

***IN VITRO* EFFECT OF SELECTED  
MEDICINAL PLANTS ON  $\beta$ -AMYLOID  
INDUCED TOXICITY IN NEUROBLASTOMA  
CELLS**

EMMANUEL ADEKANMI ADEWUSI

A thesis submitted in partial fulfillment of the requirements for the degree

**PHILOSOPHIAE DOCTOR**

in

**PHARMACOLOGY**

in the

FACULTY OF HEALTH SCIENCES

at the

UNIVERSITY OF PRETORIA

Supervisor: Prof V. Steenkamp

Co-supervisor: Prof G. Fouche

March 2012



## Declaration

I (full names): Emmanuel Adekanmi Adewusi

Student number: 10680684

Subject of the work: *In vitro* effect of selected medicinal plants on  $\beta$ -amyloid induced toxicity in neuroblastoma cells.

### Declaration

1. I understand what plagiarism entails and am aware of the University's policy in this regard.
2. I declare that this project (e.g. essay, report, project, assignment, dissertation, thesis etc) is my own, original work. Where someone else's work was used (whether from a printed source, the internet or any other source) due acknowledgement was given and reference was made according to departmental requirements.
3. I did not make use of another student's previous work and submitted it as my own.
4. I did not allow and will not allow anyone to copy my work with the intention of presenting it as his or her own work.

Signature \_\_\_\_\_

## **Publications and Presentations**

### **Publications**

Adewusi EA, Moodley N, Steenkamp V (2010). Medicinal plants with cholinesterase inhibitory activity: A Review. *African Journal of Biotechnology* 9: 8257-8276. (Appendix A).

Adewusi EA, Moodley N, Steenkamp V (2011). Antioxidant and acetylcholinesterase inhibitory activity of selected southern African medicinal plants. *South African Journal of Botany* 77: 638-644. (Appendix B).

Adewusi EA, Steenkamp V (2011). *In vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from southern Africa. *Asian Pacific Journal of Tropical Medicine* 4: 829-835. (Appendix C).

### **Submitted**

Adewusi EA, Fouche G, Steenkamp V. Protective effect of several southern African medicinal plants against amyloid- $\beta$  induced neurotoxicity. Submitted to *Natural Products Research*.

### **In Preparation**

Cytotoxicity and acetylcholinesterase inhibitory activity of an isolated crinine alkaloid from *Boophane disticha*.

Cycloeucaleanol – a new cycloartane triterpene from the bulbs of *Boophane disticha*.

## **Scientific Conferences**

### **International**

**2011 Joint International Research Conference, International Convention Centre, East London, South Africa. 17-19 August 2011.**

Paper presented: *In vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from southern Africa.

### **National**

**South African Congress for Pharmacology and Toxicology (SACPT 2010), Cape Town Lodge, Cape Town, South Africa. 3-6 October 2010.**

Paper presented: Antioxidant and acetylcholinesterase inhibitory activity of selected southern African Medicinal plants.

**South African Association of Botanists (SAAB), 38<sup>th</sup> Annual Conference, University of Pretoria, South Africa. 15-18 January 2012.**

Paper presented: *In vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from southern Africa.

## Acknowledgements

- Prof. Vanessa Steenkamp and Prof. Gerda Fouche for all their support, assistance, encouragement and guidance throughout the study; you both played a very important role in making me a well-polished scientist.
- Prof. Paul Steenkamp for his assistance with the Mass spectrometry analysis
- Dr. Cromarty for his assistance, support, encouragement and making time to help.
- The National Research Foundation and Department of Pharmacology, University of Pretoria for funding.
- Prof. Odukoya for her financial support, encouragement and helping me develop a passion for research.
- My parents and siblings, Niyi, Demola and Esther, for whom there are not enough words for me to express my gratitude, for their endless love, patience, continuous support and encouragement.
- My fiancé, Thobela for her endless love, support and encouragement.
- My friends and colleagues at the Department of Pharmacology, University of Pretoria and the Council for Scientific and Industrial Research for their help in and out of the laboratory, of special mention is Dr. Gisela Joonè, Jeremiah Senabe, Tsholofelo Mokoka, Teresa Faleschini, Gugulethu Ndlovu, Itumeleng Setshedi, Mitchell Enslin, Nial Harding, Werner Cordier and Japie van Tonder.
- My loving Heavenly Father Jehovah, who daily strengthens me and has given me the ability and understanding to obtain this degree. I glorify Him with all my achievements.

