Research Paper



Determination of Tannin Content in Banana (Musa spp) Midribs: a Comparative Study

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Abstract— Ecological concerns emanating from the use of inorganic tanning agents in leather processing have been on the rise, leading to increased research on alternative tanning agents. This study aimed at determining the tannin content in selected banana leaf midrib samples and their tanning strengths. Soxhlet method was used to extract the tannins from the banana leaf midrib samples. Different solvents were investigated in order to determine the best candidate for the extraction of tannins. Chemical tests were used to determine the nature of tanning strength. The extracted banana species. Hide powder method made it possible to quantify the tannins present and their tanning strength. The extracted samples were further analyzed using FTIR to establish the functional groups present. Highest yields were obtained using distilled water as solvent at 14.51 ± 0.17% and 7.14 ± 0.15% for sweet banana species (*Musa sapentium Linn.*) and 'Muraru' (AA genome) midribs respectively. *Musa sapentium Linn.*) and 'Muraru' (AA genome) midribs respectively from the hide powder method. Both species had a tanning strength greater than the recommended minimum value of 1.5. However, the data from the study showed that only sweet banana leaf midrib tannins can be commercially viable in leather tanning since they have a tannin content above the required minimum value (>10%).

Keywords- Banana, Banana leaf midribs, Mimosa, Musa sapentium Linn, 'Muraru', Tannins

1. Introduction

Armand Seguin introduced the term tannins to define plant extracts that are astringent, hydrophilic phenolic hydroxyl substances, and largely found in vascular compounds [1]. Organic tannins are polyphenols with molecular weight ranging between 500 - 30,000 Da and apart from being used in tanning, they also have a wide range of applications in the beverage, pharmaceutical and adhesive industries [2].

Tannins are broadly categorized into four groups: condensed tannins, hydrolysable tannins, complex tannins and phlorotannins [3-5]. The phlorotannins and the complex tannins are not considered important in leather tanning. The complex tannins are thought to be a blend of hydrolysable and condensed tannins based on their molecular weight while the phlorotannins are largely found in sea weeds [6]. Hydrolysable tannins and mixed groups. Gallo tannins when hydrolyzed by acid and bases give glucose and gallic acid whereas ellagitannins are esters of glucose and ellagic acid, an example being chebulic acid. However, the considered hydrolysable tannins are the gallotannin esters of gallic acid [7].

Hydrolysable tannins consist of a D-glucose at the centre of their molecules and their hydroxyl groups are partly esterified with fragments of gallic acid and ellagic acid for gallotannins and ellagitannins respectively [8]. They are found in plants or trees such as dividivi, chestnut, smoke tree, myrtle, valonia oak and alepo oak [6]. Condensed tannins on the other end are non-branched and their molecular weight is greater compared to that of hydrolysable tannins as they range in the 1000 - 20000 Da [9]. They are also referred to as proanthocyanins and they are made up of oligomeric and polymeric chains grounded by polyhydroxyflavan units. Their typical monomers are the stereo isomeric compounds (+)-catechin and (-)- epicatechin, all that vary in stereochemistry [10].

For years, leather tanning has been conventionally tanned using chromium salts. However, this mineral tannage process leads to increased levels of chrome effluent, which is environmentally harmful [11]. Other ecological concerns have also emanated from the use of chromium salts in tanning due to their toxicity and carcinogenic nature [2]. Studies have been carried out on different organic tannins from plants and trees to try and find alternative substitutes to fully or partially reduce the use of chrome tanning. Vegetable tannins have

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been used solely or combined with other mineral tannages to reduce the impact of chrome tanning in leather tanning [2].

Studies have been carried out on bananas with varied aims and objectives, targeting different parts of the plant. Value addition of banana fruits has been carried out, resulting in production of banana wine, juice, beer and banana chips. Crafted items have also been made from the stalks [12]. Parts of the plantain plant have been used to treat a range of ailments such as diarrhoea and inflammation [13]. Other studies have been conducted to characterize and quantify the phytochemical present in different banana species.

