purified compounds with existing antituberculosis drug INH, was significant, it will be worthwhile evaluating the efficacy of purified compounds in combination with TB-drugs in pre-clinical studies.

List of Abbreviations

AIDS	Acquired immune deficiency syndrome
ATCC	American type culture collection
CFU	Colony forming units
CMI	Cell-mediated immunity
CNS	Central Nervous System
CR	Complement receptors
CSF	Cerebrospinal fluid
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
DOT	Direct Observed Therapy
EMB	Ethambutol
FAS	Fatty acid synthase
FIC	Fractional inhibitory concentration
GI	Growth index
GPC	Gel permeation chromatography
GSK	GlakoSmithKline
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIV	Human immunodeficiency virus
HPLC	High performance liquid chromatography
ICL	Isocitrate lyase
INH	Isoniazid
INT	2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl
LADH	Lipoamide dehydrogenase
LAM	Lipoarabinomannan
LD_{50}	Lethal dose, 50%
LJ	Lowënstein-Jensen
MBC	Minimal bactericidal concentration
MDR	Multidrug-resistant
MHC	Major histocompatibility complex

List of Abbreviations

MIC	Minimal inhibitory concentration
MR	Mannose receptors
MRC	Medical Research Council
MSH	Mycothiol
MSSM	Mycothiol disulfide
MTR	Mycothiol reductase
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
OD	Optical density
PAS	Para-aminosalicylic acid
PBS	Phosphate-buffered saline
POA	Pyrazinoic acid
PZA	Pyrazinamide
RIF	Rifampicin
rRNA	Ribosomal ribonucleic acid
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute
SD	Standard deviation
SDS	Sodium dodecyl sulfate
STR	Streptomycin
TB	Tuberculosis
TH cell	T helper cell
TLC	Thin layer chromatography
TMP	Traditional medicinal practitioners
U937	Human leukemic monocyte lymphoma cell line
UV	Ultra violet light
WHO	World Health Organization
XDR	Extremely drug resistant

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(c) ^{13}C NMR of (*E*)-2',4'-dihydroxychalcone

(d) ^{1}H NMR of (*E*)-2',4'-dihydroxychalcone

(e) ^{13}C NMR of (*E*)-3,2',4'-trihydroxychalcone

(f) ^{1}H NMR of (*E*)-3,2',4'-trihydroxychalcone

(g) ^{13}C NMR of (*E*)-3,2',4'-trihydroxy-3'-methoxychalcone

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Chemical structures of isolated compounds from the ethanol extract of *G. africana*

(a) (2*S*)-5,7,2'-trihydroxyflavanone

(b) (*E*)-2',4'-dihydroxychalcone

(c) (*E*)-3,2',4'-trihydroxychalcone

(d) (*E*)-3,2',4'-trihydroxy-3'-methoxychalcone

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