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Tannins and Saponins in Two Tropical Legumes and Measurement of their Biological Activity

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Authors' contributions

This work was carried out in collaboration between all authors. Authors LACG and AFCR designed the study. Author LSS wrote the protocol, anchored the field study, gathered the initial data and performed preliminary data analysis. Authors AFCR and DABA managed the statistical analysis of data. Author MRSC managed the literature searches. Authors LACG and AFCR produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Raw tropical legumes have abundant amounts of tannins and saponins which are usually eliminated with high temperature once they are cooked. These biomolecules may represent new alternatives, to allopathic medicine, in order to prevent the accumulation of free radicals affecting the cells, or to prevent thrombosis in the blood stream respectively. Therefore the aim of this study was to evaluate the antioxidant and antithrombotic activity attributed to tannins and saponins in whole seeds flour (WSF) and fractions resulting in three types of components: endosperm (flour without hulls –Fw/oH-), protein concentrate (PC) and hulls, of two types of tropical legumes *Vigna unguiculata* (Cowpea) and *Mucuna pruriens* (Velvet bean). Analysis of nutritional value and also tannins and saponins content was carried out. Finally, antioxidant and antithrombotic activity was also estimated.

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Place and Duration of Study: Experiments were carried out in the laboratory of Food Science of the Faculty of Chemical Engineering of the Autonomous University of Yucatán, located in Mérida, Yucatán. México, during 2011-2012.
Study Design: Variables were analyzed with descriptive statistics and using the method of Generalized Linear Model to detect the effect of the type of legume, type of component and their interaction.
Methodology: Analysis of nutritional value and also tannins and saponins content was carried out. Finally, antioxidant (5093 Trolox equivalent units, TEAC) and antithrombotic activity (measurement of the capacity to inhibit the aggregation of blood platelets) was also estimated (n =3).
Results: Type of legume did not affect protein content (p>.05), but there were differences in the content of moisture, fat, ash and carbohydrate (p<.05); different seed fractions exhibited different nutritional profile. Tannin content was greater in *M. pruriens* (0.63%) compared to *V. unguiculata* (0.38%) (p<.01); among the components tested, a higher concentration was detected in WSF (1%)(p<.01). The greatest antioxidant activity was observed in *V. unguiculata* (p<.01) whereas in the fractions, the highest concentration was found in the hulls (5937 TEAC) (p<.01). These values are considered high. Saponin content was higher in *V. unguiculata* (25.8 mg / g) (p<.01); as far as the components tested, the hulls had a higher content (22.7 mg / g) (p<.01). Antithrombotic activity was similar in both legumes and as far as the components is concern, the most abundant amount was found in the Fw/oH. In all cases, values were found to be low.
Conclusion: *V. unguiculata* hulls showed high antioxidant activities, which were inversely related to tannin content. Saponin content apparently was not associated with antithrombotic activity since it was found to be low in both legumes.

Keywords: Vigna unguiculata; Mucuna pruriens; antioxidant activity; antithrombotic activity.

1. INTRODUCTION

Legumes contain secondary metabolites such as flavonoids, polyphenols, saponins, among others, in different amounts depending on the species of the plant. These metabolites can be toxic or beneficial to humans, depending on their concentration [1].

Tannins and saponins, in small amounts can have antioxidant [2] and antithrombotic [3] activity respectively. Antioxidant activity reduces free radicals in human metabolism and helps to prevent diseases like cancer, premature aging, heart problems, and fertility problems, among others [4]. Antithrombotic activity reduces the incidence of ischemic heart disease (IHD), the first cause of death in México in 2012 [5]. IHD is induced by overweight and according to the National Nutrition Survey of México, carried out in 2012 [6], the combined prevalence of overweight and obesity in México is 73% in women and 69% men; this elevated percentage underlines the fact that it is a public health problem.

Vigna unguiculata (Cowpea) and *Mucuna pruriens* (Velvet bean) are two tropical legumes that have been underutilized in México. *Vigna* is included in the diet of rural families in Yucatán, México. *Vigna* nutritional contribution to the diet is primary protein, B vitamins and minerals, among other nutrients [7].

Mucuna has an agronomic potential as a cover crop and for replenishing soil fertility; it is also high in protein and it is comparable to soybean in terms of amino acid and mineral contents [8]. It also has being used in the treatment of Parkinson disease [9].

Based on the above, the purpose of the present study was to assess the antioxidant and antithrombotic activity attributed to tannins and saponins in whole grains, endosperm, hulls and protein concentrates resulting from fractioning *Vigna unguiculata* and *Mucuna pruriens*. Such biological activity may help to prevent the accumulation of free radicals affecting the cells, or thrombosis in the blood stream.

