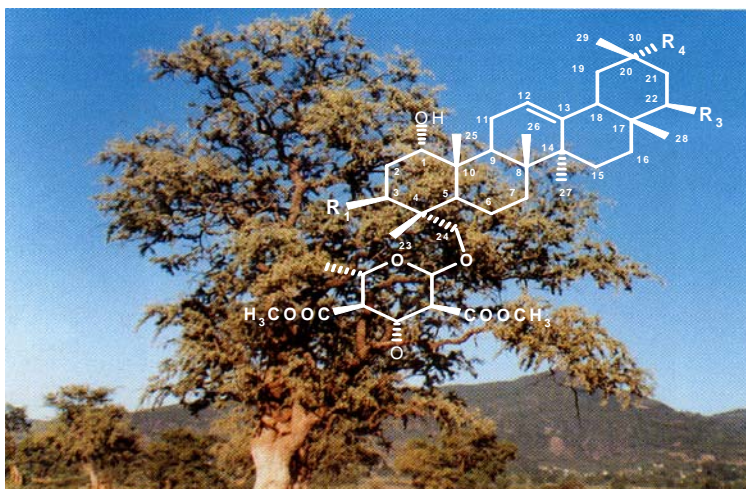
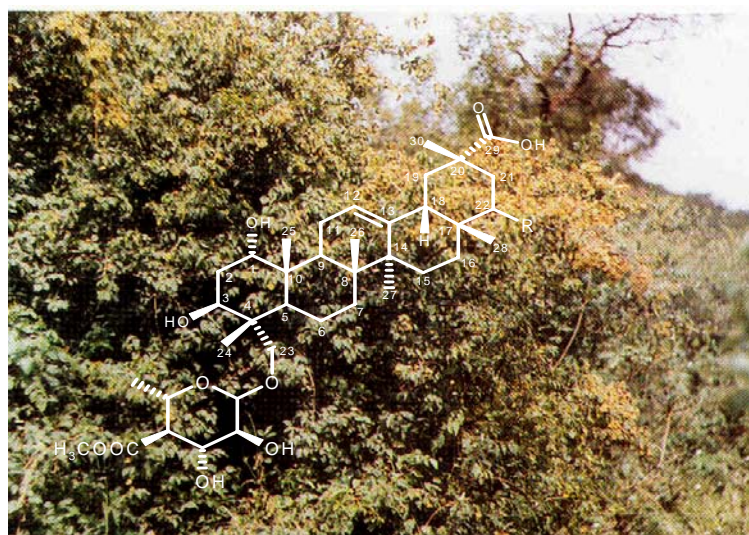


Isolation and characterization of anti-bacterial compounds present in members of *Combretum* section, **Hypocrateropsis**



Combretum imberbe



Combretum padoides

08-11-05

J.E Angeh

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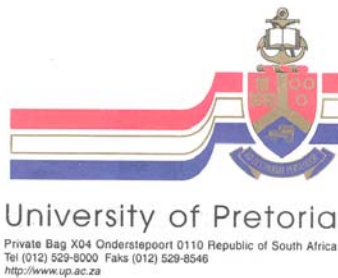
J.E Angeh

B.Tech, MSc (Bauchi)

**Submitted in fulfilment of the requirements for the degree of Doctor of
Philosophy (PhD)**

in the

**Phytomedicine Programme, Department of Paraclinical Sciences
Faculty of Veterinary Science**



Promoter: Prof. J.N. Eloff

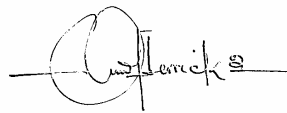
Co-Promoter: Prof. G.E. Swan

Date of submission: November 2005

DECLARATION

The experimental work described in this thesis was conducted in the Phytomedicine Programme, Department of Paraclinical Science, Faculty of Veterinary Science, University of Pretoria between June 2002 to April 2005 and at the Department of Molecular Natural Products Research, Hans-Knöll Institut für Naturstoff Forschung (Hans-Knöll Institute for Natural Product Research) Jena, Germany from March 2003 to June 2003, under the supervision of Prof. JN Eloff, Prof. G. Swan and Dr. Isabel Sattler.

These studies are the result of my own investigations, except where the work of others is acknowledged, and has not been submitted in any other form to another University. I declare the above statement to be true.

A handwritten signature in black ink, appearing to read 'J.E. Angeh' with a date '03' at the end. The signature is written over a horizontal line.

.....
JE Angeh

ACKNOWLEDGEMENTS

I would like to express special thanks to my supervisors, Prof. JN Eloff and Prof. GE Swan for supervising this research, and for their invaluable advice and encouragement during the time we have worked together. Many thanks to the Hans-Knoll Institute (HKI) Jena, Germany for determining some of the assays (cytotoxicity, anti-inflammatory and antiviral assays) that were needed for this research. The research staff of (HKI), especially members of the Molecular Natural Product Research group are gratefully acknowledged for their time and expertise that was always generously given during my research stay in HKI from March 2004 to June 2004.

Special appreciation is due to my friends Dr. S. Huang of HKI, Dr. David Katerere and Dr. Lyndy McGraw for their generous support during my research period. My greatest thanks go to the National Research Foundation (NRF), Germany Academic Exchange Service (DAAD), Phytomedicine Programme and the Faculty of Veterinary Science, University of Pretoria for financial support during these studies.

Special thanks to my supervisor's wife (Mrs Christna Eloff) for her motherly support to my family during this period and my most profound appreciation goes to my loving wife Irene, my daughter Sandy and my late father (Angeh David Ekwa) and mother (Lydia Anchi Angeh) who had been a springboard in my academic support.

