ISOLATION AND IDENTIFICATION OF COMPOUNDS FROM

THE LEAF EXTRACTS OF *Barleria dinteri*, *Grewia flava* and

*Jatropha lagarinthoides*

BY

GOLOLO SECHENE STANLEY

Submitted in fulfilment of the requirements for the degree of DOCTOR OF

PHILOSOPHY (BIOCHEMISTRY), in the Faculty of HEALTH SCIENCES, at the

SEFAKO MAKGATHO HEALTH SCIENCES UNIVERSITY, South Africa

Supervisor: Prof. M.A. Mogale

Co-supervisors: Prof N.M. Agyei

Dr L.J. Shai

NOVEMBER 2015
DECLARATION

I declare that the thesis hereby submitted to the Sefako Makgatho Health Sciences University, for the degree of Doctor of Philosophy in Biochemistry has not previously been submitted by me for a degree at this or any other University; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

Signature…………………………..            Date……………………………………
DEDICATION

I would like to dedicate this thesis for the degree of *Doctor of Philosophy in Biochemistry* to my uncle, Mr Maesela Jan Gololo, who has instigated in me the belief that education is the right way for one to overcome challenging socio-economic circumstances and the entire Gololo family for their invaluable support.
ACKNOWLEDGEMENTS

Firstly I would like to thank my God for giving me strength, perseverance and wisdom to conceptualize and carry-out this work to completion.

*I would like to express my sincere gratitude and appreciation to:*

- My supervisors: Prof M.A. Mogale, Prof N.M. Agyei and Dr L.J. Shai, for believing in my capabilities and their guidance throughout this challenging journey.
- My partner: Keletso Angelina, for the unwavering support and tolerating my absence from home in pursuit of my academic endeavours.
- My boys: Kegaugetswe, Mogau and Kgotsi, for providing me with the will and drive to pursue and achieve more in my life.
- My mom: Ramadimetja Paulina, for being everything and more one could ask from a mother.
- My late dad: Karel Piet, for instigating in me the conviction of remaining humble regardless of achievements.
- My friend and grandmother: Raisibe Rosina Kantoro, for being such an inspiration in my life and an example to always do well to others.
- My brothers, sisters and cousins: Klaas, Tinos, Reuben, Joeman, Furi, Lindiwe, Nomhle, Rosy, Marcia, *late* Tshepo, Prince, Taelo, Trinity and Kholofelo for always reminding me how important family is.
- My nephews and nieces: Genny, Seipei, Tebogo, Bafana, Siyanda, Princess, Thabang, Mosa and Kgethi, for bringing out the best in me to be a responsible uncle.
• My uncle: Khambane Jantjie Daniel, sometimes I don’t know what to refer you as because of how close we are. Whether as an uncle, brother or friend? May God bless you.

• My late grandfather: Madimetja Bop Frans, I always wish you were around to witness the fruits from the seeds you sowed.

• My late grandfather’s brother: William Lesetja Sebakeng, for sharing with me the knowledge of traditional medicinal plants and as such giving me an insight into the family heritage.

• My late grandfather’ sister: Shannana Linah and family, words can never be enough to describe your compassion.

• My friends: Laiti and John, for your support.

• Prof P.W. Mashela for when my life was in darkness you swung light into my path.

• Mr M.J. Nkhuna and Mr H. Ledwaba for teaching me how important it is for one to be engaged into community activism.

• My colleagues: Sis Debrah, Sebo, Annanias, Emelinah, Isaac, Freddy, Mutendela, Lesibana, Mr Ndlovu, Mr Mofokeng, Dr Debeila and Dr Maseko, for your support and making our workplace home away from home.

• Dr Makhosazana Gamedze, Mr Isaac Masilela, Mr Lesibana Sethoga and Mr Freddy Makhubela for assistance with acquiring NMR spectra.

• Dr Edward Bassey for assistance with structural elucidations of the compounds.

• My Honours students: Sandy, Briget, Nadhira, Keneilwe, Gerald, Thandi, Mashudu, Karabo and Nkhanedzeni, for your assistance in data collection. Thank you very much.