Banana leaf midribs have been shown to contain high dietary fibre and are also enriched with inorganic salts like potassium, calcium, phosphorus and magnesium. They also have various phytochemicals such as the phenolic compounds, antioxidants including polyphenols as well as tannins and oxalates among others [14]. Research done on Kepok banana (Musa paradisiaca L.) indicated that the tannin content in their leaves, trunks and peels fluctuate between 3.7 - 5.5%. As a result, they may be utilized as an organic tanning agent source [15]. Musa acuninata balbisianacolla leaf midrib has been estimated to contain tannins the range of 12 -16% [16]. This study is aimed at extracting tannins from the banana leaf midribs from different species and characterizing the extracted tannins using FTIR and selected chemical tests. This will help in evaluation of the possibility of using these banana tannins as alternative vegetable tanning agents.

2. Related Work

Research work related to the analysis of banana tannins and the evaluation of different organic tannins to tan leather have been carried out over the years. Binling *et al.* examined the tannin content of banana stems. The crushed material was boiled in a water bath to extract the tannins. Then, spectrophotometric analysis of the extracted tannins was performed [17].

On the other hand, research conducted by Onyango *et al.* focused on examining the differences between the Musa AA and AAB genomes by utilizing microsatellite markers to determine how useful they are in distinguishing between the two varieties. The results of the study revealed that the SSR markers were successful in distinguishing between the accessions [18]. Maryati and co-workers utilized kepok banana bunches to tan rabbit skins in comparison with Mimosa tannins. Results from the data showed that the tanned leathers from the banana bunches were of comparable quality with the control one [15]. Studies on the *Musa paradisiaca L.* midrib's therapeutic effects on gigantic gourami by Dinammella *et al.* revealed that the banana midribs contained more flavonoids than any other phytochemicals in their composition [19].

Similarly, different studies have been carried out on the use of vegetable tannins to tan leather. Musa *et al.* demonstrated the use of henna (*Lawsonia inermis*) leaves tannin extracts in combination with syntans in leather tanning. The results showed improved properties on the resultant leather [20]. In order to ascertain the tannin percentage in the various portions of the trees, Elgailani *et al.* analyzed three acacia trees in Sudan [1]. Extracted tannins were measured via protein precipitation. According to the study, the tree's barks contained more tannins than the other sections. The current study aims at evaluating the banana leaf midrib phytochemicals and assessing their potential as tanning agents.

3. Experimental Method

3.1 Materials

Mature banana leaf midribs of both 'Muraru' (AA genome) and *Musa sapientum Linn*. were collected from a farm in Gikondi, Mukurwe-ini Sub-County, Nyeri County, Kenya. The samples were shade-dried for two weeks and powdered using a star mill. Samples that passed through 300- and 90micron sieves were collected and stored in self-zipping plastic bags. The powdered materials were oven-dried at 100 °C to constant weight before use during the subsequent steps except for the moisture content determination step. Commercially available *Mimosa pudica* tanning agent was used as a control [21]. All the chemicals used in the extraction processes were of reagent grade.

3.2 Methods

3.2.1 Banana tannin extraction

Extraction of phytochemicals from the powdered material was carried out using the Soxhlet apparatus. Exactly 15 g of the ground samples were placed in thimbles and placed in the extraction chamber. The process was run for approximately 6 h using 250 mL of the solvent. The three solvents used in this process were ethanol, methanol and distilled water [22]. All experimental procedures were carried out in triplicates. Total yields of the extracts were calculated based on the weight of the dry ground material used.

3.2.2 Phytochemical analysis

For this purpose, 10 g of 'Muraru' (AA genome) materials were boiled in 100 mL of distilled water, solution filtered and cooled. A portion of the extract was added to a test tube and two drops of ferric chloride solution were added to investigate the presence of tannins. The change in colour was noted. Additionally, 10 g of the ground material were boiled in 100 mL ethanol, cooled, and filtered. A portion of the extract was used to screen for flavonoids using two drops of lead acetate solution. Aqueous KOH (0.1 M) was used to determine whether the tannins were of condensed or hydrolysable type in the ethanolic extract solution [23]. This process was repeated for *Musa sapentium Linn*. ground materials.

