CHEMOTAXONOMIC DISTINCTION OF SELECTED CLOSELY RELATED *ACACIA* SPECIES USING CHEMICAL PROPERTIES OF THEIR GUM EXUDATES

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ABSTRACT

Although Acacia senegal var. senegal and Acacia senegal var. leiorhachis are regarded as being closely related botanically, their gum exudates have shown to possess different properties. The properties of the gum exudate from A. senegal var leiorhachis differ from that obtained from A. senegal var. senegal (widely accepted as the source of commercial gum arabic) by being much more viscous, less acidic and having higher proportions of insoluble gel fraction and nitrogen contents. The specific optical rotation values of the gum exudates from these two species have also been found to be different. It is proposed that some of the physicochemical properties of the plant's gum exudates should be included in their taxonomic descriptions to provide an unambiguous distinction of the species. The properties of the gum exudates from A. sieberana var. woodii and A. sieberana var. sieberana, which are also considered to be closely related botanically are similar and it is justifiable to retain them as variants of the same species.

INTRODUCTION

The species *A. senegal* is extremely variable. *A. senegal* var. *senegal* shows a wide range of variation in terms of indumentum, armature, flower size and general habit. *A. senegal* var. *leiorhachis* differs from *A. senegal* var. *senegal* solely by its glabrous inflorescence axis, a difference considered as a minor variation by Brenan (1959). *A. senegal* var. *kerensis* which is found in Kenya also seems not to be uniform but its bushy habit is distinctive in the field. It has been suggested, however, that the status of these variants of *A. senegal* is quite uncertain (Brenan 1959). It is not yet known whether they represent a response to an unusual habitat, exceptions in an otherwise normal population, or just distinct local races.

Morphologically, *A. sieberana* var. *woodii* and *A. sieberana* var. *sieberana* can be differentiated by their crown only. *A. sieberana* var. *woodii* has a characteristic mushroom-shaped crown of great width in proportion to the length of the bole, which contrasts sharply in the field with the ascending branching of *A. sieberana* var. *sieberana*.

The use of analytical data to provide chemotaxonomic evidence to distinguish closely related species and/or variants of species has previously been suggested by some workers (Anderson 1976, 1977 a). It has also recently been shown by Baldwin *et al.* (1999) that gums harvested from different subspecies may have very distinct chemical compositions. Idris *et al.* (1998) working on eight authenticated samples, reported that no obvious trends could be observed with respect to age or source of the tree for the monosaccharide compositon, protein and amino acid content and optical rotation for gum exudates obtained from *Acacia senegal* from two different areas in Sudan. Viscosity, specific optical rotation, methoxyl content, insoluble gel, acid equivalent weight and nitrogen content are among parameters which are useful in chemotaxonomy. This paper presents the physicochemical properties of gum exudates from *A. senegal* var. *senegal*, *A. senegal* var. *leiorhachis* and *A. sieberana* var. *woodii* and presumably will contribute in the unambigous identification of the closely related species.

METHODS

Origin of samples

The gum samples were collected from central Tanzania in the following locations:

1. *A. senegal* var *senegal*	I	63 km from Dodoma on the Dodoma to Morogoro road.
	II	As above.
2. *A. senegal* var *leiorhachis*	I	37 km from Morogoro on the Morogoro to Dodoma road.
	II	As above.
3. *A. sieberana* var *woodii*		3 km from Singida on the Singida to Mlandara road, Mandawa village

Botanical vouchers (branches, twigs, fruits, pods, seeds, etc.) from each of the species were also collected and deposited in the Herbarium, Botany Department, University of Dar es Salaam. Confirmation of the species was obtained from the Royal Botanic Gardens (Kew, UK).

