CHARACTERIZATION OF *ACACIA NILOTICA* AS AN INDIGENOUS TANNING MATERIAL OF SUDAN

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MAHDI, H., PALMINA, K. & GLAVTCH, I. 2006. Characterization of *Acacia nilotica* as an indigenous tanning material of Sudan. Tannins from four indigenous and exotic woody plant species were studied by different methods with objectives of evaluating the quantity and quality of extractable tannins for comparison with standard *Acacia mearnsii* (wattle) tannins. The results showed that of the seven materials studied six had > 10% tannin content and were thus suitable for commercial exploitation. Thin layer chromatography indicated and confirmed the differences of the chemical nature of the materials as mixed and condensed tannins. The protein precipitation behaviours confirmed complexity and differences in their nature and potentiality for tanning or other uses compared with *A. mearnsii*. The tannin type of *A. nilotica* species was of hydrolysable-condensed while that of *A. mearnsii* was of condensed type.

Keywords: Tannins, wood, garad, wattle, astringency

MAHDI, H., PALMINA, K. & GLAVTCH, I. 2006. Pencirian *Acacia nilotica* sebagai bahan samak tempatan di Sudan. Tanin daripada empat spesies pokok berkayu yang asli dan eksotik dikaji menggunakan empat kaedah berbeza. Objektif utama kajian adalah untuk menilai kuantiti dan kualiti tanin boleh ekstrak supaya dapat dibandingkan dengan tanin standard daripada *Acacia mearnsii*. Keputusan menunjukkan bahawa daripada tujuh bahan yang dikaji, enam mempunyai kandungan tanin > 10% dan oleh itu, dianggap sesuai untuk kegunaan komersial. Kromatografi lapisan nipis menunjuk dan mengesahkan perbezaan dalam kandungan kimia bahan yang dikaji sebagai tanin campuran serta terkondensasi. Kelakuan mendakan protein mengesahkan kekompleksan dan perbezaan dalam sifat bahan-bahan itu dan kemungkinan bahan tersebut diguna untuk menyamak atau kegunaan lain berbanding tanin *A. mearnsii*. Tanin *A. nilotica* adalah daripada jenis boleh hidrolisis-terkondensasi sementara *A. mearnsii* adalah jenis terkondensasi.

INTRODUCTION

Tannins are water soluble high molecular weight polyphenolic compounds and rich in phenolic group. Industrially tannins are used in the production of leather, adhesive material, dye stuff and ink. Also, owing to their astringent properties, tannins are used as medicinal materials which promote rapid healing and formation of new tissues on wound and inflamed mucosa (Ayoub 1982).

In Sudan there are many indigenous and exotic plant species which contain tannins in different quantities. Some of them were analyzed by Kaith (1968), but no systematic screening has been reported. The Sudanese leather industry uses mainly imported tannin from *Acacia mearnsii* or minerals. Local vegetable tannins such as garad from the pods of *A. nilotica* are abundant, but they do not produce the same quality of leather as wattle, obtained from the bark of *A. mearnsii*. A mix of equal proportions of spray-dried extracts from *A. nilotica* (garad husks) and *Azadirachta indica* (barks) with tannin content of 45–50%, gave leather comparable to that obtained from *A. mearnsii* bark (Rao 1967), but this approach has not been adopted commercially.

Acacia nilotica is traditionally used for tanning and retanning in the tropical Africa, and is one of the most important tanning materials in Northern India (Sarkar 1991). Three subspecies of *A. nilotica* occur in Sudan and all of them have high tannin content:

(1) subsp. *nilotica* with glabrous pods strongly constricted between the seeds
(2) subsp. *adansonii* with pods only slightly constricted between the seeds
(3) subsp. *tomentosa,* necklace-like pods narrowly and regularly constricted between seeds.

