



## Characterization of Stem Bark of *Mangifera Indica* and Its Tanning Potentials

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### ABSTRACT

The stem bark of mangifera indica was dried, ground and extracted with methanol at 32 °c. The extract was fractionated using hexane, ethyl acetate and acetone, which were characterized via UV, IR, H-NMR & C-NMR spectroscopy. The solid methanolic extract was analyzed for its tannin content based on tannic acid and folincioalted methods, which gave 34.84±2.2 and 32.33 ± 1.4 respectively. The two way ANOVA of results for these two methods shows that the mean difference for the two methods is not significant. The chromatographic separation gave RF values of 0.12 and 0.13 using 6% acetic acid as mobile phase gave RF values of 0.88, and 0.36, thus implicating methyl –M- digallate, mangiferin, B – Penta –O- galloy –D- glucose and Chromatographic separation gave Rf values of 0.12 and 0.13 using 6% acetic acid as mobile phase, while n-butano1-acetic/H<sub>2</sub>O (14:1:5) as mobile phase gave Rf values of 0.88 and 0.36, Thus implicating methy-M-digallate, mangiferin, B-penta-o-galloy-D-Glucose and 1.6 digalloy1-D-glucose as components of mangifera indica. IR analysis conducted on the fractions of the plant materials shows the following bands (cm<sup>-1</sup>) at 3382.29, 2929.97, 1629.90, 1493.92 and 1261.49. Proton NMR shows signals (ppm) at 7.68 – 6.19, 5.20 while carbon-NMR shows signals (ppm) at 176.36, 167.81 – 93.91. The leather produced from stem bark of mangifera indica was supple, soft, exhibit good compressibility and water-resistant characteristics. The physicochemical carried out for the leather was favourable for the production of leather products such as car upholstery, football, belt, shoe upper and insole.

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### INTRODUCTION

Tannin is a large polyphenolic compound containing sufficient hydroxy1 and another suitable group such as carboxyls to form strong complexes with proteins and other macromolecules. The tannins are widely distributed in many species of plants where they play a role in protection from predators, and perhaps also as pesticides

and in plant growth regulation (Kantie and Richard, 2006). Tannin can be grouped according to their molecular weight. Those whose molecular weight is less than 500 are regarded as non-tans, and as the name suggest, they cannot tan but help in the solubilization of other tannins. Between 500 to 3,000 are tannins that can tan, while those above 3,000 are generally referred to as



gums, which cannot tan. Tannins can be classified into two main groups based on their structural composition: Hydrolysable and condensed tannins (Li Jingge *et al.*, 1998)). In the hydrolysable tannins, we have sub-groups; gallotannins and ellagitannins. The gallotannins are the smallest of the group and more astringent since the hydroxyl groups are very close within the molecule than the ellagitannins. On the other hand, condensed tannins have distinctive flavonoid ring structure.

The common features of these tannins are their ability to stabilize the collagens of the hides and skins against putrefaction. Tannins are important ingredients in the process of tanning leather tan bark from oak, mimosa, chestnut, quebracho and gambier tree are the primary source of tannery tannins, through inorganic tanning agents are also in use and accounts for 90% of the world's leather production. The anti-oxidative effect is due to redox properties, which emanate from their free radical scavenging activity, transition metal chelating activity and singlet oxygen quenching capacity (Luiz *et al.*, 2000). Mineral tannage especially chrome-tanned leathers are the most popular due to their durability and availability, while vegetable-tanned leathers are less stable when compared to chrome tanned leather. However, due to toxicity of chromium salts and other minerals, strict restrictions are enforced on the use of chrome-tanned leather and other mineral tannages, hence vegetable tannage is given more attention for being environmentally friendly.

However, much of the tannins consumed in Nigeria today are imported majorly from south Africa, United Kingdom, France, Switzerland, Netherland, USA and Japan (Ihuoma, 1993). Tannins of *Parkia Clappertonia* (Dorowa) and *Acacia nilotica* var *adsenii* (Bagaruwa) are the only known tannins produced in Nigeria and the quantity produced is grossly inadequate and

can only serve to augment the imported ones. Hence the discovery and introduction of new tannin regimes will help to boost our local tannin content with the consequent impact of foreign exchange conservation. Therefore, the present effort is to characterize the tannins of stem bark of mangifera indica and to determine the suitability of its tannins for tannin hides and skins.

