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Quantification of Tannins in Four Species of Genus *Mucna* Seeds

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Abstract

In order to make *Mucuna* seeds consumable, seeds are treated according to the following procedures: boiling, soaking followed by boiling in the presence of 0.5% KOH and finally soaking for a long time followed by boiling in the presence of 0.5% KOH, so that tannins can be removed. Preliminary studies done on the raw seeds of four *Mucuna* species revealed the presence of tannins. The results showed that the crushed seeds contained all high tannin with the content of 0.370 g GAE/100g DM, 0.336 g GAE/100g DM; 0.266 g GAE/100g DM and 0.203 g GAE/100g DM for *Mucuna deerigiana*, *Mucuna nagaland*, *Mucuna cochinchinensis* and *Mucuna rajada* respectively. The different methods of seed treatment significantly reduced the tannin content in *Mucuna* seeds. The method of soaking the seeds for 12 hours and then scalding in the presence of 0.5% KOH reduced tannins in *Mucuna deerigiana*, *Mucuna nagaland*, *Mucuna cochinchinensis*, and *Mucuna rajada* seeds by 47.29%; 48.21%; 66.16%; and 15.76% respectively. This method gave the highest tannin reduction rate.

Keywords

Quantification, Tannins, Reduction Rate, *Mucuna*, Burkina-Faso

1. Introduction

Plant tissues contain several substances with phenolic functions and, the most common are flavonoids, tannins and phenolic acids [1]. In the large group of compounds called tannins, a variety of chemical structures are possible, and the

number of plant species containing these compounds is immense [2] [3]. Hydrolysable tannins consisting of simple phenolic acids such as gallic acid esterified with polyols, usually glucose, and condensed tannins are polymers of flavonoid units [4]. Several basic human foods, including fruits, beverages, and some seeds contain condensed and hydrolysable tannins [4].

Mucuna is seasonal legume, and is cultivated for the good quality of its fodder, its seeds, its ability to fix atmospheric nitrogen at ground level. It is a creeping and climbing plant that adapts to the temperature of 15˚C to 35˚C [5].

Mucuna leaves are given as a supplement to cows to increase the quality and quantity of milk and to other livestock, especially ruminants. The seeds can be combined with animal feed after processing [5].

However, it is known that the seeds of *Mucuna* species us contain anti-nutritional factors, including tannins, which can present an intoxication danger in the case of direct feeding of ruminants [6]. However, these seeds are crushed to be used as a complementary food for draught cattle, cattle, or sheep for fattening [6].

This study aimed to determine the tannin content in untreated and treated seeds of the four *Mucuna* varieties.

2. Material and Methods

2.1. Plant Material

The study focuses on the untreated seeds of the four *Mucuna* varieties and the seeds are treated according to four protocols. The species studied are the seeds of *Mucuna deerigiana*, *Mucuna nagaland*, *Mucuna cochinchinensis* and *Mucuna rajada* harvested in Burkina—Faso. To better identify the part of the seed containing more tannins, the different parts, namely the epicarp, the starch and the crushed seed set, of each of the four untreated species were studied. The tannin determination for the treated species was done on the whole seed set.

2.2. Seed Treatment

The different seed processing methods [7] for four *Mucuna* species are as follows:

- *Mucuna* seeds are boiled directly for 2 hours;
- *Mucuna* seeds are soaked for 12 hours in water followed by boiling for 2 hours;
- *Mucuna* seeds are boiled in the presence of KOH (0.5%) for 2 hours;
- *Mucuna* seeds were soaked in water for 12 hours followed by scalding in the presence of KOH (0.5%) for 2 hours.

2.3. Extraction of Phenolic Compounds

Different species of the seed were finely ground in a mortar. Then, 5 g of powder of each species were weighed and put into 30 mL of acetone-water system (80: 20, v/v) and kept in the refrigerator at 4˚C for three days [8]. The extracts were

filtered and the filtrates kept in the refrigerator at 4˚C for subsequent tannin determination.

