Comparative study of Antioxidant properties, Polyphenols and Flavonoid contents of the tuber and seed extracts of Marama bean (*Tylosema esculentum*)

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Abstract

Tylosema esculentum, also known as the marama bean, is an underutilized legume from Southern Africa. Marama seeds and tubers are used as food and traditional medicine. The antiviral properties of the tuber and seeds have already been explored and the present work provides a first time report on the antioxidant activity and total phenolic content of marama tuber. Marama tuber extract, rich in phenolic compounds, exhibited the highest antioxidant activity compared to the seeds extract. IC_{50} values obtained for DPPH free radical scavenging were 95.62 ± 7.08 μg/ml and >1000 μg/ml for marama tuber and seed extracts, respectively. There was a positive correlation between the total phenolic content and antioxidant activity in the marama tuber and seed samples. In conclusion, the overall findings of this study suggest that the marama tuber could be a potential source of natural antioxidants.

Keywords: Marama bean; antioxidant; phenolics; flavonoids; free radical scavenging.

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1 Introduction

Tylosema esculentum, also known as the marama bean, belongs to the family Fabaceae and subfamily Caesalpinioideae. The marama bean plant occurs naturally in drier areas of Southern Africa, including Botswana and Namibia, where it is harvested as a wild plant for human consumption (Nepolo et al., 2009). The species produces nutritious and edible seeds called the marama bean. The plant has not been fully exploited in terms of its potential use as a food source or a non-food product which can be beneficial to the health sector (Kayitesi et al., 2012). *T. esculentum* stems grow along the ground up to 3 m in length, its bi-lobed leaves are green at maturity and can produce an oval pod of 5-6 cm long, usually bearing two (but up to 6) round seeds of about 2 cm in diameter (Bower et al., 1988). Traditionally, the seeds are first roasted before consumption and this is due to their unpleasant taste when eaten raw (Holse et al., 2010).

Porridges and flours composited with partially defatted marama flours reportedly have higher total phenolic content and antioxidant activity than porridges and flours composited with full fat marama flours (Kayitesi et al., 2012). Phenolic compounds are vastly attributed to the antioxidant properties of most food products (Chingwaru et al., 2011; Kayitesi et al., 2012; Nandutu et al., 2007). Free radicals are known to contribute to the development of many diseases (Kayitesi et al., 2012). Antioxidants scavenge free radicals and therefore protect cell components against oxidative damage by reducing the risk of various degenerative diseases associated with oxidative stress (Gil del Valle et al., 2013). Diets rich in antioxidants may play a role in the prevention of various diseases associated with oxidative stress such as cancer, cardiovascular and neurodegenerative diseases (Valko et al., 2006). There are no known studies on the antioxidant properties of the marama tuber. The present study was designed to investigate the unexplored antioxidant properties of *T. esculentum* tuber and seed extracts which are both edible parts of the Namibian marama plant and provide information that could be beneficial to the health sector by adding nutritional value to the marama bean. The specific objectives of this study were to carry out a comparative analysis of the phenolic and flavonoid contents as well as antioxidant activity of the marama seeds and tuber extracts. Phytochemical screening was also conducted to determine the major classes present. This study contributes to the nutritional value of the marama bean plant, a food product with great commercial value especially in Southern Africa and the rest of the world.

2 Experimental Section

2.1 Collection of *T. esculentum* Tuber and Seed samples

Marama tubers (3 months old) were collected from a botanical garden in Okahandja, Namibia. Marama seeds were obtained in Gobabis, Namibia.

2.2 Sample preparation and extraction

Tubers were washed with distilled water and peeled before extraction. Roasted marama seeds were prepared as previously described by Kayitesi et al. (2012) with some modifications. Seeds were roasted at 180°C for 15 min using a conventional oven. The seeds were cracked to remove the seed coat prior to milling. Extraction was carried out at room temperature (25°C). Briefly, 100 g of the ground sample was extracted with 500 ml ethanol for 48 h. The extracts were vacuum filtered through Whatman No.1 filter paper and concentrated using a rotary evaporator (Buchi, Switzerland) at 50°C. The extracts were stored at room temperature until further analysis.

2.3 Phytochemical screening

Marama tuber and seed extracts were subjected to qualitative phytochemical screening using standard procedures (Soni and Sosa, 2013).

