# COMPARATIVE STUDY OF TANNINS OF *ACACIA NILOTICA* AN INDIGENOUS TANNING MATERIAL IN SUDAN WITH *ACACIA MEARNSII*

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## Abstract

**Tannins from four indigenous and exotic woody plant species were investigated by different methods with the objectives of comparing and evaluating the potential for commercial utilization. All the three subspecies of *Acacia nilotica* (garad) which were studied contained more than 10% tannin necessary for commercial interest. The tannins of the *Acacia nilotica* subspecies were of the hydrolysable- condensed types while that of *Acacia mearnsii* (wattle) was of the condensed type. All four species contained tannin quantities of commercial interest.**

**Keywords: Tannins, garad, wattle, astringency, tanning**

## Introduction

Sudan has many indigenous and exotic woody plant species, which contain tannins in different quantities. Some of them were analysed by Kaith (1968), but no systematic screening has been reported. The Sudanese leather industry uses mainly imported vegetable (*A. mearnsii*) and mineral tanning materials (chrome). 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 (*A. mearnsii* bark is used).

A mix of equal proportions of spray-dried extracts from *A. nilotica* (garad husks) and *Azadirachta indica* (barks) with a tannin content of 45 - 50%, gave leather comparable with that obtained with *A. mearnsii* (wattle) (Rao, 1967), but this approach has not been adopted commercially.

The *A. nilotica* is traditionally used for tanning and retanning in tropical Africa, and is one of the most important tanning materials in Northern India (Sarkar, 1991). It is tree of moderate sized, spiny, and evergreen confined to flooded areas, depressions and river-beds (Thirakul, 1984; EL Amin, 1990).

Three subspecies of *A. nilotica* dominated in Sudan:

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- Sub sp. *nilotica* with glabrous pods, strongly constricted between the seeds.
- Sub sp. *adansonii* with the pods only slightly constricted between the seeds
- Sub sp. *tomentosa* with the pods necklace-like narrowly and regularly constricted between the seeds.

The present work evaluated the tannins from three subspecies of *A. nilotica* (garad) of central and western Sudan, in the hope that they could be used in place of *A. nilotica* (wattle) for commercial purposes.

## Materials and Methods

### Preparation of Samples

The raw materials investigated were the bark and pods (0.5 - 2.5 kg), collected fresh from plant species around El Obeid and Khartoum. The Soba Forestry Research Centre Herbarium confirmed the species identity.

The samples were air-dried and reduced to powder with a star mill. The fractions passing through 40-mesh and retained on 85- mesh sieves were collected, thoroughly mixed and kept in tightly closed containers.

### Extraction and Analysis of Tannins

The cold water extracts (2 L) were obtained with an ALCA-Palsy apparatus (Doat, 1978). The presence of tannins was detected by the gelatin-salt test, and testing with iron-alum and formaldehyde-HCl identified their types (SLTC, 1965). The extracts were quantitatively analysed for total solids and soluble, ash, non-tannins and tannins by the official hide-powder method (ALCA, 1957; Jamet, 2000) (hide-powder batch C28).

A modification of the hide-powder method (Combined method) by Swain and Goldstein (1964) with a simpler procedure and less time was also used. The catechin number was determined with Stiasny reagent (Yazaki and Hillis, 1998).

The total phenolics content was determined following Hagerman and Butler (1978), and also by the Folin and Denis (1915) method. The Folin and 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 2 h and making it up to 2 l with distilled water.

The raw material for chromatographic analysis (5 g) was hydrolysed with 2 M HCl by refluxing for 30 min, cooled, filtered and the filtrate was extracted with ethyl acetate. The aqueous layer was heated to remove any traces of the solvent and extracted with a small volume of amyl alcohol. The solvent extracts were concentrated to thick syrup under vacuum (Harborne, 1973).

The paper chromatography was done on Whatman No. 1 paper with Forestal solvent system (acetic acid-Conc. HCl- water, 10:3:30) (Harborne, 1973). The chromatographs were developed by ascending method at room temperature (30 - 36$^{o}$C) to a height of 7 - 15 cm and the 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 (Stahl, 1969).

