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

BY

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GLOSSARY OF ABBREVIATIONS

2A:3MDC	Acetone:methylene dichloride (2:3)
Acetone-d_6	Deuterated acetone
ATCC	American Type Culture Collection
BEA	Benzene:ethanol:ammonia (9:1:1%)
BuOH	Butanol
CCl_4	Carbon tetrachloride
C_5D_5N	Deuterated pyridine
CDC	Centre for Disease Control
$CDCl_3$	Deuterated chloroform
CEF	Chloroform:ethyl acetate:formic acid (5:4:1)
C_6H_{14}	Hexane
$CHCl_3$	Chloroform
CL	Chemiluminescence
COSY	^{1}H-^{1}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_6	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_2O	Water

HMBC	^{1}H-^{13}C-Heteronuclear Multiple Bond Connectivity
HMQC	^{1}H-^{13}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 geidentifiseer 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. Toksisiteits toetse op menslike limfosiete het gewys dat die geisoleerde 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 geisoleerde komponente is getoets teen ‘n wyer spektrum bakteriee en swamme.

Asetoon as ekstraeermiddel, het 14 fraksies met antimikrobe 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 polêr 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 onsuiwer en te min vir struktuur uitklaring.

KMR was gebruik vir identifikasie van die geisoleerde 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 triterpenoide gelewer maar die opbrengs was te min vir identifikasie. Deur van ander tegnieke gebruik te maak is daar in hierdie studie sewe flavonoide met antimikrobe-aktiwiteit geekstraeer. Drie van hierdie verbindings was flavone, nl. apigenin, genkwanin en 5-hydroksie-7,4’-dimetoksieflavoon en vier was flavonole nl. kaempferol, rhamnocitrin, rhamnazin en quercetin-5,3’-dimetieleter. Alhoewel hierdie verbindings algemene flavonoide is, is hulle almal vir die eerste keer in *Combretum erythrophyllum* geraporteer en in party gevalle in die familie Combretaceae.

Bioessaeering 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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