

**THE ISOLATION AND CHARACTERISATION  
OF ANTIBACTERIAL COMPOUNDS FROM  
*COMBRETUM ERYTHROPHYLLUM* [BURCH.] SOND.**

**BY**

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**Dissertation submitted to the Faculty of Health Sciences,  
Department of Pharmacology, University of Pretoria, PRETORIA, in fulfilment  
of the requirements for the degree of**

**DOCTOR OF PHILOSOPHY**

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**Date of submission: December 2001**

## ACKNOWLEDGEMENTS

I was once told that if anything could make you religious, a PhD could. I have no doubt.

The person who told me this is also the person I wish to give my first and most important word of thanks to, Dr David Katerere. He is the one person who has motivated me enough to complete this thesis and arrived at *just* the right time. Not only has David helped me with isolation and spectroscopic analysis but also he was wise enough to allow me to struggle through it on my own. Besides his amazing insight and knowledge he managed to emit humour into the darkest of moments and for this I thank you.

I am grateful to Dr J.N. Eloff for taking on the role as promoter and mentor. Without the constant words of encouragement and support this would have been much harder to achieve. I am particularly grateful to him for organising conference participation where I was introduced to many interesting and important people.

Many thanks to Dr Inge von Teichmann for organising things so efficiently and for her eagerness to help as well as lending her ear regardless of how much it was bent. I am especially thankful to Mrs Maryna Steinmann who, besides being a friend and confidant, was always willing to help where possible and give advice where necessary.

Thank you to Prof. JR Snyman and Prof. De K Sommers, as well as the members of the Pharmacology Department at the University of Pretoria, for leading much support and encouragement, especially when it was needed the most.

I am particularly indebted to the following people for their kind help: Dr. Anet Theron (Immunology Dept., University of Pretoria) for help with toxicity assays; Dr. T Laurens (Chemistry Dept., University of Pretoria) for GC-MS analysis; Mr E. Palmer (Chemistry, U.P.) for help with NMR analysis and interesting sense of humour and Dr. P. Boshoff (Cape Technikon) for the speedy MS analysis.

Special thanks to Robin at Microbiology for her enthusiastic help with anti-fungal testing as well as to Mrs Amelita Lombard for supplying me with microorganisms. Also to the library staff at the pre-clinical building for their help in sourcing my “urgent” articles.

I am particularly grateful to my loving family for always lending their support and unconditional love as well as giving advice and a shoulder when things seemed overwhelming. Without your presence this wouldn't have meant as much.

Finally to all those that have consciously or sub-consciously helped me, friends, acquaintances and colleagues, thank you.

## GLOSSARY OF ABBREVIATIONS

2A:3MDC	Acetone:methylene dichloride (2:3)
Acetone-d <sub>6</sub>	Deuterated acetone
ATCC	American Type Culture Collection
BEA	Benzene:ethanol:ammonia (9:1:1%)
BuOH	Butanol
CCl <sub>4</sub>	Carbon tetrachloride
C <sub>5</sub> D <sub>5</sub> N	Deuterated pyridine
CDC	Centre for Disease Control
CDCl <sub>3</sub>	Deuterated chloroform
CEF	Chloroform:ethyl acetate:formic acid (5:4:1)
C <sub>6</sub> H <sub>14</sub>	Hexane
CHCl <sub>3</sub>	Chloroform
CL	Chemiluminescence
COSY	<sup>1</sup> H- <sup>1</sup> H-CORrelation SpectroscopY
COSY-LR	Long Range COSY
CSIR	Centre for Scientific and Industrial Research
CT	Carbon tetrachloride
DCM	Dichloromethane
DMSO	Dimethyl sulphoxide
DMSO-d <sub>6</sub>	Deuterated DMSO
DNA	Deoxyribonucleic acid
EMW	Ethyl acetate:methanol:water (45:5:4.4)
ESBLs	Extended Spectrum β-lactamases
EtOAc	Ethyl acetate
EtOH	Ethanol
FLD	Fluorescence detector
GC-MS	Gas Chromatography-Mass Spectrometry
GMOs	Genetically Modified Organisms
H <sub>2</sub> O	Water