2.4 Determination of total phenolic content

Total phenolic content was determined using Folin-Ciocalteau reagent as previously described by Thomas et al. (Thomas et al., 2012), with minor modifications. In a test tube, 1 ml aliquots (1 mg per ml of ethanol) of the extracts, 0.25 ml of Folin-Ciocalteau reagent and 1 ml of 20% (w/v) sodium carbonate (Na_2CO_3) were mixed. The tubes were placed in a boiling water bath for a minute and were cooled to room temperature. The absorbance was measured at 650 nm with a Spectromax microplate reader. Results were expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g).

2.5 Determination of total flavonoid content

A colorimetric protocol (Kubola et al., 2011) was used to determine total flavonoid content of marama tubers and roasted seeds. Briefly, 0.5 ml of the extract was mixed with 2.25 ml of distilled water and 0.15 ml of 5% sodium nitrite ($NaNO_2$) solution in a test tube. After 6 min, 0.3 ml of 10% aluminium chloride hexahydrate ($AlCl_3.6H_2O$) solution was added and further incubated for another 5 min before addition of 1 ml of 1M sodium hydroxide (NaOH). The mixture was vortexed and the absorbance was measured at 510 nm using a SpectraMax microplate reader. Results were expressed as mg quercetin equivalents in 1 g of dried sample (mg QE/g)

2.6 Antioxidant Activity

2.6.1 Reducing Power Assay

The Fe^{3+} reducing power of marama tuber and seed extracts was determined by the method of Valvi et al. (2011) with minor modifications. Different concentrations of the extracts (120 μl) were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 7.4) and 2.5 ml of potassium ferricyanide (1%), followed by incubating at 50°C for 20 min. The reaction was stopped by adding 2.5 ml of trichloroacetic acid (10%, w/v) and then centrifuged at 3000 rpm for 10 min. A 2.5 ml aliquot of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%), and the absorbance of the resultant mixture was measured at 700 nm using a SpectraMax microplate reader. The higher the absorbance value the greater the reducing power. All measurements were done in triplicates.

2.6.2 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) Radical Scavenging Assay

DPPH is a stable free radical with a purple color and on scavenging, these free radicals turn to yellow. The free radical scavenging activity of the extract was evaluated using a modified method previously described by Kapewangolo et al. (2013). Briefly, dissolved tuber or roasted bean extract was mixed with DPPH. Incubation of extracts with DPPH was done in the dark at room temperature for 30 min. The absorbance of the resulting solution was measured at 520 nm using a SpectraMax plate reader. Ascorbic Acid was used as a standard control.

2.7 Statistical Analysis

All the experiments were carried out at least four times ($n = 4$) and statistical mean was calculated ± SD using Graph Pad prism program (Graph Pad Software Inc., USA). The same statistical program was used to calculate IC50 values of DPPH assay.

3 Results and Discussion

3.1 Phytochemical Screening

Phytochemicals are natural occurring plant compounds which have potential disease inhibiting capabilities (Cragg and Newman, 2013). The results of the phytochemical studies are summarized in Table 1.

Table 1: Phytochemical composition of *T. esculentum* roasted seeds and tuber extracts

Phytochemicals	Tuber extract	Seed extract
Flavonoids	+	-
Alkaloids	-	+
Terpenoids	+	-
Polyphenols	+	+
Quinones	-	-
Tannins	+	-
Cardenolides/Cardiac glycosides	+	+

Interpretation of the test results are indicated by (-): absent; (+): present

Marama tuber extract showed the presence of flavonoids, terpenoids, polyphenols, tannins and cardenolides while the roasted seed extract only revealed the presence of alkaloids, polyphenols and cardenolides. The difference in phytochemical constituents could be attributed to the difference in growth conditions of the two plant parts. The tuber, found underground, is reportedly high in water and starch contents (E Nepolo, unpublished doctoral thesis) while the leguminous seeds endure harsh growth conditions above ground (Travlos et al., 2008).

3.2 Analysis of Total Phenolic and Flavonoid contents

Total flavonoid and phenolic contents of ethanolic extract of *T. esculentum* tuber and seeds were determined and the results are displayed in Table 2.