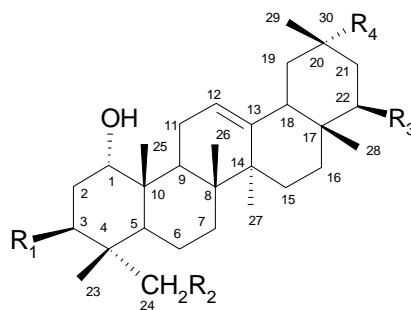
LIST OF ABBREVIATIONS USED

1D	1-dimentional
2D	2-dimentional
CC	Cytotoxic concentration
COSY	Correlation spectroscopy
DCM	Dichloromethane
DEPT	Distortionless enhancement by polarization transfer
DMSO	Dimethyl sulfoxide
EI	Electron impact
ESI	Electron spray impact
GI	Growth inhibition
HMBC	Heteronuclear multiple bond correlation
HMQC	Heteronuclear multiple quantum coherence
IC	Inhibition concentration
IUPF	Indigenous Plant use Forum
MIC	Minimum inhibitory concentration
MS	Mass spectrometer
NMR	Nuclear magnetic resonance
NOESY	Nuclear overhauser enhancement spectroscopy
n-phase	normal phase
NCCLS	National committee for clinical laboratory standards
TDH	Threonine dehydrogenase
B1	<i>Bacillus subtilis</i> ATTC 6633 (IMET) NA
B2	<i>Bacillus subtilis</i> ATTC 6633(IMET) AS
B3	<i>Staphylococcus aureus</i> (IMET 10760) SG 511
B4	<i>Escherichia coli</i> SG 458
B9	<i>Pseudomonas aeruginosa</i> K799/61
M2	<i>Mycobacterium smegmatis</i> SG 987 (HK10056)
M4	<i>Mycobacterium vaccae</i> IMET 10670
H4	<i>Sporobolomyces salmonicolor</i> SBUG 549
H8	<i>Candida albicans</i> BMSY 212
P1	<i>Penicillium notatum</i> JP 36
HKI	Hans-Knoll Institute

VLC	Vacuum liquid chromatography
TLC	Thin layer chromatography
HPLC	High performance liquid chromatography
EMW	Ethyl acetate, methanol, water (40:5.4:4)
BEA	Benzene, ethyl acetate, ammonia (90:10:1)
CEF	Chloroform, ethyl acetate, formic acid (5:4:1)
INT	p-iodonitrotetrazolium violet
C.I	<i>Combretum imberbe</i>
C.P	<i>Combretum padoides</i>
C.Cs.C	<i>Combretum celastroides</i> ssp. <i>celastroides</i>
C.Cs.O	<i>Combretum celastroides</i> ssp. <i>orientale</i>
TDH	Threonine dehydrogenase
EtOAc	Ethyl acetate
R _f	Retention factor
SA	<i>Staphylococcus aureus</i>
EF	<i>Enterococcus faecalis</i>
EC	<i>Echerichia coli</i>
PA	<i>Pseudomonas aeruginosa</i>
ssp	Subspecies

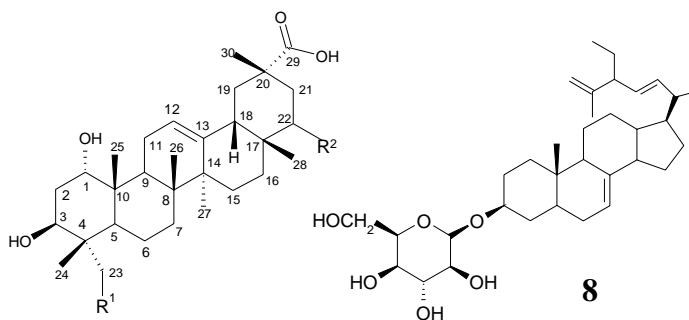
ABSTRACT

Combretum imberbe (leadwood, hardekool) has been used for medicinal purpose and several studies have been carried out to investigate the chemical compounds present in the bark of this plant. Preliminary experiments indicated that the leaves of this plant contain antibacterial compounds that do not occur in other *Combretum* species. Leaves of *C. imberbe* and the closely related *C. padoides* belonging to the *Combretum* section, Hypocrateropsis of the African Combretaceae were extracted and fractionated by bioassay-guided fractionation yielding 5 compounds.