The thin layer chromatography was done with sheets precoated with silica gel 60f - 254 (0.2 mm thick) and polyamide 6 (0.1 mm thick), and Whatman Cellulose Powder C41. The solvent systems used were (a) acetone- propanol-water (5:4:1) (Stahl, 1969); (b) Forestal system; (c) toluene-ethyl acetate-formic acid (4:4:1) (Stahl, 1969). The standards used were tannic acid, catechin, gallic acid, epicatechin, fisetin, dihydrofisetin and robinetin.

Tannin precipitation capacity (astringency factor) was determined using the method of Hagerman and Butler (1978). Bovine serum albumin (BSA), prepared from fraction V albumin (5 g/l solution in a $CH_3COOH$/NaOH buffer at pH 4.9, kept at 4$^{o}$C), was used as a precipitant. Sodium dodecyl sulphate/triethanolamine (SDS/TEA) reagent contained 5 g SDS and 25 ml TEA per litre. Ferric chloride reagent 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$^{o}$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 a blank. The samples were mixed thoroughly, left to stand for 15 min, and then centrifuged at 5,000 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 absorbance of the sample was measured at 510 nm for 20 min after zeroing the spectrophotometer with the blank. Standard curves for the absorbance of different BSA concentrations were used to calculate the astringency factor (Mugedo and Waterman, 1992).

For the relative astringency tests (Harborne, 1984) the raw material (10 g) was extracted with 50% methanol and the extracts were concentrated under vacuum and taken up in a minimum volume of water (Bate-Smith, 1972). An aliquot of 1 ml of the extract was mixed with 1 ml fresh human blood (from a fingertip, diluted in proportion 1:50 with distilled water). The mixture obtained was immediately shaken and centrifuged for 5 min at 3,000 rpm. The residual hemoglobin was determined by its absorbance versus the concentration standard curve obtained for tannic acid.

## Results and Discussions

From the iron alum test (Table 1) all the subspecies of *A. nilotica* screened were of mixed, hydrolysable-condensed (gallo-catechol) type, and *A. mearnsii* (wattle), which was used as standard, is of a condensed (catechol) type only. The Stiasny (catechin number) and gallic acid test results support these assignments. The quantitative data indicated that 6 parts (barks and pods) of three subspecies of *A. nilotica* and only bark part from *A. mearnsii,* when extracted, contained more than 10% (oven-dry basis) of tannin, the level of commercial interest. Of these four species, three 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 good, ≥ 0.6, for all subspecies of *A. nilotica*. 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.

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

| Parameters | *A. nilotica* ssp *adansonii* | | *A. nilotica* ssp *tomentosa* | | *A. nilotica* ssp *nilotica* | | *A. mearnsii* |
|---|---|---|---|---|---|---|---|
| **Part extracted** | **Bark** | **Pods** | **Bark** | **Pods** | **Bark** | **Pods** | **Bark** |
| Total solids (TS) | 25.2 | 58.6 | 33.4 | 63.1 | 16 | 40.8 | 51.8 |
| Soluble solids (SS) | 25.1 | 50.4 | 32.1 | 62.1 | 15.2 | 39.3 | 48.7 |
| pH | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Tannin (T), % | 16 | 28.9 | 23.7 | 39.4 | 9.6 | 26.6 | 39.8 |
| Non-tannin (NT) % | 9.1 | 27.5 | 8.5 | 22.7 | 5.7 | 12.7 | 8.9 |
| Extraction ratio (T/NT) | 1.8 | 1.1 | 2.9 | 1.7 | 1.7 | 2.1 | 4.5 |
| Catechin number | 18.8 | 34.8 | 27.9 | 36 | 9.2 | 28.2 | 45.7 |
| Gallic acid | + | + | + | + | + | + | - |
| Tannin present | HC | HC | HC | HC | HC | HC | C |
| Purity (T/SS) | 0.6 | 0.6 | 0.7 | 0.6 | 0.6 | 0.7 | 0.8 |

**H = Hydrolysable tannin; C = Condensed tannin**

Different parts of a species, bark, pods or leaves, had the same type of tannin but in different proportions. Usually the 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).

Paper and thin-layer chromatography with different solvent systems (Table 2) confirmed the presence of catechin and gallic acid, and showed that tannic acid, fisetin, epicatechin and some unidentified phenolics were present, while dihydrofisetin and robinetin were not detected.

