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Tannin Analysis of Selected Plants from Laikipia County, Kenya

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Summary

Due to stringent environmental regulation concerning the disposal of tannery waste products there is continuous research on discovering eco-friendly tanning reagents. Vegetable tannins are less polluting to the environment than chromium. A study has been conducted to evaluate the tannin content and tanning strength of selected vegetable tanning materials found in Laikipia County and their suitability for tanning hides and skins. The hide powder method was used in determining the amounts of tannins. *Acacia nilotica, Acacia xanthophloea* and *Hagenia abyssinica* had percentage tannins of 16.8%, 23.8%, 11.73% respectively and all of them had a tanning strength of more than 1.5.

INTRODUCTION

Tannins are defined as water soluble polyphenolic compounds with molecular weights of 500-20000 Da and have the ability to form reversible and irreversible complexes with proteins, polysaccharides, alkaloids, nucleic acid and minerals.[1,2] Tannins play a major role in plants as a defense mechanism against pathogens, herbivores and hostile environmental conditions and their effectiveness depends on the type of tannin, their chemical structure and the molecular weight.[3]

Tannins are the fourth most abundant plant constituents after cellulose, hemicellulose and lignin and 90% of all tannins produced in the world are used in leather production.[4] There are various methods used in the detection and qualitative and quantitative analysis of tannins from plants extracts and in food and beverages.[5] Some of the methods used include, hide powder method, Folins Denis method, Reverse-phase High pressure liquid chromatography (HPLC) with UV detections, Mass spectrophotometry (MS), circular dichroism (CD), and nuclear magnetic resonance (NMR).[6] Relying upon these modern analytical techniques make it possible to detect the low molecular weight composition of tannins and their derivatives and provides comprehensive understanding of their structure and chemical properties.[7,8]

Tannins are grouped into condensed and hydrolysable tannins according to their structural features.[9] Hydrolysable tannins contain glucose esterified with Gallic acid or with hexahydroxy diphenic acid and have a molecular weight of 500-3000 Da.[3,10,11] Hydrolysable tannins have been further subdivided into two groups which are ellagitannins and gallotannins.[11,12] Gallotannins on hydrolysis yield gallic acid and glucose. Ellagitannins on hydrolysis give ellagic acid and glucose. The condensed tannins have a molecular weight of 1000-20000 Da.[3] Condensed tannins are non-branched polymers of flavonoid units. Typical monomers are catechin and epicatechin that differ in their stereochemistry in position 2 and 3.[10,13] Condensed tannins are also called proanthocyanidins because they release red anthocyanidin pigments when heated in acidic media.[6] Condensed tannins are not prone to hydrolysis but are liable to oxidation and polymerization to form insoluble products known as Tannins reds/phlobaphenes.[13]

Although hydrolysable and condensed tannins are structurally different, both tannins are capable of forming strong complexes with certain types of protein.[14] The higher affinity of tannins to proteins is associated with the number of phenolic groups as this is the binding point to carboxyl carbon at the peptide bond in protein.[15] Condensed tannins are more preferred in leather processing than hydrolysable tannins but a mixture of the two produce good leathers. The higher the number of phenolic groups a tannin carries the higher is its binding affinity to proteins.[16] The high molecular weight and flexibility in the structure of both condensed and hydrolysable tannins makes them bind easily to proteins with an open flexible structure rich in proline, glutamate and glycine such as collagen.[3,17] During tanning, tannins improve the hydrothermal stability and mechanical properties of collagen. Tannin/collagen interactions are most frequently based on covalent, ionic hydrogen and hydrophobic bonds.[18] The conditions under which tannins and collagen combine has been shown to cause considerable variations in strength of that interaction.

Tanning properties of vegetable tanning agents vary, during the extraction of a tannin, soluble non-tannins

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are also extracted, although these have no tanning power their presence in the tanning liquor helps to control the rate of the tanning and imparts many qualities to the finished leathers. The distribution of the tannins throughout the thickness of the leather and also the rate of the tanning processes are largely controlled by the non-tannin content of the tan liquors.[19] For an extract to be considered suitable for tanning, its tanning strength (ratio of tannins to soluble non-tannins) must be higher than 1.5. A non-tannin content higher than the tannin content means that the tannin is considered unsuitable for tanning.

MATERIALS AND METHODS

Collection and preparation of samples

5kg of dry bark from each of *Acacia nilotica, Acacia xanthophloea* and *Hagenia abyssinica* were obtained from tanners in Laikipia County. The samples were further air dried and reduced to powder with a star mill. The samples passing through a 1.4mm sieve and retained in a 600 micron sieve were collected, thoroughly mixed and kept in air-tight containers.

Extraction

2L of extract were obtained with a Procter extractor. A sufficient quantity of dry bark was used to give about eight grams of tannin per 2L. A few drops of 10% ferric chloride solution added to a 25% solution of the extract was used to confirm the presence of tannins. The conditions of extraction were those specified by The Indian Bureau of Standards, Method of Test for Vegetable Tanning Materials CHD 17 (1627).

Quantitative analysis of tannins

The extracts were analysed for total and soluble solids, non-tannins and tannins by the official hide powder method. The tanning strength (ratio of tannins /soluble non-tannins), purity ratio (ratio of tannins/total solubles) and pH were also determined. The hide powder was made from an ox-hide by soaking, washing in salt solution and also in plain water. The washed hide was limed, bated, degreased and dehydrated with acetone and finally ground by machine.[13]

Chromed hide powder preparation

Each analysis used a multiple of hide powder containing 6.25g of dry matter which, with ten times its weight of distilled water was stirred for one hour. 1ml of chrome alum solution (3%) for each gram of air-dry hide powder was added, stirred frequently for several hours and then allowed to stand overnight. Next morning the chromed powder was transferred to clean linen, drained and squeezed. The cloth containing the powder was placed in a beaker, and the cloth opened out to pour on to the powder a quantity of water equal to 15 times the weight of the air-dry hide powder. The powder and water was mixed thoroughly and digested for 15 minutes, after which the cloth and powder was lifted out and immediately drained and squeezed, so that the powder contains approximately 75 percent moisture. The powder was digested three more times in the same way with distilled water. The cake of chromed powder was thoroughly broken up and mixed until uniformly free from lumps and its weight taken.

Determination of moisture and total solids

5g of finely ground tanning material was transferred into a tared wide-mouth weighing bottle and weighed accurately. It was dried at about 98.5-100°C in an oven for 3 to 4 hours, cooled in a dessicator for about 20 minutes and weighed again accurately. The process of drying and weighing was repeated until two weights at an interval of one hour did not differ by more than 2mg.

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

$$\text{Total solid content (by weight)} = \frac{W_2 \times 100}{W_1} \ \%$$

Where:
W_1= Weight in grams of the material taken for test,
W_2= Weight in grams of the residue left after drying.

Determination of total soluble solids

1g of kaolin was added at the base of a filter paper (Whatman No.11) and the filtrate collected in a beaker as soon as it became optically clear. The paper was kept full and the funnel and the collecting vessels were also covered at the time of filtration. 50ml of the filtrate at 27 ± 2°C was pipetted in a porcelain basin for evaporation. It was dried and weighed until a constant weight was obtained.

$$\text{Total soluble solids (by weight)} = \frac{W_2}{W_1} \times \frac{V_1}{V_2} \times 100$$

Where:
W_2 = weight in grams of the residue left after drying,
W_1 = weight in grams of the tanning material taken,
V_1 = volume of the test solution in ml made up originally,
V_2 = volume in ml of the test solution taken or pipetted out.

Determination of non-tannins

The dry hide powder (6.25 grams) was weighed and put in a bottle of 150ml capacity containing 100ml of the unfiltered prepared tannin infusion. Twenty ml of distilled water was then added. The bottle was closed tightly with a stopper and shaken vigorously first by hand for 15 seconds and then transferred to a mechanical rotary shaker and shaken for exactly 10 minutes at 50 to 65rev/min. The powdered solution was poured on to a clean dry linen filter cloth supported by a funnel, drained and squeezed by hand. One gram of kaolin was added to the filtrate and poured into a single 15cm pleated filter paper until it was clear. 50ml of the filtrate was evaporated in a tared porcelain dish and dried at 100 ± 2°C. It was cooled and weighed until a constant weight was observed. To correct for the 20ml of water of dilution introduced by the wet hide powder into 100ml of tannin solution, the residual weight was multiplied by 1.2.

$$\text{Non-tannins, (by weight)} = \frac{W_2}{W_1} x \frac{V_1}{V_2} x100$$

Where:
W_2 = weight in gram of the residue left after dying,
W_1 = weight in gram of the tanning materials taken,
V_1 = volume in ml made up originally,
V_2 = volume in ml of the test solution taken.

Determination of tannin content

The tannin content was determined from the difference between the total soluble solids and the soluble non-tannins.

Determination of pH

The pH of the solution prepared was determined by adjusting the relative density to 1.05g/ml at 27°C with cold water, by using a suitable pH meter.

RESULTS

The results for the total solubility, soluble non-tannins, tannins, tanning strength, purity ratio and pH are recorded in Table I.

The results indicate the variations between the four vegetable tanning materials are shown in Figure 1.

DISCUSSION

After evaluating the amounts of tannins in the four vegetable tanning materials, it was found that commercial mimosa had the highest level of tannins compared with the barks of *Acacia nilotica, Acacia xanthophloea* and *Hagenia abyssinica.* They had tannin contents of: Mimosa (63%), *Acacia nilotica* (16.8%), *Acacia xanthophloea* (23.8%) and *Hagenia abyssinica* (11.73%). This data indicates that the barks of the trees species have more than the 10 % tannins required for commercial extractions.[11] All four tanning materials contain condensed tannins while *Acacia nilotica* had both condensed and hydrolysable tannins. This confirmed the results by Sikanikore who stated that condensed and hydrolysable tannins can occur in the same plant.[18] Condensed tannins are preferred during tanning process compared to hydrolysable tannins as they have a higher affinity for collagen due to their high molecular weights and a greater number of phenolic groups providing many points at which bonding may occur with carbonyl group of peptide.[1] When the tanning strength (ratio of tannins to soluble non-tannins) was calculated, the results showed that all the extracts from the three species had an acceptable ratio of >1.5 with *Acacia xanthophloea*