## MATERIALS AND METHODS

### Equipment

All weighings were carried out using Mettler Toledo analytical balance. Coleparmer rotatory evaporator was used to concentrate the solution of tannins extracted from the stem bark of mangifera indica. The ultraviolet visible spectrum was obtained on Jenway 630 UV-Visible spectrophotometer using quartz cells at the Nigeria Institute of Leather Science and Technology, Zaria, Nigeria. The absorption maxima were given in nanometers (nm). Infrared spectra were recorded with FTIR-8400 Fourier Transform Infrared in NARICT, Zaria, Nigeria using KBr discs and absorption values were given in per cm ( $\text{cm}^{-1}$ ). Proton and carbon nuclear magnetic resonance (NMR) spectra were determined with Bruker Avance III (400 MHz) at Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Scotland. Column and paper chromatography was conducted to fractionate the tannin solid obtained from stem bark of mangifera indica and to separate the tannin into components respectively. The flexing machine IUP/20, model STM 409, and SMS materials test model No. SP<sub>2</sub>-4300 was used to conduct Endurance test at the Department of Textile/Leather Laboratory Standard Organization of Nigeria, Kaduna. All reagents were of analytical grade and were used as supplied unless otherwise stated.



### **Plant Materials**

Stem bark of mangifera indica – fresh bark of mangifera indica was obtained from the mango trees planted within the University of Nigeria, Nsukka. The barks were cautiously and carefully harvested to avoid damage to the cambium. The barks were identified at the Herbarium unit of plant Science and Biotechnology Department, University of Nigeria, Nsukka. The freshly harvested barks were air dried for 21 days. The dried bark was crushed in a mortar and further crushed with a blender to a particle size that were retained in a 5 mm micron sieve.

### **Extraction and Fractionation**

The air-dried powdered materials weighing 500g was macerated with 3.0l of 70% methanol at room temperature for 48 h with agitation. The filtrate was evaporated in vacuum at reduced temperature (40°C) and pressure to yield crude extract. The dried extract (10g) was fractionated in a glass column (150cm x 1.5m) packed with 500g of a slurry of silica gel (70 -230 mesh). The column was eluted in succession with 1.5 L n- hexane, 2.0 L ethyl acetate, and 1.5 L acetone to obtain n- hexane (HF), ethyl acetate (EF) and acetone (AF) fractions.

### **Paper Chromatography Separation**

The Pure tannin extract obtained through Reverse Phase Chromatography was subjected to qualitative analysis to classify the tannin. Iron (III) chloride, formalin and concentrated hydrochloric acid according to method of Dashe et al was used. The tannin was hydrolysed with 2M hydrochloric acid at 40°C and further extracted with ethyl acetate (Ukoha, 2008). The resulting extract were assayed by one dimensional paper chromatography using n-butane l/ acetic acid/H<sub>2</sub>O(14:1:5) and 6% aqueous acetic acid as mobile phase. He presence of the separated of the separated spots as evidenced on the paper (cellulose)

were identified by their characteristic colours in chromogenic reagents such as 1% ferric – cyanide and ferric chloride (1:1) and potassium iodate solution.

### **Quantitative Analysis**

The amount of tannin in the Mangifera indica was determined through tannic acid (Ukoha *et al.*, 2008) (fr) and folin-Ciocalteu methods.

### **Tannic Acid Method**

Five different molar concentrations of tannic acid (4,8,12,16 & 20)gdm<sup>-3</sup> were prepared as standard each molar concentration was reacted with few drops of 1% FeCl<sub>3</sub> solution in 50 cm<sup>-3</sup> methanol at pH 7.12. The reaction mixture was allowed to stand at room temperature for 10 minutes and then absorbance were measured using jenway 630 uv-visible spectrophotometer at 570nm using water/methanol as blank. These absorbances were plotted against the respective concentrations to obtain a standard calibration curve which was used to extrapolate concentration of the plant tannin.

### **Folin-Ciocalteu Method**

Suitable aliquots of the tannin containing extract (i.e 0.02, 0.05, 0.1)cm<sup>3</sup> were taken in test tubes and corresponding volumes made up to 0.5cm<sup>3</sup> with distilled water, followed by addition of 0.24cm<sup>3</sup> of folin -Ciocalteu reagents and then 1.25cm<sup>3</sup> of sodium carbonate solution. The tubes were vortexed and absorbance at 725nm was taken after 40min. The amount of total phenol as equivalent of tannic acid is extrapolated from the calibration curve.

### **Tanning Trials**

The tanning trials using tannin extract from stem bark of mangifera indica were conducted on wet salted pickled goat skin at the Tannery Department of the National Institute of Leather and Science



Technology, Zaria, Nigeria. The laboratory-scale trials were conducted using 20 – 24 percent pure vegetable tannins. The trial process involves skin soaking, unhairing, liming, deliming, bating, pickling, tannin and fat-liquoring. *Acacia nilotica* van adsenii (Bagaruwa) tannin extract was used as control. Leather analysis was carried out according to the Society of leather Technologist and Chemist Official methods.

