



**University of Fort Hare**  
*Together in Excellence*

# **Phytochemical Analyses and Brine shrimp (*Artemia Salina*) Lethality Studies on *Syzygium cordatum* (Hoscht ex. Krauss)**

---

**By**

**Herbert Chiguvare**

**(201108127)**

**Dissertation in fulfilment of the requirements for the degree**

**Masters of Science (Organic Chemistry)**

**Department of Chemistry**

**University of Fort Hare**

**Alice**

**Supervisor : Dr O Oyedeji**

## **Declaration**

I declare that this thesis that I hereby submit for the award of the degree of

### **Masters of Science in Chemistry**

is my original work apart from the acknowledged assistance from my supervisors. It has not been submitted to any other university other than the University of Fort Hare (Alice).

Candidate.....on this .....day of.....

Signature.....

Supervisor.....on this .....day of.....

Signature.....

## **Acknowledgements**

- Firstly I would like to give glory and thanks to the Lord God the Almighty for allowing this research to be a success and giving me the strength and perseverance to see it through.
- I would like to express my sincere gratitude to my supervisor Dr O. Oyedeji for his guidance and inspiration throughout the project.
- And to Prof A.O Oyedeji, whom the university denied her role of completion as my co-supervisor, thank you for your inspiring supervision. May God richly bless you.
- To Gloria Ndukwe all I can say is, may God bless you. Words fail to express how grateful I am.
- Many thanks go to the Walter Sisulu University Natural Products Research Group, University of Fort Hare academic staff, Dr Tichagwa, Mr Manene to name but a few for the invaluable support.
- To Manya Magwaca, thanks for the support and trouble I took you through.
- I would also like to thank Govan Mbeki Research, University of Fort Hare for research funding.
- Lastly to my family it is from you where I always get the strength and power to fight on and strive for the best. God bless you.

## Conference and Publications

### Conferences

1. Chiguvare H, Oyedeji O, Oyedeji AO **(2012)**: Bioactive Isolates of *Syzygium cordatum* Hoscht ex C. Krauss. Oral Presentation at the 2012 Walter Sisulu University Joint International Research Conference, 22 - 24<sup>th</sup> August, 2012, East London, South Africa.
2. Chiguvare H, Oyedeji O, Oyedeji AO **(2012)**: Isolation of Triterpenoids from the leaves of *Syzygium cordatum* Hochst. ex C Krauss and its Cytotoxicity Assay. Oral presentation at South African Chemical Institute (SACI) Eastern Cape Province Annual Regional Conference, East London, 12<sup>th</sup> October, 2012.

### Publications

1. Isolation of Triterpenoids from the leaves of *Syzygium cordatum* Hochst ex C Krauss and its Cytotoxicity Assay. Manuscript submitted to Natural Product Research (2012).
2. Essential oil content and composition of the leaves of *Syzygium cordatum* Hochst. ex C Krauss. Manuscript submitted to Journal of Essential Oil bearing Plant (2012).

### Award

1. Awarded 2nd Best Presenter: SACI Eastern Cape Province Regional Annual Conference, East London (2012) Isolation of Triterpenoids from the leaves of *Syzygium cordatum* Hochst. ex C Krauss and its Cytotoxicity

## Abstract

*Syzygium cordatum* Hoscht ex. C Krauss, also known as water berry, is normally used by the people of South Africa for respiratory ailments including tuberculosis, stomach complaints, treatment of wounds and as emetics. An extract of the leaves can be used as a purgative for diarrhoea treatment. The leaves of *Syzygium cordatum* Myrtaceae were obtained from the Eastern Cape Province of South Africa, air dried and sequential solvent extraction was done to obtain various non volatile crude extracts.