Experimental procedures

(a) Cold water insoluble gel (CWIG)

A gum sample (5 g) was accurately weighed and stirred in 125 cm^3 of distilled water for 2 hours. The mixture was then transferred quantitatively into centrifuge tubes and the insoluble fraction was separated by centrifugation at 1200 g for 10 minutes (Gallenkamp Centrifuge 200). The clear supernatant liquid was removed and the insoluble fraction was washed by adding distilled water into the centrifuge tubes, stirred for one minute and re-centrifuged for 10 minutes at 1200 g. The washing was repeated four times after which the insoluble fraction was transferred quantitatively into a porcelain dish which had previously been dried at 105½C for 10 minutes, cooled in a dessicator and weighed accurately. The insoluble fraction was then dried in an oven for 12 hours at 105½C, cooled in a dessicator and weighed. Duplicate determinations were carried out, averaged and corrected for moisture and insoluble matter to obtain CWIG.

(b) Hot water insoluble gel (HWIG)

An accurately weighed gum sample (5 g) was stirred in distilled water (125 cm^3) for 1 hour and then heated in a water bath at 92-95½C for two hours. Distilled water was added at regular intervals to keep the total volume of the mixture constant. The mixture was allowed to stand at room temperature for 12 hours. Separation and drying of the insoluble fraction and calculation of the HWIG was done as for the CWIG. Determinations were carried out in duplicate and averaged.

Tannin content

(c)Calibration of the colorimeter for tannin *content determination:*

Tannic acid was used as a standard to prepare a series of standard aqueous solutions, in the concentration range of 0.005-0.2 g kg^{-1}. To 10 cm^3 aliquot of each standard solution, 0.1 cm^3 of a ferric chloride solution (9 g ferric chloride hexahydrate made up to 100 cm^3 using distilled water) were added and the absorbance at 430 nm measured using a Griffin Model 40 colorimeter. A plot of absorbance against the concentration of tannic acid yielded a straight line graph which was used as the calibration curve for tannin content determination of the gum solutions.

(d) Determination of tannin content:
The tannin content was determined by photometric colorimetry using a Griffin Model 40 colorimeter. The absorbance of a 20 g kg^{-1} gum solution at 430 nm was used as a reference. 0.1 cm^3 of ferric chloride solution (see above) was added to 10 cm^3 of the gum solution. The absorbance of this mixture is the total absorbance contributed by ferric-tannin complex, gum solution and the ferric chloride solution added. The absorbance of 0.1 cm^3 ferric chloride solution (see above) added to 10 cm^3 distilled water was used as a blank. The determinations were done in duplicate and averaged.

(e) Viscosity
An approximate weight of gum was stirred in distilled water (50 cm^3) for one hour, and then heated in a water bath for two hours at 92-95½C. The suspension was agitated frequently and the volume kept constant by adding distilled water at regular intervals. The solution was then allowed to cool and settle at room temperature for twelve hours. The clear soluble fraction was separated by centrifugation at 1200 g for 10 minutes followed by filtration through a porosity 1 crucible .

The concentration of the gum solution was determined by aliquoting 5 cm^3 of the stock solution into a porcelain dish, which had previously been heated at 105 ½C for ten minutes, cooled and weighed. The gum solution was dried in an oven at 105 ½C for twelve hours. The dish was then cooled in a dessicator, weighed accurately and the concentration of the solution calculated from the weight and volume of the solution used. For each sample duplicate determinations were carried out and averaged. Subsequently, the stock gum solution was diluted with distilled water to give appropriate solutions for viscosity measurements. The viscosities (in centipoise) of the dilute solutions were determined at 30 °C using ubbleholde suspended level capillary viscometers of appropriate capillary width. For each solution, a viscometer which gave a flow time of not less than 200 seconds was chosen.

(f) Determination of other parameters
Methoxyl content was determined by the method recommended by JECFA, (1983) whilst nitrogen content was determined by the Kjeldahl method. A Model AA-10 automatic polarimeter manufactured by Optical Activity Ltd., UK was used to measure specific rotations. Atomic absorption spectrophotometry (Perkin Elmer Model 2380 double beam instrument) was used for the determination of all the metals except sodium and potassium, which were determined by the flame emission technique with external calibration using the same instrument. The acid equivalent weights (AEW) were determined by the method published by Jefferies *et al.* (1977 a).