### RESULTS AND DISCUSSION

**Qualitative Analysis:** Results of the qualitative tests carried out on the tannin extract of the stem bark of *Mangifera indica*

places the tannin of the stem bark within the class of hydrolysable tannin.

**Quantitative Assay:** Results of the quantitative determination of tannin content were based on two methods; folin-ciocalteu and tannic acid. The results are presented in

The analysis of variance performed for the result in table 1 is given in table 2. All results were the mean replicate measurements. From the tables, the mean difference is not significant for both folin-Ciocalteu and Tannic acid methods since the F calculated is less than the F tabulated.

**Table 1:** Concentration (%) of Tannins in *Mangifera indica*

	Methods	
	Folin – Ciocalteu	Tannic Acid
Mangifera indica	32.33 = 1.4	34.84=2.2

**Table 2:** Analysis of variance for percentage of Tannins extracted from *Mangifera indica* using different methods.

S/No	Method of Analysis	% Tannin		
1.	Folin Ciocalteu	30.93	32.33	33.73
2.	Tannic acid method	32.65	34.85	37.05

**Table 2b:** Anova; Two-factor without replication

Summary	Count	Sum	Average	Variance
Row 1	3	96.99	32.33	1.96
Row 2	3	104.55	34.85	4.84
Column 1	2	63.58	31.79	1.4792
Column 2	2	67.18	33.59	3.1752
Column 3	2	70.78	35.39	5.5112

**Table 2c:** Anova

Source of Variation	SS	Df	MS	F	P-value	F crit
Rows	9.5256	1	9.5256	29.7675	0.03199	18.51282
Columns	12.96	2	6.48	20.25	0.047059	19
Error	0.64	2	0.32			
Total	23.1256	5				

### Paper Chromatography Separation

Separation and formation of discrete spots was achieved by first

hydrolyzing the tannin extract of *Mangifera indica* with HCl, then extracting with ethyl acetate. One dimensional ascending paper



chromatography was then employed in the separation of individual polyphenols. One dimensional paper chromatography of the mangifera indica on whatman number 1 paper using n-butanol/acetic acid/water

(14:1:5) as the mobile phase yielded one visible spot, Using 6% acetic acid only, two spots were visible. The spots had brown and purple blue colours.

**Table 3:**  $R_f$  values of phenolic Components of Ethyl Acetate Extracts of Acid-hydrolyzed stem bark of *Mangifera indica*.

Components	A	B	Colour	
	6% Acetic Acid	n-butanol/acetic acid/H <sub>2</sub> O (14:1:5)	K <sub>3</sub> [Fe(CN) <sub>6</sub> ] <sup>-</sup> FeCl <sub>3</sub> (1:1)	UV Lamp Colour
Methyl-m digallate	-	0.88	Blue	Purple Blue
B-penta-O- galloyl-D- glucose	0.12	-	Blue	Brown
1,3-di-O-galloyl glycerol	0.13	-	Blue	Brown

From the  $R_f$  values in table3, methyl-m-digallate (Methyl 3,4-dihydroxy-5-(2-oxo-2-(3,4,5- trihydroxy phenyl)ethyl benzoate, has been implicated as the spot with a value of 0.88 in n-butanol/acetic acid/water. This assertion is in accord with what has been reported elsewhere. Also B-penta-o-galloyl-D-glucose (3,4,5-trihydroxybenzoyl)methyl)tetrahydro-2H-pyran-2,3,4,5 tetrahydro-2,3,4,5 tetrahydro-2,3,4,5 trihydroxybenzoate), has been implicated as a major constituent of the tannin with  $R_f$  values 0.12 in the 6% acetic acid solvent. Also 1,3,-di-o-galloyl-glycerol has been implicated as the constituent with  $R_f$  0.13 in the same solvent A.

#### Electronic Spectra of tannins

The ultra violet absorption bands of stem bark of mangifera indica and that of tannic acid was run in methanol. The spectra are presented in table 4. The spectra were compared with UV spectra of authentic sample of tannic acid. The UV spectrum of stem bark of mangifera indica showed striking similarities as its broad peak at 570nm is the same as that of tannic acid. This infers that the two tannins are the same which implies that the tannin of mangifera indica is likely to be gallotannin and hydrolysable type.