• The Sefako Health Sciences University Faculty of Health Sciences for financial assistance.
TABLE OF CONTENTS

Title
Declaration
Dedication
Acknowledgements
List of Abbreviations
List of Figures
List of Tables
Abstract

CHAPTER 1

Introduction, Background, Research problem and Aim of study

1.1. Introduction and Background
1.2. Research Problem
1.3. Research Questions
1.4. Significance of the Study
1.5. Purpose of the Study 4

1.5.1. Aim of the study 4

1.5.2. Study Objectives 4

1.6. Organization of the thesis 5

CHAPTER 2 7

Literature Review

2.1. Introduction 7

2.2. Nature and Classification of Phytochemical 7

2.2.1. Phenolic Compounds 10

2.2.1.1. Phenolic acids 11

2.2.1.2. Coumarins 13

2.2.1.3. Chalcones 14

2.2.1.4. Flavonoids 17

2.2.1.5. Tannins 21

2.2.2. Terpenes 22

2.2.3. Alkaloids 26
2.2.4. Glycosides

2.2.4.1. Saponin glycosides

2.2.4.2. Anthraquinones glycosides

2.2.4.3. Reducing sugars

2.3. Effect of seasonal variation on the phytochemical composition of medicinal plants

2.4. Medicinal plants under current study

2.4.1. Description of medicinal plants under current study

2.4.1.1. Barleria dinteri

2.4.1.2. Grewia flava

2.4.1.3. Jatropha lagarinthoides

2.4.2. Medicinal plant concoctions

2.4.3. Extraction of medicinal plants constituents

2.4.3.1. Extraction techniques

2.4.3.2. Choice of extraction solvents

2.4.4. Phytochemical screening of medicinal plants extracts

2.5. Isolation and identification of bioactive compounds from medicinal plant extracts
2.5.1. Bioassays

2.5.1.1. Antioxidant activity bioassays

2.5.1.2. Antibacterial activity bioassays

2.5.1.2.1. Disc diffusion method

2.5.1.2.2. Bioautography method

2.5.1.2.3. Microdilution method

2.5.2. Purification and identification of bioactive compounds

2.5.2.1. Bioassay-guided fractionation

2.5.2.2. Identification of isolated compounds

2.6. Conclusion

CHAPTER 3

Research Design and Methodology

3.1. Research design

3.2. Methodology

3.2.1. Sample collection and Identification

3.2.2. Experimental Procedure
3.2.2.1. Qualitative Phytochemical analysis

3.2.2.2. Effect of season on phytochemical compositions of the three medicinal plants

3.2.2.2(a). Quantitative determination of phenols (simple phenols)

3.2.2.2(b). Quantitative determination of Flavonoids

3.2.2.2(c). Quantitative determination of Tannins

3.2.2.2(d). Quantitative determination of Alkaloids

3.2.2.2(e). Quantitative determination of Saponins

3.2.2.3. Biological activities of the medicinal plants’ extracts

3.2.2.3(a). Antioxidant activity

3.2.2.3(b). Antibacterial activity

3.2.2.4. Isolation and identification of bioactive compounds

3.2.2.4(a). Isolation of compounds

3.2.2.4(b). Structure elucidation of the isolated compounds

3.2.2.4(c). Antibacterial activity of the isolated compounds

3.2.2.4(d). Cytotoxicity assessment of the isolated compounds

3.2.3. Statistical analysis

3.2.4. Reliability and validity
3.2.5. Bias 71

3.2.6. Ethical considerations 71

CHAPTER 4 72

Results 72

4.1. Phytochemical composition of the leaves of the medicinal plants 72

4.1.1. Qualitative phytochemical composition 72

4.1.2. Effect of seasonal variation on phytochemical composition 73

4.1.3. Yearly phytochemical yield and mass yield of the different plant extracts 80

4.2. Biological activities of the leaves of B. dinteri, G. flava and J. lagarinthoides 82

4.2.1. Antioxidant (free radical scavenging) activity 82

4.2.3. Antibacterial activity of the leaf extracts

of B. dinteri, G. flava and J. lagarinthoides 87

4.3. Isolation and identification of compounds 92

4.3.1. Isolation of compounds from the leaf extracts of J. lagarinthoides 92

4.3.2. Isolation of compounds from leaf extracts of G. flava 107

4.3.3. Isolation of compounds from the leaf extract of B. dinteri 118
4.3.4. Antibacterial activity of the isolated compounds 133