The tannin content determined by the official hide-powder method (Table 3) was highest (39.8%) for *A. mearnsii* (wattle) followed by *A. nilotica* ssp *tomentosa* pods (garad) (39.4%). These data were compared with those from the spectroscopic method (Combined Method) of Swain and Goldstein (1964) (Table 3) and also with two methods for total phenolics (Hagerman and Butler, 1978; Folin and Denis, 1915) (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 phenolics content by the Hagerman and Butler method was approximately half that with Folin and Denis assay, but the correlation between the two assays was still high ($R^2$ = 70.9%, n = 24, P < 0.01).

A modification of the hide-powder method (Combined method) by Swain and Goldstein, (1964) gave also slightly lower values and extraction rate (Tables 3 and 4). 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.

Howes's (1953) value for the ratio of tannin/non-tannin for *Acacia mearnsii* (wattle) tannin (2.5) was much lower than the 4.5 for our sample determined by the official hide-powder method.

**Table 2. Thin layer (TLC) * and paper (PC)* * chromatography of hydrolyzed bark extracts**

| Species | Part | Extracted with | Standard, Rf x 100 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 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 | | |
| *A.nilotica* | Bark | Amyl alcohol | - | - | - | - | 77 | 67 | - | 64 | - | - | 67 | - |
| ssp | | Ethyl acetate | 81 | 63 | - | - | - | - | - | - | - | - | - | - |
| *adansonii* | Pods | Amyl alcohol | - | - | - | - | 77 | 67 | 67 | 64 | - | - | - | - |
| | | Ethyl acetate | 82 | 63 | - | - | - | - | - | - | - | - | - | - |
| *A.nilotica* | Bark | Amyl alcohol | - | - | - | - | 78 | 66 | - | 64 | - | - | 55 | - |
| ssp | | Ethyl acetate | 81 | 63 | - | - | - | - | - | 65 | - | - | - | - |
| *nilotica* | Pods | Amyl alcohol | - | - | - | - | 77 | 67 | - | 65 | - | - | - | 51 |
| | | Ethyl acetate | 82 | 63 | - | - | - | - | - | - | - | - | - | - |
| *A.nilotica* | Bark | Amyl alcohol | - | 62 | - | - | 77 | 67 | 67 | 66 | - | - | 55 | - |
| ssp | | Ethyl acetate | - | - | - | - | - | - | - | - | - | - | - | - |
| *tomentosa* | Pods | Amyl alcohol | - | - | - | - | - | 66 | - | - | - | - | - | - |
| | | Ethyl acetate | - | 62 | - | - | 79 | - | - | - | - | - | - | - |
| *A.mearnsii* | Bark | Amyl alcohol | - | - | - | - | 77 | 67 | 66 | 66 | 65 | 15 | 64 | - |
| | | Ethyl acetate | - | - | - | - | - | - | - | - | - | - | 33 | 8 |

* Adsorbent: Polyamide precoated plate (10 x 10 cm); solvent system: acetone-propanol-water (5/4/1); detection: UV/254 nm; $FeCl_3$.

** Adsorbent: Whatman paper no. 2; solvent system: acetic acid-conc. HCl- water (10/3/30); detection: UV/254 nm; strong ammonia vapor.

Most of the species, with a tannin extraction ratio of 1.7 and above (the level of potential commercial interest), are fast growing and fairly widespread in the arid and semi-arid regions studied in Sudan.

The relative astringency (RAs) values (Table 3) for most of these tannins were quite close to that of *A. mearnsii* tannin (wattle).

The protein precipitation curve (Figure 1) for the tannins from *A. mearnsii* bark (wattle) and the three *A. nilotica* subspecies pods and bark (garad) reflected their different natures and relative astringency. The fairly gradual solublization 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 material would have to be controlled by selection of pH and concentration, and the resulting leather might be hard and coarse. In contrast the less astringent tannin of the three *A. nilotica* subspecies (pods) (Figure 2) should penetrate the hide more extensively and the reaction should not be weaker in terms of poorer tanning or greater vulnerability to microbiological damage.

## Conclusions

All the species studied contain more than 10% tannin needed for commercial exploitation. The richest exotic species, which is of limited distribution in Sudan, was *A. mearnsii* (bark), followed by the three indigenous subspecies of *A. nilotica* (pods and bark). All the tannin studies contained catechin, but most were of the mixed gallo-catechol type.

**Table 3. Tannin content by alternative methods, and relative astringency to tannic acid of the tannin extract**

| species | Part | Tannin content, % in oven-dry part extracted | | Extraction ratio (Tannin/non-tannin) | | Relative astringency to tannic acid (RAs) |
|---|---|---|---|---|---|---|
| | | Hide-powder method | Combined method | Hide-powder method | Combined method | |
| *A. nilotica* ssp *adansonii* | Bark | 16.0 | 15.9 | 1.8 | 0.5 | 0.12 |
| | Pods | 28.8 | 27.5 | 1.1 | 0.8 | 0.12 |
| *A. nilotica* ssp *nilotica* | Bark | 9.5 | 9.1 | 1.7 | 0.3 | 0.12 |
| | Pods | 26.6 | 25.3 | 2.1 | 0.6 | 0.11 |
| *A. nilotica* ssp *tomentosa* | Bark | 23.6 | 22.3 | 2.8 | 0.6 | 0.12 |
| | Pods | 39.4 | 38.9 | 1.7 | 0.6 | 0.11 |
| *A. mearnsii* | Bark | 39.8 | 38.1 | 4.5 | 2.7 | 0.16 |

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

| Species | Part | Total phenolics, % in oven-dry part extracted with cold water | | | Astringency factor (AsF) |
|---|---|---|---|---|---|
| | | Combined method | Folin-Denis method | Hagerman-Butler method | |
| *A. nilotica* ssp | Bark | 41.2 | 16.1 | 8.0 | 0.02 |
| *adansonii* | Pods | 45.6 | 27.9 | 13.9 | 0.04 |
| *A. nilotica* ssp | Bark | 57.3 | 11.5 | 5.7 | 0.02 |
| *nilotica* | Pods | 58.8 | 25.9 | 12.9 | 0.04 |
| *A. nilotica* ssp | Bark | 52.8 | 22.8 | 11.4 | 0.01 |
| *tomentosa* | Pods | 82.7 | 38.9 | 11.6 | 0.09 |
| *A. mearnsii* | Bark | 72.8 | 35.6 | 17.8 | 0.06 |

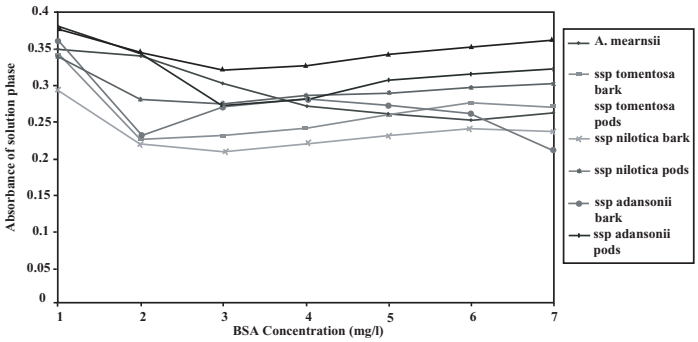


**Figure 1. Protein precipitation curves obtained for the phenolics in the tannin extracts from *Acacia mearnsii* bark; *A. nilotica* ssp *adansonii* (bark and pods); *A. nilotica* ssp *nilotica* (bark and pods); and *A. nilotica* ssp *tomentosa* (bark and pods)**

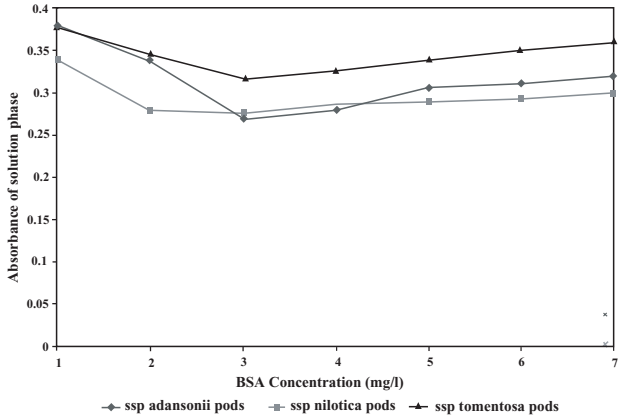


**Figure 2. Protein precipitation curves obtained for the phenolics in the tannin extracts from *A. nilotica* ssp *adansonii* (pods); *A. nilotica* ssp *nilotica* (pods); and *A. nilotica* ssp *tomentosa* (pods)**

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