## Abstract

Neurodegenerative diseases occur as a result of the breakdown and deterioration of the neurons of the central nervous system (CNS). They are commonly found in elderly people and are a major cause of morbidity and mortality, thereby imposing severe strains on the social welfare systems. Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder. Cholinergic deficit, senile plaque/amyloid- $\beta$  peptide deposition and oxidative stress have been identified as three main pathogenic pathways which contribute to the progression of AD. The current therapeutic options cause several side-effects and have problems associated with bioavailability. Therefore, the need arises to search for new compounds from natural products with potential to treat AD.

Seventeen plants were selected for this study based on their documented ethno-medicinal use in improving memory, to treat insomnia, calm agitated people, and other neurological disorders. The plants were screened for inhibition of acetylcholinesterase (AChE) using the TLC and microtiter plate method. A dose-dependent inhibition of the enzyme was observed and 4.5% of all the plants showed low (<30% inhibition) AChE inhibition. The ethyl acetate extracts of the roots of *Crinum bulbispermum*, *Xysmalobium undulatum*, *Lannea schweinfurthii*, *Scadoxus puniceus* and bulbs of *Boophane disticha* had the best AChE inhibition. Although the  $IC_{50}$  of these plant extracts were higher than that of the positive control, galanthamine (0.00053 mg/ml), they showed good AChE inhibitory activity considering they are still mixtures containing various compounds.

The antioxidant activity of the plant extracts was determined by their ability to scavenge ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) and DPPH (1,1-diphenyl-2-picryl-

hydrazyl) radicals. The dichloromethane/methanol (1:1) extracts of *Chamaecrista mimosoides* (root), *Buddleja salviifolia* (whole plant), *Schotia brachypetala* (root and bark), water extracts of *Chamaecrista mimosoides* (root), *Buddleja salviifolia* (whole plant), *Schotia brachypetala* (root and bark) and methanol extracts of the roots of *Crinum bulbispermum*, *Piper capense*, *Terminalia sericea*, *Lannea schweinfurthii* and *Ziziphus mucronata* all showed good antioxidant activity (>50%), in both assays.

*B. disticha* contained very promising AChE inhibition and was subjected to isolation of active compounds using thin layer chromatography, column chromatography and preparative thin layer chromatography. Two compounds, 6-hydroxycrinamine (a crinine-type alkaloid) and cycloeucalenol (a cycloartane triterpene), were isolated for the first time from the bulbs of this plant. 6-Hydroxycrinamine, and two fractions, EAM 17-21 21,22 and EAE 11 (which could not be purified further due to low yield), were found to inhibit AChE with IC<sub>50</sub> values of  $0.445 \pm 0.030$  mM,  $0.067 \pm 0.005$  mg/ml and  $0.122 \pm 0.013$  mg/ml, respectively.

Cytotoxicity of the isolated compounds and two active fractions was determined on human neuroblastoma (SH-SY5Y) cells using the MTT and neutral red uptake assays. 6-hydroxycrinamine and fraction EAM 17-21 21,22 were found to be toxic with IC<sub>50</sub> values of 54.5  $\mu$ M and 21.5  $\mu$ g/ml as determined by the MTT assay. The isolated compounds and fractions did not show any protective effect against cell death induced by A $\beta$ <sub>25-35</sub> possibly due to the poor antioxidant activity of *B. disticha* bulbs.

Cytotoxicity was also determined for the methanol extracts of the roots of *C. bulbispermum*, *T. sericea*, *L. schweinfurthii* and *Z. mucronata*, as they contained promising antioxidant activity. *C. bulbispermum* was the most toxic, reducing cell viability by <40% at the highest concentration

tested. *Z. mucronata* and *L. schweinfurthii* were the least toxic with IC<sub>50</sub> values exceeding 100 µg/ml, the highest concentration tested. Three concentrations of the plant extracts that were not toxic, or presented low toxicity, were selected to evaluate their possible protective effect against cell death induced by Aβ<sub>25-35</sub>. Pretreatment with *Z. mucronata* and *T. sericea* roots showed a dose dependent inhibition of cell death caused by Aβ<sub>25-35</sub>. Pre-treatment with *L. schweinfurthii* roots resulted in an optimum dose for inhibition of Aβ<sub>25-35</sub> induced cell death at 25 µg/ml, while still maintaining 80% viability. The roots of *C. bulbispermum* at non-toxic dose still maintained >50% viability.