2.4. Determination of Tannins

The method adopted to determine the tannins of the different *Mucuna* species is the reference method used to quantify the tannins of sorghum [9].

Ferric ammonium citrate (III) and ammonia were added to part of the acetone phase and the absorbance of the resulting solution was measured spectrophotometrically at 525 nm.

The determination of the tannin content is done using a calibration curve obtained from gallic acid as reference.

2.5. Identification Tests of Tannins ($FeCl_3$)

2.5 g of powder of the plant material were added into 50 mL of boiling water and left to infuse for 15 min before filtering. A few drops of $FeCl_3$ are added to the infused, precipitation with green, brown or blue coloration shows:

- The presence of gallic tannins if the color is dark blue;
- The presence of catechic tannins if the color is green brown [10].

2.6. Determination of Total Tannins

After extraction of phenolic compounds by chloroform, then comes the processing of the total tannin determination.

1 mL of the acetone phase was measured with a pipette and introduced into a test tube N˚ 1. 5 mL of distilled water and 1 mL of ammonia were added and the whole was stirred for a few seconds with a vortex mixer.

A second sample of 1 mL of the acetone phase is taken in a test tube N˚ 2 by adding 5 mL of distilled water and 1 mL of the iron (III) ammonium citrate solution while stirring for a few seconds with a vortex mixer.

The two solutions from tubes N˚ 1 and N˚ 2 are transferred to the measuring vessels (1 cm) for measurement of absorbances with a spectrophotometer (UV-1700: UV-Visible spectrophotometer—SHIMADZU) at 525 nm against water, 10 min after operations 1 and 2. The result is based on differing absorbances.

The establishment of the calibration curve was done as follows:

- The gallic acid solution was pipetted into 6 test tubes 0, 1, 2, 3, 4, and 5 mL, respectively, and then each test tube was supplemented with acetone such that 10 mL of solution was obtained.
- 1 mL of each of these 6 solutions was pipetted into 6 other test tubes and successively supplemented with 5 mL of distilled water, 1 mL of the iron (III) ammonium citrate solution. The whole was shaken for a few seconds with a vortex mixer.
- The curve was plotted with the absorbance values on the ordinates and the corresponding concentrations on the abscissa.

The resulting solutions are transferred to the measuring cessels and the absorbances are measured 10 minutes later at 525 nm against water.

2.7. Statistical Analysis

The statistical study was carried out by Excel software at the 5% probability threshold. All experiments were performed in triplicate. The comparison of means was performed by analysis of variance (ANOVA). Results are expressed as mean ± standard deviation. Values of $p < 0.05$ are considered statistically significant [11].

3. Results

3.1. Qualitative Analysis

The results of the phytochemical screening on the tannins of the *Mucuna* different species seeds are shown in Table 1.

3.2. Quantitative Analysis

A calibration curve was plotted with gallic acid at different concentrations. The tannin content was determined from Equation (1):

$$y = 0.0234x + 0.0911 \quad (1)$$

With the correlation coefficient $R^2 = 0.9985$.

3.3. Dosage of Untreated Seeds

The results of tannin determination in different parts of untreated seeds of the four *Mucuna* species are shown in Table 2.

Table 1. Results of tannin identification tests four species of *Mucuna* seeds.

Material Plant	Results	Staining
Mucuna rajada	+	Green
Mucuna nagaland	++	Dark green
Mucuna deerigiana	++	Dark green
Mucuna cochinchinensis	+	Green

++: intense coloring and +: less intense coloring.

Table 2. Estimated values of tannins in the different parts of untreated *Mucuna* seeds.

Part of a seed	Concentration in g Gallic Acide Equivalent/100g DM			
Species of genus *Mucuna*	Deerigiana	Nagaland	Cochinchinensis	Rajada
Epicarp	0.0512 ± 0.0002^{c}	0.0142 ± 0.0002^{d}	0.0897 ± 0.0001^{a}	0.0586 ± 0.0002^{b}
Starch	0.286 ± 0.0027^{a}	0.150 ± 0.0067^{b}	0.159 ± 0.0031^{b}	0.157 ± 0.0013^{b}
Crushed whole	0.370 ± 0.0044^{a}	0.336 ± 0.0027^{a}	0.266 ± 0.0013^{b}	0.203 ± 0.0027^{c}

Superscripted values with the same letters in the lines were not significantly different ($p < 0.05$) according to Duncan's multiple comparison test.

