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Standardization of *Commiphora Abyssinica* Engl. Gum Resin From Kajiado, Kenya

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Abstract: *Information on the physical and chemical characteristics of Commiphora abyssinica gum resin is scanty. The aim of this work was to establish the composition and physicochemical properties of above mentioned gum resin and on that basis propose its standard specifications for commercial use. Samples from three sites in Kajiado in Kenya were characterized in terms of parameters commonly used in the evaluation of the quality of crude oleogumresins and their products. There was no substantial variability with collection location observed on most physical properties including pH, density, refractive index, viscosity and optical rotation. Composition parameters such as moisture, ash, nitrogen, metals, extraneous impurities, essential oils, ethanol and water soluble matter varied from location to location. The ethanol-soluble matter which is crucial in the formulation of traditional gum resin products varied significantly and ranged between 26.37 and 47.79%. The gum-resin was found to have 1.31 to 1.87% essential oils and the yield of water soluble matter was high at 82.25-84.50%. In general, the gum resin had relatively low content of extraneous impurities (2.27-3.17%) and ash (2.24-3.04%) in comparison with gum resins from related Commiphora species. Saponification value, acid value as well as free fatty acids also varied with location. Values of parameters obtained in this study were found suitable for proposing standard specifications of local Commiphora abyssinica gum resin from the location. From the results, standard specifications that can be used in commerce for identification and quality specification of Commiphora abyssinica gum resin sourced in Kenya were suggested. Magnesium could be used to identify the gum resin from the area due to its very low variability. It was also recommended that freshly harvested material be stored for a few months before processing.*
Keywords: *Commiphora abyssinica; ethanol extract; essential oil; gum resin; myrrh; standard specifications.*

I. Introduction

Commiphora abyssinica Engl. also known as *Commiphora habessinica* (O.Berg) Engl. and *Commiphora madagascariensis* Jacq., is widely distributed in the arid and semi-arid areas of Kenya, especially in Kajiado, Laikipia and Isiolo [1]. It produces a yellow to brown oleo-gum resin rich in essential oil, gum and resin components [2]. These products form a vital part of the non-wood forest products and have an established international market that provide income supplement to largely marginalized and poor rural communities. Due to the surface activity, medicinal, anti-microbial and other properties of gum resin components, they are used industrially in cosmetics, pharmaceuticals and foods [3, 4].

International quality standards are important to dealers and users of natural raw materials such as oleo gum resins. Standardization and grading of a natural raw material is essential for its pricing and marketing [5]. The physical properties used in quality evaluation of gums and oleogumresins include moisture, total ash content, volatile matter, nitrogen content, optical rotation and elemental composition. In the case of gums used in food processing for example gum Arabic, international specifications have been established [6,7]. Some of the characteristics used to evaluate the suitability of oleo-chemicals for application as surfactants include saponification, ester, acid, free fatty acids and iodine values.

Manufacturers using non-wood forestry products as raw materials are concerned about supply reliability, quality consistency including chemical and toxicological quality. Deliberate adulteration, lack of knowledge and skills and, adverse environment conditions are major causes of poor quality [7]. Gum resins from *Commiphora* species are susceptible to mixing with related species growing in the same habitat, leading to quality variation between different batches [8]. Furthermore, the quality of the same raw material from a given species may vary with collection location.

Industrial processing and chemical application of *Commiphora abyissinica* gum resin potentially obtainable from large areas of Kajiado County is important for the improvement of income base of the local people. In order to facilitate this commercialization, the physical and chemical characteristics of the crude gum resin and its various extracts were determined for the purpose of proposing standard specifications.

II. Methods

2.1 General experimental procedures

Analytical grade reagents were used for extraction and analysis. Rotary evaporation was done in vacuo using Rotavapor 11, Buchi, Switzerland. The experiments were carried out in triplicate.

2.2 Plant material

The plant materials (gum resin) were collected from three different sites in Kajiado County namely Nolokwa (NL), Kilonito (KL) and Kudu Hills (KH), approximately 100 km from Nairobi city. In Nolokwa, a gum resin that looked slightly different from the rest was also sampled and labeled NL 2. The plant material was identified by a senior technologist from the Department of Chemistry, National Museums of Kenya where voucher specimen were deposited. The gum resin of *Commiphora abyssinica* (1 kg) from different locations were air dried under shade, ground into coarse and fine particles using a Willy mill at the Department of Chemistry, University of Nairobi.