2. MATERIALS AND METHODS

Experiments were carried out in the laboratory of Food Science of the Faculty of Chemical Engineering of the Autonomous University of Yucatán, located in Mérida, Yucatán. México, during 2011-2012.

2.1 Preparation and Extraction of Legumes and Initial Analysis

Legume beans of *Vigna unguiculata* and *Phaseolus lunatus* were obtained in the local market (creole variety). They were cleaned manually, removing impurities. In the case of *M. pruriens* two types of grains were obtained depending on their color: beige and mottled. Five kg of each legume were crushed manually and the hulls were separated. To obtain a finer powder a grinding mill was used (Cyclotec 1093, Tecator Sweden) with a mesh number 80 and then 100. The following products and byproducts were obtained:

Whole seeds flour (WSF) and fraction resulting components: endosperm (flour without hulls, Fw/oH), hulls and protein concentrate (PC). The PC was obtained using the method described by Chel et al. [10].

The following analysis were conducted (n= 3).

Weende analysis of samples; moisture (drying the sample at 105ºC for 4 h), ash (ashing in oven at 550ºC during 4 h), crude protein (Kjeldahl), ether extract (Soxtec™ 8000, Foss), crude fiber (double digestion in caustic soda and acid. Fibertec™, Tecator) and nitrogen-free extractives (NFE, calculated as the difference of previous results from 100), was determined. The methods used were those quoted by the Official Methods of Analysis of the Association of Official Analytical Chemist [11].

2.2 Quantification of Tannin and Saponin

Tannins were estimated by the method ISO [12] and dimethylformamide was used for their extraction; quantification was determined using an spectrophotometer at 525 nm using tannic acid as a reference. Saponins were extracted with methanol (80%) four times; quantification was carried out using a spectrophotometer at 520 nm, using dosgenine as a reference; this method was described by Hiai et al. [13].

2.3 Assay of Antioxidant and Antithrombotic Activity

The antioxidant activity assay relies on the ability of antioxidants in the sample to inhibit the oxidation of ABTS (2.2'-Azino-di-3-ethybenzthiazoline sulphonate) to $ABTS^{\bullet+}$ by metmyoglobin. The amount of ABTS produced can be monitored by reading the absorbance at 750 nm. Under the reactions condition used, the antioxidants in the sample cause suppression of the absorbance to a degree which is proportional to their concentration. The capacity of the antioxidant in the sample to prevent ABTS oxidation is compared with that of Trolox®, a water soluble tocopherol analogue, and is quantified as milimolar of Trolox® equivalent antioxidant capacity [14].

Anti-thrombotic activity measured in the capacity to inhibit the aggregation of blood platelets; it was expressed as a percentage. It was carried out by means of the methods proposed by Miyashita et al. [15] and Chrono-Log Corporation [16].

2.4 Statistical Analysis of Data

The data dealing with nutritional value were analyzed using descriptive statistics (mean and standard deviation). Subsequently, the results of nutritional value, tannins, saponins, antioxidant and antithrombotic activity were statistically analyzed using the method of least squares, using the following fixed effects linear model:

$$y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \varepsilon_{ijk}$$

where:

y_{ijk}= observation *k* in *i* type of legume *A* and *j* fraction of the seed *B*
μ = the overall mean
A_i= the effect of *i,* type of legume (*V. unguiculata* or *M. pruriens*)
Bj= the effect of *j,* fraction of the seed (type of component) (WSF, Fw/oH, hulls and PC; considering the combination of both legumes)
$(AB)_{ij}$= the effect of the interaction of *j* of *A* with *j* of *B*
ε_{ijk}= random error with mean 0 and variance σ^2

Analyses were performed using a statistical package [17] by means of the routines GLM and Means. When significant effects were detected in any variable, means were compared by the multiple range test of Duncan [18]. Correlation analyses were also estimated.

3. RESULTS AND DISCUSSION

Nutrient content in each fraction of *V. unguiculata* and *M. pruriens* are shown in Tables 1, 2, 3 and 4. Statistical analysis of data (%), in general, indicated that the type of legume did not affect protein content (33, 28 and 23) or NFE (52.0, 46.4 and 46.6) (p >.05); there were differences in the content of moisture (8.0, 7.3 and 7.4), ether extract (1.3, 6.1 and 6,2), crude fiber (10.0,15.0 and 13.9) and ash (3.1, 3.7 and 4.1) (p<.05), for *V. unguiculata, M. pruriens* mottled and beige respectively. These results are similar to those found by other authors [19, 20].