The present work investigated the quantity and quality of tannins from the three subspecies of *A. nilotica* (garad) 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. Planted all over the country in vast areas for various purposes, *A. nilotica* is thus a good substitute for *A. mearnsii.*

MATERIALS AND METHODS

Preparation of sample

Fresh bark and pods (0.5–2.5 kg) of *A. nilotica* growing around El Obeid and Khartoum were used for this study. 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 air-tight 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 (Doat 1978). The presence of tannins was detected by the gelatin-salt test and their types were identified using the iron-alum and formaldehyde-HCl test (SLTC 1965).

Qualitative analysis

Paper chromatography was done on Whatman No. 1 paper with forestal solvent system (concentrated acetic acid:HCl:water, 10:3:30) (Harborne 1973). The chromatography was developed by ascending method at room temperature (30–36 °C) 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 by exposing to ammonia vapour (Stahl 1969).

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) (Stahl 1969). 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 2 M HCl 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 (Harborne 1973).

Quantitative analysis

The extracts were quantitatively analyzed for total and soluble solids, non-tannins and tannins by the official hide-powder method (Jamet 2000) (hide-powder batch C28). A modification of the hide-powder method, i.e. the combined method (Swain & Goldstein 1964) was also used. Total phenolic materials in the extract were measured using the Folin-Denis method (Folin & Denis 1915). 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 (1998). 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 °C). Then the precipitate was filtered on a tared crucible (G4) before being dried to constant weight at about 100 ± 5 °C to obtain the weight of catechin.

Contents of total phenolics were determined following the methods by Folin and Denis (1915), Hagerman and Butler (1978), and Swain and Goldstein (1964). We used these three different methods to compare their efficiencies and to see the performance of tannins under different extraction conditions. The Folin-Denis reagent was prepared by mixing 85.5 g sodium tungstate, 15.7 g phosphomolybdic acid, 40 ml phosphoric acid (85%) and 600 ml distilled water, refluxing for two hours and making it up to 2 l with distilled water. A weighted sample of freeze-dried tannin (500 mg) was dissolved in 100 ml distilled water and used as stock solution. The stock solution was then subjected to a series of dilutions to give concentrations ranging from 5–100 mg l^{-1}. An aliquot (1 ml) of each of those dilutions was added to 60 ml distilled water in a volumetric flask (100 ml). Folin-Denis reagent (5 ml) was added followed, after precisely 3 min, by 2 ml of saturated sodium carbonate solution (60 g l^{-1}). The contents were further diluted to 100 ml with distilled water, and after 20 min, the UV absorbency was measured at 725 nm.

Tannin precipitation capacity, i.e. astringency factor (a degree by which tannins bind to the proteins and it differs from one plant to another), was determined according to the method by Hagerman and Butler (1978). Bovine serum albumin (BSA), prepared from fraction V albumin (5 g l^{-1} solution in a CH_3COOH/NaOH buffer at pH 4.9, kept at 4 °C), was used as precipitant. The sodium dodecyl sulphate/triethanolamine (SDS/TEA) reagent used contained 5 g SDS and 25 ml TEA per litre. The ferric chloride reagent used was a solution of anhydrous $FeCl_3$ (1.62 g) in 0.1 M HCl (2 l). Stock solutions (0.1%) of freeze-dried tannin extracts in the buffer were prepared, stored at 4 °C and warmed to room temperature before use. Pairs of 10 test tubes containing 0–4 ml BSA were made up to 5 ml with the buffer. A 1 ml aliquot of the stock tannin solution was added to one tube of each pair and 1 ml of the buffer to the other tube as blank. The samples were mixed thoroughly, left to stand for 15 min, and then centrifuged at 5000 rpm for 10 min. The supernatant solutions were decanted into clean tubes and ferric chloride (1 ml) and SDS/TEA (2 ml) reagents were added. The samples were left to stand for 20 min after which the absorbance was measured at 510 nm, using the blank to zero the spectrophotometer. Standard curves for the absorbance of different BSA concentrations were used to calculate the total phenolic contents, and the minimum point on that curve was used to calculate the astringency factor (ASF).