3.2.3 Quantitative analysis of tannins

The tannins and the non-tannins present in the 'Muraru' (AA genome) and *Musa sapientum L*. together with the control tanning agent were measured using the hide powder method. The hide powder was obtained from a hide material that was pretreated up to pickling stage then treated with acetone

solution, dried and ground [24]. One litre of tannin solution was prepared using 100 g banana leaf midrib powder and distilled water in the ratio of 1:10 (w/v) for use in the analysis. The solution was boiled for 1 h in a water bath and left to stand for 72 h before use [25].

3.2.4 Chromed hide powder preparation

In this experiment, 6.25 g of the dry chromed hide powder was blended with distilled water ten times its weight and agitated for 1 h. Chrome alum solution of the same weight as the dry matter was added, agitated for 2 h and left to sit overnight. The chromed powder was later filtered using a clean white cloth and the powder filled cloth placed in a beaker, opened out and then rinsed with distilled water 15 times the weight of the dry powder. The two were then blended together and mixed for about fifteen minutes then the cloth removed from the beaker. It was drained and squeezed to ensure only about 75% of moisture remains. The powder was then rinsed for another three runs with distilled and lastly the weight of the chromed hide powder measured [26].

3.2.5 Determination of moisture and total solids

For this purpose, 5 g of each of the powdered tanning materials were placed in a weighing bottle and their weights measured and recorded. The samples were then oven-dried at a 100 °C for 4 h and cooled in a desiccator for twenty minutes. After cooling the weight was measured and recorded. The analysis was done until two runs on a given sample did not exceed more than two grams in weight [27]. The percentage moisture content and total solids were determined using Equations (1) and (2) respectively.

% Moisture (by weight) =
$$\frac{W_1 - W_2}{W_1} \times 100$$
 (1)

% Total solids (by weight) =
$$\frac{W_2}{W_1} \times 100$$
 (2)

where W_1 = weight of wet sample, W_2 = weight of dry sample [24].

3.2.6 Determination of total soluble solids (TSS)

In order to determine the TSS in the extracts, unfiltered 200 mL tannin solutions prepared as outlined in Section 3.2.2 were mixed with 1 g of kaolin and filtered until a clear filtrate was collected. The residue weights were then calculated after 50 mL of the filtrates had been pipetted and placed into a crucible for drying and evaporation. The process was repeated until a constant dry weight was achieved [28]. Equation (3) was used to calculate the total soluble solids as a percentage.

TSS % (by weight) =
$$\frac{W_2}{W_1} \times \frac{V_1}{V_2} \times 100$$
 (3)

where W_2 = weight of the dry residue, W_1 = weight of the tannin material taken, V_1 = volume of the test solution in milligrams made and V_2 = volume in mL of the pipetted solution. The mean values were compared against mimosa by paired samples test.

3.2.7 Determination of Non-tannins (NT)

For each banana sample and control agent, the chromed hide powder previously prepared (Section 3.2.4) was placed in a 150 mL wide mouth flask and mixed with 100 mL unfiltered tannin infusion and 20 mL distilled water. The flask was securely sealed with a stopper and then manually shaken for 15 seconds. After that, it was placed on a rotary shaker and agitated at a speed of 50-65 revolutions per minute for 15 minutes. The solution was filtered on a linen cloth and the filtrate further filtered until the solution was clear. Exactly 20 mL of the filtrate was evaporated and dried at 100 °C. It was weighed until it reached a steady weight. The residual weight was multiplied by 1.2 to account for the wet hide powder 20 mL water dilution in the 100 mL of tannin solution [28]. The percentage by weight of non-tannins was calculated using Equation (4) below [24].

NT % by weight =
$$\frac{W_2}{W_1} \times \frac{V_1}{V_2} \times 100$$
 (4)

where W_1 is the weight in grams of the tanning material, W_2 is the weight in grams of the residue after drying, V_1 is the volume in millilitres made up originally and V_2 is the volume in millilitres of the test solution taken.

3.2.8 Determination of tannin content

The tanning content determined by calculating the difference between the TSS and the NT as described in literature [29].