HMBC	$^1\text{H}$ - $^{13}\text{C}$ -Heteronuclear Multiple Bond Connectivity
HMQC	$^1\text{H}$ - $^{13}\text{C}$ -Heteronuclear Multiple Quantum Correlation
HPLC	High Performance Liquid Chromatography
HREIMS	High Resolution Electron Impact Mass Spectrometry
INT	p-Iodonitrotetrazolium violet
LDH	Lactate dehydrogenase
LPC	Lysophosphatidylcholine
MCW	Methanol:chloroform:water (12:5:3)
MDC	Methylene dichloride
MHB	Mueller Hilton Broth
MIC	Minimum Inhibitory Concentration
MWD	Multiple Wavelength Detector
NCCLS	National Committee for Clinical Laboratory Standards
NMR	Nuclear Magnetic Resonance
PBS	Phosphate Buffered Saline
PTLC	Preparative Thin Layer Chromatography
RNA	Ribonucleic acid
RP	Reverse phase
SOZ	Serum opsonized zymosan
TLC	Thin Layer Chromatography
TMSI	N-Trimethyl silylimidazole
UV	Ultraviolet
WHO	World Health Organisation
W/M	Water/methanol

## SUMMARY

Previous studies [Martini, 1998] on the leaves of *Combretum erythrophyllum* (Combretaceae) confirmed the antimicrobial activity but the compounds responsible for the activity could not be identified due to insufficient material. The main aim of this study was therefore to isolate and identify the antimicrobial compounds from *Combretum erythrophyllum* leaf material. Trees around the Pretoria region were tested for variation in activity and a small difference in bioactivity between plants was noted. Leaf extracts were also tested for free radical scavenging activity exhibiting good antioxidant activity and possible anti-inflammatory activity. Toxicity tests using human lymphocytes showed that compounds isolated were not toxic to these human cells.

For preliminary testing four standard organisms were used to compare the activity of antimicrobial components, namely *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*. These are isolates recommended by the National Committee for Clinical Laboratory Standards. The pure compounds isolated were tested using a wider spectrum of bacteria and fungi.

Acetone as solvent extracted 14 antibacterial inhibitors in previous studies and was hence used for the crude extraction. The extracts were complex and group separation by solvent/solvent extraction yielded six fractions. The hexane and chloroform fractions were selected for further study. The hexane fraction contained mainly non-polar compounds and the chloroform fraction both non-polar and polar compounds. Both these fractions showed activity against *S. aureus*.

Methods used for compound isolation were mainly column chromatography, preparative TLC and HPLC using solvents with different polarities and selectivity. Column chromatography produced the best results, yielding a number of pure compounds. Although PTLC was not as effective, seven highly active compounds were isolated from the hexane fraction but were too impure and in insufficient quantities for structure elucidation.

NMR was the method used for identification of isolated compounds and confirmed by MS. The hexane fraction yielded primarily waxes and fatty acids. Although these compounds are known to exhibit good antimicrobial activity, they have not been used in medicinal research due to their poor pharmacokinetic properties and as a result further research with this fraction was abandoned. Previous work with the chloroform fraction produced triterpenoids but these were in insufficient quantity for identification. This study yielded seven antibacterial flavonoids from the same fraction, possibly due to different extraction techniques. Three of these compounds were flavones, i.e. apigenin, genkwanin and 5-hydroxy-7,4'-dimethoxyflavone and four flavonols were identified i.e. kaempferol, rhamnocitrin, rhamnazin and quercetin-5,3'-dimethylether. Although all these compounds are fairly common flavonoids they are all reported for the first time in *Combretum erythrophyllum*, and in some cases in the family Combretaceae.

Bioassays showed selective antibacterial activity between different microorganisms. In some cases MIC values ranged in the order of 25-100 µg/ml with the overall best activity against *Vibrio cholerae*. For some of these compounds this is the first report of antibacterial activity.