**Table 4:** Absorption Wavelength and Absorbance in UV spectra of the Tannins @ 12mg/1

	Max <sub>1</sub> /nm	Abs max <sub>1</sub>	Max <sub>2</sub> /nm	Abs.max <sub>2</sub>
M. indica	-	-	570	2.5
Tannic acid	-	-	570	0.6

**Table 5:** Proximate Analysis of mangifera indica tannins

S/no	Parameters	%
1.	Moisture content	1.52
2.	Total Soluble	74.13
3.	Total Solid	98.08
4.	pH	6.39
5.	Total Insoluble	23.95
5.	Total acid	0.62

**Table 6:** Chemical Properties of Resultant Leather from stem bark of mangifera indica

Properties	Leather		Sample	
	[A] (20% mang)	[B] (20% Bag)	[C] (24% mang)	[D] 24% Bag
Volatile matter (%)	9.82±0.80	11.32±1.62	11.52±1.50	12.92±1.30
Water Soluble	17.38±1.04	20.23±1.72	17.74±1.30	20.15±0.30
Fatty Substance (%)	2.0±0.58	2.50±0.35	2.25±0.40	2.68±0.52
Nitrogen (%)	1.17±0.5	1.68±0.7	1.16±0.40	1.83±0.62
Hide Substance	48.80±1.40	51.03±1.64	47.83±1.22	49.06±1.05
Insoluble Ash	0.94±0.01	0.88±0.20	0.92±0.03	0.87±0.06
pH	5.40±0.20	5.05±0.50	5.62±0.42	4.94±0.30
Fixed Tannins (%)	29.29±1.38	24.85±1.24	30.23±1.45	26.02±1.04
Degree of Tannage	52.14±1.70	49.94±1.07	54.06±1.52	52.84±1.63
Moisture Content	16.00±0.50	16.99±0.75	16.52±0.32	17.24±0.62
Water-Soluble Inorganic Matter	14.25±0.70	14.17	14.85±0.80	14.32±0.53

**Table 7:** Physical Characteristics of Resultant Leather from stem Bark of mangifera indica

Leather sample	A	B	C	D
Shrink age Temperatur (°C)	70	72	72.5	74.1
Tensile Strength (Kgf/Cm <sup>2</sup> )	308.72	424.60	306.64	416.16
Stitch Tear Strength (Kgf/Cm <sup>2</sup> )	117.41	112.99	119.02	114.00
Elongation at Break (%)	34.32	32.40	34.82	33.05
Grain Distribution at Crack (MN)	6.02	6.72	6.52	6.84
Load at Crack (Kgf)	53	51	58	56
Apparent Density (g/cm <sup>3</sup> )	2.0X10 <sup>-1</sup>	1.8X10 <sup>-1</sup>	2.32X10 <sup>-1</sup>	1.9 X10 <sup>-1</sup>
Indentation Index (1/100m)	25	22	27	24
Resistance to Compression (kgf/cm <sup>2</sup> )	2.6 X 10 <sup>-1</sup>	2.4X10 <sup>-1</sup>	2.92X10 <sup>-1</sup>	2.72X10 <sup>-1</sup>
Light Fastness (grey Scale)	3/4	2/3	3/4	2/3
Water Vapour Abdorption (mg/cm <sup>2</sup> )	1.27 X10 <sup>6</sup>	1.29X10 <sup>6</sup>	1.30X10 <sup>6</sup>	1.32X10 <sup>6</sup>
Fullnet(Kg/c)	10.54	9.980	10.74	10.09

**Table 8:** Characterization of Components implicated from paper chromatographic separation of stem bark of Mangifera indica

S/no	Name of Components	Structure of Components	IR Spectra	H-nmr spectra	13C-nmr
1	Methyl-M-Digallate		Streching at 3382cm <sup>-1</sup> , bending at 1629cm <sup>-1</sup> signifying the presence of OH	The Strong signal at 2.12 ppm is due to strong carbonyl	Aromatic carb 167.81 – 93.91 sign at 176.36 ppm indicative of C = O



			<p>group.          Aromatic C-H          Stretching at 2929cm<sup>-1</sup>, bending frequency at 1493cm<sup>-1</sup> indicates aliphatic OH. The strong band observed at 1261 is due to the C-O stretching</p>	<p>signal.          Aromatic protons (7.68 - 6.19) ppm</p>	
2	Mangiferin		<p>Stretching at 3382cm<sup>-1</sup> bending at 1629cm<sup>-1</sup> signifying the presence of OH group.          Aromatic C-H stretching at 2929-1, bending frequency at 1493cm<sup>-1</sup> indicates aliphatic OH. The strong band observed at 1261 is due to the C-O stretching</p>	<p>Aromatic protons appeared at 6.88ppm . the peak at 3.68 ppm indicates the presence of CH, while the peak at 5.3ppm indicates OH protons</p>	<p>Aromatic carbo appeared between (10-168) ppm, while peak at (56-61) ppm indicates the presence of aliphatic carbon</p>