The volatile extract, that is the essential oil was extracted from the leaves using hydrodistillation and analysis of compounds was done by GC/MS for composition. 32 compounds were obtained from the fresh leaves and 18 compounds were obtained from the dry leaves. The fresh oil contains  $\beta$ -caryophyllene (11.8%) and caryophyllene oxide (11.1%) as the main sesquiterpene component.  $\alpha$ -Pinene(5.0%) was the only monoterpene compound identified in the fresh oil in substantial amount. The dry leaves oil had  $\alpha$ -copanene (17.0%),  $\beta$ -Caryophellene (26.0%), cubenol (6.5%) and caryophellene oxide (14.2%) as the dominant constituent of the oil. Summary of the classes of compounds in the oil revealed that the chemical profile of both oils were dominated by sesquiterpenoid compounds. This is the first time that terpenoids compounds are being identified in both the fresh and dry leaf oil of *S. cordatum*.

Hexane leaf extract was selected due to the interest in the terpenoid compounds. Column chromatography of the hexane crude gave five (5) of which two are fully reported. The isolates were fully elucidated using spectroscopic methods to be  $\beta$ -Sitosterol (HC3) and Friedela-3-one (HC1A/HC1D). Cytotoxicity analysis was carried out on the crude using the Brine shrimps assay. Isolates 1C and 1D showed significant lethality using the brine shrimps assay with lethality values (LC50) of 4.105mg/ml for HC1C and 4.11mg/ml for 1D/1A respectively.

### **List of Abbreviations**

|                   |                                                  |
|-------------------|--------------------------------------------------|
| Mb                | Mobile phase                                     |
| R <sub>f</sub>    | Retardation factor                               |
| NMR               | Nuclear magnetic resonance                       |
| COSY              | Correlation spectroscopy                         |
| NOESY             | Nuclear Overhauser enhancement spectroscopy      |
| HSQC              | Hetero nuclear single quantum correlation        |
| HMBC              | Hetero nuclear multiple quantum correlation      |
| DEPT              | Distortionless electron by polarisation transfer |
| CDCL <sub>3</sub> | Deutriated Chloroform                            |
| 2D-NMR            | 2 Dimension Nuclear Magnetic Resonance           |
| 1D-NMR            | 1 Dimension Nuclear Magnetic Resonance           |
| FTIR              | Fourier Transform Infrared spectroscopy          |
| UV                | Ultraviolet spectroscopy                         |
| LC                | Lethality concentration                          |
| <sup>13</sup> C   | Carbon-NMR                                       |
| J                 | Coupling constant                                |
| <sup>1</sup> H    | Proton-NMR                                       |
| TLC               | Thin layer chromatography                        |
| Ppm               | parts per million                                |
| S, d, t, q, m     | singlet, doublet, triplet, quartet, multiplet    |
| EtoAc: hex        | ethyl acetate: hexane                            |

## TABLE OF CONTENTS

|                                     |             |
|-------------------------------------|-------------|
| <b>Title</b>                        | <b>i</b>    |
| <b>Declaration</b>                  | <b>iii</b>  |
| <b>Acknowledgements</b>             | <b>ii</b>   |
| <b>Conferences and Publications</b> | <b>iv</b>   |
| <b>Abstract</b>                     | <b>v</b>    |
| <b>List of Abbreviations</b>        | <b>vii</b>  |
| <b>Table of Content</b>             | <b>viii</b> |
| <b>Table of Tables</b>              | <b>xii</b>  |
| <b>Table o Figures</b>              | <b>xiii</b> |

### Chapter 1

|         |                                              |   |
|---------|----------------------------------------------|---|
| 1.      | Introduction and Literature review           | 1 |
| 1.1     | Plant metabolites                            | 2 |
| 1.2     | Therapeutic value of phytochemicals          | 3 |
| 1.3     | Classification and benefits of metabolites   | 4 |
| 1.3.1   | Flavonoids                                   | 4 |
| 1.3.2   | Phenols                                      | 4 |
| 1.3.3   | Quinones                                     | 5 |
| 1.3.4   | Tannins                                      | 6 |
| 1.3.5   | Alkaloids                                    | 7 |
| 1.3.6   | Terpenes                                     | 7 |
| 1.4     | Methods of isolating bioactive compounds     | 8 |
| 1.4.1   | Thin Layer Chromatography (TLC)              | 8 |
| 1.4.2   | High Performance Liquid Chromatograph (HPLC) | 9 |
| 1.4.3   | Gas Chromatography (GC)                      | 9 |
| 1.4.4   | Column Chromatography (CC)                   | 9 |
| 1.4.4.1 | Stationary phase                             | 9 |