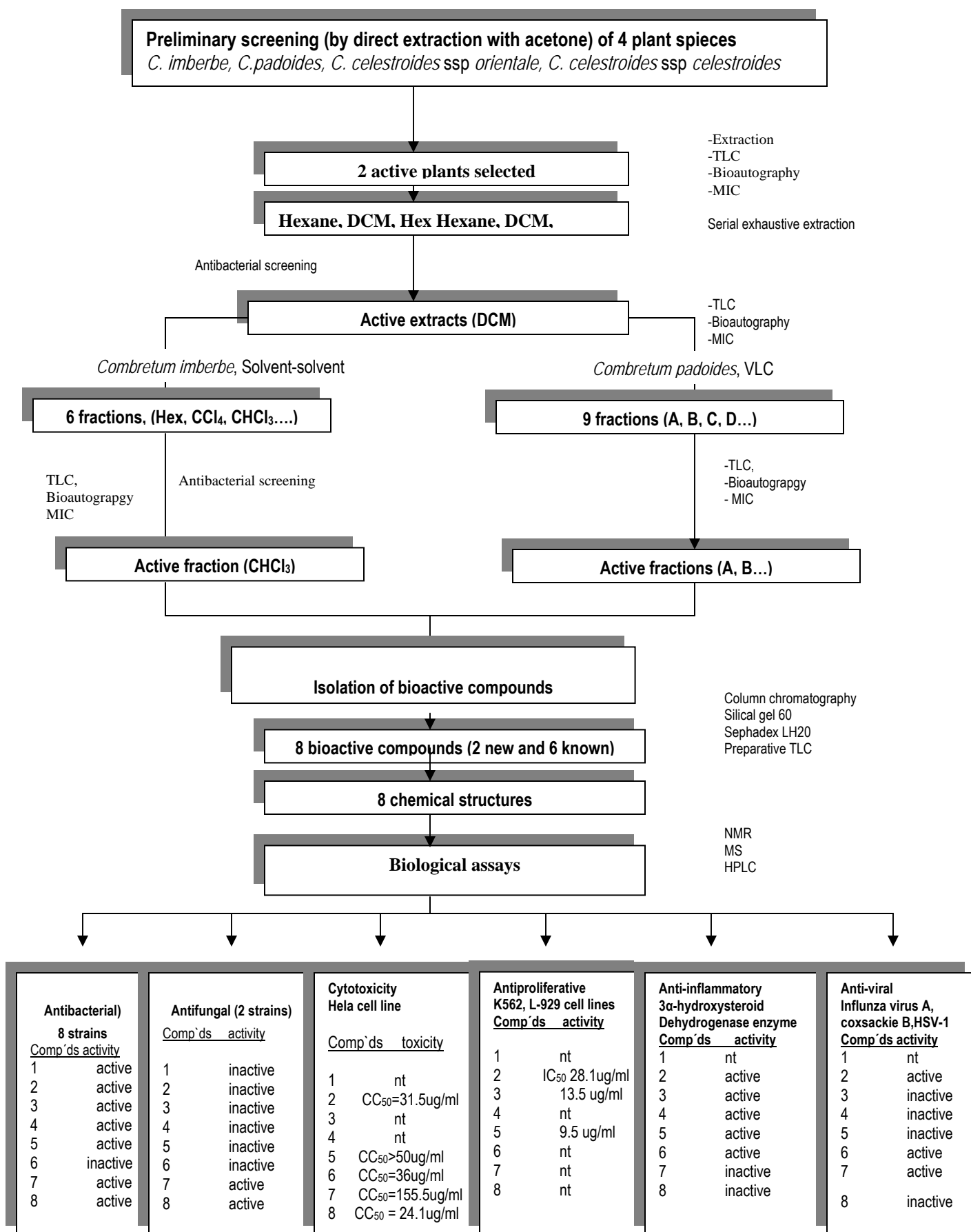


Compounds	R ₁	R ₂	R ₃	R ₄
1	OH	H	H	COOH
2	H	H	H	COOH
3	H	H	=O	CH ₂ OH
4	H	H	OH	COOH
5	2,4-Di-Ac-O-Rh	OH	H	H

Two new antibacterial pentacyclic triterpenoids (1 α , 24 β -dihydroxyl-12-oleanen-29-oic acid-3 β -O- α -2, 4-diacetylramnopyranoside and 1 α , 3 β -dihydroxyl-12-oleanen-29-oic acid-23 β -O- α -4-acetylramnopyranoside) **5** and **6** along with six known triterpenoids **1-4** (1 α , 3 β -dihydroxyoleanen-12-29-oic, 3-hydroxyl-12-olean-30-oic, 3, 30-dihydroxyl-12-oleanen-22-one, 1,3, 24-trihydroxyl-12-olean-29-oic acid **7** (1 α , 22 β -dihydroxyl-12-oleanen-30-oic acid) and **8** (24-ethylcholesta-7, 22,25-trien-3-ol-O- β -D-glucopyranoside) were isolated with the aid of closed and open column chromatography.



Compound	R ¹	R ²
6	4-Ac-O-Rh	H
7	H	OH



Activity not tested (nt), Compounds (comp'd)

All eight compounds had moderate (MIC of 60 µg/ml) to strong (10 µg/ml) antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Mycobacterium vaccae* with **2**, **5** and **7** being most active. Compound **2** and **3** also had strong anti-inflammatory activity against 3 α -hydroxysteroid dehydrogenase enzyme with an IC₅₀ of 10 µg/ml and 7.8 µg/ml as well as moderate cytotoxicity (CC₅₀ = 17.6 µg/ml and CC₅₀ = 10.5 µg/ml) against Hella cell lines. Compound **2** and **5** also had moderate anti-proliferative (GI₅₀ = 16.5 µg/ml, 13.2 µg/ml, 8.7 µg/ml) activity against K-562, L-929 cell lines.

The structures of the compounds were elucidated on the basis of 1-dimensional (1D) and 2-dimensional (2D) Nuclear Magnetic Resonance (NMR) experiments, as well as Electron Impact (EI) and Electron Spray Impact (ESI) mass spectrometric techniques.

The results of this study have added new compounds to the global database of phytochemicals, expanded our knowledge on the phytochemistry of *Combretum*, confirmed the rationale of the ethnomedicinal use of *C. imberbe*, and opened up potential new applications of extracts.

PAPERS PREPARED FROM THIS THESIS

Angeh, J. E., Eloff, J. N., Swan, G.E., 2005. Comparing two extraction methods in isolating antibacterial compounds from *Combretum* section Hypocrateropsis (Manuscript).

Angeh, J. E., Eloff, J. N., Swan, G.E., Huang S., Sattler I., 2005. Activity guided isolation of a new anti-microbial triterpenoid from *Combretum imberbe*. (Manuscript).

Angeh, J. E., Eloff, J. N., Swan, G.E., Huang S., Sattler I., 2005. Novel anti-microbial triterpenoid from *Combretum padoides* (Manuscript).

Angeh, J. E., Eloff, J. N., 2005. Synergism in isolated natural compounds (Manuscript).

CONFERENCES AND PROCEEDINGS

2003

Indigenous Plant Forum (IPUF), Kloof Avenue Rustenburg (South Africa)

Poster: JE Angeh, GE Swan, JN Eloff. Comparing two extraction methods in isolating antibacterial compounds from *Combretum Section Hypocrateropsis*.

