Tannins of the Testa of Anacardium Occidentale (Cashew) and Husk of Arachis Hypogaea (Groundnut): Characterization and Potential Applications#

by

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Abstract

Aqueous and acid-hydrolysed ethyl acetate extracts of the kernel husks (testa) of cashew (Anacardium occidentale) and groundnut (Arachis hypogaea) were analyzed for tannin content using qualitative and quantitative tests, paper chromatographic separation and UV spectroscopy. Qualitative tests indicate that the tannins of cashew nut kernel husks are mainly condensed type while those of groundnut husks are mixed tannins. UV spectra of the tannins implied that ellagitannins was present in the groundnut husks whereas the tannins of cashew nut husks contain cyanidin, quercetin, and delphinidin, among others. Chromatographic separation of the hydrolysed tannins also detected other components. Quantitative determination showed about 19.9 - 22.1 percent tannins in the husks of cashew nut and about 5.7 - 7.5 percent tannins in the husks of groundnut. Potential applications of the extracts as tanning and re-tanning agents in the production of shoe upper leathers have also been investigated.

Resumen

Extractos acuosos, y los [producidos por extracción] con acetato de etilo, obtenidos por hidrólisis ácida tanto de las cáscaras (envolturas) de las semillas del anacardo [cajú en Portugués] (*Anacardium occidentale)*, como también los de la nuez de tierra [cacahual o maní] (*Arachis hipogaea*) fueron analizados para sus contenidos de tanino por medios cuantitativos y cualitativos, utilizando separación por cromatografía de papel y por espectrometría UV. Pruebas cualitativas indicaron que los taninos de la cáscara de la nuez del anacardo son principalmente de los de tipo condensado mientras que los taninos en la cáscara del cacahual son mixtos. Los espectros por UV demostraron que taninos elágicos se encuentran en la cáscara del cacahual mientras que los taninos de la cáscara del cajú tienen cianidina, quercetina y delfinidina, entre otros. En la separación cromatográfica de los taninos hidrolizados también se detectaron otros componentes. Determinaciones cuantitativas demostraron que existe aproximadamente alrededor de 19,9-22,1 por ciento de tanino en la cáscara de la nuez del cajú y más o menos 5,7-7,5 por ciento de taninos en la cáscara del cacahual. También se investigó posibles aplicaciones de los extractos como agentes de curtido y recurtido en la producción de cueros para capelladas.

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INTRODUCTION

Vegetable tannins are polyphenolic biopolymers and are secondary metabolites widely distributed in the various sectors of the higher plant kingdom.[1,2] Tannins traditionally are used in the production of leather.[3] However; other industries are finding tannins useful. In the oil industry vegetable tannins are used as additives in drilling fluids. The food industry uses tannins to add flavor, taste, color and nutritional value to foods.[4-5] Adhesives based on reaction of tannins and formaldehyde are also being formulated. In addition, polyphenols have formed major constituents of a lot of pharmaceuticals of natural herbal medicines.[7] Tannins as antioxidants are very useful in physiological processes[8]. The antioxidative effect is due to their redox properties, which emanate from their free radical scavenging activity, transition metal chelating activity, and singlet-oxygen-quenching capacity.[9-11]

Groundnut and cashew nut are abundant in Nigeria. The cultivation is spread over all the geopolitical zones of the Country. During the processing of nuts, the kernel husks are treated as chaff and thrown away. Apart from the recent reports[12] showing that cashew nut husks contains about 19.59 percent tannins and could be used as a re-tanning agent, nothing much has been reported on the utilization of groundnut husks or even the cashew nut husks. The present effort is an attempt at the characterization of the tannins in the husks of cashew nut and groundnut. This is with a view to finding their possible utilization in the leather, pharmaceutical and food industry.