4.3.5. Cytotoxicity assessment of the isolated compounds 135

CHAPTER 5 137

Discussion, Limitation and Conclusion

5.1. Discussion 137

5.1.1. Phytochemical screening 137

5.1.2. Effect of seasonal change on phytochemical compositions 140

5.1.3. Antioxidant and Antibacterial activities 143

5.1.4. Isolation and Identification of bioactive compounds 148

5.2. Limitations and Recommendations 155

5.3. Conclusion 155

REFERENCES 157

APPENDIX 1: MREC Approval Certificate
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABTS</td>
<td>2, 2’- azinobis (3-ethylbenzothiazoline-6-sulfonic acid)</td>
</tr>
<tr>
<td>Ace</td>
<td>acetone</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BEA</td>
<td>benzene: ethanol: ammonium hydroxide (90:10:1 v/v/v)</td>
</tr>
<tr>
<td>BIA</td>
<td>benzylisoquinoline alkaloids</td>
</tr>
<tr>
<td>BPP</td>
<td>bornyl diphosphate</td>
</tr>
<tr>
<td>CAD</td>
<td>hydroxycinnamyl alcohol dehydrogenase</td>
</tr>
<tr>
<td>CEF</td>
<td>chloroform: ethylacetate: formic acid (5:4:1, v/v/v)</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>chloroform</td>
</tr>
<tr>
<td>DAAD</td>
<td>diode array detector</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DMAPP</td>
<td>dimethylallyl diphosphate</td>
</tr>
<tr>
<td>DPPH</td>
<td>2, 2- diphenyl-1-picrylhydrazyl</td>
</tr>
<tr>
<td>EC₅₀</td>
<td>fifty percent effective concentration</td>
</tr>
<tr>
<td>EMW</td>
<td>ethylacetate: methanol: water (40:5.4:5, v/v/v)</td>
</tr>
<tr>
<td>EtOAc</td>
<td>ethylacetate</td>
</tr>
</tbody>
</table>
FRAP : ferric reducing antioxidant power
GC : gas chromatography
GC-MS : mass spectrometry coupled gas chromatography
GPP : geranyl pyrophosphate
HCl : hydrochloric acid
HHDP : hexahydroxydiphenoyl
HPLC : high performance liquid chromatography
H₂O₂ : hydrogen peroxide
H₂SO₄ : sulphuric acid
INT : p-iodonitrotetrazolium violet
IPP : isopentenyl diphosphate
IR : infra-red spectrophotometry
K₄Fe(CN)₆.3H₂O : potassium ferrocyanide
LPP : linalyl diphosphate
MDA : malondialdehyde
MIA : monoterpenoid indole alkaloids
MIC : minimum inhibitory concentration
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>MREC</td>
<td>Medunsa research ethics committee</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectroscopy</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NaOH</td>
<td>sodium hydroxide</td>
</tr>
<tr>
<td>PAL</td>
<td>phenylalanine ammonia lyase</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>reverse phase high performance liquid chromatography</td>
</tr>
<tr>
<td>SEE</td>
<td>serial exhaustive extraction</td>
</tr>
<tr>
<td>SPSS</td>
<td>statistical package for the social sciences</td>
</tr>
<tr>
<td>TBARS</td>
<td>thiobarbituric acid- reactive substances</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>UV-vis</td>
<td>ultraviolet-visible</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Plant metabolites: groups of plant primary and secondary metabolites</td>
<td>8</td>
</tr>
<tr>
<td>2.2</td>
<td>The two major pathways for the production of phenolic compounds and other molecules in plants</td>
<td>9</td>
</tr>
<tr>
<td>2.3</td>
<td>Types of phenolic compounds; simple phenols (A) and polyphenols (B)</td>
<td>10</td>
</tr>
<tr>
<td>2.4</td>
<td>Summary of the biosynthesis of phenolic acids and flavonoids branch of the phenylpropanoid biosynthetic pathway</td>
<td>12</td>
</tr>
<tr>
<td>2.5</td>
<td>Gallic acid, an example of a phenolic acid compound showing reactive aromatic hydroxyl substituents</td>
<td>13</td>
</tr>
<tr>
<td>2.6</td>
<td>Examples of coumarin compounds</td>
<td>14</td>
</tr>
<tr>
<td>2.7</td>
<td>Backbone structure of chalcone compounds</td>
<td>15</td>
</tr>
<tr>
<td>2.8</td>
<td>Examples of chalcone compounds</td>
<td>16</td>
</tr>
<tr>
<td>2.9</td>
<td>General structure of flavonoids showing two aromatic rings (A and B) and a central oxygenated heterocyclic ring (C)</td>
<td>17</td>
</tr>
<tr>
<td>2.10</td>
<td>General structural backbones of some groups of flavonoid compounds</td>
<td>18</td>
</tr>
</tbody>
</table>
Figure 2.11: Flavonoid compounds (1-6) identified from litchi (*Litchi chenensis*)