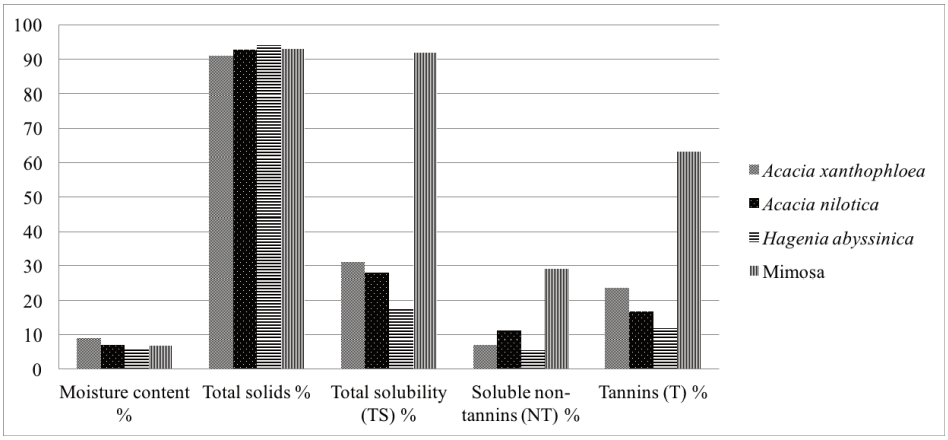

Figure 1. Characteristics of the tested vegetable tanning materials.

TABLE I
Results for three trial tannins and mimosa

Tree species	*Acacia xanthophloea*	*Acacia nilotica*	*Hagenia abyssinica*	Mimosa
Moisture content %	8.9	7.1	5.9	6.9
Total solids %	91.1	92.9	94.1	93.1
Total solubility (TS) %	31	28	17.33	92
Soluble non-tannins (NT) %	7.2	11.2	5.6	29
Tannins (T) %	23.8	16.8	11.73	63
Tanning strength (T/NT) %	3.3	1.5	2.1	2.2
Purity ratio (T/TS)	0.76	0.60	0.67	0.68
Types of tannins	C	C/H	C	C
pH	4.5	5.62	4.96	4.59

having the highest. The purity ratio (ratio of tannins/total soluble) was good for all the species. It was above the minimum recommend value of >0.5 11. The pH of their tanning liquors differed with *Acacia nilotica* having the highest pH of 5.62. *Acacia xanthophloea, mimosa* and *Hagenia abyssinica* had pHs of 4.5, 4.59 and 4.96 respectively. This pH of the tanning liquors was within the recommended value of 4-6. At pH values below 4.0 plumping of the pelt can take place if the salt contents in the liquor are very low. Plumping reduces the rate of diffusion of the tanning liquors as a result of decreasing the spaces between the fibres. Secondly, at lower pH the tendency of the tannins to combine with the collagen on the surface of the pelt increases and this leads to cracky leather with poor tannage. At pH4-6, the leather is at its isoelectric point and the tannins can penetrate well into the pelt and fixation takes place at a later stages.

Acacia nilotica is known to have a tannin content of 12-20% tannins.[20] A study conducted in Kenya indicated that *Acacia nilotica* had a tannin content of 13%[20] while another in North Sudan showed that *Acacia nilotica* had 16% tannins, 9.1% soluble non-tannins, extraction ratio of 1.8 and purity ratio of 0.6 11. Other studies in South Africa and in Kenya found *Acacia xanthophloea* to have a tannin content of 17%.[20,21] This data differed only slightly with the results obtained in this work. This may be due to the variations in the environmental factors. high temperature, water stress, extremes of light intensity and poor soil quality are known to increase the tannin contents in plants.[3] The tannins contents also vary with the bark thickness, age of the trees and from the base of the trunk upwards with the branches having low tannin contents.[22] The tannin content of *Acacia xanthophloea* and *Hagenia abyssinica* has not been intensively studied and there was not enough data to compare with this result. Although the amount of tannin is very important in selecting the type of vegetable tanning materials to use, the thickness of the bark is also very important. *Acacia xanthophloea* was preferred in the tanning process since it had a higher tanning strength and tannin content compared to *Hagenia abyssinica* and *Acacia nilotica*. Although the barks of *Acacia nilotica* had a higher tannin content compared to *Hagenia abyssinica* it had a thin bark and therefore a huge amount of the bark will be needed to extract enough tannins for economic feasibility.

CONCLUSION

The selected vegetable tanning materials from Laikipia County had more than the minimum 10% tannins required for commercial extractions. The tanning strength and purity ratio were also above the recommended values of >1.5, >0.5 respectively. It was therefore concluded that the selected vegetable tanning materials from Laikipia County have adequate tannin content for use in a tanning process.

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