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Evaluation of Vegetable Tannin Contents and Polyphenols of some Indigenous and Exotic Woody Plant Species in Sudan

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***Abstract*— This paper presents a complementary analytical approach to characterize vegetable tanning materials in different woody plant species grown in Sudan. It is described the application of hide powder and combined techniques for determination of tannin content, and three specific chemical techniques to analyze the total phenolic content present in the species. The catechin number (Stiasny number) determined according to the method by Yazaki and Hillis, the iron alum test were usedto identify condensed tannins and hydrolysable tannins types respectively. Thirteen species of commercial, or laboratory prepared vegetable tannins were analyzed and the obtained results were used for comparison. The complete analytical procedure was performed with the objectives of evaluating the quantity and quality of extractable tannins for comparison with standard *Acaciamearnsii* (wattle) tannins. The result showed that of the fifteen parts studied; fourteen had more than 10% tannin content and were thus suitable for commercial exploitation. Thin layer and paper chromatography indicated and confirmed the differences of the chemical nature of the materials as mixed (Hydrolysable-condensed) and condensed tannins. The protein precipitation performance confirmed complexity and differences in their nature and potentiality for tanning or other uses compared with *A. mearnsii*. The tannin type of the whole species studied of hydrolysable-condensed while that of *A. mearnsii* was of condensed type.**

***Index Terms*— Tannins, protein precipitation, catechin, bark, Astringency**

I. INTRODUCTION

Vegetable tannins are polyphenolic compounds widely distributed in plants which have the property to precipitate proteins [1and 2]. Since ancient times, this property has been empirically explored to transform animal skins, a proteinaceous biomaterial, into leather [3 and 4]. The process, termed vegetable tanning, is one of the oldest known leather making processes and it can be briefly described as a treatment of hides/skins with powdered barks, leaves, wood, fruits, pods or galls, or their extracts, obtained from different vegetable sources [5]. With this treatment, traditionally performed in pits, a chemical interaction between collagen protein (the main constituent of dermis) and tannins present in vegetable materials is slowly established, generating a very useful and remarkably non-putrescible material under moist and warm conditions, termed vegetable tanned leather [4 and 6]. Vegetable tanned leather was one of the most important pre-industrial materials in Western and Mediterranean Europe, very much appreciated and demanded due to its versatility. It was the main material of a wide range of artifacts and adapted to very diverse functional needs such as footwear, book bindings, saddles, harness, cases and caskets coverings or seating furniture and carriages upholstery. Beyond its utilitarianfunction, it was also used as support material for artistic and decorative paintings, wall hangings and screen coverings. Different ornamental techniques such as dyeing, painting, gilding, moulding, tooling, embroidering, cutting-out, scorching or sewing, have been often incorporated transforming vegetable tanned leather into a noble, luxurious and valuable material. Vegetable tanning materials differ greatly in chemical constitution and tanning properties. Chemically, tannins are complex and heterogeneous group of polyphenolic secondary metabolites biosynthesized by higher plants with molecular weights ranging from 500 to over 20,000 Da [7]. According to the main polyphenolic compounds present, they are classified into condensed tannins (also named proanthocyanidins) and hydrolysable tannins, which comprise two subclasses, gallotannins and ellagitannins [8–10]. Condensed tannins are oligomeric or polymeric flavonoids and hydrolysable tannins consist of a polymer containing a polyol core (d-glucose is the commonest) multi-esterified with gallic acid (gallotannins) or its oxidized derivative, ellagic acid (ellagitannins). Classification and examples of different chemical structures of tannins were extensively reviewed by Khanbabaee and van Ree [7].

The hydrolysable tannins are readily hydrolyzed by acids, alkalis or enzymes (tannases) into a sugar or a related polyhydric alcohol (polyol) and a phenolic carboxylic acid

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[11].Hydrolysis of gallotannins yields gallic acid while hydrolysis of ellagitannins yields hexahydroxydiphenic acid which is isolated as ellagic acid [11]. Hydrolysable tannins are considered as one of the most potent antioxidants from plant sources. They are ready to form complexes with reactive metals, avoiding free radical generation which results in oxidative damage of cellular membranes and DNA [12].Hydrolyzable tannins, in addition, clean free radicals within the body by neutralizing them before cellular damage occurs [11]. Thus, the in vitro antimutagenic and anticarcinogenic activity of tannic acid has been previously reported [13].

This study aims to improve the knowledge about vegetable tanning materials used in leather making by investigating the quantity and quality of tannins from twelve species of central and western Sudan, in the hope that they could be used in place of *A. mearnsii* (wattle) in the Sudanese leather industry. *Acacia mearnsii* is not available in Sudan and has to be imported at a high price.

II. MATERIALS AND METHODS

Preparation of sample

Fresh bark and pods (0.5–2.5 kg) of Acacia species growing around El Obeid and Khartoum were used for this study **(Table 1)**. The Soba Forestry Research Center Herbarium confirmed the identity of species. The samples were air-dried and reduced to powder with a star mill. The fractions passing through 40-mesh and retained on 85-mesh sieve were collected, thoroughly mixed and kept in airtight containers.

Analysis of tannins

Extraction using ALCA-Palsy method

Cold water extracts (2 litres) were obtained with an ALCA (American Leather Chemist Association)-Palsy apparatus [14]. The presence of tannins was detected by the gelatin salt test [15] and their types were identified using the iron-alum and formaldehyde-HCl test [15].

Qualitative analysis

Paper chromatography was done on Whatman No. 1 paper with forestal solvent system (concentrated acetic acid: HCl: water, 10:3:30) [16]. The chromatography was developed by ascending method at room temperature (30–36 ℃) to a height of 7–15 cm. Spots were detected first under UV light (254 nm) and then by spraying with ferric chloride reagent (2 g $FeCl_3$ in 98 ml methanol) or exposing to ammonia vapour [17]. Thin layer chromatography was done with sheets (20 × 20 cm) precoated with polyamide six layer (thickness 0.1 mm). The solvent system used was acetone-propanol-water (5:4:1) [17].

Tannic acid, catechin, gallic acid, epicatechin, fisetin, dihydrofisetin and robinetin were used as standard compounds (Rf ×100) for the above chromatographic analyses. Samples were prepared by hydrolyzing 5 g raw materials with 2M HCl 13 parts of the species (**Table 2**). However, the type of tannin present and the part extracted are also important.

using reflux for 30 min. The effluent was then cooled and filtered and the filtrate was extracted with ethyl acetate. The aqueous layer was heated to remove any trace of solvent and extracted with a small volume of amyl alcohol. The solvent extracts were concentrated to thick syrup under vacuum [16].

Quantitative analysis

The extracts were quantitatively analyzed for total and soluble solids, non-tannins and tannins by the official hide-powder method [18] (hide-powder batch C28). A modification of the hide-powder method, i.e. the combined method [19] was also used. Total phenolic materials in the extract were measured using the Folin-Denis method [20]. Freshly hydrated chromated hide-powder equivalent to 3.0 g oven-dried was prepared. Tannin was then allowed to absorb onto the hide powder, after which the remaining phenolic materials were determined. The catechin number (Stiasny number) was determined according to the method by Yazaki and Hillis [20]. For this 100 ml extract were filtered through a glass fritted funnel (G4) and poured into a conical flask. Stiasny reagent (5 ml of HCl + 10 ml of 37% formaldehyde) was added into the flask and then the mixture was allowed to stand for 24 hours at room temperature (30–35 ℃). Then the precipitate was filtered on a tared crucible (G4) before being dried to constant weight at about 100 ± 5 ℃ to obtain the weight of catechin[20].

III. RESULTS AND DISCUSSION

Tannins are phenolic compounds of relative high molecular weight. They are classified as condensed and hydrolyzable tannins. The hydrolyzable tannins are readily hydrolyzed by acids, alkalis or enzymes (tannases) into a sugar or a related polyhydric alcohol (polyol) and a phenolic carboxylic acid [11]. Depending on the nature of the phenolic carboxylic acid, hydrolyzable tannins are subdivided into gallotannins and ellagitannins. Hydrolysis of gallotannins yields gallic acid while hydrolysis of ellagitannins yields hexahydroxydiphenic acid which is isolated as ellagic acid [11]. Hydrolyzable tannins are considered as one of the most potent antioxidants from plant sources. They are ready to form complexes with reactive metals, avoiding free radical generation which results in oxidative damage of cellular membranes and DNA[12].