RESULTS AND DISCUSSION

From the iron alum and formaldehyde-HCl test, tannins from the three subspecies of *A. nilotica* screened were of mixed, i.e. hydrolysable-condensed type. *Acacia mearnsii*, which was used as standard, was of condensed type (Table 1). The Stiasny number and the results from the paper and thin layer chromatographies supported these assignments. Stiasny number is catechin number. The presence of catechin means the tannin is condensed, while the presence of both gallic acid and catechin means that the tannin is mixed. In

Table 1 Analysis of the tannin cold aqueous extracts (% oven-dry part extracted)

Species	Part	Total solid (TS)%	Soluble solid (SS)%	Tannins (T)%	Non tannins (NT)%	Extraction ratio (T/NT)	Stiasny number	Gallic acid	Tannin type	Purity (T/SS)
Acacia nilotica	Bark	25.2a	25.1a	16a	9.1a	1.8a	18.8a	+	HC	0.6a
ssp. *adansonii*	Pods	58.6b	50.4b	28.9b	27.5b	1.1b	34.8b	+	HC	0.6a
A. nilotica	Bark	33.4c	32.1c	23.7c	8.5c	2.9c	27.9c	+	HC	0.7a
ssp. *tomentosa*	Pods	63.1d	62.1d	39.4d	22.7d	1.7a	36d	+	HC	0.6a
A. nilotica	Bark	16e	15.2e	9.6e	5.7e	1.7a	9.2e	+	HC	0.6a
ssp. *nilotica*	Pods	40.8f	39.3f	26.6f	12.7f	2.1d	28.2c	+	HC	0.7a
A. mearnsii	Bark	51.8g	48.7g	39.8d	8.9c	4.5e	45.7f	-	C	0.8a
r^2		0.99	0.99	0.99	0.99	0.99	0.99			0.99
F		< 0.0001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001			< 0.001
Mean		41.21	38.9	26.28	13.58	2.26	28.66			0.66

Means with the same letter are not significant with Duncan's multiple range tests at 0.05.
H = Hydrolysable tannin, C = condensed tannin, + = Detected, - = not detected

this experiment, paper and thin-layer 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, dihydrofisetin and robinetin, which were used as standards, were not detected (Table 2).

The quantitative data indicated that all parts (bark and pods) of the subspecies studied, except bark of *A. nilotica* subsp. *nilotica*, had more than 10% (oven-dry basis) of tannin, i.e. the level of commercial interest (Table 1). Of these three subspecies, two, namely, subspecies *tomentosa* and *adansonii* had an acceptable extraction ratio (tannin to non-tannin) of 1.5–3.0.

The tannin purity or the ratio of tannin/ soluble solids was ≥ 0.6 for all subspecies of *A. nilotica* (based on the amount of non-tannins which interact positively if in considerably large amount). This is within the standard range of 0.5–1.0 (Seigler *et al* 1986). However, the type of tannin present and the part extracted are also important. If the tannin content of a bark is high, but the bark is very thin, a huge amount of bark will be needed to extract enough tannin for economic feasibility, unless the bark is already available as waste from other uses of the wood. Also different parts of a species, bark and pods may contain the same type of tannin but in different proportions. The subspecies used in this study all have thick barks, which are waste material. Our study showed that tannin content was higher in the deseeded pods than in the bark. The Stiasny numbers indicated that all the species studied contained condensed tannin in varying amounts (9.2–45.7) (Table 1).

The tannin content determined by the hide-powder method was highest (39.8%) for *A. mearnsii* followed by *A. nilotica* ssp. *tomentosa* pods (39.4%) (Table 1). These data were compared with those obtained from the spectroscopic method of Swain and Goldstein (1964) and also with two methods for total phenolics (Folin & Denis 1915, Hagerman & Butler 1978) (Table 3). 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 phenolics content by the Hagerman and Butler method (1978) 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 3). Care should be taken when comparing tannin content determined by different methods as the isolation procedures may affect the proportion and types of phenolics present.