This study confirms the neuroprotective potential of some of the plants which had AChE inhibitory and antioxidant activity. In addition, four of the plants were shown to prevent cell death caused by Aβ<sub>25-35</sub>. These plants can serve as potential leads in developing drugs relevant to treatment of AD. Furthermore, two new compounds present in the bulbs of *B. disticha* were identified. Additional investigations need to be carried out by applying QSAR studies to modify the structure of the alkaloid with the aim of reducing its observed toxicity.

Keywords: Acetylcholinesterase, Alzheimer's disease, amyloid-β, antioxidant, cytotoxicity, plant extracts, SH-SY5Y cells.

# Table of Contents

|   |             |
|---|-------------|
| <b>Declaration.....</b>   | <b>ii</b>   |
| <b>Publications and presentations.....</b>                      | <b>iii</b>  |
| <b>Acknowledgements.....</b>                                    | <b>v</b>    |
| <b>Abstract.....</b>  | <b>vi</b>   |
| <b>Table of Contents.....</b>                                   | <b>ix</b>   |
| <b>List of Figures .....</b>                                    | <b>xiv</b>  |
| <b>List of Tables.....</b>                                      | <b>xvii</b> |
| <b>List of Abbreviations.....</b>                               | <b>xix</b>  |
| <b>Chapter 1: Literature Review.....</b>                        | <b>1</b>    |
| 1.1 Neurodegeneration.....                                      | 1           |
| 1.2 Alzheimer’s disease.....                                    | 2           |
| 1.3 Pathology of Alzheimer’s disease.....                       | 3           |
| 1.4 Current therapeutic approaches for Alzheimer’s disease..... | 7           |
| 1.4.1 Acetylcholinesterase inhibitors.....                      | 8           |
| 1.4.2 Antioxidants.....   | 11          |
| 1.4.3 Statins.....  | 11          |
| 1.4.4 Non-steroidal anti-inflammatory drugs.....                | 11          |
| 1.4.5 Diet.....   | 12          |
| 1.5 Traditional Medicine.....                                   | 13          |

|  |  |           |
|--|--|-----------|
| 1.6  | Plants used traditionally to treat age-related/neurological disorders..... | 13        |
| 1.7  | Study Aim.....   | 19        |
| 1.8  | Objectives.....  | 19        |
| <b>Chapter 2: Materials and Methods.....</b> |  | <b>20</b> |
| 2.1  | Reagents and Chemicals.....  | 20        |
| 2.2  | Plant material.....  | 21        |
| 2.2.1  | Plant collection.....  | 21        |
| 2.2.2  | Extract preparation.....   | 24        |
| 2.3  | Determination of acetylcholinesterase inhibition.....                      | 24        |
| 2.3.1  | Micro-plate assay.....   | 24        |
| 2.3.2  | TLC assay.....   | 25        |
| 2.4  | Antioxidant activity.....  | 26        |
| 2.4.1  | DPPH radical scavenging activity.....                                      | 26        |
| 2.4.2  | ABTS radical scavenging activity.....                                      | 26        |
| 2.5  | Phytochemical screening.....   | 27        |
| 2.5.1  | Determination of total phenolics.....                                      | 27        |
| 2.5.2  | Determination of total flavonoids.....                                     | 28        |
| 2.6  | Compound isolation.....  | 28        |
| 2.6.1  | Column chromatography.....   | 28        |
| 2.6.2  | Thin-layer chromatography.....   | 28        |
| 2.6.3  | Preparative thin-layer chromatography.....                                 | 29        |

|  |           |
|--|-----------|
| 2.6.4 Isolation of compounds from the methanol extract.....                            | 29        |
| 2.6.5 Isolation of compounds from the ethyl acetate extract.....                       | 32        |
| 2.7 Structural elucidation of compounds.....   | 35        |
| 2.7.1 Nuclear Magnetic Resonance (NMR) Spectroscopy.....                               | 35        |
| 2.7.2 Mass Spectrometry.....   | 35        |
| 2.8 Cytotoxicity studies.....  | 36        |
| 2.8.1 Cells and cell culture.....  | 36        |
| 2.8.2 Cell viability.....  | 37        |
| 2.8.2.1 MTT assay.....   | 37        |
| 2.8.2.2 Neutral red assays.....  | 37        |
| 2.8.3 Treatment with A $\beta$ <sub>25-35</sub> .....                                  | 38        |
| 2.9 Statistical analysis.....  | 38        |
| <b>Chapter 3: Results.....</b>   | <b>40</b> |
| 3.1 Acetylcholinesterase inhibitory activity.....                                      | 40        |
| 3.2 Antioxidant activity and polyphenolic content.....                                 | 45        |
| 3.3 Isolation of compounds from <i>Boophane disticha</i> .....                         | 51        |
| 3.3.1 Structural elucidation of compound 1 (6-hydroxycrinamine).....                   | 51        |
| 3.3.2 Structural elucidation of compound 3 (cycloeucalenol).....                       | 52        |
| 3.3.3 Acetylcholinesterase inhibitory activity of isolated compound and fractions..... | 61        |
| 3.4 Cytotoxicity studies.....  | 63        |
| 3.4.1 Cytotoxicity assessment and effect of isolated compounds and active fractions on |           |

