UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

# Phenolic compounds in aqueous extracts of marama bean [*Tylosema esculentum* (Burchell) A. Schreiber] seed coat, sorghum (*Sorghum bicolor* L. Moench) bran and their bioactive properties

By

Jeremiah Sijubo Shelembe

Submitted in partial fulfilment of the requirements for the degree

PhD Food Science

in the

Department of Food Science

Faculty of Natural and Agricultural Sciences

University of Pretoria

South Africa

May 2012

# DECLARATION

I Jeremiah S. Shelembe declare that the thesis, which I hereby submit for the degree PhD Food Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE:...............................................................

DATE: May 2012

# ACKNOWLEDGEMENTS

I would like to express my sincere thanks and acknowledgement to the following:

Dr K. G. Duodu, my supervisor, for his guidance, advice and encouragement for the successful execution of this research and compilation of this thesis.

Professor Amanda Minnaar and Professor Megan Bester, my co-supervisors, for their valuable contribution towards the research and review of the contents of this thesis.

Dr Duncan Cromaty for his assistance and guidance in using the HPLC-MS system for this study.

Staff and fellow students in the Department of Food Science, Department of Anatomy and Department of Pharmacology of the University of Pretoria for their constructive discussions and sharing of ideas.

My wife Cynthia, and my children Nondumiso, Sandile, Ncamiso, Nosipho, Ntokozo and Nandi for their love and patience during my studies.

My mother, brothers and sisters for their moral support.

## ABSTRACT

Phenolic compounds in aqueous extracts of marama bean [*Tylosema esculentum* (Burchell) A. Schreiber] seed coat, sorghum (*Sorghum bicolor* L. Moench) bran and their bioactive properties

**By**

**Jeremiah S. Shelembe**

**Supervisor:** Dr. K. G. Duodu

**Co-supervisors:** Prof A. Minnaar and Prof. M. Bester

**Department:** Food Science

**Degree:** PhD Food Science

The marama bean is an underutilised legume growing wild in the arid and semi arid regions of Southern Africa. Sorghum is an important cereal crop in arid regions of the world. The seed coats of marama beans and bran of sorghum contain antioxidant phenolic compounds with potential health benefits. Aqueous extracts for possible use as antioxidant functional food ingredient were prepared from seed coats of marama beans and bran of condensed tannin sorghum by extracting with water (water extract) or with water acidified to pH 2 (acidified water extract). Aqueous extraction was used in order to obtain an extract free from organic solvents, safe for human consumption, and containing compounds representative of those that are available under aqueous conditions of the gastrointestinal tract. The extracts were analysed for total phenolic content, total flavonoid content, condensed tannin content and protein precipitation capacity using spectrophotometric methods and for individual phenolic compounds using HPLC-MS. Proanthocyanidins were characterised using thiolysis degradation in conjunction with HPLC-MS. The antioxidant activities of the extracts were measured using ABTS, DPPH and ORAC assays. Extracts were also evaluated for protective effect against free radical induced human erythrocytes haemolysis, oxidative DNA damage and human LDL oxidation.

Extracts from marama bean seed coats had significantly higher total phenolic content, total flavonoid content, condensed tannin content, protein precipitation capacity and phenolic compound concentration than equivalent extracts from condensed tannin sorghum bran. Three phenolic acids and three flavonols esterified to gallic acid were identified in the extracts from marama bean seed coats. Extracts from condensed tannin sorghum bran had six major phenolic acids, two phenolic aldehydes and three flavanones. Proanthocyanidins in extracts from marama bean seed coats were predominantly highly galloylated prodelphinidins while those in extracts from condensed tannin sorghum bran were procyanidins.

Extracts from marama bean seed coats had higher antioxidant activity and protective effects against free radical induced erythrocyte haemolysis and LDL oxidation compared to equivalent extracts from condensed sorghum bran. Extracts from condensed tannin sorghum bran showed some protective effect against oxidative DNA damage. However, extracts from marama bean seed coats gave inconclusive results probably due to prodelphinidins binding to DNA. Extraction of marama bean seed coats under acidic condition resulted in reduction in phenolic compound content, antioxidant activity and protective effect against erythrocyte haemolysis, possibly due to co-precipitation of phenolic compounds with interpolymer complex precipitate formed between highly galloylated condensed tannins and cell wall polysaccharides at pH 2. In contrast, extraction of condensed tannin sorghum bran under the acidic condition resulted in an extract with significantly higher phenolic content and protective effect against erythrocyte haemolysis than the water extract possibly due to enhanced extraction of free and esterified phenolics and release of bound phenolic compounds. Water extracts and acidified water extracts (from marama been seed coats or sorghum bran) did not show significant differences in their protective effect against oxidative DNA damage and LDL oxidation.

Extraction under acidic condition may be the preferred method for sorghum bran because it increases recovery of phenolic compounds, but not for marama bean seed coats because it causes reduction in phenolic compound content. The findings of this study show that the extracts have a potential to reduce oxidative stress which is implicated in many chronic diseases such as neurodegenerative diseases, cancer and cardiovascular disease. The extracts can be used in the development of functional foods with potential health benefits.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES