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Variations in Tannin's Contents of *Acacia nilotica* (L.) Willd. ex Del. in the Sudan

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Abstract: The present study checks the variation in tannin's contents of *Acacia nilotica* in the Sudan according to subspecies, tissues, pod's developmental stage and site of growth. Samples were collected from six different sites and Hyde powder method was used for the determination of the percentages of total tannin's contents, while thin layer chromatography test (a polyamide paper and methanol and water 40:60 solvent) was carried for qualitative comparison. The study revealed that tannin's contents varied significantly among the three subspecies in the Sudan, especially at Elfasher, Elgazaire and Elfaw sites, where subspecies *nilotica* being best followed by *tomentosa* and then *adansonii*. Pods were generally significantly better than bark in their tannin's contents and the premature pods were always better than the mature ones. The highest amount (54%) was recorded from the premature pods of subspecies *nilotica* at Elfaw site. Qualitatively, there were variations in the fractionation ratio (Rf) values of the tannin compounds among the three subspecies according to tissue and pod's developmental stage. The values were increasing from initial to the premature stage of pods, while the sites were generally in the order: Shambat; Elgazaire, Elfaw and Nayala and then Elfasher, for the compounds of mature pods.

Key words: Tannin, *Acacia nilotica*, subspecies, pods, bark, sites

INTRODUCTION

Acacia nilotica (Sunut in Arabic) is a member of subfamily *Mimosoideae* of leguminous trees. It is of multiple uses in the Sudan, Africa and many Arabic countries. The tree is readily distinguished by the long white spines, yellow head inflorescence and the grey necklace-like pods. Three subspecies are commonly found in the Sudan, namely *tomentosa* that characterized by the pubescent pods and grow throughout Sudan, *nilotica* that characterized by the glabrous pods and grow along the White Nile and *adansonii* that characterized by the broad pods and grow in western Sudan^[1].

In public medicine, the fruits of subspecies *tomentosa* are used in Guinea for treating cough^[2], in Ghana for toothache^[2], while bark is used in Nigeria to combat dysentery^[3] and different parts of subspecies *nilotica* are used as tonic^[4]. In Sudan, the fruits of the different subspecies are used against chest diseases, bleeding and diarrhea and in Yemen to reduce sugar levels for diabetics.

Many researches have indicated that tannins and related compounds have different astringent effects^[5] and an inhibitory effects on pathogens^[6]. This accounts for their medicinal properties. Accordingly, pods of *Acacia*

nilotica were found to have algicidal^[7], anti-protozoal^[8], antibacterial^[9] and antifungal^[10] effects. Tannin as group of complex hydrocarbon substance in the plants, consist of two types of polyphenolic systems, namely hydrolysable or condensed. Many investigators^[7,11-13] indicated the presence of tannins in the fruits and/or bark of *Acacia nilotica*.

However, no literature were found concerning the variations of tannin's contents according to subspecies sites and tissues.

To test the hypothesis that there are considerable differences in Tannin's contents of *Acacia nilotica* according to the subspecies, site, tissue and its stage of development, the present study has been conducted. The main objectives were, to compare between the tannin's contents among the different subspecies at the same site, the different parts of the tree of the same species and sites, the different stages of pod development and the different sites for the same subspecies.

MATERIALS AND METHODS

The experiments of the present study were carried during the period 2000-2001 at Shambat area, University of Khartoum, Sudan. Six sites were chosen randomly as

source of samples to study the effects of site on the tannin's contents. These are, Elobied in western Sudan (latitude 13°N and longitude 30°E) with rainfall of about 600 mm/year and generally sandy clay loam soil, Nayala (Latitude 13°N and Longitude 24°E) with main rainfall of about 720 mm/year and generally sandy clay soil, Elfasher (Latitude 13°N and longitude 26°E) with main rainfall of about 680 mm/year and generally sandy clay soil, Elfaw (latitude 14°N and longitude 34°E) with main rainfall of about 400 mm/year and generally silty and sandy clay soils, Elgazaire (latitude 13°N and longitude 33°E) with main rainfall of about 850 mm/year and generally heavy clay soil and Shambat (latitude 15°N and longitude 30°E) with main rainfall of about 250 mm/year and generally heavy clay and alkaline soil.