Table 2: Total phenolic content (TPC) and total flavonoid content (TFC) of ethanolic extracts of marama bean (*T. esculentum*) tuber and seeds

Ethanolic extract	TPC, (mg GAE/g)	TFC, (mg QE/g)
Marama bean tuber	4.48 ± 0.09	0.93 ± 0.001
Marama bean seeds	0.87 ± 0.75	0.0 ± 0.0‡

‡Mixture was turbid and there was no change in color. GAE, gallic acid equivalents; QE, quercetin equivalents

Ethanolic extract of marama tuber was found to contain the highest amounts of both polyphenols and flavonoids as evidenced by its total phenolic (TPC) and flavonoid (TFC) contents, which were 4.48*pm*0.09 mg/g gallic acid equivalents (GAE) and 0.93±0.001 mg/g quercetin equivalents (QE), respectively. TPC of the marama seeds was lower than that of the tuber and TFC was not detectable in the seed extract.

Flavonoids have many health promoting effects such as antioxidant and anti-allergic potential (Halliwell and Gutteridget, 1986). Plant extracts that contain a high amount of polyphenols reportedly exhibit antioxidant activity. Oxidative stress has been associated with cardiovascular diseases, certain cancers and neurodegenerative diseases (Gulcin, 2012). Dietary antioxidants such as phenolic compounds provide bioactive mechanism to reduce these lifestyle related diseases. Marama bean extract also showed the presence of tannins which when combined with proteins, starches, and digestive enzymes can reduce the nutritional value of foods (Chung et al., 1998; Serrano et al., 2009). Proximate composition of marama bean flours was previously reported (Amarteifio and Moholo, 1998; Holse et al., 2010; Kayitesi et al., 2012).

3.3 Reducing Power

The reducing power of marama bean extracts serves as a reflection of its antioxidant activity (Ferreira et al., 2007). In this Fe^{3+} to Fe^{2+} assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each sample (Soni and Sosa, 2013). The presence of reducers (antioxidants) causes the conversion of the Fe^{3+}/ferricyanide complex to the ferrous form. The higher the absorbance of the reaction mixture the higher the reductive potential of the plant extracts (Soni and Sosa, 2013).

Marama tuber extract, illustrated in Fig. 1, and the standard ascorbic acid (Fig. 2) had a higher absorbance that accounts for their high reducing potential.

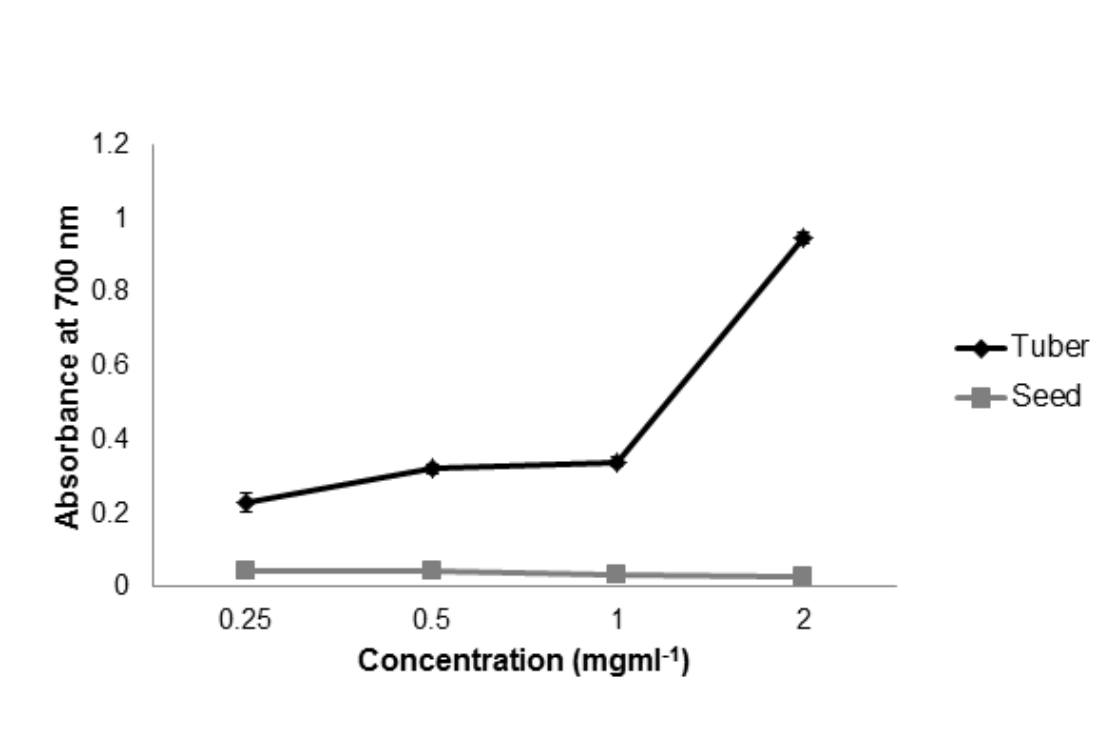

Figure 1: Reducing power of ethanolic extracts from *T. esculentum*. Each value is expressed as mean ± standard deviation ($n = 3$).

