UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

IN VITRO EFFECT OF SELECTED MEDICINAL PLANTS ON β-AMYLOID INDUCED TOXICITY IN NEUROBLASTOMA CELLS

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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

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March 2012

Declaration

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

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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-β induced neurotoxicity. Submitted to Natural Products Research.

In Preparation

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

Cycloeucalenol – 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), 38th 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-β 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_{50} values of 0.445 ± 0.030 mM, 0.067 ± 0.005 mg/ml and 0.122 ± 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_{50} values of 54.5 µM and 21.5 µg/ml as determined by the MTT assay. The isolated compounds and fractions did not show any protective effect against cell death induced by $A\beta_{25-35}$ 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_{50} 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 $A\beta_{25-35}$. Pretreatment with *Z. mucronata* and *T. sericea* roots showed a dose dependent inhibition of cell death caused by $A\beta_{25-35}$. Pre-treatment with *L. schweinfurthii* roots resulted in an optimum dose for inhibition of $A\beta_{25-35}$ 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 $A\beta_{25-35}$. 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.

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List of Abbreviations

^{13}C	carbon 13
1D	one dimensional
^{1}H	proton
2D	two dimensional
5-HT	5-hydroxytryptamine
Aβ	Amyloid-β
ABTS	2,2´-azinobis-3-ethylbenzothiazoline-6-sulfonic acid
ACh	Acetylcholine
AChE	Acetylcholinesterase
AChEIs	Acetylcholinesterase inhibitors
AD	Alzheimer's disease
APP	Amyloid-β precursor protein
ATCC	American cell type collection culture
ATCI	Acetylthiocholine iodide
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxyl toluene
$CDCl_3$	deuterated chloroform
CD_3OD	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^+	Electrospray positive mode
EtOAc	Ethyl acetate
FCS	Fetal calf serum
GABA	γ-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_{50}	50% Inhibitory concentration
J	spin-spin coupling in Hertz
LC-MS	Liquid chromatography-mass spectrometry
m/z	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_f	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