3.	B-Penta-o-galloyl-D-glucose		<p>Stretching at 3382-1 bending at 1629cm-1 signifying the presence of OH group. Aromatic C – H stretching at 2929cm-1 bending frequency at 1493cm-1 indicates aliphatic OH. The strong band observed at 1261 is due to the C-O stretching</p>	<p>The peak at 7.60ppm indicates the presence of aromatic protons while the peak at 4.16ppm signifies the presence of Oh protons</p>	<p>The peak at 162.3 – 115.6ppm are indicative of aromatic carbons, while the peak at 56 73.1 signifies the aliphatic carbon</p>
4.	1,3-digalloyl D- glucose		<p>Stretch at 3382cm-1, bending at 1629cm-1 signifying the presence of OH group. Aromatic C – H stretching at 2929cm-1 bending frequency at 1493cm-1 indicates aliphatic OH. The strong band observed at 1261 is due to the C – O stretching.</p>	<p>The strong at 2.60ppm in due to glucose proton, while aromatic protons appeared at 7.68ppm and 6.19ppm</p>	<p>In the C-nmr the aromatic carbon appears between (167.81 – 93.91)pp m while the peak at 176.33 ppm indicative of C – O function group</p>





### **Physical and Chemical Properties of Stem Bark of mangifera indica tanned Leather**

The physical and chemical characteristics of leathers are quite significant in terms of preparing production recipes and determining the field of utilization of leathers. Leathers are put to a variety of uses which is inherently linked to its properties or its characteristics. These properties are dependent on the physical structure and chemical composition, and mechanical operations during the manufacture of the leather. Physical structure decides the properties of the finished products to a large extent. For the purpose of quality control, the properties of mangifera indica tanned-leather were determined in parallel with that of pods of *Acacia nilotica* in the (Bagaruwa) which have been a familiar trusted tanning materials for most tanneries in the northern part of the country. Aside from *Acacia nilotica* van adsenii (Bagaruwa), comparison was further made with leathers tanned with mimosa, quebracho, gambier, sumac and parkia, to ascertain if qualities of mangifera indica tanned leathers produced to conform with the general properties for mangifera indica tanned-leather indicates a perfect correlation of properties with that of *Acacia nilotica* van adsenii (Bagaruwa). The implication is that mangifera indica tanned leather can be used as substitute especially in areas of products and materials where *Acacia nilotica* van adsenii has gained currency. The values recorded for moisture content, pH total water soluble, insoluble ash, nitrogen and hide substance, degree of tannage, have collectively placed mangifera indica tanned leather as a safe material devoid of inherent environmental challenges. On the physical side, the low apparent density value implies lower weight with concomitant of greater comfort. The high tensile value of 308.72 and 306.64 recorded for mangifera indica tanned leather against the generally accepted

lowest benchmark value of 200, are clear indication that the leather materials is suitable to produce wide range of leather products, such as shoe uppers, insole, sole upholstery, football and belts. The mangifera indica tanned leather has a better light fastness rate that *Acacia nilotica* van adsenii, as such it tends to give a better background colour for leather to be dyed into various light colours. Other parameters like indentation index, hydrothermal stability, resistance to compression, water vapour permeability, flexing endurance, stitch for strength, shrinkage temperature and their corresponding values as presented in the table, have collectively assured that mangifera indica tanned leather is a good quality material that can be used to produce multipurpose leather products.

### **CONCLUSION**

The research has succeeded in placing the stem bark of mangifera indica as hydrolysable tannin. This was further confirmed by its shrinkage temperature that was maintained between 70 – 72°C, a characteristic temperature of hydrolysable tannins whose shrinkage temperature do not exceed 80°C. The tanning trials conducted on mangifera indica has revealed it as an alternative and promising tanning materials that could be applied to augment the existing stock of tannins available in the leather industry. The fact that the tannin was obtained from a plant which is a renewable if carefully and properly harvested, will no doubt ensure the availability of it tannin at all times. Thus it is recommended that this plant should be planted on a large scale, since our tropical weather is favorable for its production. Finally, the huge foreign exchange hitherto committed into importation of tannins, will be favorably mediated if its tannins is made available to the leather industry.



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