Faculty day, Faculty of Veterinary Science, University of Pretoria.

Poster: JE Angeh, GE Swan, JN Eloff. The significance of serial exhaustive extraction in isolating antibacterial compounds from *Combretum imberbe*.

2004

Indigenous Plant Forum (IPUF), Clanwilliam (South Africa).

Paper: JE Angeh, GE Swan, JN Eloff, S. Huang, I. Sattler. Bioassay-guided isolation of pentacyclic triterpenoids from *Combretum imberbe*.

Faculty day, Faculty of Veterinary Science, University of Pretoria.

Paper: JE Angeh, GE Swan, JN Eloff, S. Huang, I. Sattler. Novel biologically active triterpenes from *Combretum imberbe* and *Combretum padoides*

2005

Annual conference of Society for Medicinal Plants Research, Florence, Italy

Paper: J. Angeh, J. Eloff, G. Swan, S. Huangi I, Sattler. Novel biologically active triterpenoids from the African Combretaceae

Indigenous Plant Use Forum (IPUF), Gramhamstown

Paper: J. Angeh, J. Eloff. Synegetic effect of triterpenoids isolated from *Combretum* species.

TABLE OF CONTENT

DECLARATION	i
ACKNOWLEDGEMENTS	ii
LIST OF ABBREVIATIONS USED	iii
ABSTRACT.....	iv
PAPERS PREPARED FROM THIS THESIS	v
CONFERENCES AND PROCEEDINGS.....	vi
CHAPTER 1. INTRODUCTION.....	1
1.1 Introduction.....	1
1.2 Hypothesis.....	2
1.3 Aim of research.....	3
1.3.1 Objectives.....	3
CHAPTER 2: LITERATURE REVIEW.....	4
2.1 Introduction.....	4
2.2 Antibiotic resistance.....	5
2.3 Natural products in drug discovery.....	6
2.4 Plants as a potential source of antibiotics.....	9
2.5 Plants and antibacterial production.....	11
2.6 The family Combretaceae.....	13
2.6.1 Taxonomy.....	13
2.6.2 Taxonomy of the section Hypocrateropsis.....	14
2.6.2.1 <i>Combretum imberbe</i> Engl. & Diels.....	14
2.6.2.2 <i>Combretum padoides</i> Engl. & Diel.....	15
2.6.2.3 <i>Combretum celastroides</i> Welw Ex M.A. Lawson.....	16
2.7 Ethnopharmacology of Combreteceae.....	17
2.8 Phytochemistry/chemistry and biological activity of Combretaceae.....	18
2.8.1 Tannins.....	19
2.8.1.1 Classes of tannins.....	19
2.8.1.1.1 Hydrolysable tannins (HTs).....	19

2.8.1.1.2	Proanthocyanidins (Condensed tannins).....	20
2.8.2	Tannins chemistry of Combretaceae.....	22
2.8.3	Stilbenoids.....	22
2.8.4	Flavonoids.....	25
2.8.4.1.2	Flavonoids chemistry of Combretaceae.....	26
2.8.5	Terpenoids.....	27
2.8.5.1	Classes of terpenes.....	28
2.8.5.1.1	Terpene essential oils.....	28
2.8.5.1.2	Diterpenoids and Gibberellins.....	28
2.8.5.1.3	Triterpenoids and Steroids.....	29
2.8.5.1.4	Tetraterpenoids (Carotenoids).....	29
2.8.5.2	Terpenoids chemistry of Combretaceae.....	30
2.8.6	Alkaloids.....	32
2.8.6.1	Classes of alkaloids.....	33
2.8.6.1.1	Ornithine and lysine derivatives.....	33
2.8.6.1.2	Phenylalanine and tyrosine derivatives.....	33
2.8.6.1.3	Tryptophan derivatives.....	34
2.8.6.2	Combretaceae alkaloids chemistry.....	34
2.8.7	Other constituents.....	34
2.9	Methods developed and results obtained in the phytomedicine programme.....	35
2.9.1	Bioautography and MIC methods.....	35
2.9.2	Overview of activities in Combretaceae research in the phytomedicine group.....	35
2.9.2.1	Selection of plants.....	35
2.9.2.2	Selection of best extraction procedure.....	36
2.9.2.3	Selection of best purification procedures.....	36
2.9.2.4	Developing a novel way of determining antibacterial activity.....	36
2.9.2.5	Antibacterial activity of <i>C. erythrophyllum</i>	36
2.9.2.6	Antibacterial activity and stability of 27 members of Combretaceae.....	36
2.9.2.7	Stability of antibacterial activity in <i>C. erythrophyllum</i>	36
2.9.2.8	A proposal for expressing antibacterial activity.....	37
2.9.2.9	Isolation of antibacterial compound from <i>C. woodii</i>	37
2.9.2.10	Isolation of antibacterial compounds from <i>C. erythrophyllum</i>	37
2.9.2.11	Other biological activities of <i>Combretum</i> species.....	37

2.9.3 Other work done on <i>C. imberbe</i> and <i>C. padoides</i>	38
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CHAPTER 3: PLANT COLLECTIONS, EXTRACTION AND ANALYSIS.....44

3.1 Introduction.....	39
3.2 Material and Methods.....	39
3.2.1 Experimental design for bioassays.....	39
3.2.2 Plant collection.....	40
3.2.3 Plant preparation and extraction.....	40
3.2.3.1 Direct extraction with acetone.....	40
3.2.3.2 Serial exhaustive extraction.....	41
3.2.4 Analysis of plant extracts for preliminary screening.....	42
3.3 Results.....	43
3.3.1 Extraction.....	43
3.3.1.1 Direct extraction with acetone.....	43
3.3.1.2 Serial exhaustive extraction.....	43
3.3.2 TLC analysis of plant extracts for preliminary screening.....	44
3.3.3 TLC analysis of plant extracts from serial exhaustive extraction.....	45
3.4 Discussion and conclusion.....	48
3.5 Summary.....	49