MATERIALS AND METHODS

Equipment: Electronic spectra of tannins were obtained on UNICO-UV2102 Spectrophotometer inter-phased with a computer and printer. Also absorbencies of solutions were read on Bausch and Lomb Spectronic 20. Tanning trials using the tannins and leather analysis were done at the Pilot Tannery of Federal College of Chemical and Leather Technology, Zaria, Nigeria. All reagents were of analytical grade and were used as supplied unless otherwise stated.

Plant Materials: Cashew fruits were purchased from the plantation farmers at Okigwe, Imo State of Nigeria and from Samaru in Zaria, Kaduna State Nigeria. The choice of the zones for collection was to ensure geographical spread as Zaria is in the Northern Savannah whereas Okigwe is in the Southern rain forest Zone of the country. Also, groundnut was collected from the two areas and identical (small nuts) species used. All samples were identified at the Botany Department of the University of Nigeria. Cashew nut husks were removed from the kernels after heating to remove the tough outer shell. The husk was air-dried before extraction. The groundnuts were either air-dried or partially roasted to avoid denaturation of the tannins to ease removal of the husks. The husks were also air-dried before extraction.

Extraction and Isolation: The dried husks were ground into powder and extracted with 70% aqueous acetone. Acetone (70 percent) has been reported[13] to be a more effective extractant than alcoholic solvents. Acetone is thought to inhibit tannin-protein interaction. The acetone extracts were reddish brown in color. Semidry tannins were obtained after removal of the solvent using a Rotary Evaporator. Also extraction of the tannins with water was carried out for purposes of tanning.

Paper Chromatographic Separation: Experience has shown that application of aqueous acetone extracts in paper chromatograph yields very few fractions.[14] Therefore the tannins were hydrolyzed with dilute HCl at 40°C, cooled, filtered and further extracted with ethyl acetate following the method of Adewoye and Ajayi.[15] The resulting extracts were assayed by one dimensional, two-dimensional and ascending two-dimensional paper chromatography using n-butanol/acetic acid/water (4:1:5), glacial acetic acid/conc.HCl/water (30:3:10) and 6 percent aqueous acetic acid as mobile phases. The presence of the separated components on the paper (cellulose) chromatograms was identified by their characteristic colors in daylight or under UV light. They were also modified by spraying with ammonia vapor or other chromogenic reagents; one percent ferricyanide, ferric chloride, ethanol solution of toluene-p-sulphonic acid or Vanillin-HCl reagent.

Qualitative Tests: Aqueous extracts of the tannins were tested for their class of tannins using $FeCl_3$, bromine water, limewater, concentrated HCl, formalin and $NaNO_2$, as reported elsewhere.[16]

Quantitative Assay: The Folin-Dennis method as modified by the Folin-Ciocalteau method[17] was employed for the determination of total soluble phenolics. Phosphomolybdic acid oxidation of the phenols was monitored using a spectrophotometer. Standard curve based on absorbances of various concentrations of phosphomolybdic acid solutions was used to extrapolate concentration of tannins. As much as possible, interfering materials like ascorbic acid were eliminated using parallel methods to determine their concentrations and subtracting it from that determined by the Folin-Ciocalteau method. Also comparative determination following two other methods, viz: reaction of the methanol solution of tannins with $FeCl_3$ and determining the concentration by recording their absorbance at 570nm. In this case,[18] known concentrations of tannic acid were reacted with $FeCl_3$ in methanol and used to prepare the standard curve. Also the traditional Shakes method[12] using hide powder was employed in determining the percentage tannins in the plant materials.

Tanning Trials: Laboratory scale tanning trials were conducted using between 20-24 percent pure vegetable tannins on wet salted, pickled goatskins. The trials were conducted

according to a method that involved skin soaking, unhairing, pit liming and reliming, deliming, pickling, tanning, and fatliquoring.[19] Also chrome-tanned leathers were re-tanned using 12-18 percent tannins to produce shoe upper leathers. Mimosa extract was used as control. Leather analysis was carried out according to the Society of Leather Technologists and Chemists official methods.[20]

RESULTS AND DISCUSSION

Qualitative Analysis: Results of the qualitative tests carried out on the tannins places cashew nut husks tannins within the class of condensed tannins (catechols) whereas tannins of the groundnut husks are most probably a mixture of pyragallols and catechols. Earlier reports[12] had indicated the presence of condensed tannins in cashew nut husks. No reports on the tannins of groundnut husks exist so far to the knowledge of the authors.