|         |                                                       |    |
|---------|-------------------------------------------------------|----|
| 1.4.4.2 | Mobile phase                                          | 10 |
| 1.4.4.3 | Column size                                           | 10 |
| 1.4.5   | Development of a TLC system for column chromatography | 11 |
| 1.5     | Volatile components: Essential oils                   | 11 |
| 1.5.1   | Introduction: Composition and chemical constituents   | 11 |
| 1.5.2   | Uses and importance                                   | 11 |
| 1.5.3   | Essential oil extraction methods                      | 12 |
| 1.5.3.1 | Expression                                            | 12 |
| 1.5.3.2 | Supercritical fluid extraction                        | 12 |
| 1.5.3.3 | Solvent Extraction                                    | 13 |
| 1.5.3.4 | Hydrodistillation                                     | 13 |
| 1.6     | <i>Syzygium Cordatum</i> Myrtaceae                    | 13 |
| 1.6.1   | Botanical description and distribution                | 13 |
| 1.6.2   | Parts used and medicinal uses                         | 14 |
| 1.6.3   | Literature report of <i>Syzygium cordatum</i>         | 15 |
| 1.7     | Research gap                                          | 17 |
| 1.8     | Aims                                                  | 17 |
| 1.9     | Objectives                                            | 18 |

## Chapter 2

|       |                                                            |    |
|-------|------------------------------------------------------------|----|
| 2     | Volatile extractives of <i>Syzygium cordatum</i> Myrtaceae | 19 |
| 2.1   | Extraction method                                          | 19 |
| 2.1.1 | Plant Material                                             | 19 |
| 2.1.2 | Extraction method for volatile compounds                   | 19 |
| 2.2   | Analysis of the essential oil                              | 20 |
| 2.2.1 | GC/MS analysis                                             | 20 |
| 2.2.2 | Identification of compounds                                | 21 |
| 2.3   | Results and Discussion                                     | 21 |

|     |            |    |
|-----|------------|----|
| 2.4 | Conclusion | 26 |
|-----|------------|----|

### Chapter 3

|         |                                                                 |    |
|---------|-----------------------------------------------------------------|----|
| 3       | Non volatile extractive of <i>Syzygium cordatum</i> Myrtaceae   | 28 |
| 3.1     | Plant material                                                  | 28 |
| 3.2.    | Extraction of crude                                             | 28 |
| 3.2.1   | Extraction procedure                                            | 28 |
| 3.3     | Isolation                                                       | 29 |
| 3.3.1   | Pre- column preparation                                         | 29 |
| 3.3.2   | Column chromatography                                           | 30 |
| 3.3.3   | Purification of isolates                                        | 30 |
| 3.3.3.1 | Recrystallisation                                               | 30 |
| 3.3.3.2 | Recrystallisation procedure for compounds 1A, 1C, 1D            | 31 |
| 3.3.3.3 | Purification of compound 2 and 3 by small column chromatography | 32 |
| 3.4     | Results and Discussion                                          | 32 |
| 3.4.1   | Yields from the extraction of crude                             | 32 |
| 3.4.2   | Isolates obtained from column chromatography                    | 33 |
| 3.4.3   | Characterisation and analysis of the isolates                   | 34 |
| 3.4.3.1 | Introduction: Melting Point Determination                       | 34 |
| 3.4.3.2 | Melting point procedure followed: Capillary Melting Point       | 35 |
| 3.4.4   | Introduction: Spectroscopic Techniques                          | 36 |
| 3.4.4.1 | Nuclear Magnetic Resonance Spectroscopy (NMR)                   | 36 |
| 3.4.5   | Structural elucidation                                          | 37 |
| 3.4.5.1 | Compound 1A                                                     | 37 |
| 3.4.5.2 | Compound 1D                                                     | 41 |
| 3.4.5.3 | Compound 1C                                                     | 41 |
| 3.4.5.4 | Compound 3                                                      | 45 |

|     |            |    |
|-----|------------|----|
| 3.5 | Conclusion | 50 |
|-----|------------|----|

