Phytochemical Profiles and Antioxidant Activity of Legumes Consumed in Botswana

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Abstract: Legume consumption has been consistently linked with lower risk of cardiovascular disease (CVD) and Coronary heart disease (CHD), as a result from their unique phytochemicals. Studies investigating phytochemical profiles and antioxidant activity of legumes in Botswana are limited. Five legume varieties were studied. All the legumes showed a significant amount of total phenolic acids and flavonoids ranging from 64.83 to 828.69 mg of gallic acid equiv/100 g of sample, DW and from 85.36 to 410.99 ± 21.24 mg of catechin equiv/100 g of sample, DW respectively. Their antioxidant activity ranged from 50.7 to 114.6 mg vitamin C /100g of DW. In this study, there was a positive correlation between TPCs and PSC value of the samples ($R^2=0.9940$, $P<0.01$). The higher TPCs resulted in higher antioxidant activity, an indication that phenolics were the major contributors to antioxidant activities. Chlorogenic, caffeic, $p$-coumaric, and ferulic acid were detected in all Cowpea varieties (Cowpea-Thamagana Speckle, Cowpea-Inia, and Cowpea-Red). The results from the study emphasize the importance of these legumes as a source of phenolic acids and antioxidants which could contribute to their health promoting properties and prevention of some diseases.

Keywords: Legumes, Phytochemicals, Phenolics, Flavonoids, Antioxidant Activity, HPLC

1. Introduction

Legumes belong to the family Leguminosae [1] that are used as food and play an important role in the traditional diets in developing countries, especially in Sub-Saharan African countries where they complement the lack of proteins from cereals, roots, and tubers [2].

As the shortage of food continues to be a major problem in Africa, these legumes are being promoted more than before in order to alleviate the protein-energy malnutrition [3, 4]. In addition to proteins, legumes are also considered a good source of complex carbohydrates, displaying a low glycemic index and high content of fibers, polyunsaturated fatty acids (PUFAs), dietary fiber, contain significant amounts of vitamins and minerals [5, 6], and low in fats [7].

Research has shown that legumes are rich in phytochemicals, that contain many bioactive compounds which are beneficial to health in addition to the identified nutrients such as proteins, vitamins and minerals [8]. The dominant phenolic compounds present in leguminous seeds are the flavonoids, phenolic acids and procyanidins [9]. These compounds inhibit many chronic diseases linked with cancer, inflammation, atherosclerosis, and aging caused by free radicals [8, 9, 10]. Thus, regular legume consumption has been associated with 22% and 11% lower risk of coronary heart disease and CVD [11]. Furthermore, consumption of legumes with high phenolic content is correlated to a number of positive health benefits such as hypocholesterolemia, and antiatherogeni [12]. Legumes also possess a hypoglycemic effect, reducing the increase in blood glucose after a meal. Legumes therefore are included in the diet of insulin dependent diabetics. Furthermore, consumption of legumes helps prevent osteoporosis [13] and reduces body lipid accumulation [14].

In Botswana, various varieties of legumes are cultivated and consumed as a source of dietary protein. Their presence therefore could be taken advantage of in addressing both macro and micronutrient deficiencies. However, much data has not been obtained on phytochemical constituents in legumes. So far, there has been no report on the
2. Materials and Methods

### Table 1. Descriptions of Legume Varieties Used in this Study.

| Common name            | Scientific name                        | Varieties                  | Moisture content (%) | Common use                                 |
|------------------------|----------------------------------------|----------------------------|----------------------|--------------------------------------------|
| Bambara groundnut      | Vigna Subterranea (L.) Verdc           | Keledi                     | 8.80                 | They are eaten whole, consumed directly as a snack or combined with other foods e.g. samp (crushed maize). |
| Groundnut              | Arachis hypogaea (L.)                   | Mokgalo                    | 8.59                 | They can be boiled, roasted and consumed as a snack or ground into flour that has a wide variety of use e.g. peanut butter, and in preparing of traditional delicacies. |
| Cowpea                 | Vigna unguiculata (L.) Walp             | Selie                      | 5.31                 | They are eaten whole/mashed, used in soups, stews, salads, may be eaten alone or used in eating with local staples like sorghum and maize meal porridge. |
| Mung bean              | Vigna radiate var. radiata (L) R. Wileczek | INIA 37                    | 8.62                 | Used in preparation of soups, stews or may be eaten alone and consumed as a snack or used in eating with local staples. |
| Tepary bean            | Phaseolus acutifolius (A. Gray)         | Thamagana speckled         | 9.38                 | May be eaten alone or combined with other foods. |

#### 2.2. Moisture Content of Legumes

The moisture content was determined by using the oven-dry method. A mass of 2 g of sample was dried in an oven at 105 °C to a constant weight. The measurements were expressed as percent of dry weight in triplicate (Table 1).