Quantitative Assay: Results of the quantitative determination of tannin content based on the three methods: Shake's, Folin-Ciocalteau and Tannic acid method are presented in Table 1. The values in parenthesis are percent tannins in samples obtained from Zaria, in the Northern Part of the country. The analysis of variance performed for the result in Table 1 for the Zaria samples is given in Table 2. All results are the mean of replicate measurements. From the Table, the mean difference

TABLE 1

Concentration (%) of Tannins in Cashew Nut Testa and in Groundnut Husks.

	Methods		
Samples	**Shakes**	**Folin-Ciocalteau**	**Tannic acid**
Cashew nut testa	19.87 ±1.1 (19.80)	21.01±0.5 (22.0)	22.10± 1.0 (22.50)
Groundnut husks	5.65± 2.0 (5.80)	6.10± 0.8 (6.15)	7.52± 2.1 (7.60)

( ) percent (%) tannins in samples obtained from Zaria

TABLE 2

Analysis of Variance for the Percentage of Tannins Extracted using Various Methods

Dependent Variable	(I) Parameters	(J) Parameters	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Cashew testa	1.00	2.00	-1.1400	.73998	.174	-2.9507	0.6707
		3.00	-2.2300*	.73998	.024	-4.0407	-.4193
	2.00	1.00	1.1400	.73998	.174	-.6707	2.9507
		3.00	-1.0900	.73998	.191	-2.9007	0.7207
	3.00	1.00	2.2300*	.73998	.024	.4193	4.0407
		2.00	1.0900	.73998	.191	-.7207	2.9007
Groundnut	1.00	2.00	-0.4533	1.42042	.760	-3.9290	3.0223
		3.00	-1.8700	1.42042	.236	-5.3456	1.6056
	2.00	1.00	0.4533	1.42042	.760	-3.0223	3.9290
		3.00	-1.4167	1.42042	.357	-4.8923	2.0590
	3.00	1.00	1.8700	1.42042	.236	-1.6056	5.3456
		2.00	1.4167	1.42042	.357	-2.0590	4.8923

*The mean difference is significant at the 0.05 levels.

is only significant for Shakes and Tannic acid methods at the 0.05 level for tannins extracted from cashew husks. The slight deviations are likely due to method variation. The mean difference is not significant for Folin-Ciocalteau method for tannins extracted from cashew husks and for all the three methods for tannins extracted from groundnut husks. It is also our considered opinion that in the determination, there were no significant differences between the concentrations of tannins in the samples obtained from Zaria in the Northern part of Nigeria and that from Okigwe in the Southern part of the country. The result indicates that the concentration of tannins in cashew nut husks is likely between 20 and 22%, and in groundnut husks about 6.5%. Earlier reports[12, 21] based on the Shake's method gave the amount of tannins in cashew nut kernel husks to be between 19.59 and 25 percent.

Paper Chromatographic Separation: Separation and formation of discrete spots was achieved by first hydrolyzing the tannins with HCl then extracting with ethyl acetate. One dimensional, two-dimensional and two dimensional ascending paper chromatography were then employed in the separation of individual polyphenols. One dimensional paper chromatography of the cashew nut husks on Whatman number 1 paper using Forestall (acetic acid, HCl and H_2O; 30:3:10) as the mobile phase yielded four spots. These spots showed up as pink, yellow, light brown and brown colors under UV light. However, 2D paper chromatogram of the same tannins gave seven spots when viewed with UV light in the presence of ammonia vapor. A summary of the retardation factor (R_f) values is given in Table 3. The same results were obtained using 6% aqueous acetic acid as the mobile phase. In n-butanol-acetic acid-water (4:1:5), seven spots were visible under UV light in the presence of ammonia vapor for both 2D and ascending 2D system. Using 6 percent acetic acid only, five spots were visible. The spots had yellow brown, fluorescent yellow, pink and purple-blue colors. From the R_f values in Table 3, quercetin has been implicated as the spot with a value of 0.02 in 6 percent acetic acid, 0.71 in n-butanol /acetic acid/ water and 0.50 in Forrestal. The assertion is in accord with what has been reported elsewhere. [14-16]

Also catechin has been implicated as a major constituent of the tannins with R_f values 0.53 in solvent A, 0.62 (solvent B) and 071 (solvent C). Delphinidin and cyanidin are also likely components of the tannins with R_f values of 0.37 and 0.54 in Forestal and 0.35 and 0.52 in solvent B, as shown in Table 3. Most of the spots gave blue-black coloration on spraying with freshly prepared $FeCl_3 – K_3[Fe(CN)_6]$ solution (1:1).

Phenolic components of the husks of groundnut were subjected to the same treatments as those of cashew nut husks and the R_f values presented in Table 4. In comparison with earlier reports on tannins of Eucalyptus citriodora[16] and parkia

TABLE 3

R_f Values of Phenolic Components of Ethyl Acetate Extracts of Acid-hydrolyzed Anacardium Occidentale Testa.

	A	B	C	Colour		
Components	6% Acetic Acid	n-butanol/ acetic acid/ H_2O(4:1:5)	ACH/conc.HCl /H_2O (30:3:10)	K_3-[Fe(CN)$_6$]-$FeCl_3$ (1:1)	UV	UV+NH_3 vap.
Quercetin	0.02	0.71	0.50	Blue	Bright-yellow	Brilliant yellow
Catechin	0.53	0.62	0.71	Blue	Yellow	Purplish-yellow
Myricetin	0.03	0.56	0.35	Blue	Bright-yellow	Green yellow
Azaleatin	0.28	0.45	0.55	Blue	Fluorescent yellow	Fluorescent yellow
Epicatechin	0.44	0.58	0.65	Blue	Light-brown	Yellow brown
Cyanidin	___	0.52	0.54	Blue	*Pink	Red
Delphinidin	___	0.35	0.37	Blue	*Pink	Pinkish red

*Sprayed with vanilin – HCl reagent.

TABLE 4

R_f Values of Phenolic Components of Ethyl Acetate Extracts of Acid-hydrolyzed Husks of Arachis hypogaea*

Components	6% acetic acid	n-butanol ACH/H_2O (4:1:5)	ACH/ conc. HCl /water (30:3:10)	Day light	UV	UV+NH_3 vapor	$FeCl_3$-K_3 $[Fe(CN)_6]$
Myricetin	0.07	0.45	0.36	Light yellow	Bright yellow	Bright yellow	Blue
Fisetin	0.03	0.75	0.68	Yellow	Golden yellow	Golden yellow	Blue
Tricin	0.40	0.76	0.77	Brown	Brownish yellow	Pink	Blue
UI*	0.55	0.27	0.03	Yellow	Brown	Red brown	Blue

*Unidentified

TABLE 5

Absorption Wavelength and Absorbance in UV spectra of the Tannins @ 12mg/l

	λ_{max1}/nm	$Abs.max_1$	λ_{max2}/nm	$Abs.max_2$
A. occidentale	202	0.60	280	0.464
A. hypogaea	218	0.55	260	1.01
Tannic acid	205	0.79	281	0.50

clappertoniana,[15] the spots have been assigned to likely presence of myricetin, fisetin and tricin. In 6 percent acetic acid the spots showed as light brown, yellow and pink under NH_3 vapor and UV light. 2D Paper chromatography of ethyl acetate extract of the acid hydrolyzed tannins in Forrestal also gave four spots of R_f values 0.03, 0.36, 0.68 and 0.77 with colors, brown, bright yellow, golden yellow and pink respectively in NH_3 vapor and UV. Spraying the spots with fresh one percent ferric-potassium ferriyanide mixture revealed the spots by the characteristic blue implicating them as polyphenols.

The spot with R_f values 0.07 (6 percent acetic acid), 0.45 (n-butanol-acetic acid-water) and 0.36 (Forrestal) has been assigned to the presence of myricetin. This is corroborated by what has been reported[15] for the tannins of parkia clappertoniana. Following the same argument and in line with R_f values reported for Anogeissus schimperi[14] and exudates of Eucalyptus citriodora,[16] tannin spots with R_f values 0.03 (6 percent acetic acid), 0.75 (butanol-acetic acid-water) and 0.68 (Forrestal) are probably due to the presence of fisetin which is a flavonol glycone. Also the spot with R_f values 0.40 (6 percent acetic acid), 0.76 (n-butanol/ acetic acid/ water) and 0.77 (Forrestal) is an indication of the presence of tricin, which is a flavone. The spots with R_f values 0.55 (6percent acetic acid), 0.27 (butanol, acetic acid, water) and 0.03 (Forrestal) and having significantly yellow color in daylight and UV in the presence of NH_3 fumes could not be accounted for based on literature.

Electronic Spectra of Tannins: The ultraviolet absorption bands of cashew and groundnut husk extracts were recorded in ethanol and are presented in Table 5. These spectra were also compared with the UV spectra of authentic sample of tannic acid (Chinese gallotannin) (Table 5). Cashew extract has peaks at 202.0 and 280.0 nm, respectively with absorbencies of 0.60, and 0.464, respectively, while groundnut husk extract absorbs at 218 and 260nm.

TABLE 6

Chemical Properties of Resultant Leather*

Properties/Leather Samples	(A) (20% Cas)	(B) (20% Mim)	(C) (24% Cas)	(D) (24% Mim)	(E) (12% Cas)	(F) (12% Mim)	(G) (18% Cas)	(H) (18% Mim)
Volatile Matter (%)	10.80±0.90	13.75 ±1.22	11.10 ±1.00	13.86 ±1.5	10.56 ±2.0	13.35 ±2.0	10.52 ±1.50	13.21 ±1.2
Water Soluble (%)	5.4 ±0.2	5.1 ±0.3	5.3 ±0.2	5.2 ±0.30	4.9 ±0.5	4.8 ±0.6	4.9 ±0.8	5.0 ±0.4
Fatty Substance (%)	6.58 ±0.52	6.15 ±0.25	6.02 ±0.21	6.25 ±0.31	8.90 ±0.42	7.68 ±0.42	6.70 ±0.54	6.92 ±0.61
Nitrogen (%)	8.90 ±1.20	9.15 ±0.85	8.76 ±0.52	8.87 ±0.61	9.30 ±1.0	8.92 ±0.25	8.40 ±0.21	8.10 ±0.22
Hide Substance (%)	49.86 ±1.20	50.65 ±1.30	49.10 ±1.32	49.85 ±2.20	51.20 ±1.50	50.53 ±1.50	46.10 ±2.10	45.00 ±1.20
Insoluble Ash (%)	0.98 ±0.02	0.81 ±0.05	0.92 ±0.02	0.75 ±0.22	1.80 ±0,05	1.82 ±0.08	1.97 ±0.06	1.75 ±0.07
pH	4.9 ±0.5	5.2 ±0.5	4.9 ±0.3	4.4 ±0.5	4.1 ±0.4	4.2 ±0.2	4.4 ±0.4	4.3 ±0.2
Fixed Tannins (%)	26.61 ±1.12	23.81 ±0.85	27.80 ±0.92	25.00 ±1.20	22.90 ±0.80	22.35 ±0.92	31.00 ±1.15	29.25 ±1.05
Degree of Tannage	53.35 ±1.62	47.00 ±1.25	56.80 ±2.20	51.00 ±1.50	44.80 ±2.22	44.10 ±2.25	67.90 ±1.50	65.10 ±1.55
Cr_2O_3	-	-	-	-	2.20 ±0.200	2.18 ±0.20	2.40 ±0.30	2.32 ±0.15

* i. Tables A-D are full vegetable tanned leather, and E-H chrome retanned leathers
ii. Cas = Cashew and Mim = Mimosa

TABLE 7

Physical Characteristics of Resultant Leather

Leather Sample	Shrinkage Temperature (°C)	Tensile Strength (kgf/cm²)	Stitch Tear Strength (kgf/cm²)	Elongation at Break (%)	Grain Distribution at Crack (mm)	Load at Crack (kgf)
A	85.0	375	129	36.50	6.01	55
B	81.0	384	131	34.70	6.96	57
C	87.5	370	133	36.83	6.42	61
D	80.5	371	134	35.00	6.75	63
E	105.0	425	145	45.91	6.61	64
F	102.5	428	147	45.05	6.85	64
G	106.5	453	151	44.25	7.05	67
H	106.0	458	153	43.00	6.88	66

Please note that the footnote in Table 6 also applies to Table 7 in terms of the meanings of 'A' - 'H'.

It has been shown that using UV spectra, it is possible to determine the type of vegetable tannins.[22] The λ_{max1} and λ_{max2} absorptions normally fall within 200 – 280 nm. These peaks have been assigned to K(E_2) $\Pi \rightarrow \Pi^*$ (λmax_1) transitions and the B-absorption band (λ_{max2}).[23] Ellagitannins and gallotannins usually have their λ_{max1} peak at longer wavelength and λ_{max2} peaks at shorter wavelength when compared to condensed tannins.[22] Comparison of the tannins of groundnut husk with tannic acid shows that λ_{max1} of groundnut is 218 and λ_{max2} is 260 nm, whereas that of tannic acid occurs at 205nm (λ_{max1}) and 281nm (λ_{max2}). This places the tannins of groundnut in the class of ellagitannins. The absorption of the tannins of cashew husks compare very well with that of mimosa and tamarind which are condensed tannins. Moreover, the λmax_1 peak of cashew husks is of shorter wavelength when compared to groundnut husk and the λmax_2 is of longer wavelength than that of groundnut husk. This therefore suggests that the tannins of cashew husks are of the condensed type[22] and is in conformity with the deductions made from the qualitative tests.

Tanning Trials: Wet salted goatskins were processed for full cashew nut husks leathers using the aqueous extracts. Tables 6 and 7 show the chemical and physical properties of resultant leathers. The leathers were full with orange-yellow color and slight red tint. The color of the leathers did not change on standing for four months. This is indicative of high degree of resistance of the tannins to air oxidation. The tannins showed good penetration rate when compared to mimosa, which was used as the control. The solo cashew leathers had shrinkage temperature of 86.25 ± 1.25°C while the chrome retan leathers had shrinkage temperature of 105.75 ± 0.75°C.

Full vegetable tannage with the groundnut tannins gave poor quality leathers with average shrinkage temperature of 75.60 ± 1.4°C. The leathers were light brown in color and the color tended to degrade in a short period (≤2 months). Chrome retan leather produced with groundnut tannins gave shrinkage temperatures of about 96.5 ±1.0°C. The use of groundnut tannins in tannage is unwieldy because of very low percentage of tannins in the material.

CONCLUSION

Evidences drawn from the qualitative analysis, UV spectroscopy and tanning trials indicate that the tannins of cashew nut testa are mainly condensed while those of groundnut husk are likely mixed hydrolysable and condensed tannins. The cashew nut testa tannins performed well in the manufacture of full vegetable leathers while groundnut husks tannin leathers performed poorly. Overall, the tannins of cashew nut testa have very good potential as tanning agents.

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