The relative astringency values for most of these tannins were quite close to that of *A. mearnsii* tannin (Table 3). This shows that *A. nilotica* can be used in place of *A. mearnsii* because the degree of relative astringency or the ability of their tannin to combine with protein is close to that of *A. mearnsii*; in other words *A. nilotica* can give leather with characteristics comparable with *A. mearnsii*.

The protein precipitation curve for the tannins from *A. mearnsii* bark and the three *A. nilotica* subspecies pods and bark reflected their different nature and relative astringency (Figure 1). The fairly gradual solubilization of wattle and garad tannins 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 penetration of 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 obtained from the pods of the three *A. nilotica* subspecies should penetrate the hide more extensively and the reaction should not be weaker in terms of poorer tanning or greater vulnerability to microbiological damage.

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Table 2 Results of thin layer (TLC) and paper (PC) chromatography of hydrolyzed bark extracts

	Part	Extracted with	Standard, RF × 100															
			Gallic acid		Tannic acid		Catechin		Epicatechin		Fisetin		Dihydrofisetin		Robinetin		Unknown	
			TLC	PC	TLC	PC	TLC	PC	TLC	PC	TLC	PC	TLC	PC	TLC	PC	TLC	PC
			82	63	56	32	78	64	66	64	66	15	67	20	72	35		
A. nilotica	Bark	Amyl alcohol	-	-	-	-	77	67	-	64	-	-	-	-	-	-	67	-
ssp. *adansonii*		Ethyl acetate	81	63	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Pods	Amyl alcohol	-	-	-	-	77	67	67	64	-	-	-	-	-	-	-	-
		Ethyl acetate	82	63	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A. nilotica	Bark	Amyl alcohol	-	-	-	-	78	66	-	64	-	-	-	-	-	-	55	-
ssp. *nilotica*		Ethyl acetate	81	63	-	-	-	-	-	65	-	-	-	-	-	-	-	-
	Pods	Amyl alcohol	-	-	-	-	77	67	-	65	-	-	-	-	-	-	-	51
		Ethyl acetate	82	63	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A.nilotica	Bark	Amyl alcohol	62	-	-	-	77	67	67	66	-	-	-	-	-	-	55	-
ssp. *tomentosa*		Ethyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Pods	Amyl alcohol	-	-	-	-	-	66	-	-	-	-	-	-	-	-	-	-
		Ethyl acetate	-	-	-	-	79	-	-	-	-	-	-	-	-	-	-	-
A. mearnsii	Bark	Amyl alcohol	-	-	-	-	77	67	66	66	65	15	-	-	-	-	64	-
		Ethyl acetate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	33	8

Table 3 Tannin content, total phenolics and relative astringency of the tannin extract by different methods

Species	Part	Tannin content, % in oven dry part extracted		Extract ratio Tannin/non-tannin		Total phenols, % in oven dry part			
		Hidepowder method	Combined method	Hidepowder method	Combined method	Combined method	Folin-Denis method	Hagerman Butler method	Relative astringency
A. nilotica	Bark	16a	15.9a	1.8a	0.5a	1.8a	0.5a	0.5a	0.5a
ssp. *adansonii*	Pods	28.8b	27.5b	1.1b	0.8a	1.1b	0.8a	0.8a	0.8a
A. nilotica	Bark	9.5c	9.1c	1.7a	0.3a	1.7a	0.3a	0.3a	0.3a
ssp. *nilotica*	Pods	26.6d	25.3d	2.1a	0.6a	2.1a	0.6a	0.6a	0.6a
A. nilotica	Bark	23.6e	22.3e	2.8c	0.6a	2.8c	0.6a	0.6a	0.6a
ssp. *tomentosa*	Pods	39.4f	38.9f	1.7a	0.6a	1.7a	0.6a	0.6a	0.6a
A. mearnsii	Bark	39.8f	38.1f	4.5d	2.7b	4.5d	2.7b	2.7b	2.7b
r^2		0.987	0.987	0.987	0.987	0.987	0.987	0.987	0.987
F		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Mean		26.24	25.3	2.24	0.87	2.24	0.87	0.87	0.87

The means with the same letter are not significant with Duncan's multiple range tests at 0.05.