2.3 Determination of moisture, ash, nitrogen, protein content and elemental composition

The moisture quantitative analysis was done using a Memmert U400 oven at 105±2°C for 5 to 6 hours to constant weight. Moisture was also determined after 3 and 4 months storage of the gum resins. The ash content was obtained by heating the sample in a Nabertherm, LH15/14 programmable muffle furnace at 550ºC for over 1 hour to constant weight while nitrogen content was determined using a Gerhardt-Vapodest nitrogen distiller. Elemental analysis was done using Bulk Scientific Model 210VGP-USA atomic absorption spectrophotometer.

2.4 Extraction procedure for essential oils

Extraction of essential oils was done using both hydrodistillation and steam distillation methods. In the hydrodistillation process, 200g of ground gum resin was soaked in 300ml distilled water and then extracted for 6 hours using Clevenger apparatus. The resultant distillate consisted of an emulsion of creamish essential oil and water, which was partitioned with *n*-hexane. Steam distillation was achieved by placing similar amount of ground gum resin into a wire mesh/grid above boiling water in a distillation flask. Using the Clevenger apparatus, the resultant essential oil was similarly partitioned with *n*-hexane. The *n*-hexane layers were separated using a separating funnel.

2.5 Extraction procedure of the gum resin ethanol extract

To extract the ethanol soluble matter, 200g of the ground gum resin sample was macerated in 200ml ethanol. The mixture was heated under reflux for 3 hours in a water bath maintained at 80ºC. The first extract was decanted and the residue extracted repeatedly with fresh 200ml ethanol for 2 hours, until no significant change in the colour of the solvent was observed. The extracts were then combined, filtered through Whatman No.1 filter paper and concentrated *in vacuo* using a rotary evaporator.

2.6 Extraction procedure of the gum resin aqueous extract

To extract the water soluble matter, 100 ml distilled water was added to 5g of finely homogenized gum resin, mixed well and filtered to remove insoluble matter and debris. The water was removed by means of a freeze drier.

2.7 Determination of refractive index and optical rotation of crude gum resin and the pH, density, and viscosity of the gum resin extracts

Refractive index and optical rotation of 1% gum resin solutions were determined using a Zeiss Abbe refractometer and a Bellingham and Stanley ADP220 polarimeter with 10 cm length cell. Density, pH and viscosity of distilled water, 1% ethanol and aqueous extract (after ethanol extraction) solutions using a 2 ml pycnometer, a Hanna 240 glass electrode microprocessor and a Technico Ostwald viscometer respectively.

2.8 Determination of acid, saponification and ester values of the gum resin

The acid value was determined by dissolving 0.5g sample in neutralized ethanol and then titrating with 0.1N KOH. To determine the saponification value, the sample was dissolved in 5ml ethanol after which 25ml of 0.5N alcoholic KOH was added and the mixture warmed under reflux for about an hour until the solution was clear. The solution was then titrated with 0.5M HCl. A blank run was similarly treated. Ester value was determined as the difference between saponification and acid values.

III. Results And Discussion

3.1 Moisture, ash, nitrogen and elemental content of the gum resin

The data for the moisture, ash, nitrogen, protein contents and elemental composition of the gum resin are presented in Table 1.

Table 1: Physico-chemical properties of *C. abyssinica* gum resin

Location	Moisture	Ash	Nitrogen	Protein	Elemental Composition (ppm)							
	(%)	(%)	(%)	(%)	Mg	K	Ca	Zn	Fe	Cu	Mn	Na
NL	9.15	2.31	1.60	10.00	4.48	63.67	399.04	1.93	1.70	0.70	1.00	3.01
NL 2	8.90	2.56	1.78	11.13	4.48	113.56	398.14	0.94	1.26	1.12	1.08	3.09
KL	10.59	2.24	1.45	9.06	4.48	45.37	201.65	ND	2.55	0.62	2.43	2.27
KH	10.28	3.04	1.59	9.94	4.51	102.82	243.81	0.10	4.00	0.63	1.77	2.67

There were observed variations in moisture content which ranged between 8.90 and 10.59% and this could be attributed to factors such as the degree of vitrification and variations in environmental conditions [9]. These values are similar to those reported by Mwendwa [1] and Kyalo [6], for *C. schimperii* gum resin from the same location. The moisture decreased with storage time from an average of 9.73% to 7.69% after 3 months and 6.50% after 4 months. It was also noted that freshly harvested gum resin was sticky and difficult to grind. Hence, storage under suitable conditions is recommended before processing the gum resin.

The ash content of a gum or gum resin is a measure of its purity; the lower the ash content, the higher is its degree of purity [9]. The ash values for the gum resins from all three locations were determined to be below 4% which is the maximum limit recommended for use in food and pharmaceutical formulations [3]. Kudu Hills sample showed the highest ash content (3%). Nitrogen content was in the range 1.45 to 1.78% which was found to be similar to 1.69% for gum resin from *Commiphora myrrha* reported by Yasser [10] also within the recommended specifications for use in food and pharmaceutical formulations.