CHAPTER 4: BIOLOGICAL ASSAYS FOR PRELIMINARY SCREENING50

4.1 Introduction.....	50
4.2 Materials and methods.....	50
4.2.1 Antibacterial assay.....	50
4.2.1.1 Test organisms.....	50
4.2.2 Bioautography for preliminary screening.....	50
4.2.3 .Microplate dilution assay for preliminary screening.....	51
4.2.4 Determination of total activity.....	52
4.3 Results.....	53
4.3.1 Antibacterial assays.....	54
4.3.1.1 Bioautography.....	57
4.3.1.2 Bioautography of serial exhaustive extraction extracts.....	57

4.3.2	Minimum inhibitory concentration of extracts from serial exhaustive extraction.....	68
4.3.3	Total activity of extracts.....	60
4.4	Discussion and conclusion.....	60
4.5	Summary.....	61
CHAPTER 5: PRELIMINARY SEPARATION AND ISOLATION OF BIOACTIVE COMPOUNDS.....		63
5.1	Introduction.....	63
5.2	Materials and methods.....	63
4.3.4	Solvent-solvent fractionation.....	63
5.2.2	Column chromatography.....	63
5.2.2.1	Vacuum Liquid chromatography.....	65
5.2.2.2	Gravity gradient column chromatography.....	66
5.2.2.3	Sephadex LH-20.....	67
5.2.3	Conventional Preparative TLC.....	68
5.2.4	Analysis and Grouping of fractions.....	68
5.2.4.1	Combination of fractions.....	69
5.2.5	Dereplication.....	69
5.3	Results and discussions.....	69
5.3.1	Solvent-solvent fractionation of the DCM extracts.....	69
5.3.1.1	Complexity of fractions.....	70
5.3.2	Vacuum liquid chromatography.....	71
5.3.2.1	Fractions from VLC process of <i>C. imberbe</i>	74
5.3.3	Gravity gradient chromatography fractions from solvent-solvent fractionation process of <i>C. imberbe</i>	74
5.3.4	Sephadex LH-20.....	75
5.3.5	Conventional preparative TLC.....	76
5.3.6	Gravity gradient chromatography.....	76
5.3.7	Bioautography and MIC.....	78
5.3.8	Bioautography and MIC of VLC fractions.....	81
5.3.9	Dereplication of compounds.....	82
5.4	Summary.....	83

CHAPTER 6: INSTRUMENTAL ANALYSIS AND STRUCTURAL ELUCIDATION OF ISOLATED COMPOUNDS.....	84
6.1 Introduction.....	84
6.2 Nuclear magnetic resonance spectroscopy [NMR].....	84
6.2.1 NMR sample preparation.....	84
6.2.2 One-dimensional spectroscopy.....	85
6.2.2.1 ¹ H-NMR.....	85
6.2.2.2 ¹³ C-NMR.....	86
6.2.2.3 DEPT.....	86
6.2.3 Two-dimensional spectroscopy.....	86
6.2.3.1 Homonuclear correlations.....	86
6.2.3.1.1 ¹ H- ¹ H Correlation Spectroscopy (COSY).....	86
6.2.3.2 Heteronuclear correlations.....	86
6.2.3.2.1 Heteronuclear Multiple Quantum Coherence (HMQC) (¹ H- ¹³ C COSY).....	87
6.2.3.2.2 Heteronuclear Multiple Bond Connectivity (HMBC) (Long range ¹³ C- ¹ H COSY).....	87
6.2.3.2.3 NOESY spectroscopy.....	87
6.3 Mass spectrometry.....	88
6.3.1 Sample preparation.....	88
6.3.2 Gas Chromatography - Mass Spectrometry (GC-MS).....	88
6.3.2.1 Electron Impact Spray MS (EIS-MS).....	88
6.4 IR.....	88
6.5 Results and Discussions.....	89
6.5.1 Identification of compounds.....	89
6.5.1.1 Compound 1.....	89
6.5.1.2 Compound 2.....	94
6.5.1.3 Compound 3.....	95
6.5.1.4 Compound 4.....	97
6.5.1.5 Compound 5.....	99
6.5.1.6 Compound 6.....	104
6.5.1.7 Compound 7.....	110
6.5.1.8 Compound 8.....	114
6.6 Summary.....	119

CHAPTER 7: BIOLOGICAL CHARACTERIZATION OF ISOLATED COMPOUNDS.....	121
7.1 Introduction.....	121
7.2 Material and methods.....	121
7.2.1 Anti-microbial activity.....	121
7.2.1.1 Antibacterial activity (Microplate dilution assay).....	121
7.2.1.2 Antibacterial and antifungal activity (Agar diffusion assay).....	121
7.2.1.3 Antimicrobial effect of some combined compounds.....	122
7.2.2 Anti-inflammatory activity.....	122
7.2.2.1 Preparation of Cytosol.....	123
7.2.2.2 Preparation of Purified 3 α -Hydroxysteroid Dehydrogenase	123
7.2.2.3 Enzyme Assays.....	123
7.2.2.4 Inhibition Studies.....	124
7.2.3 Antiproliferative and cytotoxicity assay.....	124
7.3 Results and Discussion.....	125
7.3.1 Antibacterial activity (Microplate dilution assay).....	125
7.3.2 Antimicrobial activity (Agar diffusion assay).....	126
7.3.3 Combined antimicrobial effect of mixtures of isolated compounds.....	128
7.3.4 Anti-inflammatory activity.....	130
7.3.5 Anti-proliferative effect and Cytotoxicity.....	131
7.3.5 Structure activity relationship of isolated compounds.....	132
7.4 Summary	132
CHAPTER 8: GENERAL CONCLUSION.....	134
8.1Introduction.....	134
8.2 Evaluation on the best preliminary fractionation procedure.....	134
8.3 Isolation and chemical characterization of antibacterial compounds.....	135
8.4 Biological characterization of plant species and isolated compounds.....	136
8.5 Evaluation of how well phytochemistry agrees with taxonomy based on anatomy.....	137
CHAPTER 9: REFERENCES.....	138-146