From the formaldehyde-HCl and iron alum test, the whole 12 species screened were of the mixedhydrolysable-condensed(gallo-catechol) type. The gallic acid and catechin number test results supported these assignments (**Table 2**). The quantitative data indicated that 13 parts (bark,leaves, and fruits) of 12 species, when extracted, contained more than 10% (oven-dry basis) of tannins, the level of commercial interest. Of these 12 species, 13 parts had an acceptable extraction ratio (tannin to non-tannin) of 1.1-4.2. The tannin purity or the ratio of tannin/soluble solids was good, >0.5, for

Different parts of species bark, leaves, and fruits had the same type of tannin but in different proportions. Usually the tannin

content was higher in the barks and leaves (*Anogessus leiocarpus, Pithecello biumdulce,*and *Terminalia brownii*) (**Table 2**). The catchin numbers indicated that all the studied species contained condensed tannin in varying amounts (1.1-32.3), while the presence of both gallic acid and catechinmeans that the tannin is of mixed type (**Table 2**).

Table 1
Collection data for the tanniferous species studies

Species	Part	Age	Collection site	Air-dried Material
Acacia mearnsii	Bark	25	Jebel Marra	2.0
Albeizzialebbek	Bark	15	Soba	0.5
Anogessusleiocarpus	Bark	30	Dalang natural Forest	2.0
	Leaves	30	Dalang natural Forest	2.0
Azadirachtaindica	Bark	30	El Obeid area	2.0
Casuarinaequistifolia	Bark	10	Soba	0.3
Cassia fistula	Bark	10	Soba	0.3
Combretumhartmannianum	Bark	15	shambat	0.3
Eucalyptus camaldulensis	Bark	10	Soba	0.3
E. tereticornis	Bark	10	Khartoum East	0.3
Pithecellobiumdulce	Bark	18	Blue Nile	0.3
Tamarixaphylla	Bark	25	El Obeid area	2.0
Terminaliabrownii	Bark	20	El Obeid area	2.0
	Fruits	20	El Obeid area	2.0
Zizyphusspina-christi	Bark	20	El Obeid area	2.0

Thin-layer and paper chromatographies with different solvent systems confirmed the presence of catechin and gallic acid, and showed that tannic acid,fisetin, epicatechin and some unidentified phenolics were present. However, dihydrofisetinand robinetin, which were used as standards, were not detected (**Table 3**).

The tannin content determined by the hide-powder method was highest (28.8%) for *Pithecellobiumdulce* barkfollowed by *Anogessusleiocarpus*leaves and*Terminaliabrownie* bark(20.8% & 20.5% respectively) (Table 2). These data were compared with those obtained from the spectroscopic method of Swain and Goldstein [21] and also with two methods for total phenolic [22-24] (**Table 4**). In the first comparison, the correlation between total phenolics and tannin content was high (r^2= 98.7%, n = 24, p < 0.01). In the second case, the phenolic content by the Hagerman and Butler method [23 and 24] was approximately half that of Folin-Denis assay, but the correlation between the two assays was still high (r^2= 70.9%,n = 24, p < 0.01). The combined method also gave slightly lower values of tannin content and extraction rates (Table 4). Care should be taken when comparing tannin content determined bydifferent methods as the isolation procedures may affect the proportion and types of phenolic present (this due to different method have different ways of determination and isolation). The relative astringency values for most of these tannins were quite close to that of *A. mearnsii* tannin, but much higher values were obtained for *Azadirachtaindica* bark. However, the*Azadirachtaindica*bark has low tannin contents (16.7%) (**Table 4**).

Astringency values shows that the *Pithecello biumdulce* bark (0.15), *Tamarix aphylla* bark (0.14),*Terminalia brownii* bark (0.13),and *Zizyphus spina-christi* bark (0.13) could be used in place of *A. mearnsii* (0.16) because the degree of relative astringency or the ability of their tannin to combine with protein is close to that of*A. mearnsii*; in otherwards these five species can give leather with characteristics comparable with that of *A. mearnsii*.

The protein precipitation curve for the tannins from *A. mearnsii* bark and the *Pithecello biumdulce* bark,*Tamarix aphylla* bark, *Terminalia brownii* bark,and *Zizyphus spina-christi* barkreflected their different nature and relative astringency (**Figure 1**). The fairly gradualsolubilizationof *A. mearnsii*tannins (wattle) and *Pithecellobiumdulce*bark,*Tamarixaphylla* bark, *Terminaliabrownii* bark, and *Zizyphusspina-christi* barktannins indicated greater reactivity. It seemed probable that the highly astringent and strongly binding tannin would react with animal hide protein so firmly and rapidly that the penetrationof the materials would have to be controlled by selection of pH and concentration. Thus, the resulting leather might be hard and coarse. In contrast the less astringent tannin (mixed type) obtained from the *Pithecellobiumdulce*bark and *Terminaliabrownii* bark mixed with *Azadirachtaindica* barkshould penetrate the hide more extensively and the reaction should not be weaker in terms of poorer tanning or greater vulnerability to microbiological damage.

Table 2
Analysis of the tannin cold aqueous extracts (% oven-dry part extracted)

Species	Part	Total solids (TS)%	Soluble solids (SS)%	pH	Tannins, (T)%	Non-Tannins,(NT)%	Extraction Ratio (T/NT)	Catechin number	Gallic acid	Tannin type	Purity (T/SS)%
Acacia mearnsii	Bark	51.8	48.7	6	39.8	8.9	4.5	45.7	-	C	0.8
Albeizzia lebbek	Bark	28.5	21.5	6	14.0	7.5	1.9	17.4	+	HC	0.7
Anogessus leiocarpus	Bark	23.0	21.9	6	14.5	7.4	2.0	5.7	+	HC	0.7
	Leaves	36.2	34.4	6	20.8	13.6	1.5	7.2	+	HC	0.6
Azadirachta indica	Bark	25.1	24.9	6	16.8	7.6	2.2	22.4	+	HC	0.7
Casuarinaequistifolia	Bark	16.7	14.9	6	10.2	4.7	2.2	12.3	+	HC	0.7
Cassia fistula	Bark	41.0	28.2	6	19.3	8.9	2.2	32.3	+	HC	0.7
Combretum hartmannianum	Bark	27.1	27.0	6	14.2	12.8	1.1	4.6	+	HC	0.5
Eucalyptus camaldulensis	Bark	17.7	16.9	6	10.5	6.5	1.6	10.6	+	HC	0.6
E. tereticornis	Bark	18.1	16.6	6	10.4	6.2	1.7	11.8	+	HC	0.6
Pithecello-biumdulce	Bark	38.9	35.7	6	28.8	6.9	4.2	26.6	+	HC	0.8
Tamarix aphylla	Bark	27.2	26.8	6	15.8	11.0	1.4	10.7	+	HC	0.6
Terminaliabrownii	Bark	28.2	28.1	6	20.5	7.6	2.7	4.2	+	HC	0.7
	Fruits	11.4	11.3	6	3.5	7.8	0.4	1.1	+	HC	0.3
Zizyphusspina-christi	Bark	18.9	17.6	6	12.2	5.4	2.3	12.8	+	HC	0.7

H = Hydrolysable tannin, C = condensed tannin, + = Detected

Table 3
Thin layer (TLC)* and paper (PC) ** chromatography of hydrolyzed bark extracts

Species	**Part**	**Extracted with**	**Gallic acid**		**Tannic acid**		**Catechin**		**Epicatechin**		**Fisetin**		**Unknown**	
			TLC	PC	TLC	PC	TLC	PC	TLC	PC	TLC	PC	TLC	PC
			82	63	56	32	78	64	66	64	66	15		
Acacia mearnsii	*Bark*	Amyl alcohol	-		-		77	67	66	66	65	15	-	-
		Ethyl acetate	-		-		-	-	-	-	-	-	64	-
Albeizzialebbek	Bark	Amyl alcohol	-	-	-	-	77	67	-	-	-	-	-	-
		Ethyl acetate	83	62	-	-	-	-	-	-	-	-	-	-
Anogessusleiocarpus	Bark	Amyl alcohol	-	-	-	-	77	67	-	-	-	-	-	-
		Ethyl acetate	-	62	-	-	-	-	-	-	-	-	-	-
	Leaves	Amyl alcohol	-	-	-	-	-	-	66	-	-	-	-	-
		Ethyl acetate	81	62	56	32	77	67	-	-	-	-	-	-
Azadirachtaindica	Bark	Amyl alcohol	-	-	-	-	77	67	-	-	-	-	-	-
		Ethyl acetate	-	62	-	-	78	-	-	-	-	-	-	-
Casuarinaequistifolia	Bark	Amyl alcohol	-	-	-	-	78	64	-	-	-	-	-	-
		Ethyl acetate	-	62	-	-	-	-	-	-	-	-	-	-
Cassia fistula	Bark	Amyl alcohol	-	-	-	-	77	67	-	-	-	-	-	-
		Ethyl acetate	82	62	-	-	-	-	-	-	-	-	-	-
Combretumhartmannianum	Bark	Amyl alcohol	-	-	-	-	78	64	-	-	-	-	-	-
		Ethyl acetate	-	62	-	-	-	-	-	-	-	-	-	-
Eucalyptus camaldulensis	Bark	Amyl alcohol	-	-	-	-	78	62	-	63	-	-	-	51
		Ethyl acetate	-	65	-	-	-	-	-	-	-	-	-	-
E. tereticornis	Bark	Amyl alcohol	-	-	-	-	78	67	-	-	-	-	-	-
		Ethyl acetate	-	63	-	-	-	-	-	-	-	-	-	-
Pithecellobiumdulce	Bark	Amyl alcohol	-	-	-	-	78	62	-	-	-	-	-	-
		Ethyl acetate	83	65	-	-	-	-	-	-	-	-	-	-
Tamarixaphylla	Bark	Amyl alcohol	-	-	-	-	77	66	-	-	-	-	-	-
		Ethyl acetate	82	64	-	-	-	-	-	-	-	-	64	-
Terminaliabrownii	Bark	Amyl alcohol	-	-	-	-	79	66	-	-	-	-	70	-
		Ethyl acetate	83	64	-	-	-	-	-	-	-	-	-	-
	Fruits	Amyl alcohol	-	-	-	32	77	67	-	64	-	-	44	-
		Ethyl acetate	81	64	-	32	-	-	-	-	-	-	44	-
Zizyphusspina-christi	Bark	Amyl alcohol	-	-	-	-	79	66	-	64	-	-	-	-
		Ethyl acetate	83	63	-	-	-	-	-	-	-	-	-	-

* Adsorbent: Polyamide precoated plate (10x10 cm); solvent system: acetone- propanol- water (5/4/1); detection: UV/254nm; $FeCl_3$.
**Adsorbent: Whatman paper no.2; solvent system: acetic acid-conc. HCl- water (10/3/30); detection: UV/254nm; strong ammonia vapor.

Table 4
Total phenolics content in tannin extract by different methods and astringency factor