3.4. Dosage of Treated Seeds

The tannin content values in the treated seeds of the different *Mucuna* species are recorded in Table 3.

3.5. Tannin Reduction Rate

The percentages of tannin reduction in the treated seeds of the four *Mucuna* varieties are recorded in Table 4.

4. Discussion

4.1. Qualitative Analysis

Intense staining was observed in the tubes containing extracts of *Mucuna deerigiana* and *Mucuna nagaland* seeds. This implies a strong presence of tannins in the seeds of these *Mucuna* species. The tubes containing the extracts of *Mucuna rajada* and *Mucuna cochinchinensis* seeds are less colored indicating a light presence of tannins in the seeds of these two species.

The action of iron (III) chloride on the aqueous extract showed that all four seed varieties contain tannins (Table 1). This corroborates to the work of Agbafor and Nwachukwu [12] and later Murthy *et al.* [13].

Table 3. Estimated values of tannins in *Mucuna* seeds after different treatments.

Material plant	Concentration in g Gallic Acid Equivalent /100g DM			
Treatment	A†	B†	C†	D†
Deerigiana	0.278 ± 0.001^{a}	0.270 ± 0.007^{a}	0.339 ± 0.003^{a}	0.195 ± 0.002^{a}
Nagaland	0.192 ± 0.001^{c}	0.183 ± 0.001^{b}	0.231 ± 0.001^{c}	0.174 ± 0.002^{b}
Cochinchinensis	0.217 ± 0.001^{b}	0.179 ± 0.001^{b}	0.262 ± 0.002^{b}	0.090 ± 0.004^{c}
Rajada	0.195 ± 0.002^{c}	0.191 ± 0.001^{b}	0.195 ± 0.001^{d}	0.171 ± 0.001^{b}

Superscripted values with the same letters in the columns were not significantly different ($p < 0.05$) according to Duncan's multiple comparison test. †A: seeds scalded directly for 2 hours; †B: seeds soaked for 12 hours in water followed by scalding for 2 hours; †C: seeds boiled in the presence of 0.5% KOH for 2 hours; †D: seeds soaked in water for 12 hours followed by scalding in the presence of KOH (0.5%) for 2 hours.

Table 4. Rate of tannin reduction in *Mucuna* seeds according to the treatments performed.

Material plant	Percentage of tannin reduction (%)			
Treatment	A†	B†	C†	D†
Deerigiana	24.86 ± 0.002	27.02± 0.002	8.37± 0.003	47.29± 0.002
Nagaland	42.85 ± 0.001	45.53 ± 0.007	31.25 ± 0.003	48.21 ± 0.002
Cochinchinensis	18.42 ± 0.002	32.27 ± 0.001	1.50 ± 0.002	66.16 ± 0.002
Rajada	3.94 ± 0.003	5.91 ± 0.001	3.94 ± 0.001	15.7 ± 0.003

4.2. Determination of Untreated Seeds

The test results of the different parts of the untreated seeds (Table 2) revealed, on the one hand, that the extract of the starch contains more tannins than the extract of the epicarp. On the other hand, for the crushed seed set, the results show that the *Mucuna deerigiana* seed extract is richer in tannins (0.370 g GAE/100g DM). It is followed by the seed extracts of *Mucuna nagaland*, *Mucuna cochinchinensis* and *Mucuna rajada* with the respective values of 0.336 g GAE/100g DM, 0.266 g GAE/100g DM, and 0.203 g GAE/100g DM thus confirming the results of the identification test. These values are similar to those found by Murthy *et al.* [13] which was 0.306 g GAE/100g DM for *Mucuna cochinchinensis* (white seeds) but lower than the value of 0.577 g GAE/100g DM found for *Mucuna cochinchinensis* from black seeds [13].