LIST OF FIGURES

Figure 2-1: The sub generic classification for South Africa Combretaceae according to Carr (1988)	14
Figure 2-2: <i>C. imberbe</i> Leadwood/hardekool (Steyn, 1994).....	15
Figure 2-3: <i>C. padoides</i> (Steyn, 1997).....	16
Figure 2-4: <i>C. celastroides</i> ssp. <i>orientale</i> (Steyn, 1994).....	17
Figure 3-1: Percentage of material extracted from <i>C. imberbe</i> and <i>C. padoides</i> by each solvent in the serial exhaustive extraction process.....	43
Figure 3-2: Percentage of material extracted from <i>C. imberbe</i> and <i>C. padoides</i> by each solvent in the serial exhaustive extraction process.....	45
Figure 3-3: TLC chromatograms (viewed under UV 365 nm and vanillin sulphuric acid spray reagent) of extracts from direct extraction with acetone in preliminary screening process.....	45
Figure 3-4: TLC chromatogram (viewed after spraying with vanillin sulphuric acid spray reagent) of serial exhaustive extraction extracts of <i>C. imberbe</i> and <i>C. padoides</i> developed with EMW. Areas on the chromatogram cycled indicate UV active compounds.....	46
Figure 3-5: TLC chromatogram (viewed after spraying with vanillin sulphuric acid spray reagent) of serial exhaustive extraction extracts of <i>C. imberbe</i> and <i>C. padoides</i> developed with CEF. Areas on the chromatogram cycled indicate UV active compounds.....	47
Figure 3-6: TLC chromatogram (viewed after spraying with vanillin sulphuric acid spray reagent) of serial exhaustive extraction extracts of <i>C. imberbe</i> and <i>C. padoides</i> developed with BEA. Areas on the chromatogram cycled indicate UV active compounds.....	47
Figure 4-1: INT, coupling reagent for the colorimetric assay (reaction pathway for the assay of TDH).....	51
Figure 4-2: Bioautograms of acetone extracts of <i>C. imberbe</i> , <i>C. padoides</i> , <i>C. celastroides</i> ssp. <i>orientale</i> and <i>C. celastroides</i> ssp. <i>celastroides</i> . TLC developed in EMW and sprayed with actively growing <i>S.</i> <i>aureus</i> cultures and later sprayed with INT. White areas indicate zones of growth inhibition.....	54
Figure 4-3: Bioautograms of acetone extracts of <i>C. imberbe</i> , <i>C. padoides</i> , <i>C. celastroides</i> ssp. <i>orientale</i> and <i>C. celastroides</i> ssp. <i>celastroides</i> . TLC developed in EMW and sprayed with actively growing <i>P.</i> <i>aeruginosa</i> cultures and later sprayed with INT. White areas indicate zones of growth inhibition.....	54

Figure 4-4: Bioautograms of acetone extracts of <i>C. imberbe</i> , <i>C. padoides</i> <i>C. celastroides</i> ssp. <i>orientale</i> and <i>C. celastroides</i> ssp. <i>celastroides</i> . TLC developed in BEA and sprayed with actively growing <i>S. aureus</i> cultures and later sprayed with INT. White areas indicate zones of growth inhibition.....	55
Figure 4-5: Bioautograms of acetone extracts of <i>C. imberbe</i> , <i>C. padoides</i> , <i>C. celastroides</i> ssp. <i>orientale</i> and <i>C. celastroides</i> ssp. <i>celastroides</i> . TLC developed in BEA and sprayed with actively growing <i>E. coli</i> cultures and later sprayed with INT. White areas indicate zones of growth inhibition.....	56
Figure 4-6: Bioautography of serial exhaustive extraction extracts on <i>S. aureus</i> sprayed with INT sprayed reagent. White areas indicate zones of inhibition.....	57
Figure 4-7: Bioautography (BEA solvent system) of serial exhaustive extraction extracts against <i>S. aureus</i> sprayed with INT spray reagent. White areas indicate zones of inhibition.....	57
Figure 4-8: Bioautography of serial exhaustive extraction extracts against <i>E. coli</i> , using EMW solvent system and sprayed with INT spray reagent. White areas indicate zones of inhibition.....	58
Figure 4-9: Bioautography of serial exhaustive extraction extracts against <i>E. coli</i> , using BEA solvent system and sprayed with INT spray reagent. White areas indicate zones of inhibition.....	58
Figure 4-10: MIC of the hexane and DCM extracts of <i>C. imberbe</i> and <i>C. padoides</i> on <i>S. aureus</i> on microtitre plates. White wells indicate inhibition and purple wells indicate bacterial growth.....	58
Figure 4-11: MIC of the hexane and DCM extracts of <i>C. imberbe</i> and <i>C. padoides</i> against <i>E. coli</i>	58
Figure 5-1: Diagrammatic summary of the isolation routes.....	62
Figure 5-2: The protocol used for the solvent-solvent fractionation of the components in the DCM extract of <i>C. imberbe</i> and <i>C. padoides</i>	64
Figure 5-3: Vacuum liquid chromatography apparatus (Houghton, 1998)	65
Figure 5-4: Separation of components present in the different fractions obtained by solvent-solvent extraction by EMW and sprayed with vanillin-sulphuric acid. Lanes from left to right: hexane, water, n-butane, carbon tetrachloride, chloroform and 35% water in methanol fractions. In each case 50 µg of 5 mg/ml stock solution was chromatographed.	71
Figure 5-6: Chromatogram ran in CEF indicating number of test tubes collected in the VLC process.....	71
Figure 5-7: Grouped fractions resulting from Vacuum Liquid Chromatography of DCM extract of <i>C. imberbe</i> ran with CEF solvent system. Lane from left to right: DCM crude extract, fractions A, B, C, D, E, F and G.....	72
Figure 5-8: Chromatogram from the combined fractions E, F and G ran on CEF solvent system.....	72
Figure 5-9: Quantities obtained from 6.55 g of chloroform fraction with the solvent-solvent fractionation process of the DCM extract of <i>C. imberbe</i>	73