Marama tuber extract exhibited the highest reducing activities due to high levels of phenolic components, including flavonoids, as revealed by the total phenolic assay. Marama seed extract had low absorbance and exhibited the lowest reducing activities when compared to the tuber extract. Phytochemical analysis of the seed extract revealed low levels of phenols compared to the tuber as well as the absence of flavonoids and this could be attributed to the low reducing power absorbance observed here.

Plant extracts with reducing power indicate that they are electron donors and can scavenge free radicals found in the human body that causes cardiovascular diseases, certain cancers and neurodegenerative diseases (Soni and Sosa, 2013).

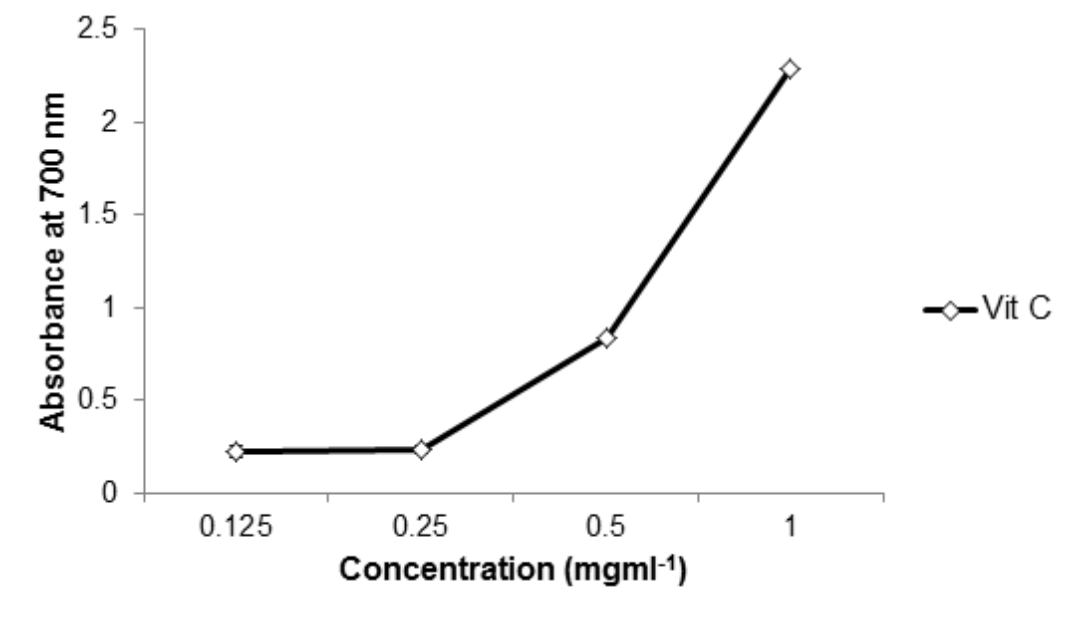

Figure 2: Reducing power of the standard control, ascorbic acid (vitamin C). Each value is expressed as mean ± standard deviation ($n = 3$).

3.4 DPPH Radical Scavenging Activity

DPPH radical scavenging is a rapid and sensitive way to survey the antioxidant activity of plant extracts. In the presence of an antioxidant, a stable DPPH radical is reduced to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine which is quantified using a spectrophotometer (Gulcin, 2012).

Marama tuber extract (Fig. 3) exhibited the highest antioxidant activity compared to the seed extract with IC_{50} values of 95±7.1 μg/ml for the tuber and >1000 μg/ml for the roasted seed extract.

The good antioxidant properties observed here for the tuber extract, similar to what was observed with the reducing power data, could also be attributed to high levels of phenolic content obtained in the present study. Phenolic compounds are known to have strong DPPH free radical scavenging activity (Soni and Sosa, 2013). The low antioxidant activity observed with the marama seed extract correlated with low levels of polyphenols revealed during phytochemical analysis.