Figure 2.12: Classification of tannins into four major groups

Figure 2.13: Monoterpene-forming carbocation rearrangements

Figure 2.14: Examples of some synthesized complex terpene compounds

Figure 2.15: Examples of earlier identified alkaloid compounds

Figure 2.16: Some alkaloid compounds used in cancer therapy

Figure 2.17: Examples of glycosidic compounds showing a combination of a sugar and non-sugar molecules

Figure 2.18: Examples of saponin compounds

Figure 2.19: Different classes of saponin aglycones classified according to the biosynthesis of the carbon backbones

Figure 2.20: General backbone structure of anthraquinones showing carbon numbering

Figure 2.21: Biosynthetic pathways of anthraquinone backbone in plants

Figure 2.22: Examples of compounds belonging to the anthraquinone class of glycosides
Figure 2.23. *Barleria dinteri* 38

Figure 2.24. *Grewia flava* 39

Figure 2.25. *Jatropha lagarinthoides* 40

Figure 2.26. Schematic representation of a general bioassay-guided fractionation procedure for isolation and purification of natural compounds from plant extracts 52

Figure 3.1. Flow chart outlining the analysis and determination of the phytochemical composition and biological activities as well as the isolation of bioactive compounds of the leaves of *B. dinteri, G. flava and J. lagarinthoides* 55

Figure 4.1a: Effect of seasonal variation on the percentage yield of alkaloids in the leaves of *B. dinteri, G. flava and J. lagarinthoides*. 77

Figure 4.1b: Effect of seasonal variation on the percentage yield of flavonoids in the leaves of *B. dinteri, G. flava and J. lagarinthoides*. 78

Figure 4.1c: Effect of seasonal variation on the percentage yield of phenols in the leaves of *B. dinteri, G. flava and J. lagarinthoides*. 78

Figure 4.1d: Effect of seasonal variation on the percentage yield of saponins in the leaves of *B. dinteri, G. flava and J. lagarinthoides*. 79

Figure 4.1e: Effect of seasonal variation on the percentage yield of tannins in the leaves of *B. dinteri, G. flava and J. lagarinthoides*. 79

Figure 4.2: Average yearly yield of different phytochemicals from
the leaves of *B. dinteri*, *G. flava* and *J. lagarinthoides*.

**Figure 4.3a:** Free radical scavenging activity of the extracts of the leaves of *B. dinteri*, *G. flava* and *J. lagarinthoides* against DPPH with the chromatogram developed using BEA (90:10:1 v/v/v) mobile phase

**Figure 4.3b:** Free radical scavenging activity of the extracts of the leaves of *B. dinteri*, *G. flava* and *J. lagarinthoides* against DPPH with the chromatogram developed using CEF (5: 4:1 v/v/v) mobile phase

**Figure 4.3c:** Free radical scavenging activity of the extracts of the leaves of *B. dinteri*, *G. flava* and *J. lagarinthoides* against DPPH with the chromatogram developed using EMW (40:5.4:5 v/v/v) mobile phase

**Figure 4.4a:** Percentage DPPH scavenging activity of acetone extracts of *B. dinteri*, *G. flava*, *J. lagarinthoides* and BGJ (concoction of the three medicinal plants), with Vitamin C as a standard

**Figure 4.4b:** Percentage DPPH scavenging activity of methanol extracts of *B. dinteri*, *G. flava*, *J. lagarinthoides* and BGJ (concoction of the three medicinal plants), with Vitamin C as a standard

**Figure 4.5:** Bioautographic determination of the antibacterial activity of the leaf extracts of *B. diteri*, *G. flava* and *J. lagarinthoides*

**Figure 4.6:** Summary of the bioassay-guided fractionation for the isolation of compounds, CPD1JH and CPD2JA, from the leaf extracts of *J. lagarinthoides*.