Figure 5-10: Chromatogram of fractions resulting from the wash of crystallized test tubes of fraction A and B run along the crude DCM extract.....	73
Figure 5-11: Separation of components present in 50 µg of 12 different fractions resulting from 3 x 35 cm silica gel 60 column using EMW as eluent and vanillin-sulphuric acid spray reagent.....	75
Figure 5-12: Quantities obtained from 6.55 g of chloroform fraction with the solvent-solvent fractionation process of the DCM extract of <i>C. imberbe</i>	75
Figure 5-13: Chromatogram of the separation of 2.5 g of F ₈ -F ₁₂ combined fraction on Sephadex LH-20 ran with chloroform/methanol (9:1) solvent system and sprayed with vanillin sulphuric acid spray reagent.	75
Figure 5-14: Compounds 4 and 5 isolated from <i>C. imberbe</i> by Sephadex LH-20 column.....	76
Figure 5-15: Gravity based separation of components of the chloroform fraction resulting from the solvent-solvent fractionation of the DCM fraction of <i>C. padoides</i> ran in EMW and sprayed with vanillin sulphuric acid.....	76
Figure 5-16: Separation of re-grouped fractions of the gravity based separation of components of the chloroform fraction resulting from the solvent-solvent fractionation of the DCM fraction of <i>C. padoides</i>	77
Figure 5-17: Chromatogram (developed in EMW sprayed with vanillin sulphuric acid spray reagent) of compounds isolated from <i>C. padoides</i>	78
Figure 5-18: Bioautogram of DCM extracts of <i>C. imberbe</i> (left) and <i>C. padoides</i> (right) leaves separated into different fractions by solvent-solvent extraction. TLC plate developed in EMW and sprayed with <i>S. aureus</i> culture incubated overnight and then sprayed with INT. Growth inhibition is indicated by colourless zones on TLC plates. Lanes from left to right: hexane, water, n-butanol, carbon tetrachloride, 35% water in methanol, chloroform fractions.	79
Figure 5-19: Bioautogram of DCM extracts of <i>C. imberbe</i> (left) and <i>C. padoides</i> (right) leaves separated into different fractions by solvent-solvent extraction. TLC plate developed in BEA and sprayed with <i>S. aureus</i> culture incubated overnight and then sprayed with INT. Growth inhibition indicated by colourless zones on TLC plates. Lanes from left to right: hexane, water, n-butanol, carbon tetrachloride, 35% water in methanol, chloroform fractions.	85
Figure 5-20: Bioautogram of the group separation (silica gel 60) of 6.55 g of the chloroform resulting from the solvent-solvent separation of DCM extract of <i>C. imberbe</i> on EMW.	80
Figure 5-21: Bioautogram of the DCM extract of <i>C. imberbe</i> separated into different fractions in a Vacuum Liquid Chromatography process. TLC plate developed in CEF (as best separating system) and sprayed with <i>S. aureus</i> culture, incubated overnight and then sprayed with INT. Growth inhibition	

indicated by colourless zones on TLC plates. Lanes from left to right: fractions A, B, C, D, E, F and G.	81
Figure 5-22: Chromatogram of the crude extract of <i>C. imberbe</i> compared with isolated compounds to dereplicate isolated compounds from the crude extract. Lanes from left to right: Crude extract of <i>C. imberbe</i> , compounds 1, 4, 5 and 3.	82
Figure 5-23: Identification of isolated compounds from the crude extract of <i>C. padoides</i>	82
Figure 6-1: Skeletal unit of olean-12-ene type of pentacyclic triterpenoid isolated from <i>C. imberbe</i>	89
Figure 6-2: Fragmentation pattern of compound 1.....	91
Figure 6-3: EI-MS spectrum of compound 1.....	91
Figure 6-4: ¹ H NMR spectrum of Compound 1.....	92
Figure 6-5: ¹³ C NMR spectrum of Compound 1.....	93
Figure 6-6: ¹³ C NMR spectrum of Compound 2.....	95
Figure 6-7: Fragmentation pattern of compound 3.....	96
Figure 6-8: Electron impact mass spectrum of Compound 3 indicating the m/e of each fragment.....	97
Figure 6-9: ¹ H NMR spectrum of Compound 3	97
Figure 6-10: ¹ H NMR spectrum of Compound 4.....	98
Figure 6-11: DEPT NMR spectrum of Compound 4 differentiating CH ₃ -, CH ₂ -, and CH- carbons.....	99
Figure 6-12: ¹ H NMR spectrum of compound 5.....	102
Figure 6-13: DEPT spectrum of Compound 5 indicating the presence of CH ₃ -, CH ₂ - and CH- carbons in the compound.....	103
Figure 6-14: ¹ H NMR spectrum of compound 6.....	107
Figure 6-15: ¹³ C NMR of Compound 6.....	108
Figure 6-16: HMBC spectrum of Compound 6.....	109
Figure 6-17: ¹ H NMR of compound 7.....	111
Figure 6-18: DEPT spectrum of Compound.....	112
Figure 6-19: ¹³ C NMR of Compound 7.....	113
Figure 6-20: ¹ H-NMR of Compound 8.....	115
Figure 6-21: DEPT spectrum of Compound.....	116
Figure 6-22: Compounds 1-5 isolated from <i>C. imberbe</i>	119
Figure 6-23: Compounds 6-8 isolated from <i>C. padoides</i>	120