3.2.9 Determination of pH

The pH of the solution made was measured using a pH meter (Hanna Model 2221). For this purpose, 25 mL buffer solutions with pH levels of 4.0 and 7.0 were used to calibrate the pH meter and the pH values set for the different buffers respectively [30]. After the two-point calibration the pH of the tannin solutions were then measured by inserting the electrode into the solutions until they measured a stable value [30].

3.2.10 Fourier Transform Infrared (FTIR) analysis of Banana tannins

A Jasco FT/IR- 4700 spectrometer was used to record the FT-IR spectra's for the extracted tannins samples in the range of 4000 cm⁻¹ to 600 cm⁻¹. Samples were prepared in line with the manufacturer's specifications and according to the procedure described in literature [26]. The obtained spectra were analyzed in comparison with the information available in literature to determine the nature of tannins present in the banana leaf midribs.

4. Results and Discussion

4.1 Extraction of Banana tannins

The results of the extraction of the tannins from different banana species over a period of 6 h using methanol, ethanol and distilled water as solvents are shown in Table 1.

Table 1. Percentage tannin yield for selected banana species

Solvent Media	Musa sapentium Linn.	'Muraru' (AA	
Solvent Media	(%)	genome) (%)	
Methanol	11.40 ± 0.20	6.61 ± 0.12	
Ethanol	10.20 ± 0.15	4.83 ± 0.25	
Distilled water	14.57 ± 0.17	7.14 ± 0.15	

The highest yield for the Musa sapentium L. tannins was obtained with distilled water at $14.51 \pm 0.17\%$ and lowest vield obtained with ethanol at $10.20 \pm 0.15\%$ whereas for the 'Muraru' (AA genome) tannins highest yield was obtained with distilled water at $7.14 \pm 0.15\%$ and lowest yield with ethanol at $4.83 \pm 0.25\%$. In both cases distilled water gave the highest yield since it is more polar compared to the other solvents and hence penetrated the extraction environment more, giving better results [31]. The observed variations in the extract yields from the different plant materials in this study can be attributed to the varying availability of extractable compounds, which is dependent on the chemical composition of the plants [32]. The success of the extraction process and the solubility of the plant's compounds in the chosen solvent will both have a substantial effect on the amount of antioxidants extracted from the plant material. However, a previous study by Nuriana and co-workers indicated that banana midrib tannins had a higher yield of 16.25% with methanol [16]. Another study indicated that banana midrib have 6.10% tannins [19]. The differences in tannins may be associated with the species and maturity stage of the plant species used and the ecological differences [33].

4.2 Phytochemical screening of tannin extracts

The tannins and flavonoids presence was performed as per the standard analysis method [34] and the results are presented in Table 2 below. All the extracts from the banana samples were found to contain tannins and flavonoids, an observation that was also made for the control. The tannins were found to be of condensed type given that the solutions developed a dark red colour on addition of few drops of KOH solution, in agreement to observations made earlier in literature [29]. None of the analysed samples contained hydrolysable tannins since no observable colour change was made.

 Table 2. Phytochemical screening of extracts

Phyte	ochemical	Test method	Colour	Musa sapenti um Linn	'Murar u' (AA genom e)	Mimo sa
Т	annins	FeCl ₃	Blue green	+	+	+
Fla	wanoids	$\begin{array}{c} Pb(C_2H_3\\O_2)_2 \end{array}$	Yellow precipit ate	+	+	+
Tann in type	Hydrolysa ble	KOH	No change	-	-	-
	Condense d	КОН	Dark red	+	+	+

Key: +present

-Absent

4.3 Quantitative tanning analysis

Comparison of the tannin content in the different plant extracts obtained using distilled water was determined and the

different parameters tested and results are as shown in Table 3.