Field trips were carried to the different sites in the appropriate season to collect the samples required for the experiment. The collected samples of pods and bark were dried under shade for three days, purified and ground by 0.3 mm fineness grinder (seeds were discarded from pods prior to grinding)

Hyde powder method was adopted. Five grams were weighed for each sample into a conical flask, diluted with 150 mL water and heated over boiling water bath for 30 min. The mixture was cooled and diluted to 250 mL into a volumetric flask. The solids allowed to settle and filtered.

To determine the total water extractives (T) 50 mL of the drug extracts was evaporated and the residue was dried in an oven at 105°C for 4 h and weighed. To get the water extractives not bound with hyde powder (T), 80 mL of the drug extracts was mixed to 600 g of the hyde powder and the mixture was filtered after shaking and 50 mL of the clear filtrate was evaporated to dryness and then the residue was dried in an oven at 150 and weighed. To determine the water soluble of hyde powder, 6 g of the hyde powder were added to 80 mL water, shaken and filtered. Fifty milliliter of the clear filtrate were evaporated in an oven at 105°C and weighed. The tannin's percentage was calculated from the following formula:

$$\{T-(T+T)\} \times 500/W$$

All data obtained were subjected to statistical analysis. Analysis of variance was performed separately for each site. Duncan's Multiple Range Test was used for the separation of means.

The preparation of the samples for the Chromatographic tests was done as follows:

First, the fingerprints were done in the same way of the hyde powder i.e. 5 g of each sample were put into a conical flask, diluted with 150 mL of water, heated over

boiling water bath for 30 min and the mixture was cooled and diluted with water to 250 mL in a volumetric flask, filtered and the filtrate was used for the test.

Secondly, for the polyamide papers, polyamide plates (6×12 cm) were used. Simple spots of each sample extract were applied using capillary pipettes as application devices. Standard separating tanks of different solvents system were used and the plates were allowed to develop. The solvent system (40:60 water to methanol) front was then allowed to travel for 10 cm from the base line, after which the plates were viewed in day light and under ultraviolet light (254/365) and then the plates were sprayed with ferric chloride.

The ratio of fractionation (Rf value) of each compound of the samples was calculated as the ratio of the distance traveled by the compound and that traveled by the solvent.

RESULTS

Considerable variations in tannin's contents of *Acacia nilotica* were observed according to subspecies, part of the tree (pods, bark), pods developmental stage and site of collection.

For Elfasher, Elgazaire and Elfaw sites, mean separation showed that the best combination is the pod of subspecies *nilotica* at the premature stage. However, the second best result was the mature pods of the same subspecies (Table 1).

However, for Nayala site, premature pods of subspecies *adansonii* gave the best result, while the premature pods of subspecies *tomentosa* gave the second best result (Table 1)

For Shambat site, where the comparison was within the subspecies *tomentosa*, the initial pods gave the best result, followed by premature pods, mature pods and then the bark (Table 2). While for Elobied site, where the comparison was within the subspecies *adansonii*, the premature pods gave the best result and no significant differences shown between the results of the mature pods and bark (Table 2).

The qualitative tests also show variations among the subspecies, sites, tissues and their developmental stages.

Concerning subspecies *tomentosa*, data on (Table 3) show differences in Rf values for the different sites. At station 1, samples from Nayala, Elgazaire and Elfaw have had the same value (0.0750) whereas Shambat and Elfasher were different at station 1.

Data obtained from the test show differences in the Rf values among the three pods developmental stages at site Shamabat where four mobile stations were studied (Table 4). The values are increasing from the initial stage

Table 1: The variation in tannin's contents according to the subspecies and tissues at Elfasher (1), Elfaw (2), Elgazaire (3) and Nayala (4) sites

Bark	Mature	Pods: premature	Subspecies
A	A	A	(1) <i>nilotica</i>
33.666c	45.333b	53.000a	
A	B	B	(1) <i>tomentosa</i>
33.666c	38.333b	45.333a	
A	A	A	(2) <i>nilotica</i>
29.333c	46.000b	45.000a	
A	B	B	(2) <i>tomentosa</i>
29.000c	37.666b	42.000a	
A	A	A	(3) <i>nilotica</i>
15.333c	38.333b	39.666b	
A	B	B	(3) <i>tomentosa</i>
13.333c	27.000b	36.333a	
A	A	A	(4) <i>adansonii</i>
24.000c	32.333b	58.333a	
A	B	B	(4) <i>tomentosa</i>
30.333c	31.000b	41.333a	

Table 2: The variation in total tannin content of *Acacia nilotica* subspecies *tomentosa*-tissues (Shambat site) and *adansonii*-tissues (Elobied site)

Sub species	Pods: initial stage	Premature stage	Mature stage	Bark
<i>Tomentosa</i>	A	B	C	D
	53.000	42.667	37.000	25.000
<i>Adansonii</i>		A	B	B
		53.333	24.333	25.000

Table 3: The effect of site on Rf values for the tannin compounds of mature pods subspecies *tomentosa*

Sambat	Elfazaire	Elfaw	Nyala	Elfasher
0.0500	0.0750	0.0750	0.0750	0.0875
0.0875	0.3750	0.3750	0.3875	0.3875
0.4000	*	*	*	*
0.7250	*	*	*	*

*No separated compound

Table 4: The variation in the ratio of fraction (Rf values) for tannin compounds of pods developmental stages of *Acacia nilotica* sub species *tomentosa* (Shambat)

Spot No.	Initial stage	Premature stage	Mature stages
1	0.0875	0.0750	0.0500
2	0.2625	0.1000	0.0875
3	0.4000	0.4000	0.4000
4	0.7625	0.7375	0.7250

(0.0875) to the mature stage (0.0500) at station 1, while other stations seem to have the same value.

As far as the bark is concerned, it is of lower value, where it is comparable (site Nayala) than the pods, at station 1 (Table 5).

For subspecies *nilotica*, all data obtained (indicated three mobile stations) show that the three sites of Elfasher, Elfaw and Elgazaire differ only at stage 1 concerning tannin's contents of premature and mature stages of pods (Table 6). Similar results were obtained for the subspecies *tomentosa* (Table 7).

However, for subspecies *adansonii*, Elobied and Nayala sites do not differ in tannin's contents of premature and mature of pods, while the bark samples seem to be different (Table 8).

Table 5: The variation in Rf values for tannin compounds of subspecies *tomentosa*, pods-developmental stages-and bark (Nyala)

Spot No.	Premature stage	Mature stage	Bark
1	0.0875	0.0875	0.3000
2	0.3500	0.3500	0.3750
3	0.4250	0.4250	*
4	0.7625	*	*

*No separated compounds

Table 6: The effect of sites on the Rf Values for tannin compounds of subspecies *nilotica*, pods at premature and mature stages

	Elfasher	Elfaw	Elgazaire
Premature stage			
	0.0750	0.1125	0.0875
	0.2750	0.2750	0.2750
	0.3620	0.3620	0.3620
Mature stage			
	0.0625	0.0875	0.0750
	0.2500	0.2500	0.2500
	0.3620	0.4125	0.3620

Table 7: The variation in Rf of sub species *tomentosa* tissues-pods (mature and premature) at Elfaw, Elgazaire and Elfasher sites

Site and Spot No.	Premature stage	Mature stage
Elfaw: 1	0.0875	0.0750
2	0.3750	0.3750
Elgazaire: 1	0.0875	0.0750
2	0.3750	0.3750
Elfasher: 1	0.1000	0.0875
2	0.3875	0.3875

Table 8: The effects of sites on the Rf values of subspecies *adansonii*, pods (premature and mature stages) and bark

	Nayala	Elobied
Premature pods		
	0.0875	0.0875
	0.3500	0.3500
	0.4250	0.4250
	0.7625	0.7625
Mature pods		
	0.0875	0.0875
	0.3500	0.3500
	0.4250	0.4250
Bark		
	0.3000	0.0875
	0.3750	0.1750

DISCUSSION

Considerable variations in tannin's contents of *Acacia nilotica* were observed according to subspecies, part of the tree (pods, bark), pods developmental stage and site of collection. This confirmed the hypothesis and fulfilled the objectives set for the experiment.

The results, in general, are in agreement with those of Cannos^[13] which indicated the amount of tannin synthesized by Olants varied considerably with plant species, subspecies, cultivar and tissue. This may be related to ontogenetic changes.

The present study revealed that tannin's contents decrease from early stages of pods to mature stage, thus confirm the study of Giner^[14] who reported that the quantity of tannin decreases as the fruits ripen.

The present study also indicates differences in tannin's contents of the same subspecies according to different sites. This is in accordance with Dilleman^[15] which might be attributed to environmental modifications.

Data obtained from the chromatography test show that the Rf values of the major spots on the chromatographic fingerprints were varying in their component according to the subspecies, part of the tree and the stages of pod's development. Many previous studies^[13,16,17], on different tree species, resulted in similar findings. However study on subspecies *tomentosa* and *nilotica*, respectively had isolated the components^[7,18] similar to the present study.

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