LIST OF TABLES

Table 2-1: Plant-derived drugs widely employed in Western medicine (Adapted from Farnsworth, 1984).....	4
Table 2-2: Plants containing chemotherapeutic activity.....	7
Table 2-3: Some plant-derived preparations for medicinal use	10
Table 2-4: Derivatives of stilbenes (Combrestatins) isolated from Combretaceae.....	23
Table 2-5: Derivatives of dihydrostilbenes (Combrestatins) isolated from Combretaceae.....	23
Table 2-6: Derivatives of phenanthrenes isolated from Combretaceae.....	24
Table 3-1: Quantity extracted at each batch (A, B, C, D, E, and F) in a serial exhaustive extraction from 500 g of <i>C. imberbe</i> leaves.....	44
Table 3-2: Quantity extracted at each batch (A, B, C, D, E and F) in a serial exhaustive extraction from 500 g of <i>C. padoides</i> leaves.	44
Table 3-3: Total number of UV and vanillin spray reagent active compounds resulting from direct extraction with acetone of <i>C. imberbe</i> and <i>C. padoides</i>	46
Table 3-4: Total number of UV and vanillin sulphuric acid spray reagent active compounds resulting from the serial exhaustive extraction extracts of <i>C. imberbe</i> and <i>C. padoides</i> in EMW solvent system.	48
Table 4-1: Bioautography (TLC in EMW) and Minimum Inhibitory Concentration (MIC) results of preliminary screening of <i>C. imberbe</i> , <i>C. padoides</i> , <i>C. celastroides</i> ssp. <i>orientale</i> and <i>C. celastroides</i> ssp. <i>celastroides</i> acetone extracts against <i>S. aureus</i> (SA), <i>E. faecalis</i> (EF), <i>E. coli</i> (EA) and <i>P. aeruginosa</i> (PA)	56
Table 4-2: Bioautography, MIC and total activity values of serial exhaustive extracts (<i>S. aureus</i> (SA), <i>E. faecalis</i> (EF), <i>E. coli</i> (EC) and <i>P. aeruginosa</i> (PA)).....	60
Table 5-1: Gradients of solvent used in VLC for the separation of the dichloromethane extract of <i>C.imberbe</i>	65
Table 5-2: Quantity (g) and percentage of initial mass (10 g) of <i>C. imberbe</i> and <i>C. padoides</i> DCM extracts, extracted by different solvents in a solvent-solvent fractionation process.....	70
Table 5-3: Quantity obtained, colour and complexity of fractions resulting from a VLC process of the DCM extract of <i>C. imberbe</i>	73

Table 5-4: Quantity of fractions derived through gravity gradient column chromatography of the chloroform fraction obtained from solvent-solvent fractionation of the DCM extract of <i>C. padoides</i>	77
Table 5-5: Number of antibacterial compounds and MIC values of fractions resulting from the solvent-solvent fractionation process of the DCM fraction of <i>C. imberbe</i> and <i>C. padoides</i>	80
Table 5-6: Quantity obtained, no of compounds, and MIC of VLC fractions of DCM extract of <i>C. imberbe</i>	81
Table 5-7: Dereplication, R _f values of isolated compounds.....	83
Table 6-1: ¹³ C (300 MHz) and ¹³ C (75.4 MHz) NMR data of 1-4 (CD ₃ OD)	101
Table 6-2: ¹ H (300 MHz) and ¹³ C (75.4 MHz) NMR data of 6 (CD ₃ OD).....	104
Table 6-3: HMBC correlations for methyl groups in compound 5.....	105
Table 6-4: ¹³ C (75.4 MHz) and ¹ H (300 MHz) NMR data of 7 band 8 in CD ₃ OD.....	117
Table 6-5: ¹ H (300 MHz) and ¹³ C (75.4 MHz) NMR data of 6(CD ₃).....	118
Table 7-1: Bacteria and Fungi tested for efficacy in the Agar diffusion assay.....	122
Table 7-2: MIC of compounds isolated from <i>C. imberbe</i> and <i>C. padoides</i> [<i>S. aureus</i> (SA), <i>E. faecalis</i> (EF), and <i>E. coli</i> (EC), <i>P. aeruginosa</i> (PA)]	125
Table 7-3: Zone of inhibition of compounds isolated from <i>C. imberbe</i> and <i>C. padoides</i> against several bacterial and fungal organisms (<i>B. subtilis</i> ATTC 6633 (IMET) NA (B1), <i>B. subtilis</i> ATTC 6633 (IMET) NS (B2), <i>S. aureus</i> (IMET 10760) SG511 (B3), <i>E. coli</i> SG 458 (B4), <i>P. aeruginosa</i> K 799/61 (B9), <i>M. smegmatis</i> SG 987 (HKI0056) (M2), <i>M. vaccae</i> IMET 10670 (M4) H4 H8 & P1?	127
Table 7-4: MIC (µg/ml) values of individual and mixtures of isolated compounds.....	129
Table 7-5: Anti-inflammatory activity of compounds isolated from <i>C. imberbe</i> and <i>C. padoides</i>	130
Table 7-6: Anti-proliferative and cytotoxic effect of compounds isolated from <i>C. imberbe</i> and <i>C. padoides</i> ...	131