**Figure 4.7:** TLC chromatograms of the Hexane: CHCl₃ fractions from hexane extract of *J. lagarinthoides* developed in BEA (90:10:1, v/v/v)
**Figure 4.8.** TLC-chromatogram fingerprints of the elution fractions of 2:8, v/v (Hexane: CHCl₃) fraction on silica gel 60 column monitored through antioxidant activity

**Figure 4.9.** TLC chromatogram to check purity and free radical scavenging activity of the fraction of test tubes (70 – 104) which were pooled together

**Figure 4.10.** TLC chromatogram monitoring the re-elution of a fraction of pooled test tubes (70 – 104) by free radical scavenging activity

**Figure 4.11.** Free radical scavenging activity (A) and vanillin visualization of a compound (CPD1JH) isolated from the Hexane: CHCl₃ (2:8, v/v) fraction of the hexane extract of *J. lagarinthoides*

**Figure 4.12.** Free radical scavenging activity of the EtoAc: MeOH (1:0- 0:1, v/v) fractions of the acetone extract of *J. lagarinthoides*

**Figure 4.13.** Free radical scavenging activity (A) and vanillin reaction (B) of a compound (CPD2JA) isolated from the EtoAc: MeOH (7:3, v/v) fraction of the acetone extract of *J. lagarinthoides*

**Figure 4.14.** ¹H PROTON NMR spectrum of CPD1JH

**Figure 4.15.** ¹³C CARBON NMR spectrum of CPD1JH

**Figure 4.16.** COSY NMR spectrum of CPD1JH

**Figure 4.17.** HMBC NMR spectrum of CPD1JH

**Figure 4.18.** TOCSY NMR spectrum of CPD1JH

**Figure 4.19.** Stigmasterol (a sterol), whose NMR spectra profile was concluded to be consistent with that of CPD1JH
Figure 4.20.: $^1$H NMR spectrum of CPD2JA

Figure 4.21.: Summary of bioassay-guided fractionation for isolation of compounds, CPD3GM and CPD4GM, from the leaf extracts of *G. flava*

Figure 4.22.: Free radical scavenging activity of the CHCl$_3$: MeOH fractions of the methanol extract of the leaves of *G. flava* against DPPH

Figure 4.23.: A compound (CPD3GM) with antioxidant activity isolated from the CHCl$_3$: MeOH (7:3, v/v)

Figure 4.24.: CPD4GM from fraction CHCl$_3$: MeOH (0:1 v/v) of the MeOH extract of *G. flava*

Figure 4.25.: $^1$PROTON NMR spectra of CPD3GM

Figure 4.26.: $^{13}$CARBON NMR spectra of CPD3GM

Figure 4.27.: COSY NMR spectra of CPD3GM

Figure 4.28.: gHSQC NMR spectra of CPD3GM

Figure 4.29.: TOCSY NMR spectra of CPD3GM

Figure 4.30.: Kaempferol-3-O-rutinoside-7-O-β-glucoside (a glycosidic flavonoid), whose NMR spectra profile was concluded to be consistent with that of CPD3GM

Figure 4.31.: Summary of bioassay-guided fractionation for isolation of compounds, CPD5BA and CPD6BA, from the leaf extracts of *B. dinteri*
Figure 4.32.: Summary of bioassay-guided fractionation for isolation of a compound, CPD8BM from the leaf extracts of *B. dinteri* 119

Figure 4.33.: Free radical scavenging activity of the CHCl$_3$: MeOH fractions of the acetone extract of the leaves of *B. dinteri* against DPPH 120

Figure 4.34.: A compound (CPD5BA) with antioxidant activity isolated from the CHCl$_3$: MeOH (1:1, v/v) 121

Figure 4.35.: A compound (CPD6BA) with antioxidant activity isolated from the CHCl$_3$: MeOH (7:3, v/v) 121

Figure 4.36.: A compound (CPD8BM) with antioxidant activity isolated from the MeOH fraction obtained through solvent-solvent extraction of the MeOH extract of the leaves of *B. dinteri* with hexane: A- showing reaction with vanillin in sulphuric acid and B- is showing reaction with DPPH 122