## **Chapter 4**

|     |                                                 |    |
|-----|-------------------------------------------------|----|
| 4   | Bioactivity test                                | 51 |
| 4.1 | Cytotoxicity: Brine shrimp Lethality Assay      | 51 |
| 4.2 | Bioactivity screening and determination         | 52 |
|     | 4.2.1 Procedure used for the brine shrimp assay | 52 |
|     | 4.2.1.1 Materials                               | 52 |
|     | 4.2.1.2 Serial dilutions                        | 52 |
| 4.3 | Results                                         | 53 |
| 4.4 | Discussions                                     | 58 |
|     | 4.4.1 Bio-potential analysis of crude           | 58 |
|     | 4.4.2 Bio-potential analysis isolates           | 58 |
| 4.5 | Conclusion                                      | 59 |
|     | References                                      | 60 |
|     | Appendix                                        | 65 |

## Table of Tables

|           |                                                     |    |
|-----------|-----------------------------------------------------|----|
| Table 2.1 | Summary of essential oil results                    | 22 |
| Table 2.2 | Chemical composition of essential oil               | 24 |
| Table 2.3 | Summary of the chemical profiles of the oil samples | 26 |
| Table 3.1 | Percentage yield of various solvent extracts        | 32 |
| Table 3.2 | TLC Results of Isolates after purification          | 33 |
| Table 3.3 | Yields from the purification process                | 33 |
| Table 3.4 | Melting points results of isolates                  | 36 |
| Table 3.5 | NMR Data for Compound HC1A                          | 40 |
| Table 3.6 | NMR Data for Compound HC1C                          | 44 |
| Table 3.7 | NMR Data for Compound HC 3                          | 49 |
| Table 4   | Appendix 5                                          | 88 |

## Table of Figures

|              |                                                                                |    |
|--------------|--------------------------------------------------------------------------------|----|
| Figure 1     | <i>Syzygium cordatum</i> Myrtaceae                                             | 15 |
| Figure 2.1   | (a) Clevenger apparatus for hydrodistillation;(b) Volatile extractive obtained | 20 |
| Figure 2.2   | GC Chromatogram of fresh leaves essential oil                                  | 22 |
| Figure 2.3   | GC Chromatogram of dry leaves essential oil                                    | 23 |
| Figure 3.1   | Sequential extraction of plant material                                        | 29 |
| Figure 3.2   | TLC profiles of the crude extracts                                             | 30 |
| Figure 3.3   | TLC of purified isolates with OA and BA                                        | 34 |
| Figure 3.4.1 | <sup>1</sup> HNMR for HC1A                                                     | 38 |
| Figure 3.4.2 | <sup>13</sup> C NMR for HC1A                                                   | 39 |
| Figure 3.5.1 | <sup>1</sup> HNMR for HC1C                                                     | 42 |
| Figure 3.5.2 | <sup>13</sup> C NMR for HC1C                                                   | 43 |
| Figure 3.6.1 | <sup>1</sup> HNMR for HC 3                                                     | 47 |

|              |                                    |    |
|--------------|------------------------------------|----|
| Figure 3.6.2 | $^{13}\text{C}$ NMR for HC3        | 48 |
| Figure 4.1   | LC50 graph of EtoAc Crude          | 54 |
| Figure 4.2   | LC50 graph of Hexane Crude         | 54 |
| Figure 4.3   | Cytotoxicity graph of EtoAc Crude  | 55 |
| Figure 4.4   | Cytotoxicity graph of Hexane Crude | 55 |
| Figure 4.5   | LC50 graph of HC1C                 | 56 |
| Figure 4.6   | Cytotoxicity graph of HC1C         | 56 |
| Figure 4.7   | LC50 graph of HC1A(HC1D)           | 57 |
| Figure 4.8   | Cytotoxicity graph of HC1A(HC1D)   | 57 |