The gum resins had high levels of calcium and potassium at 310.66 and 81.36ppm, respectively. These high levels were attributed to the close proximity of the collection points to a limestone mining area (Athi River Mining). Magnesium was 4.4875±0.0087ppm and can therefore be used for identification of *C. abyssinica* gum resin from the location.

3.2 Percentage yields of essential oils, ethanol and water soluble matter of the gum resin

The % yields of various extracts were location-dependent with gum resin from Kudu Hills yielding the least percent of essential oil. Steam distillation gave higher yields of essential oils (1.31-1.87%) compared with hydrodistillation (1.12-1.51%) as shown in Table 2. These results are consistent with those reported by Ali et al. [11] which showed a yield of 1.1% for *C. abyssinica*. The % yield of the essential oils from *C. myrrha* was reported by Hanus et al. [12] to range from 2-10 % which is much higher than that obtained in this study from *C. abyssinica*.

Table 2: Yields of essential oils, **ethanol and** water extracts of the gum resin

Collection Location	% Essential oil		% ethanol soluble matter		% water soluble matter	% water and ethanol insoluble matter
	Hydro-distillation	Steam distillation	Coarse particles	Fine particles		
Nolokwa	1.32	1.62	33.24	37.65	84.50	2.27
Nolokwa 2	1.51	1.72	47.41	47.79	83.75	3.11
Kilonito	1.37	1.87	44.13	46.52	84.25	3.54
Kudu Hills	1.12	1.31	24.78	26.37	82.25	3.77
Average	1.33	1.63	37.39	39.58	83.69	3.17

The yields of the ethanol extracts varied significantly with collection location. The % yield of the ethanol soluble matter was dependent on particle size. The highest yields of the ethanol extract were observed for Nolokwa 2 (47.79%) and Kilonito (46.52%) with Nolokwa and Kudu Hills having lower yields of 37.65% and 26.37%, respectively. According to Chikamai and Banks [13], gum properties are affected by a number of factors including soil type, age of tree and climatic conditions. Typically, myrrh gum resins yield between 25 and 40% but some species have higher yields; *C. holtziana* has been reported to have a yield of 41-44% [14]. From these observations, the yields of ethanol extracts of *C. abyssinica* from the two geographical locations *ca* Nolokwa 2 (47.79%) and Kilonito (46.52%) are within the recommended specifications of commercial myrrh gum resins.

The gum resin was found to be highly water-soluble yielding between 82.2 and 84.50% which is higher than the typical 60% yield for other *Commiphora* species [2]. There were no substantial variations in solubility in water of the gum resin with geographical locations. The water- and ethanol-insoluble matter is usually a measure of contamination by foreign matter. This component ranged between 2.27 and 3.77% which compared well with typical levels of 3-4% [15]. The main impurities were tree bark, dry leaves and soil particles.

3.3 Optical rotation and refractive index of C. abyssinica gum resin

The optical rotation and refractive index of 1% gum resin solutions are shown in Table 3.

Table 3: Optical rotation and refractive index of 1% *C. abysinica* gum resin solution

Parameter	Nolokwa	Nolokwa 2	Kilinito	Kudu Hills	Distilled water
Optical rotation (20 ^{o})	-49	-51	-44.5	-49.5	-
Refractive index	1.334	1.334	1.334	1.334	1.333

As observed in Table 3, the gum resin solutions were levorotatory, and had the same refractive indices. The physical properties did not vary significantly with location and can therefore be used for quality specification of *C. abyssinica* gum resin. Hanus et al. [12] and Selvamani et al. [16] have reported latex of *C. abyssinica* containing 6.5% of a steroid fraction with optical rotation of -37^o. Furthermore, the mass spectra of this fraction showed the presence of cholest-5-en-3β-ol, Δ-campestan-3β-ol and Δ-sitostan-3β-ol in the ratio of 13.6:1.8:1 and the three compounds exhibited optical rotations of -39^o, -33^o and -36^o respectively.

3.4 Density, pH and viscosity of the crude gum resin and its extracts.

The values obtained for above physical properties for the raw gum resin, its ethanol extract and the water extract of the residue obtained after ethanol extraction are shown in Table 4.