#### 2.3. Extraction of Phenolic Compounds

Free phenolic compounds in legume samples were extracted following a procedure adapted from [15]. Briefly, 2 g of legume flour was blended in Waring blender using 50 mL of 80% chilled acetone for 5 min and samples were homogenized with a Polytron Homogenizer for 3 min. The mixture was then centrifuged at 2500 rpm g for 10 min and the supernatants were collected in a 25 mL volumetric flask. All the supernatants were evaporated until 10% of the supernatants has been retained. The phytochemical extracts were brought to 10 mL in water and were kept at -40 °C until analysis.

#### 2.4. Determination of Total Phenolic Content

The total phenolic content of legume varieties was determined using the Folin-Ciocalteu colorimetric method described by [16]. All extracts were diluted 1:20 with Milli-Q water in order to obtain readings that falls within the standard curve concentration range of 0.0– 600.0 μg gallic acid/mL. Folin-Ciocalteu reagent was used to oxidized the legume extracts and sodium carbonate was added to the mixture to neutralize the solution. The absorbance was measured at 760 nm. Total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per 100 g of dry weight (DW) of sample. Data were expressed as mean ± SD of at least triplicates for each sample.

#### 2.5. Quantification of Total Antioxidant Activity by Hydro-PSC Assay

The total antioxidant activity was determined using the hydrophilic peroxyl radical scavenging capacity (Hydro-PSC) assay, a method described by [17]. The results were calculated as milligrams of vitamin C equivalents per 100 g of DW of sample. Data were reported as the mean ± SD of at least triplicates for each sample.

#### 2.6. Determination of the Total Flavonoid Content

The total flavonoid content of each legume sample was determined using the sodium borohydride/chloranil-based (SBC) assay as described by developed by [18]. Total flavonoid content was expressed as milligrams of catechin equivalents per 100 g of DW of sample. Data was reported as mean ± standard deviation (SD) with at least triplicates.

#### 2.7. High Performance Liquid Chromatography (HPLC)

Phenolic acids of Legume extracts were separated in a Waters C18 column (5 μm, 250 mm X 4.6 mm; Grace Vydac, Baltimore, MD) on a Waters HPLC system (Waters Corp.,
Milford, MA). Pure standards used for the identification were, chlorogenic acid, caffeic acid, p-coumaric acid, and ferulic acid. The samples were identified by retention time and absorbance spectrum. Results obtained for the sample extracts were expressed as mean ± SD for triplicates.

2.8. Analysis

Statistical analysis was conducted using SPSS (Statistics for Social Science) version 18.0 for windows. Differences between means were performed using ANOVA and Turkey’s test. All graphical representations were performed using Sigmaplot version 2000 (Aspire Software International, Ashburn, VA). Statistical significance was set at p < 0.05. All the results were presented as mean ± SD for at least triplicate for each sample.

3. Results and Discussions

3.1. Total Phenolic Content of Legume Varieties

All the legumes showed a significant amount of total phenolics (Table 2). Expressed as milligrams of gallic acid equivalent per 100 gram of sample on DW basis (mg GAE/100 g DW), total phenolic contents are presented in Table 2. A wide variation was observed for phenolic contents and differed significantly with respect to this parameter. The free phenolic content ranged from 646.10 ± 7.62 (Cowpea-Thamaga Speckle) to 35.74 ± 3.81 (Tepary Bean) mg of GAE/100 g DW.