RESULTS AND DISCUSSION

The physicochemical data for the samples studied are summarized in Table 1. *Acacia* gums are known to be highly soluble in water and are different from other tree exudate gums (e. g. gum karaya) which are not completely soluble in water and form highly viscous solutions or suspensions at relatively low concentrations. The gum exudate from *A.*

sieberana var. *woodii* has a mean Cold Water Insoluble Gel (CWIG) value (Table 1) which is similar to other *Acacia* gums from the series Gummiferae, for example, *A. malacocephala* (CWIG, 5.88% w/w) (Mrosso 1996). However, its value is higher than the average obtained previously for Tanzanian commercial *Acacia* gums (CWIG, 0.27% w/w) (Mhinzi and Mosha 1995). The proportion of the insoluble gel is taken as a measure of the quality of a gum and has generally been found to vary widely among tree exudate gums. Gums with a high proportion of insoluble gel are considered to be of poor quality than gums with a low proportion of insoluble gel. In addition, gums with a high proportion of CWIG are also expected to be more viscous than gums with a low proportion of CWIG (Philips *et al.* 1980). In this work, however, the viscosity of *A. sieberana* var. *woodii* gum has been found to be similar to that of *A. drepanolobium* gum (thought to be the major source of Tanzanian commercial *Acacia* gums) reported by Mrosso (1996) (4.01 at 10%, 9.48 cP at 15% w/v) although its CWIG content is higher.

Nitrogen content is considered as one of the most useful parameters in distinguishing gums from different species (Anderson 1978). The nitrogen content of the gum specimen from *A. sieberana* var. *woodii* (Table 1) found in this work is similar to that reported for its close relative *A. sieberana* var. *sieberana* gum (0.35% w/w) (Anderson *et al.* 1984). However, the methoxyl content of these two species are different. The mean methoxyl content of *A. sieberana* var. *woodii* gum found in this work is higher than that reported for its close relative *A. sieberana* var. *sieberana* (0.74% w/w) but approaches that reported by Mghweno (2000) for the average of Tanzanian commercial *Acacia* gums (1.22% w/w). The values for the two species are, however, within the range (0.47-2.4% w/w) expected for gums from the series Gummiferae (Anderson 1977 b).

It has been suggested by Biswas *et al.* (2000) that the specific optical rotation of a polysaccharide exudate gum is a linear function of the carbohydrate composition and that exudate gums from a particular species and genus can be represented by a formula, called a Rotation Operator. JECFA/FAO (1999) has specified that gum arabic for food and pharmaceutical applications should be laevorotatory. The gum exudate from *A. sieberana* var. *woodii* is dextrorotatory (see Table 1) but its mean value of specific optical rotation (+100.8°) is similar to the average (+94.9°) obtained previously for Tanzanian commercial *Acacia* gums. It is also similar to that reported previously for its close relative *A. sieberana* var. *sieberana* (+106.0°) (Anderson *et al.* 1984).

Another parameter worth comparing in this study is the Acid Equivalent Weight (AEW). The AEW of a gum is defined as the mass of the gum that contains one equivalent of uronic acid. A high value of AEW indicates a low uronic acid content in the gum while a low value of AEW shows a high uronic acid content. The natural pH is not a very good measure or indicator of the acidity of gums because it varies with concentration. Gums from members of the series Gummiferae are known to be less acidic than those from members of the series Vulgares (Anderson 1977 b). The mean value of the AEW of *A. sieberana* var. *woodii* (2058, see Table 1) is slightly lower than that reported for *A. sieberana* var. *sieberana* gum (2300) implying a slightly higher uronic acid content for *A. sieberana* var. *woodii* gum.