## OPSOMMING

Vorige studies [Martini, 1998] op die blare van *Combretum erythrophyllum* (Combretaceae) het die antimikrobiese aktiwiteit bevestig. Die verbindings verantwoordelik vir die aktiwiteit kon egter nie geïdentifiseer word nie as gevolg van te min materiaal. Die hoof doelwit van hierdie studie was daarom om die antimikrobiese verbindings in die blare van *Combretum erythrophyllum* te isoleer en identifiseer. Bome in die Pretoria gebied was ondersoek om die moontlikheid van variasie in aktiwiteit vas te stel. Daar is nie 'n noemenswaardige verskil in bioaktiwiteit tussen verskillende plante nie. Blaar ekstrakte was ook getoets vir hul potensiaal om vry radikale op te neem. Hierdie vermoë is aanduidend van antioksidant en moontlike anti-inflammatoriese werking. Toksisiëits toetse op menslike limfosiete het gewys dat die geïsoleerde verbindings nie toksies vir hierdie selle is nie.

Vier standaard organismes is in voorlopige toetse gebruik om die aktiwiteit van die antimikrobiese verbindings te vergelyk, naamlik *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* en *Pseudomonas aeruginosa*. Hierdie isolate is deur die "National Committee for Clinical Laboratory Standards" aanbeveel. Die nuwe suiwer geïsoleerde komponente is getoets teen 'n wyer spektrum bakterieë en swamme.

Asetoon as ekstraermiddel, het 14 fraksies met antimikrobie aktiwiteit opgelewer en was dus vir die basiese ekstraksies gebruik. Die ekstrakte was kompleks en groep skeiding met vloeistof/vloeistof-ekstraksie het ses fraksies gelewer. Die heksaan en chloroform-fraksies was gekies vir verdere studie. Die heksaan-fraksie het hoofsaaklik nie-polêre verbindings bevat en die chloroform-fraksie beide polêre en nie-polêre verbindings. Beide hierdie fraksies het aktiwiteit teen *S. aureus* getoon.



Die metodes gebruik om verbindings te isoleer was hoofsaaklik kolom chromatografie, preparatiewe dun-laag chromatografie en HDVC met oplosmiddels met verskillende polariteite en selektiwiteit. Kolom chromatografie het die beste resultate gelewer met heel party suiwer verbindings. Alhoewel PDLC nie so effektief was nie, is sewe hoogs aktiewe verbindings geskei deur die heksaan fraksie. Laasgenoemde was egter te onsuiver en te min vir struktuur uitklaring.

KMR was gebruik vir identifikasie van die geïsoleerde verbindings en is bevestig deur MS. Die heksaan fraksie het hoofsaaklik wasse en vetsure gelewer. Alhoewel hierdie verbindings goeie antimikrobe aktiwiteit het, is hulle nie verder vir moontlike medisinale gebruik nagevors nie as gevolg van hul swak farmakokinetiese eienskappe. Vorige werk met die chloroform-fraksie het triterpenoïde gelewer maar die opbrengs was te min vir identifikasie. Deur van ander tegnieke gebruik te maak is daar in hierdie studie sewe flavonoïde met antimikrobe-aktiwiteit geëkstraheer. Drie van hierdie verbindings was flavone, nl. apigenin, genkwanin en 5-hidroksie-7,4'-dimetoksieflavoon en vier was flavonole nl. kaempferol, rhamnositrin, rhamnazin en quercetin-5,3'-dimetieleter. Alhoewel hierdie verbindings algemene flavonoïde is, is hulle almal vir die eerste keer in *Combretum erythrophyllum* gerapporteer en in party gevalle in die familie Combretaceae.

Bioëssaëring het selektiewe antibakteriese aktiwiteit tussen verskillende mikroorganismes getoon. In sommige gevalle is MIK waardes tussen 25-100 µg/ml gerapporteer met die beste aktiwiteit teen *Vibrio cholerae*. Sommige van hierdie verbindings was nog nooit voorheen beskryf as om oor antimikrobe aktiwiteit te beskik nie.

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