Table 2. Total Phenolic Content of Legumes. Values Expressed as Milligrams of GAE Equivalents/100 g DW (mean ± SD, n=3).

| Name          | Varieties                  | Total phenolic (mg GAE/100 g DW) |
|---------------|----------------------------|----------------------------------|
| 1. Cowpea     | Thamaga speckle            | 646.10 ± 7.62a                   |
|               | Red                        | 488.69 ± 13.13b                  |
|               | Inia 37                    | 505.39 ± 10.56b                  |
| 2. Bambara    | Mokgalo                    | 570.02 ± 8.93c                   |
|               | Keledi                      | 61.49 ± 2.96d                    |
| 3. Groundnut  | Sellie                     | 342.25 ± 7.98e                   |
|               | Peolwane                   | 312.79 ± 6.90e                   |
| 4. Mung bean  | -                          | 218.72 ± 5.30b                   |
| 5. Tepary Bean| -                          | 35.74 ± 3.81f                    |

Values with different letters in each row are significantly different (p<0.05)

3.2. Total Antioxidant Activity in Legume Varieties by PSC

Antioxidant potentials of legumes have been reported in several studies [21, 22, 23, 24]. However, antioxidant activities were measured by DPPH, TEAC, FRAP, TAC and ABTS assay and therefore difficult to compare our data to that reported in other studies [7, 9, 24, 25]. In this study the total antioxidant activity measured by the PSC assay of the different varieties of legumes was expressed as micrograms of Vitamin C equivalent per 100 grams of DW. The PSC assay is usually employed to estimate antioxidant activity of foods. This assay is simple, reliable, robust, sensitive, and precise and can produce acceptable results comparable to those obtained with similar published assays [26]. The PSC values of total antioxidants of the legume fractions are presented in Table 3. In this study, there was a positive correlation between TPCs and PSC value of the samples (R²=0.9940, P<0.01). The higher TPCs resulted in higher antioxidant activity, and therefore, phenolics were the major contributors to antioxidant activities (Tables 2 & 3). Literature shows that the total phenolic content is directly associated with antioxidant activity [7, 20, 24, 27]. Antioxidant activity of phenolics depends on the structure and substitution pattern of hydroxyl groups [28].

3.3. Flavonoid Content of Legume Varieties

Flavonoids are widespread plant secondary metabolites, including flavones, flavanols, and condensed tannins. Flavonoids present in leguminous seeds belong to flavanols, flavones, and anthocyanidins [8, 29]. As components of vegetables, fruits, and grains, they have generated interest because of their broad human health promoting effects. Many of these effects are related to their antioxidant properties, which may be due to their ability to scavenge free radicals [8, 29]. There are no reports available to compare total flavonoids of legume varieties studied here. However, a few reports on identification and quantification of flavonoids on common beans [30, 31], cowpea sprouts [31], and, pea [32] were documented. Expressed as milligrams of catechin equivalent per gram of samples on a DW basis, total flavonoids contents of Legume varieties were shown in Table 4. The flavonoid content of Groundnut-Sellie (300.85 ± 149.03mg/g) was higher (p <0.05) than those of the other
3.4. HPLC Analysis

The phenolic acids of Legume varieties were further evaluated by HPLC. Expressed as µg/g DW sample, quantities of chlorogenic, caffeic, p-coumaric, and ferulic acids were either less or not detected in legume extracts. The levels of phenolic acids varied from 17.79 ± 0.04 – 110.95 ± 3.18 µg/g DW sample for chlorogenic acid, 10.02 ± 0.12 - 39.67 ± 0.01 µg/g DW sample for caffeic acid, 1.25 ± 0.06 – 23.56 ± 0.67 µg/g DW sample for p-coumaric acid, and 4.04 ± 0.08 – 9.16 ± 0.07 µg/g for ferulic acid. Chlorogenic, caffeic, p-coumaric, and ferulic acid were detected in all Cowpea varieties (Cowpea-Thamagana Speckle, Cowpea-Inia, and Cowpea-Red). Tepary bean, exhibited the highest concentration of chlorogenic acid (110.95 ± 3.18 µg/g DW sample) while the lowest concentration was observed in p-coumaric acid (2.21 ± 0.11 µg/g DW sample). Among the four components quantified, only little amounts of ferulic acid (9.16 ± 0.07 µg/g of DW sample) were detected in Groundnut-Sellie. Bambara-Mokgalo differed from other legumes in its high concentration of caffeic acid (39.67 ± 0.01 µg/g DW sample) and its lack of other phenolic acids.

4. Conclusion

All legumes extracts showed a significant amount of phenolics and flavonoids and antioxidant activity. In this study, Cowpea-Thamagana Speckle, Cowpea-Inia 37, Cowpea-Red and Bambara Groundnut-Mokgalo had the highest antioxidant capacity and could be explained by their higher phenolic contents. Significant positive correlation was observed between phenolics and total antioxidant activity of the different legume extracts. Given the phytochemicals profiles and antioxidant activity contribution of legumes, nutritionists should make a concerted effort to encourage the public to consume more legumes in general.

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