Table 1. Physicochemical properties of gum exudates from some *Acacia* species

Parameter	*A. senegal* var. *senegal*				*A. senegal* var *leiorhachis*				*A. sieberana* var. *woodii*		
	I	II	MEAN	SD	I	II	MEAN	SD	Sample 1	Sample 2	MEAN
Moisture % w/w	15.0	14.1	14.55	0.523	15.9	15.3	15.6	0.392	14.52	14.60	14.56
Ash % w/w	4.5	3.8	4.15	0.395	5.0	5.2	5.1	0.130	2.64	2.70	2.67
Acid insoluble											
matter %w/w	0.88	0.60	0.74	0.162	0.28	0.21	0.25	0.0486	1.59	1.51	1.55
CWIG % w/w	1.57	1.83	1.70	0.158	15.0	15.67	15.34	0.400	5.07	4.97	5.02
HWIG % w/w	0.96	1.23	1.10	0.168	7.64	4.03	5.84	2.085	4.63	4.52	4.57
Methoxyl %w/w	0.17	0.26	0.22	0.0635	0.29	0.27	0.28	0.0216	1.01	1.01	1.01
Nitrogen % w/w	0.33	0.28	0.31	0.0419	0.48	0.48	0.48	0.000	0.36	0.42	0.39
$[\alpha]_D$ In H_2O, deg	-50	-55	-52.5	3.416	-22	-26	-24	2.328	+99.8	+101.8	+100.8
Viscosity											
(centipoise)											
100 gl^{-1}	4.61	2.90	3.76	1.011	64.37	79.22	71.80	8.576	4.55	4.59	4.57
150 gl^{-1}	9.44	6.14	7.79	1.917	103.89	181.86	142.88	7.210	10.76	10.82	10.79
Optical density	0.06	0.06	0.06	0.000	0.07	0.07	0.07	0.000	0.08	0.08	0.08
Tannin % w/w	0.28	0.52	0.40	0.142	0.69	0.44	0.57	0.147	0.21	0.27	0.24
Acid Equivalent											
Weight	1575	1922	1749	200.45	979	1703	1341	418.201	2055	2061	2058
pH	4.84	5.01	4.93	0.103	4.65	4.80	4.73	0.080	4.70	4.80	4.75
% Salt form	93.1	94.1	93.6	0.608	94.2	90.5	92.4	2.138	90.9	90.5	90.7

Table 2: Metal composition of gum exudates from some *Acacia* species

Sample	Metal composition % w/w				ppm			
	Na	K	Ca	Mg	Fe	Zn	Pb	Cu
A. senegal								
var. *senegal* I	0.014	0.984	0.592	0.290	98.0	19.5	4.29	6.67
A. senegal								
var. *senegal* II	0.009	0.087	0.433	0.015	59.0	4.52	0.37	3.49
MEAN	0.012	0.535	0.512	0.152	78.5	12.0	2.33	5.08
SD	0.0035	0.5175	0.235	0.150	22.60	8.67	2.26	1.83
*A. senegal*var.								
leiorhachis I	0.028	1.350	0.741	0.154	23.0	9.30	0.69	5.13
*A. senegal*var.								
leiorhachis II	0.011	1.191	0.866	0.149	22.5	6.99	2.55	5.36
MEAN	0.020	1.271	0.804	0.153	22.75	8.145	1.620	5.245
SD	0.010	0.093	0.072	0.005	0.370	1.335	1.074	0.139
A. sieberana								
var. *woodii*								
sample 1	0.258	0.374	0.607	0.435	78.360	6.927	8.437	5.257
sample 2	0.266	0.380	0.601	0.437	78.614	6.905	8.441	5.245
MEAN	0.262	0.377	0.604	0.436	78.487	6.916	8.439	5.251