|  |            |
|--|------------|
| A $\beta$ -induced neurotoxicity.....  | 63         |
| 3.4.2 Cytotoxicity assessment and effect of several medicinal plants on A $\beta$ -induced neurotoxicity.....                | 66         |
| <b>Chapter 4: Discussion.....</b>  | <b>71</b>  |
| 4.1 Acetylcholinesterase inhibitory and antioxidant activity.....  | 71         |
| 4.2 Isolation and structural elucidation of compounds from <i>Boophane disticha</i> .....                                    | 77         |
| 4.2.1 Compound 1 (6-hydroxycrinamine).....   | 78         |
| 4.2.2 Compound 3 (cycloeucalenol).....   | 81         |
| 4.2.3 Acetylcholinesterase inhibitory of isolated compound and fractions.....  | 83         |
| 4.3 Cytotoxicity studies.....  | 85         |
| 4.3.1 Cytotoxicity assessment and effect of isolated compounds and active fractions on A $\beta$ -induced neurotoxicity..... | 87         |
| 4.3.2 Cytotoxicity assessment and effect of several medicinal plants on A $\beta$ -induced neurotoxicity.....                | 89         |
| <b>Chapter 5: Conclusion.....</b>  | <b>93</b>  |
| <b>References.....</b>   | <b>97</b>  |
| <b>Appendix A.....</b>   | <b>132</b> |
| <b>Appendix B.....</b>   | <b>152</b> |
| <b>Appendix C.....</b>   | <b>159</b> |



**Appendix D.....166**

**Appendix E.....167**

# List of Figures

## Chapter 1

### **Figure 1.1**

Schematic representation of the known and proposed changes in cholinergic neurons that occur in the aged and early AD brain compared with healthy young neurons. Alterations in high-affinity choline uptake, impaired acetylcholine release, deficits in the expression of nicotinic and muscarinic receptors, dysfunctional neurotrophin support (i.e., NGF receptors), and deficits in axonal transport are represented in the early AD neuron either by a decrease in the number of symbols presented or by reduced colour intensity (Terry and Buccafusco, 2003).....6

### **Figure 1.2**

ROS production as a player in the cycle of events leading to neurodegeneration.....9

### **Figure 1.3**

Acetylcholinesterase, beta amyloid plaque formation and oxidative stress as part of a chain reaction leading to neurodegeneration.....10

## Chapter 2

### **Figure 2.1**

Schematic diagram of the fractionation and isolation of compounds from the methanol extracts of *Boophane disticha*.....31

### **Figure 2.2**

Schematic diagram of the fractionation and isolation of compounds from the ethyl acetate extracts of *Boophane disticha*.....34

## **Chapter 3**

### **Figure 3.1**

AChE inhibitory activity (%) of (A) DCM: MeOH (1:1) extracts and (B) water extracts of plants with moderate to good activity. ST, *Salvia tiliifolia* (whole plant); CM, *Chamaecrista mimosoides* (root); BS, *Buddleja salviifolia* (whole plant); SBR, *Schotia brachypetala* (root); SBB, *Schotia brachypetala* (bark); GAL, Galanthamine (positive control).....41

### **Figure 3.2**

AChE inhibitory activity (%) of (A) ethyl acetate extracts and (B) methanol extracts, of plants with moderate to good activity. AG, *Adenia gummifera* (root); LS, *Lannea schweinfurthii* (root); ZM, *Ziziphus mucronata* (root); ZD, *Zanthoxylum davyi* (root); FC, *Ficus capensis* (fruit); SPR, *Scadoxus puniceus* (root); SPB, *S. puniceus* (bulb); CBB, *Crinum bulbispermum* (bulb); CBR, *C. bulbispermum* (root); PC, *Piper capense*; XU, *Xysmalobium undulatum* (root); BDR, *Boophane disticha* (root); BDB, *B. disticha* (bulb); GAL, Galanthamine (positive control).....42