## Appendix

### Appendix 1

|               |                                                  |    |
|---------------|--------------------------------------------------|----|
| Figure 3.2.1: | TLC plates of the isolates HC-1A, C, D           | 66 |
| Figure 3.2.2: | TLC plates from ratio 8.2 to 7.3 for isolate HC2 | 67 |
| Figure 3.2.3: | TLC plates from ratio 8.2 to 7.3 for isolate HC2 | 67 |

### Appendix 2

|               |                                                                  |    |
|---------------|------------------------------------------------------------------|----|
| Figure 3.4.3: | IR spectrum of Compound 1A                                       | 68 |
| Figure 3.4.4: | $^{13}\text{C}$ -DEPT NMR for Compound 1A                        | 69 |
| Figure 3.4.5: | $^1\text{H}$ - $^1\text{H}$ COSY NMR spectrum for Compound 1A    | 70 |
| Figure 3.4.6: | $^1\text{H}$ - $^{13}\text{C}$ HSQC NMR spectrum for Compound 1A | 71 |
| Figure 3.4.7: | $^1\text{H}$ - $^{13}\text{C}$ HMBC NMR spectrum for Compound 1A | 72 |
| Figure 3.4.8: | $^1\text{H}$ - $^1\text{H}$ NOESY NMR spectrum for Compound 1A   | 73 |

### Appendix 3

|               |                                           |    |
|---------------|-------------------------------------------|----|
| Figure 3.5.3: | IR spectrum of Compound 1C                | 74 |
| Figure 3.5.4: | $^{13}\text{C}$ -DEPT NMR for Compound 1C | 75 |

|               |                                                                  |    |
|---------------|------------------------------------------------------------------|----|
| Figure 3.5.5: | $^1\text{H}$ - $^1\text{H}$ COSY NMR spectrum for Compound 1C    | 76 |
| Figure 3.5.6: | $^1\text{H}$ - $^{13}\text{C}$ HSQC NMR spectrum for Compound 1C | 77 |
| Figure 3.5.7: | $^1\text{H}$ - $^{13}\text{C}$ HMBC NMR spectrum for Compound 1C | 78 |
| Figure 3.5.8: | $^1\text{H}$ - $^1\text{H}$ NOESY NMR spectrum for Compound 1C   | 79 |

#### Appendix 4

|               |                                                                  |    |
|---------------|------------------------------------------------------------------|----|
| Figure 3.6.3: | IR spectrum of Compound 1C                                       | 80 |
| Figure 3.6.4: | $^{13}\text{C}$ -DEPT NMR for Compound 1C                        | 81 |
| Figure 3.6.5: | $^1\text{H}$ - $^1\text{H}$ COSY NMR spectrum for Compound 1C    | 82 |
| Figure 3.6.6: | $^1\text{H}$ - $^{13}\text{C}$ HSQC NMR spectrum for Compound 1C | 83 |
| Figure 3.6.7: | $^1\text{H}$ - $^{13}\text{C}$ HMBC NMR spectrum for Compound 1C | 84 |
| Figure 3.6.8: | $^1\text{H}$ - $^1\text{H}$ NOESY NMR spectrum for Compound 1C   | 85 |

#### Appendix 5

|           |                                       |    |
|-----------|---------------------------------------|----|
| Table 4.1 | Cytotoxicity results of EtoAc crude   | 87 |
| Table 4.2 | Cytotoxicity Results for Hexane crude | 88 |
| Table 4.3 | Cytotoxicity Results for Isolate HC1C | 99 |
| Table 4.4 | Cytotoxicity Results for HC1D         | 90 |