In general, Table 1 shows that *A. senegal* var. *leiorhachis* and *A. senegal* var. *senegal* produce gums with different physicochemical properties. For example, there is a notable difference in terms of solubility and viscosity. The gum from *A. senegal* var. *leiorhachis* is far less soluble (mean CWIG, 15.34% w/w) than that from *A. senegal* var. *senegal* and the average of Tanzanian commercial *Acacia* gum (CWIG, 0.27% w/w) (Mhinzi and Mosha 1995). Likewise, the viscosity of *A. senegal* var. *leiorhachis* gum is much higher than that of *A.°senegal* var. *senegal* gum at the same concentration (Table 1). The CWIG of batches of commercial gum ghatti has been shown to vary between 8-23% w/w (Jefferies *et al.* 1977 b) and the viscosities of the gum was reported to depend on the proportion of the insoluble gel fraction.

In this work, *A. senegal* var. *senegal* gum has been found to have a more negative optical rotation (mean, -52.5°) as compared to that of *A. senegal* var. *leiorhachis* gum (mean, -24°). It is also higher than the mean reported for *A. senegal* gum (-31.3°) (Karamalla *et al.* 1998) from Sudan (the major source of commercial *Acacia* gums in the world). *A. senegal* var. *senegal* is the most prevalent variety of Sudanese *A. senegal*, and the value of -31.3° for optical rotation assigned to Sudanese gum arabic is presumably that of *A. senegal* var. *senegal* gum. The differences between the values obtained in this work and the literature value might be due to variation between the exuding *A. senegal* trees as reported by Duvallet *et al.* (1993), who recorded a wider range of optical rotation with a minimum of -25° and a maximum of -62°. The mean optical rotation value of *A. senegal* var. *leiorhachis* gum found in this work is similar to that reported for gums from *A. senegal* (-31.3°) and *A. senegal* var. *kerensis* (-35°) (Chikamai and Banks 1993).

Table 1 shows that the mean nitrogen content of the gum exudates from *A. senegal* var. *leiorhachis* is higher than that of the gums from *A. senegal* var. *senegal*. The values of the latter are, however, similar to those obtained by Idris *et al.* (1998) for eight authenticated *Acacia senegal* gum samples obtained from trees of varying age and location. The mean value for *A. senegal* var. *leiorhachis* gum is similar to the mean nitrogen content obtained previously for Tanzanian commercial *Acacia* gums (Mghweno 2000). It is interesting to note that the nitrogen contents and specific rotation values of *A. senegal* var. *leiorhachis* gum found in this work are also similar to those found in *A. senegal* var. *kerensis* gum (Chikamai and Banks 1993) reflecting a close relationship between the two variants of *A. senegal*. Examination of Table 1 reveals that the mean value of the AEW for *A. senegal* var. *senegal* gum (1749) is higher than that of *A. senegal* var. *leiorhachis* gum (1341).

The metal compositions of *A. senegal* var. *senegal* and *A. senegal* var. *leiorhachis* gums found in this work are shown in Table 2. Metal ion content in plant material is thought to be a function of the composition of the soil on which the plants grow (Anderson and Morrison 1989, Chikamai and Banks 1993). Therefore, their levels are not very useful as chemotaxonomic markers in identifying different *Acacia* species. Calcium ions are known to be responsible for gel formation in some tree exudate gums such as *Khaya grandifoliola* gum, however this effect has not been reported for *Acacia* gums. Work by Kunkel *et al.* (1997) has also shown that gum arabic does not bind added magnesium.

In conclusion, the notable differences observed in this study between the properties of the gums from *A. senegal* var. *senegal* and *A.°senegal* var. *leiorhachis* suggest that it is important to incorporate selected analytical data as chemotaxonomic evidence in order to provide an unambiguous distinction of some closely related *Acacia* species. This work has shown that the properties of the gum exudates from *A. sieberana* var. *woodii* and *A. sieberana* var. *sieberana* gums are similar and it is justifiable to retain them as variants of the same species.

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