### **Figure 3.3**

ABTS radical scavenging activity of (A) DCM: MeOH (1:1) extracts, (B) water extracts, and (C) methanol extracts, of plants with good activity. ST, *Salvia tiliifolia* (whole plant); CM, *Chamaecrista mimosoides* (root); BS, *Buddleja salviifolia* (whole plant); SBR, *Schotia brachypetala* (root); SBB, *S. brachypetala* (bark); PC, *Piper capense* (root); LS, *Lannea schweinfurthii* roots; ZMM, *Ziziphus mucronata* roots; CBR, *Crinum bulbispermum* (roots); TS, *Terminalia sericea* roots; trolox (positive control).....46

### **Figure 3.4**

DPPH radical scavenging activity of (A) DCM: MeOH (1:1) extracts, (B) water extracts and (C) methanol extracts, of plants with good activity. ST, *Salvia tiliifolia* (whole plant); CM, *Chamaecrista mimosoides* (root); BS, *Buddleja salviifolia* (whole plant); SBR, *Schotia brachypetala* (root); SBB, *S. brachypetala* (bark); PC, *Piper capense* (root); LS, *Lannea schweinfurthii* roots; ZMM, *Ziziphus mucronata* roots; CBR, *Crinum bulbispermum* (roots); TS, *Terminalia sericea* roots; trolox (positive control).....47

**Figure 3.5**

HRTOFMS (ESI<sup>+</sup>) spectra for compound 1 (6-hydroxycrinamine).....53

**Figure 3.6**

Structure of 6-hydroxycrinamine showing its two epimers.....54

**Figure 3.7**

HRTOFMS (ESI<sup>+</sup>) spectra for compound 3 (cycloeucalenol).....57

**Figure 3.8**

Structure of cycloeucalenol with its stereo-isomer.....58

**Figure 3.9**

Effect of (A) 6-hydroxycrinamine, (B) cycloeucalenol, (C) EAM 17-21 21,22 and (D) EAE 11 on the viability of SH-SY5Y cell lines as measured by the MTT and neutral red uptake assays after 72 h of incubation.....64

**Figure 3.10**

Effect of the methanol extract of the investigated plant extracts on viability of SH- SY5Y cell lines as measured by the MTT and neutral red uptake assays after 72 h of incubation (A) *Z. mucronata* roots; (B) *L. schweinfurthii* roots; (C) *T. sericea* roots; (D) *C. bulbispermum* roots.....67

**Figure 3.11**

Effect of A $\beta$ <sub>25-35</sub> on SH-SY5Y cell viability.....69

**Figure 3.12**

Effect of different plant extracts on A $\beta$ <sub>25-35</sub> induced toxicity. (A) and (B) Neutral red assay; (C) and (D) MTT assay. ZM, *Z. mucronata* root; LS, *L. schweinfurthii* root; TS, *T. sericea* root; CR, *C. bulbispermum* root.....70

# List of Tables

## Chapter 2

### Table 2.1

Plants investigated in the present study with documented ethno-medicinal use in treatment of neurological disorders.....22

## Chapter 3

### Table 3.1

Acetylcholinesterase inhibitory activity of the plant extracts as represented by their IC<sub>50</sub> values.....43

### Table 3.2

Antioxidant activity of the plant extracts as represented by their IC<sub>50</sub> values.....48

### Table 3.3

Total phenol and flavonoid contents of plant extracts with antioxidant activity (>50%) in both DPPH and ABTS assays.....50

### Table 3.4

<sup>1</sup>H NMR data for 6-hydroxycrinamine in methanol-d<sub>4</sub> (CD<sub>3</sub>OD) compared to literature.....55

### Table 3.5

<sup>13</sup>C NMR data for 6-hydroxycrinamine in methanol-d<sub>4</sub> (CD<sub>3</sub>OD) compared to literature.....56

### Table 3.6

<sup>1</sup>H NMR data for cycloeucalenol in chloroform-d<sub>1</sub> (CDCl<sub>3</sub>) compared to literature.....59

### Table 3.7

<sup>13</sup>C NMR data for cycloeucalenol in chloroform-d<sub>1</sub> (CDCl<sub>3</sub>) compared to literature.....60

