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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

phytochemical and antioxidant activity of these legumes consumed in Botswana. Thus, the limited information from literature presents a scope for a comprehensive study that will make a list of legumes available with known and tested functional properties. Furthermore, knowledge of the health beneficiary of phytochemical profiles is important to increase consumers' awareness.

Against this background, the objective of this study were to assess the phytochemical profiles including total phenolic content, phenolic acids, flavonoids, and antioxidant activity in legumes consumed in Botswana.

2. Materials and Methods

2.1. Sample Preparation and Extraction of Phenolic Compounds

Five legume varieties used in this study were provided by Dr. K. Safi of the Department of Agricultural Research, Sebele, Botswana, at the Seed Multiplication Unit (Table 1). The legumes represent the best known and generally the most widely consumed legumes in Botswana especially in the rural communities. The eight legume samples were ground using a kitchen grinder into small sizes that can pass through sieve No. 72 (British Sieve Standards) and stored at -40 °C until analysis.

Free phenolic compounds in legume samples were extracted following a procedure adapted from [15].

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 Mokgalo	8.80 8.59	They are eaten whole, consumed directly as a snack or combined with other foods e.g. samp (crushed maize).
Groundnut	*Arachis hypogaea* (L.)	Sellie Peolwane	5.31 5.43	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	INIA 37 Red Thamagana speckled	8.62 9.74 9.38	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. Wilczek	-	9.29	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)	-	7.79	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 o 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 three replications.

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, chlorongenic 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 GA Equivalents/100 g DW (mean ± SD, n=3).*

Name	Varieties	Total phenolic (mg GAE/100 g DW)
1. Cowpea	Thamagana 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 ($p< 0.05$)

Table 3. *Antioxidant Activity of Legumes Expressed as PSC values Milligrams of Vitamin C Equivalents/100 g DW (mean ± SD, n=3).*

Name	Varieties	PSC values (mg vitamin C equiv/100 g DW)
1. Cowpea-	Thamagana Speckle	74.1 ± 0.9a
	Red	67.9 ± 2.1a
	Inia 37	61.1 ± 8.5a
2. Bambara Groundnut	Mokgalo	73.3 ± 4.4a
	Keledi	32.1 ± 2.3b
3. Groundnut	Peolwane	37.1 ± 2.2b
	Sellie	32.3 ± 2.8b
4. Mung bean	-	39.2 ± 1.3b
5. Tepary Bean	-	18.6 ± 1.9c

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

The bound phenolic content ranged from 260.60 ± 63.19 (Cowpea-Inia 37) to 15.00 ± 5.77 (Groundnut-Sellie) mg of GAE/100 g DW). Cowpea-Thamaga Speckle (646.10 ± 7.62 mg of GAE/100 g DW) stood out among all the studied legumes followed by Bambara Groundnut - Mokgalo (570.02 ± 8.93 mg/g). This can be explained by the fact these legumes possess red integuments which are considered high in phenolic compounds [18, 19] as compared to Tepary bean (35.74 ± 3.81 mg of GAE/100 g DW) with a white integument which had the lowest free phenolic content. The abundant phenolic content of leguminous seeds indicates that legumes are the principal sources of antioxidant activity in food. The total phenolic content of Tepary Bean extracts is similar to those observed in another study [20].

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 (R^2=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

legume varieties followed by Cowpea-Thamagana Speckle (280.11 ± 20.21mg/g). Tepary Bean had the lowest flavonoid content (47.19 ± 33.48 mg/g).

Table 4. *Total Flavonoids Content of Legumes Expressed as (Milligrams of Catechin Equivalents/100 g, DW (mean ± SD, n=3). Percentage Contribution to Total Flavonoids Content is in Parentheses.*

Name	Varieties	Total flavonoids (mg catechin/100 g DW)
1. Cowpea	Thamagana Speckle	280.11 ± 20.21a
	Cowpea-Inia 37	235.10 ± 48.39a
	Red	229.07 ± 20.84a
2. Bambara	Mokgalo	226.28 ± 39.78a
	Keledi	166.89 ± 67.97b
3. Groundnut	Peolwane	144.63 ± 82.68b
	Sellie	300.85 ± 149.03ac
4. Mung bean	-	172.93 ± 34.12b
5. Tepary Bean	-	47.19 ± 33.48d

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

Table 5. *Composition of the Phenolic Compounds in Legume Extracts Expressed as Micro Grams per Gram of DW (mean ± SD, n=3) Revealed by HPL*

Sample	Varieties	Chlorogenic acid (µg/g of DW)	Caffeic acid (µg/g of DW)	*p*-coumaric acid (µg/g of DW)	Ferulic acid (µg/g of DW)
1. Cowpea	Inia	24.95 ± 0.11	10.29 ± 0.19	5.19 ± 0.12	4.25 ± 0.03
	Red	26.90 ± 0. 12	11.02 ± 0.16	5.26 ± 0.15	4.13 ± 0.05
	Thamagana-Speckle	17.79 ± 0.04	10.02 ± 0.12	5.24 ± 0.12	4.04 ± 0.08
2. Groundnut	Sellie	ND	ND	ND	9.16 ± 0.07
	Peolwane	ND	ND	23.56 ± 0.67	6.96 ± 0.52
3. Bambara	Mokgalo	18.58 ± 0.04	39.67 ± 0.01	ND	ND
	Keledi	20.84 ± 0.25	ND	1.25 ± 0.06	ND
4. Tepary bean	-	110.95 ± 3.18	ND	2.21 ± 0.11	5.95 ± 0.11
5. Mung bean	-	ND	ND	18.12 ± 0.37	4.26 ± 0.06

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 ± 0.67µg/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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