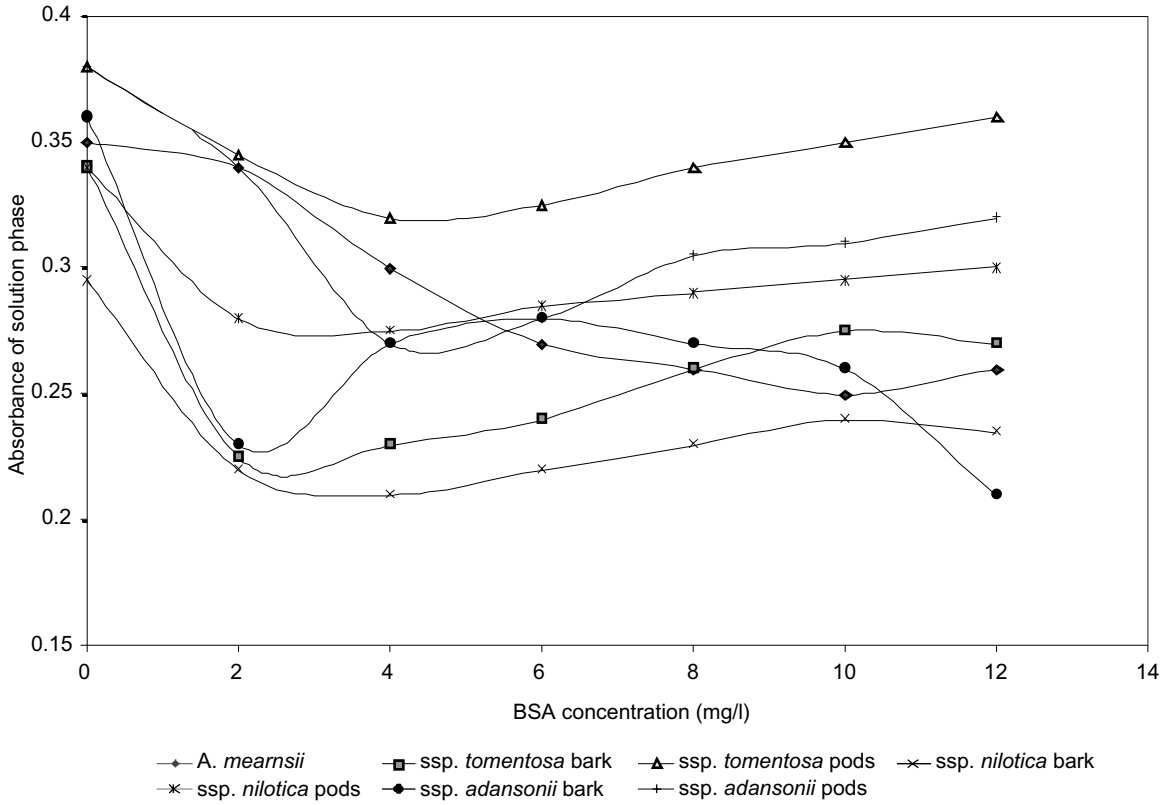

Figure 1 Protein precipitation curves obtained for the phenolics in the tannin extracts from *A. mearasii* bark, *A. nilotica* ssp. *adansonii* (bark and pods), *A. nilotica* ssp. *nilotica* (bark and pods), and *A. nilotica* ssp. *tomentosa* (bark and pods)

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