Tannins are much more concentrated in starches than in epicarps. If they were more concentrated in the epicarp, the epicarp would be removed and the starch left for feeding. Since the starch constitutes the largest part of the seed, hence the need for the treatment processing.

4.3. Determination of Treated Seeds

Determination results of extracts from treated seeds of the four *Mucuna* varieties showed an effective decrease in tannins (Table 3).

All seed treatment methods resulted in a decrease of tannin content. However, the method of soaking the seeds in water for 12 hours and boiling in the presence of KOH (0.5%) for 2 hours gave the best result.

4.4. Tannin Reduction Rates

The percentages of tannin content reduction in the treated seeds of different *Mucuna* species are recorded in Table 4. The results revealed that the method of direct boiling of seeds for 2 hours decreased the tannin content by 24.86% in *Mucuna deerigiana* seed extract, 42.85% in *Mucuna nagaland* seed extract, 18.42% in *Mucuna cochinchinensis* seed extract and 3.94% in *Mucuna rajada* seed extract. This decrease in tannins from the different seeds of four species of *Mucuna* can be explained by the increase in the temperature of the water which broke some tannin-protein bonds allowing the polar solvent to extract the tannins from these seeds.

In addition, the method of soaking the seeds for 12 hours in water followed by boiling for 2 hours reduced the tannin content in the extracts of *Mucuna deerigiana*, *Mucuna nagaland*, *Mucuna cochinchinensis* and *Mucuna rajada* by 27.02%, 45.53%, 32.27% and 5.91% respectively. It is noted that this method reduced more tannins in the seeds of the different *Mucuna* species than the previous one. The high rate of tannin reduction can be explained by the duration of the soaking of the seeds.

On the other hand, the method of treating seeds by boiling in the presence of KOH (0.5%) for 2 hours resulted in low tannin reduction rates. It resulted in

8.37% tannin reduction in *Mucuna deerigiana* seed extract, 31.25% in *Mucuna nagaland* seed extract, 1.50% in *Mucuna cochinchinensis* seed extract and 3.94% in *Mucuna rajada* seed extract. This low reduction rate can be explained by the formation of potassium salts with some tannins that are trapped.

Finally, the method of soaking seeds in water for 12 hours, then boiling in the presence of KOH (0.5%) for 2 hours gave for the extracts of the seeds of *Mucuna deerigiana*, *Mucuna nagaland*, *Mucuna cochinchinensis* and *Mucuna rajada*, the percentages of reduction of tannins respectively 47.29% , 48.21% , 66.16% and 15.76%. These results, compared to those obtained by the first treatment method which seems to be the best, indicate a clear increase in the rate of tannin reduction for the seeds of these different *Mucuna.* However, this method of soaking seeds in water for 12 hours and then boiling in the presence of KOH (0.5%) for 2 hours resulted in a high reduction of tannins in the seeds of all four species of *Mucuna.* This high reduction rate can be explained by the soaking time, which has already extracted some seeds that can form salts.

5. Conclusions

The seed extracts from the different *Mucuna* species were subjected to qualitative and quantitative analysis. Phytochemical screening revealed excessive presence of tannins in the seeds of *Mucuna deerigiana* and *Mucuna nagaland* and slight presence of tannins in the seeds of *Mucuna cochinchinensis* and *Mucuna rajada*.

The results of the tannin analysis showed that the crushed seeds as a whole contain more tannins. The tannin content in the seed extract of *Mucuna deerigiana* is high. It is followed by the seed extracts of *Mucuna nagaland*, *Mucuna cochinchinensis* and *Mucuna rajada* respectively. These results also show that tannins are much more concentrated in the seed starches of the four *Mucuna* species than the epicarps.

The different seed treatment methods were effective in reducing the tannin content in the seeds of the different *Mucuna* species. However, the method of soaking the seeds for 12 hours and then boiling them in the presence of 0.5% KOH for 2 hours was the most effective of the four methods.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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