 Table 3. Physical-chemical analysis of the banana midrib water extracts in comparison with Mimosa

Parameters	'Muraru' (AA Musa sapentium genome) Linn		Mimosa	
Powdered Moisture content (%)	8.26 ± 0.17	8.80 ± 0.33	7.16 ± 0.23	
Powdered Total solids (%)	91.74 ± 0.17	91.20 ± 0.33	92.85 ± 0.23	
Total Soluble Solids (TSS) %	10.29 ± 0.13	18.14 ± 0.46	87.61 ± 0.17	
Non-Tannins (NT) %	3.93 ± 0.10	6.43 ± 0.27	28.50 ± 0.16	
Tannins (T) % = (TSS - NT)	6.36 ± 0.19	11.71 ± 0.33	59.11 ± 0.16	
Tanning strength = (T/NT)	1.61	1.82	2.07	
Purity ratio = (T/TSS)	0.62	0.65	0.67	
pН	7.78	5.41	4.58	

There was a significant difference (p < 0.05) in moisture content with sweet banana tannins having a higher moisture content of $8.80 \pm 0.33\%$ while 'Muraru' and Mimosa had $8.26 \pm 0.17\%$ and $7.16 \pm 0.23\%$ respectively. Non-tannins aid in solubilizing the tans hence improving penetration in to the pelt matrix [35]. There was a significant difference (p < 0.05) in non-tannins between the control and 'Muraru', and between the control and *Musa sapentium* tannins. The control had the highest value of non-tannins at 28.50 \pm 0.16% whereas 'Muraru' and *Musa sapentium* had non-tannin content of $3.93 \pm 0.10\%$ and $6.43 \pm 0.27\%$ respectively.

Non-tannins, such as carbohydrates, acids, and salts influence the tanning process and the end quality of leather. They regulate the rate of tanning, as well as the way in which tannins are spread out in the leather [36]. There was a significant difference in the tannin amount present between Mimosa and 'Muraru' and Mimosa and *Musa sapentium* tannins. Mimosa had the highest yield at 59.11 \pm 0.16%, this was found to be within the recommended yield range for Mimosa extracts according to literature [37]. 'Muraru' and *Musa sapentium* extracts had tannins in the range of $6.36 \pm$ 0.19% and $11.71 \pm 0.33\%$ respectively. 'Muraru' tannins did not meet the standard threshold for commercial extraction of tannins required (> 10%) [24]. *Musa sapentium L*. were within the pH range required for vegetable tanning [35].

Previous studies carried out on determination of tannins by hide powder proved that this method was suitable for tannin quantification. One study found *Acacia nilotica* leaves to contain 11.80% tannins [1]. Studies by Seda and co-workers showed that Sodom apple fruit had 12.13% tannins by hide powder method [31]. In addition, different methods of sample treatment will yield different amounts of tannins. It can be seen from Table 1 that Soxhlet extraction (using water as a solvent) yielded 14.51 \pm 0.17% tannins for *Musa sapentium* whereas 'Muraru' (AA genome) yielded 7.14 \pm 0.15%. However, boiling the powders in water for 1 h and leaving them to stand for 72 h (Table 3) yields 18.14 \pm 0.46% and $10.29 \pm 0.13\%$ respectively. This implies that the banana leaf midrib powders continue to release more tannins over time. This is an important observation since during organic tannage, tanning agents continue interacting with the raw materials for periods of up to 2 weeks [35]. The continued release of tannins will improve the tanning action of the tanning agent (powder).

Although the samples TSS were highly varied for the samples analysed (Table 3) their purity ratios ranged from 0.62 - 0.67. This may be interpreted that most plants' extracts could easily achieve the minimum purity ratio (> 0.5) irrespective of the percentage TSS. It interesting to note that the purity ratios and tanning strengths of the banana and mimosa samples increased with the decrease in pH. This observation begs for further research in order to establish if a change in pH has an influence on TSS. That is, the pH at which extraction is carried out may influence the TSS. This data will also be of interest since tanning operations occur under different conditions other than the ones investigated herein. Vegetable tanning is usually carried out at pH range of 4 - 6, which may improve the tanning strength of different powders [38].