DPPH scavenging activities is reportedly high in plant extracts that contain high levels of phenolic components such as flavonoids, phenolic acids, and phenolic terpenes (Nandutu

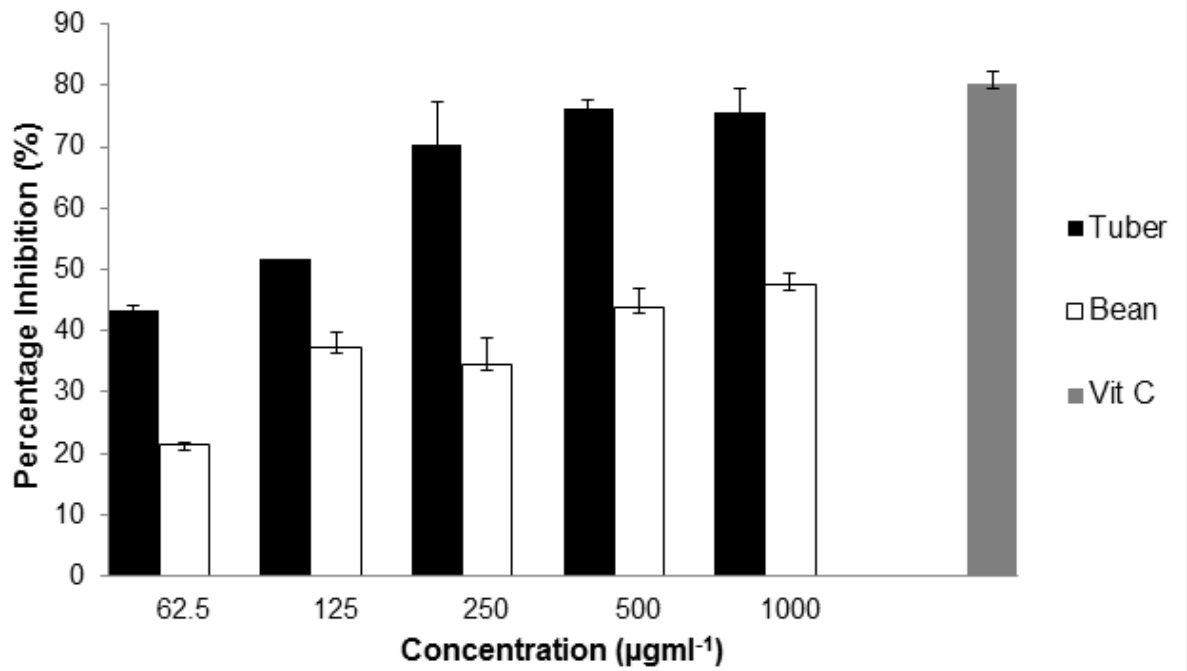

Figure 3: The antioxidant activity of the *T. esculentum* tuber and seed (bean) extracts as measured by the DPPH method. Vitamin C (Vit C), at 0.2 $mgml^{-1}$, was used as a standard control. Each value is expressed as mean ± standard deviation ($n = 3$).

et al., 2007; Soni and Sosa, 2013). These phenolic components possess many hydroxyl groups including o-dihydroxy group which have very strong radical scavenging effect and antioxidant power (Soni and Sosa, 2013). Ascorbic acid was used as a standard because of its ability to scavenge free radicals and can regenerate other antioxidants from their radical species (Halliwel and Cross, 1991). To the best of our knowledge, this is the first report of the antioxidant activity of marama tuber extract assessed using the DPPH and reducing power assays. Data presented here is in support of the epidemiological evidence that suggests that consumption of vegetables can alleviate degenerative diseases linked to oxidative stress (Sreeramulu and Raghunath, 2010). This study therefore supports the continuous consumption of marama tuber as a vegetable.

4 Conclusions

The total phenolic and flavonoid contents correlated well with the antioxidant assays. Marama tuber extract showed excellent antioxidant activity in both DPPH and reducing power as-

says. The same extract had high phenolic and flavonoid contents in comparison to the seed extract. This study is the first time report of the antioxidant properties of marama tuber extract and the findings of this study suggest that this edible tuber could be a potential source of natural antioxidants that could be of therapeutic importance in preventing or slowing the progress of oxidative stress related disorders. Further research should be done on the identification of the specific antioxidant compounds in marama tuber.

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