Species	Part	Tannin content, % in oven-dry part extracted		Extraction Ratio (Tannin/non-tannin)		Total phenols, % in oven-dry part extracted			
		Hide Powder Method	Combined Method	Hide Powder Method	Combined Method	Combined Method	Folin Denis Method	Hagerman Butler Method	Relative Stringency
Acacia mearnsii	Bark	39.8	38.1	4.5	2.7	72.8	35.6	17.8	0.16
Albeizzialebbek	Bark	14.0	14.3	1.9	0.4	49.2	14.2	6.8	0.08
Anogessusleiocarpus	Bark	14.4	14.3	2.0	0.2	69.6	14.2	4.1	0.12
	Leaves	20.8	19.9	1.5	0.3	72.0	19.9	10.9	0.10
Azadirachtaindica	Bark	16.7	15.5	2.2	0.6	46.8	16.0	8.0	0.18
Casuarinaequistifolia	Bark	10.2	10.2	2.2	1.0	47.1	10.0	5.1	0.12
Cassia fistula	Bark	19.3	19.2	2.2	3.2	45.6	18.6	9.3	0.08
Combretumhartmannianum	Bark	14.2	14.3	1.1	1.2	60.0	13.8	7.0	0.12
Eucalyptus camaldulensis	Bark	10.5	10.6	1.6	1.0	43.8	10.1	5.0	0.13
E. tereticornis	Bark	10.4	10.3	1.7	1.5	50.4	10.3	5.3	0.12
Pithecellobiumdulce	Bark	28.8	27.9	4.2	1.4	39.6	27.3	14.5	0.15
Tamarixaphylla	Bark	15.8	15.7	1.4	2.2	58.0	15.4	8.3	0.14
Terminaliabrownii	Bark	20.5	20.9	2.7	2.7	98.0	19.1	7.2	0.13
	Fruits	3.50	3.20	1.0	04	13.8	8.8	4.3	0.11
Zizyphusspina-christi	Bark	12.2	11.7	2.3	0.9	36.0	11.8	9.4	0.13

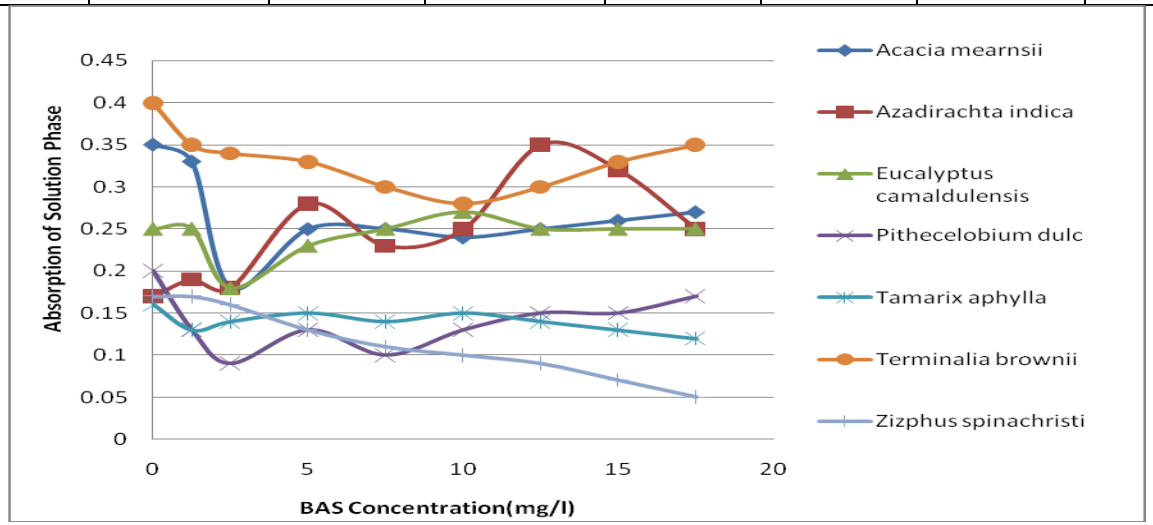

Figure 1. Protein precipitation curves obtained for the phenolics in the tannin extracts

IV. CONCLUSIONS

This study highlights the potential of an analytical approach based on absorption and chemical analysis to characterize tannins in different plant parts. Each analytical technique gives different information of complementary nature, which is useful for the knowledge and research of vegetable tannin materials formerly used for leather production.

Notwithstanding it may be difficult to interpret the methods of determination of tannin content with that one of total polyphenolic determination one, the aim of this study is also to provide the scientific community with some reference data of vegetable tanning materials for future studies.

Of the thirteen indigenous and exotic woody plant species parts studied, the whole species contained more than the 10% tannin needed for commercial exploitation except *Terminaliabrownie* fruits which has less than 10%. The richest exotic species, but of limited distribution, was *Acaciamearnsii* bark (wattle), followed by the indigenous*Pithecellobiumdulce*bark,*Anogessusleiocarpus leaves*, *Terminalia brownie bark and* the *Cassia fistula* bark.Thestudied tannins containscatechin and gallic acid which is of the mixed gallo-catechol type.

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