Type of fraction led also to find differences in the nutritional value (%) of all the samples (p< .01): moisture (9.5, 9.4, 4.1 and 7.4), protein (28.8, 27.1, 50.1 and 6.9), ether extract (3.5, 3.4, 10.4 and 0.9), crude fiber (2.0, 7.2, 0.8 and 41.8), ash (3.7, 3.4, 5.0 and 2.7), and NFE (62.1, 58.9, 25.4 and 47.0), for Fw/oH, WSF, PC and hulls respectively.

3.1 Tannin Content and Antioxidant Activity

Table 5 shows the average tannin content depending on the type of legume or component. A larger amount of tannins were found in *M. pruriens* beige type and as far as the type of component WSF averaged the largest amount compared to other components (p<.01). This amount of tannins is considered high. Previous authors [21] mentioned that the percentage of tannin ranges between 0.03 and 1.92%, depending on the type of legume, similar range to the one observed in this paper. It was expected to find a higher content of tannins in hulls of legumes since they are a first barrier of protection against insects; however, tannins were found in the inner part of the grains especially in WSF.

Highest antioxidant activity was found in *V. unguiculata* and as far as the type of component, in the hulls (p <.01) (Table 6).

Antioxidant activity in this study was related inversely to the tannin content (r= -0.70), the fewer amounts of tannins, the antioxidant activity was increased. This is clearly shown in the results obtained with the type of legume (*Vigna*) and the type of component (hulls), which were found to be lower in tannins, but provided the greatest antioxidant activity. These results suggest that other molecules may play a certain role in antioxidant activity, preventing tannins to express antioxidant activity effectively. More research is needed to confirm these observations.

3.2 Saponin Content and Antithrombotic Activity

Table 7 describes the average saponin content depending on the type of legume or component. The largest amount was found in *V. unguiculata* and in the hulls (p<.05). Saponin content in hulls in the case of *V. unguiculata* was the highest of all the samples analyzed (47.3 mg/kg); in the samples of Flour w/o hulls (17.9 mg/kg) and WSF(18.7 mg/kg), saponins decrease rather than increase as it could be expected, this was also observed in *M. pruriens*. Probably the interaction between saponins and fats help to explain such behavior, since free saponins have emulsifying properties providing them with hipo-cholesterolemian activity in the human body [22,1]. The range of saponins in legumes previously reported was found between 10 to 15mg/g [23], these quantities are similar to those reported in this study.

Antithrombotic activity was found similar in all grains (p>.05) and when type of component was analyzed, it was larger in Fw/oH (p <.05) (Table 8). The amounts found in platelet inhibition it is low, as is expressed in the range of 1-100 as a percentage.

Anti-thrombotic activity, in general was found to be low (Table 8).

Several studies were oriented to develop techniques in order to enhance the antithrombotic activity in legumes. Some authors [24] used hydrolyzed protein concentrates of *M. pruriens* flours using Pepsin-120, obtaining 72% inhibition of aggregation; others [25] used alkalase enzyme hydrolysis with *Jatropha curcas* L., obtaining 51% inhibition of platelet aggregation. This indicates that generally legumes possess lower antithrombotic activity, but it may be increased by means of enzymatic treatments. It is important to mention that in this study total saponins were analyzed and no other metabolites derived from them (steroids or triterpenoids) that could have affected this result. In all cases, values were found to be low.

Table 1. Nutrient content of *Vigna unguiculata* flour without hulls and whole seeds flour (% dry basis). Mean and standard deviation

Nutrient	Flour without hulls	Whole seeds flour
(Moisture)	(7.91)±0.10	(11.64)±0.01
Crude protein	29.27±0.52	29.44±0.03
Ether extract	1.45±0.25	2.06±0.13
Crude fiber	3.14±0.01	5.64±0.16
Ash	4.32±0.01	2.67±0.25
NFE	61.82	60.21

Table 2. Nutrient content of *Mucuna pruriens* flour without hulls and whole seeds flour (% dry basis). Mean and standard deviation

Nutrient	Flour without hulls		Whole seeds flour	
	***M. pruriens* (beige)**	***M pruriens* mottled**	***M. pruriens* beige**	***M. pruriens* mottled**
(Moisture)	(10.17)±0.07	(10.49)±0.16	(8.12)±0.21	(8.3)±0.24
Crude protein	28.96±0.08	28.10±0.45	25.16±0.10	26.88±0.53
Ether extract	4.51±0.08	4.47±0.16	3.98±0.14	4.07±0.23
Crude fiber	1.41±0.05	1.49±0.17	5.70±0.22	10.15±0.03
Ash	3.52±0.04	3.18±0.48	3.74±0.09	3.66±0.45
NFE	61.65	62.8	61.32	55.25

Table 3. Nutrient content of *Vigna unguiculata* protein concentrate and hulls (% dry basis). Mean and standard deviation