Table 4: Physical properties of the gum resin and its extracts

Solution		Density (kg/m^3) (1%, 25^{o}C)	pH (1%, 25^{o}C)	Viscosity (cP) (1%, 25^{o}C)
Distilled water		997.1	5.82	0.8937
Ethanol Extract	NL	998.1	4.21	0.9321
	NL 2	998.1	4.22	0.9512
	KL	998.1	4.12	0.9307
	KH	998.1	4.04	0.9257
Raw Gum	NL	998.4	5.31	0.9889
	NL 2	998.4	5.31	0.9672
	KL	998.3	5.26	0.9754
	KH	998.3	5.20	1.0154
Water Extract	NL	998.7	5.18	1.0433
	NL 2	998.5	5.19	1.0202
	KL	998.6	5.16	1.0456
	KH	998.5	5.12	1.0917

The densities of the ethanol and water extracts were higher than that of water. The crude gum resin and its extracts had acidic pH with the ethanol extracts having the lowest. The pH varied slightly with location with Kudu Hills samples generally being more acidic. All the solutions had higher viscosities than that of distilled water, with the water extract having viscosities in the range 1.0202-1.0917cP. Kudu Hills crude gum resin and water extract had relatively higher viscosities than those of other locations but its ethanol extract showed the lowest viscosity. As seen in Table 2, the gum resin from this location had the lowest yield of ethanol extract and hence had more of the gum component.

3.5 Saponification, acid, ester and free fatty acid values

These results are presented in Table 5. The saponification values of the *C. abyssinica* ethanol extracts from the different collection points were between 170.8 and 176.4mg KOH/g. The two resin samples from Nolokwa gave slightly higher values (176.72mg/g) than those from Kudu Hills (173.91mg/g) and Kilonito (171.11mg/g).

Table 5: Saponification value, acid value, ester value and % free fatty acids (FFA) of the ethanol extract.

Sample	Saponification Value (mgKOH/g)	Ester value (mgKOH/g)	Acid value (mgKOH/g)	% FFA
NL	176.72	172.80	3.92	1.97
NL 2	176.72	172.80	3.92	1.97
KL	171.11	166.63	4.48	2.25
KH	173.91	169.43	4.48	2.25
C. mukul		230.32-233.94	13.16-15.77	

[18]				
C.wighitii [18]		226.94-255.37	8.2-14.65	

Some gum resin extracts such as rosin are used in soap-making. According to Panda [17] typical gum rosins have saponification values between 170 and 176mg KOH/g which are similar to those of the ethanol extract of *C. abyssinica* gum resin. The acid values and free fatty acids both of which are indicators of a material's potential for rancidity were between 3.92 to 4.48 and 1.97 to 2.25mg KOH/g, respectively. These values were relatively low compared to those of *C. mukul* and *C. wighitii* [18] also presented in Table 5. Most of the fats and oils used in soap manufacture have free fatty acids below 5%; tallow 2-4%, coconut oil and palm kernel oil 3% maximum [19]. The above free fatty acids values observed for *C. abyssinica* gum resins are therefore within the recommended specifications. The ester value is a measure of saponifiable glycerides and it ranged from 166.63 to 172.80mg KOH/g, lower than that of *C. mukul* and *C. wightii*.

3.6 Proposed partial standard specifications of Commiphora abyssinica gum resin from Kajiado

By analyzing the variations of the physical parameters with location, the proposed standard specifications for *Commiphora abyssinica* from Kajiado County are shown in Table 6.

Table 6: Proposed Standards for *Commiphora abyssinica* gum resin

Parameter	Proposed specifications
Moisture,%	6-11
Ash,%	2-4
Magnesium, ppm	4-5
Nitrogen,%	1.4-1.8
Density (1% solution), g/ml	0.998-0.999
pH (1% solution)	5.2-5.4
Refractive index (1% solution)	1.334
Optical rotation (1% solution),	-40^o to -50^o
Essential oil, %	1.0-2.0
Ethanol soluble matter, %	20-50
Water soluble matter, %	80-85
Insoluble matter, %	2.0-4.0
Saponification value, mgKOH/g	170-180
Ester value, mgKOH/g	170-175
Acid value, mgKOH/g	4.0-5.0
Free fatty acids, %	2.0-3.0

IV. Conclusion

Characterization of a natural resource is prerequisite to its commercial exploitation. Results of physicochemical properties and composition of *Commiphora abyssinica* gum resin from three locations in Kajiado compared well with those of related *Commiphora* species. Most physical parameters did not show variability with collection locations. Storage of the raw material was observed to cause the lowering of moisture content and hence reduction in stickiness. In industrial extractions, storage is therefore recommended for the purpose of improving size reduction, hence yield. With respect to elemental composition, magnesium had very low variability and could therefore be used for identification of the gum resin from this area. The ethanol-soluble matter varied significantly with collection location, had low content of essential oils and high water soluble matter. From these results, standard specifications for *Commiphora abyssinica* gum resin from Kajiado County could be proposed. The findings are important for the establishment of a market and economic management of the agro-forestry resource for the benefit of the local community.

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