Figure 4.37.: PROTON NMR spectra of CPD5BA 123

Figure 4.38.: PROTON NMR profile of CPD6BA 124

Figure 4.39.: PROTON NMR spectra of CPD8BM 125

Figure 4.40.: CARBON NMR spectra of CPD8BM 126

Figure 4.41.: gHMBC NMR spectra of CPD8BM 128

Figure 4.42.: gHSQC NMR spectra of CPD8BM 129
Figure 4.43.: COSY NMR spectra of CPD8BM

Figure 4.44.: TOCSY NMR spectra of CPD8BM

Figure 4.45.: Barlerin (an iridoid glycoside), whose NMR spectra profile was concluded to be consistent with that of CPD8BM

Figure 4.46.: Bioautographic determination of the antibacterial activity of the isolated compounds against S. aureus

Figure 4.47.: Cytotoxicity assessment of some of the isolated compounds from the leaves of B. dinteri (CPD5BA), G. flava (CPD3GM and CPD4GM) and J. lagarinthoides (CPD1JH)

Figure 4.48.: LC50 values of the isolated compounds against RAW 264.7 macrophage cells determined by MTT assay
LIST OF TABLES

Table 4.1: Qualitative phytochemical composition of the leaf extracts of the three medicinal plants 72

Table 4.2a: Comparison of the amount of different phytochemicals in the leaves of B. dinteri across different seasons. 74

Table 4.2b: Comparison of the amount of different phytochemicals in the leaves of G. flava across different seasons. 75

Table 4.2c: Comparison of the amount of different phytochemicals in the leaves of J. lagarinthoides across different seasons. 76

Table 4.3.: Mass extracted (mg) (% Yield) from the ground leaves (1 g) of B. dinteri, G. flava, and J. lagarinthoides, and the three plant concoction, BGJ (3 g) 81

Table 4.4.: EC_{50} values of the acetone and methanol extracts of the leaves of B. dinteri, G. flava, J. lagarinthoides and BGJ (concoction of the three medicinal plants) against DPPH 86

Table 4.5a: MICs values of the leaf extracts of B. dinteri, G. flava and J. lagarinthoides against test organisms 89

Table 4.5b: MICs values of the concoction extracts of the three medicinal plants (BGJ) 90

Table 4.5c.: Total antibacterial activity, ml (extract mass/MIC value) of the leaf extracts of B. dinteri, G. flava and J. lagarinthoides, as well as concoction extracts (BGJ) (at 24 h incubation period). 91
Table 4.6: $^1$H and $^{13}$C NMR chemical shift values (ppm) of compound CPD1JA compared with the ones from the literature. Assignments made using COSY and HMBC correlations).

Table 4.7: $^1$H and $^{13}$C NMR chemical shift values (ppm) of compound CPD3GM compared with the ones from the literature. Assignments made using COSY and gHSQC correlations).

Table 4.8: $^1$H and $^{13}$C NMR chemical shift values (ppm) of compound CPD8BM compared with the ones from the literature. Assignments made using COSY, gHMBC and gHSQC correlations).

Table 4.9: MICs of the isolated compounds against *E. coli*, *E. faecaelis*, *P. aeruginosa* and *S. aureus* determined overnight
ABSTRACT

BACKGROUND:

World-wide, medicinal plants continue to be relied upon as solutions to health problems. The medicinal value of plants emanates from their inherent biological activities that are attributed to their phytochemical compositions. Three medicinal plants, *Barleria dinteri*, *Grewia flava* and *Jatropha lagarinthoides*, are among the frequently used medicinal plants in traditional medicine by local communities of the Zebediela subregion, Limpopo province (South Africa). However, these plants have never been investigated for their phytochemical composition and biological activities.

AIM OF THE STUDY:

The primary aim of the study was to determine the phytochemical composition, the antioxidant and antibacterial activities, to isolate and identify phytochemicals responsible for the antioxidant properties of the leaf extracts of *B. dinteri*, *G. flava* and *J. lagarinthoides* collected from Bolahlakgomo village, Zebediela subregion of the Limpopo province (South Africa).