Nutrient	Protein concentrate	Hulls
(Moisture)	(4.17)±0.17	(8.14)±0.18
Crude protein	63.47±0.02	9.93±1.00
Ether extract	0.54±0.01	1.16±0.22
Crude fiber	1.31±0.54	29.96±0.67
Ash	4.41±0.01	3.22±0.02
NFE	30.29	55.75

Table 4. Nutrient content of beige and mottled *Mucuna pruriens* protein concentrate and hulls (% dry basis). Mean and standard deviation

Nutrient	Protein concentrate		Hulls	
	***M. pruriens* (beige)**	***M pruriens* (mottled)**	***M. pruriens* (beige)**	***M pruriens (mottled)***
(Moisture)	(3.73) ± 0.09	(4.26) ±0.09	(7.52)±0.20	(6.53) ±0.49
Crude protein	55.80 ± 0.53	56.09±0.04	6.73±0.05	4.00±0.01
Crude fiber	15.30 ± 0.27	15.39±0.26	1.06±0.42	0.63±0.05
Ether extract	0.62±0.33	0.60±0.25	47.67±0.52	47.70±0.49
Ash	6.13±0.33	4.31±0.35	3.16±0.07	3.79±0.64
NFE	22.17	23.62	41.45	43.91

Table 5. Average tannin content (%) according to the type of legume and type of component. Mean and standard deviation*

Type of legume		
***Mucuna pruriens* mottled**	***Vigna unguiculata***	***Mucuna pruriens* beige**
0.59^{b}±0.45	0.38^{c}±0.45	0.63^{a}±0.36

Type of component			
Flour w/o hulls	**WSF**	**Protein concentrate**	**Hulls**
0.68^{b}±0.48	1.00^{a}±0.11	0.28^{c}±0.21	0.18^{d}±0.13

**Different letters in the same row indicate, p<.05 (Duncan test)*

Table 6. Average antioxidant activity depending on the type of legume and type of component (TEAC Trolox Equivalent). Mean and standard deviation*

Type of legume			
***Mucuna pruriens* mottled**	***Vigna unguiculata***	***Mucuna pruriens* beige**	
2280^{c}±1795	5093^{a}±4668	3279^{b}±3034	
Type of component			
Flour w/o hulls	**WSF**	**Protein concentrate**	**Hulls**
5044^{c}±5007	1002^{d}±360	2220^{a}±923	5937^{b}±2626

**Different letters in the same row indicate, p<.05 (Duncan test)*

Table 7. Average saponin content depending on the type of legume and type of component (mg /g). Mean and standard deviation*

Type of legume			
***Mucuna pruriens* mottled**	***Vigna unguiculata***	***Mucuna pruriens* beige**	
15.3^{c}±6.0	25.8^{a}±13.3	17.1^{b}±4.2	
Type of component			
Flour w/o hulls	**WSF**	**Protein concentrate**	**Hulls**
20.2^{b}±2.4	15.0^{c}±2.9	19.7^{b}±1.9	22.7^{a}±19.2

**Different letters in the same row indicate, p<.05 (Duncan test)*

Table 8. Average antithrombotic activity depending on the type of legume and type of component (% inhibition). Mean and standard deviation*

Type of legume			
***Mucuna pruriens* mottled**	***Vigna unguiculata***	***Mucuna pruriens* beige**	
4.40^{a}±1.63	3.87^{a}±2.53	3.32^{a}±1.73	
Type of component			
Flour w/o hulls	**WSF**	**Protein concentrate**	**Hulls**
5.30^{a}±1.53	3.36^{bc}±2.06	4.78^{ab}±1.46	2.01^{c}±1.08

**Different letters in the same row indicate, p<.05 (Duncan test)*

Results obtained in this experiments show the potential important biological activity of *V. unguiculata* and in lesser extent, of *M. pruriens* as antioxidants, associated in a negative trend, with their tannin content. Further research is needs to clarify the relationship between antioxidant activity and the amount of tannins, where other molecules may be interacting. Since antithrombotic activity was found to be low, the use of the previously mentioned two legumes for such purpose is not promising.

4. CONCLUSION

Quantification of tannins was higher in *M. pruriens* compared to *V. unguiculata*. Tannin content according to the staining was bigger in *M. pruriens* beige compared to mottle. Contrary to expected, they were more abundant in whole seed flour, instead of the hulls. Nevertheless, the strongest antioxidant activity was mainly observed in *V. unguiculata* hulls.

In both *V. unguiculata* as *M. pruriens,* saponins were quantified. Higher amounts were found in hulls; however, it was observed that they are widely distributed in all other components of the grains. Antithrombotic activity of *V. unguiculata* and *M. pruriens* showed low intensity, with the highest, found in the flour without hulls. There was no relationship between these two variables.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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