**Table 3.8**

*In vitro* AChE inhibitory activity of the isolated compound and fractions.....62

**Table 3.9**

IC<sub>50</sub> values of isolated compounds and fractions on SH-SY5Y cell lines.....65

**Table 3.10**

IC<sub>50</sub> values of the methanol extracts of the investigated plants on SH-SY5Y cell line.....68

## List of Abbreviations

|                        |  |
|------------------------|--|
| $^{13}\text{C}$        | carbon 13  |
| 1D                     | one dimensional                                      |
| $^1\text{H}$           | proton   |
| 2D                     | two dimensional                                      |
| 5-HT                   | 5-hydroxytryptamine                                  |
| A $\beta$              | Amyloid- $\beta$                                     |
| ABTS                   | 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid |
| ACh                    | Acetylcholine  |
| AChE                   | Acetylcholinesterase                                 |
| AChEIs                 | Acetylcholinesterase inhibitors                      |
| AD                     | Alzheimer's disease                                  |
| APP                    | Amyloid- $\beta$ precursor protein                   |
| ATCC                   | American cell type collection culture                |
| ATCI                   | Acetylthiocholine iodide                             |
| BHA                    | Butylated hydroxyanisole                             |
| BHT                    | Butylated hydroxyl toluene                           |
| $\text{CDCl}_3$        | deuterated chloroform                                |
| $\text{CD}_3\text{OD}$ | deuterated methanol                                  |
| CNS                    | Central Nervous System                               |
| COSY                   | Correlation Spectroscopy                             |
| COX-2                  | Cyclooxygenase-II                                    |
| CSIR                   | Council for Scientific and Industrial Research       |
| DCM                    | Dichloromethane                                      |

|                  |  |
|------------------|--|
| DEPT             | Distortionless Enhancement by Polarisation Transfer            |
| DMSO             | Dimethyl sulfoxide   |
| DNA              | Deoxyribonucleic acid  |
| DPPH             | 1,1-diphenyl-2-picryl-hydrazyl                                 |
| DTNB             | 5,5'-bisdithionitrobenzoic acid                                |
| ESI              | Electrospray negative mode                                     |
| ESI <sup>+</sup> | Electrospray positive mode                                     |
| EtOAc            | Ethyl acetate  |
| FCS              | Fetal calf serum   |
| GABA             | $\gamma$ -aminobutyric acid                                    |
| HMBC             | Heteronuclear Multiple Bond Correlation                        |
| HPLC             | High Performance Liquid Chromatography                         |
| HRTOFMS          | High resolution time-of-flight mass spectroscopy               |
| HSQC             | Heteronuclear Single Quantum Coherence                         |
| IC <sub>50</sub> | 50% Inhibitory concentration                                   |
| <i>J</i>         | spin-spin coupling in Hertz                                    |
| LC-MS            | Liquid chromatography-mass spectrometry                        |
| <i>m/z</i>       | mass to charge ratio   |
| MAO              | Monoamine oxidase  |
| MeDi             | Mediterranean diet   |
| MeOH             | Methanol   |
| MMP              | 1-methyl-4-phenylpyridinium ion                                |
| MS               | Mass spectrometry  |
| MTT              | 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide |
| NIST             | National Institute of Standards and Technology                 |

|                |   |
|----------------|---|
| NMR            | Nuclear Magnetic Resonance                    |
| NO             | Nitric oxide                                  |
| NOE            | Nuclear Overhauser Effect                     |
| NR             | Neutral red                                   |
| NSAIDs         | Non-steroidal anti-inflammatory drugs         |
| OSI            | Oxidative stability instrument                |
| PBS            | Phosphate Buffered Saline                     |
| PC-12          | Rat pheochromocytoma cells                    |
| Ppm            | parts per million                             |
| QSAR           | Quantitative structure activity relationship  |
| QTOF           | Quadrupole Time-of-Flight                     |
| R <sub>f</sub> | Retention value                               |
| ROS            | Reactive oxygen species                       |
| SANBI          | South African National Biodiversity Institute |
| SD             | Standard deviation                            |
| SDS            | Sodium dodecyl sulphate                       |
| SH-SY5Y        | Human neuroblastoma cells                     |
| TLC            | Thin layer chromatography                     |
| TMS            | Tetramethylsilane                             |
| UPLC           | Ultra Performance Liquid Chromatography       |
| UV             | Ultraviolet                                   |
| WHO            | World Health Organisation                     |
| XO             | Xanthine oxidase                              |