METHODOLOGY:

In this descriptive cross-sectional study, the leaves of the medicinal plants were collected from Bolahlakgomo village in Zebediela subregion of the Limpopo province (South Africa), dried at room temperature and ground to powder. Phytochemical compositions of the ground powder as well as the effect of seasonal change on the amount of the phytochemicals were determined.
using standard procedures and compared seasonally. The ground leaves of the medicinal plants were then extracted with \textit{n}-hexane, dichloromethane, acetone and methanol using a sequential exhaustive extraction (SEE) procedure. Antioxidant and antibacterial activities of the leaf extracts of the plants were determined by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and TLC- bioautographic method, respectively. The 50 \% effective concentrations (EC_{50}) of the leaf extracts of the medicinal plants were determined spectrophotometrically and compared to known standard, Vitamin C. The minimum inhibition concentration (MIC) values of the leaf extracts were determined against \textit{Staphylococcus}, \textit{Enterococcus faecaelis}, \textit{Escherichia coli} and \textit{Pseudomonas aeruginosa} using micro-dilution plate procedure with gentamycin (0.1 \%) used as positive standard. Compounds with antioxidant activity were isolated from the leaf extracts of the medicinal plants silica gel 60 column chromatography. The isolated compounds were characterized by NMR spectroscopy. Protons and carbons peak signals were assigned based on 2D NMR spectra correlations and comparison with spectral data of compounds found in the literature. Finally, the MIC values of the isolated compounds were subsequently determined using the micro-dilution procedure and their cytotoxicity was assessed on RAW 264.7 macrophage cells.

\textbf{RESULTS:}

Leaf extracts of all three plant species under investigation contained flavonoids, saponins, alkaloids and tannins. Anthraquinones and reducing sugars were present in leaf extracts of \textit{G. flava} and \textit{J. lagarinthoides} but were absent in the leaf extracts of \textit{B. dinteri}. Amounts of tannins and alkaloids were higher during colder seasons while the amounts of flavonoids and saponins were higher in warmer seasons. The leaf extracts of the three medicinal plants exhibited
antioxidant activity, with stronger activity demonstrated by the acetone and methanol extracts as suggested by EC$_{50}$ values. The methanol leaf extracts of *B. dinteri* (EC$_{50}$ value of 82 µg/ml) and *G. flava* (EC$_{50}$ value of 105 µg/ml) demonstrated stronger antioxidant activity than the used standard, Vitamin C (EC$_{50}$ value of 190 µg/ml). Most of the leaf extracts had good antibacterial activity of moderate strength, as indicated by their MIC values (for the purpose of this study, MIC values lower than 1.00 mg/ml were considered good activity), against *Staphylococcus aureus*, *Enterococcus faecaelis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Both the antioxidant and antibacterial activities appeared to be stronger in the concoction (mixture of the three plants) extracts, which had lower EC$_{50}$ (79 µg/ml) and MIC values (ranging from 0.023 to 0.188 mg/ml) to compared to individual plant extracts. Seven compounds with antioxidant activity were isolated from the leaf extracts of the three medicinal plants, of which three were identified. A compound (CPD1JA) isolated from the hexane leaf extract of *J. lagarinthoides* was identified as stigmasterol (3-β-Hydroxy-24-ethyl-5, 22-cholestadiene). A compound (CPD3GM) with antioxidant and antibacterial activity isolated from the methanol leaf extract of *G. flava* was identified as kaempferol-3-O-rutinoside-7-O-β-D-glucoside. A compound (CPD8BM) with antioxidant activity isolated from the methanol leaf extract of *B. dinteri* was identified as barlerin (8-O-Acetyl shanzhiside methyl ester). With the exception of compounds isolated from the methanol leaf extract of *G. flava* (MIC values lower than 1.00 mg/ml), other compounds had low antibacterial activity (MIC values higher than 1.00 mg/ml) compared to the extracts. Cytotoxicity assessment on the isolated compounds, except CPD8BM from the methanol leaf extract of *B. dinteri* that was not assessed due to insufficient quantity, showed that the compounds are not toxic towards RAW 264.7 cells.
CONCLUSION:

The study has successfully established the phytochemical composition and the antioxidant and antibacterial activities of the leaf extracts of *B. dinteri*, *G. flava* and *J. lagarinthoides*. Furthermore, bioactive compounds with antioxidant and antibacterial activities were isolated from the leaf extracts of the plants and identified. The phytochemical compositions of the leaves of these medicinal plants, as well as the antioxidant and antibacterial properties shown by their extracts support the usage of these medicinal plants in traditional medicine and suggest that their leaves may serve as sources for potential pharmaceutical agents.