Mimosa, *Musa sapentium Linn* and 'Muraru' liquors had pH values of 4.58, 5.41 and 7.78 respectively. The pH level of the tanning liquid has a major impact on the quality of the leather produced. Liquors with high acidity and low salt content will produce a leather that is stiff and rigid, while those with low acidity and high salt content will create a leather that is soft and pliable [35]. On the other hand, the ratio of tan to non-tan in different samples varies, even when samples are taken from the same plant. This is due to factors such as the age, season, and locality of the tree from which it was harvested [39]. As can be seen from Table 3, the ratios of tan to non-tan for all the analysed samples were above the recommended value of 1.5 [28], implying that they all met the minimum set standard.

4.4 Spectroscopic analysis

Fourier transform infrared (FTIR) technique was used to study the functional groups present in the extracted phytochemicals from banana leaf midribs, in the range of 4000 cm⁻¹ to 600 cm⁻¹ and the results are as shown in Figure 1 and Figure 2 below. Bands in the regions above 3000 cm⁻¹ and below 1750 – 700 cm⁻¹ implied the presence of tannins in the material analyzed [31]. Polymerization process may have occurred in the banana extracts as suggested by the -OH band present in the tannins in the range of 3700 to 3000 cm⁻¹ [40]. Bands in the regions of 3269.72 cm⁻¹ and 3781.69 in 'Muraru' tannins, and 3701.68 cm⁻¹ and 3363.35 cm⁻¹ in *Musa sapentium* tannins respectively were associated with the -OH stretching vibrations of the hydrogen bonds. The C-H stretching of saturated (sp³) carbons of the flavanol groups were observed at 2922.58 cm⁻¹ and 2856.35 cm⁻¹ in *Musa sapentium* tannins.

Band at 1701.87 cm⁻¹ in *Musa sapentium* tannins was associated with the C=O stretching of the carboxylic acids of the hemicellulose [41]. Bands at 1584.24 cm⁻¹ and 1386.57 cm⁻¹ in 'Muraru' tannins and 1590.99 cm⁻¹ in *Musa*

sapentium tannins were associated with C=C stretching vibrations of the aromatic bonds [42]. Bands at 1378.95 cm⁻¹ and 1209.15 cm⁻¹ in *Musa sapentium* tannins were associated with the presence of lignin in the banana leaf midribs. The C-O stretches were present in both spectra at 1028.84 cm⁻¹ and 1041.37 cm⁻¹ respectively. The above information also confirmed that the extracted tannins were of condensed type.

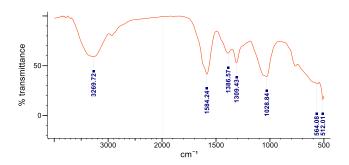


Figure 1. 'Muraru' (AA genome) FTIR spectrum

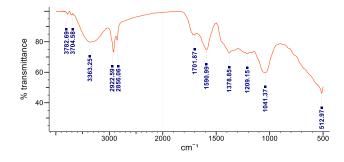


Figure 2. Musa sapentium Linn FTIR spectrum

5. Conclusion and Future Scope

Selected banana (Musa spp) leaf midribs were found to contain condensed tannins. From this study, it was observed that Musa sapentium Linn. leaf midrib tannins can be commercially utilized for tanning since they have a tannin value of $14.51 \pm 0.17\%$ higher than that required for commercialization (>10%). 'Muraru' (AA genome) tannins can also be utilized for tanning light vegetable tanned leathers as they had $6.36 \pm 0.19\%$ tannins which compared with previous research according to literature [43]. Observations in Table 1 and Table 3 showed that boiling for 1 h and leaving the solution to stand for 72 h led to higher total soluble solids than for 4 h in the Soxhlet this can be attributed to the increased interaction time. Extracts from the selected samples had a tanning strength ratio above the standard (> 1.5) and purity ratio (> 0.5). In all the parameters tested, there was a significant difference (p < 0.05) between the selected banana leaf midrib tannins with mimosa tannins. Although 'Muraru' (AA genome) did not meet the threshold, these tannins can be used for other purposes such as in the cosmetic industry and for medicinal purposes. Additional studies should be carried out to determine the variation in tannin content from these species at different maturity stages. Further studies should also be carried out to compare the tanning ability of the

banana leaf midribs evaluated herein and determine their suitability as substitutes to mimosa tanning agent.

Conflict of Interest

Authors declare that they do not have any conflict of interest.

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