

PHYSICOCHEMICAL PROPERTIES OF *TERMINALIA* *AVICENNIoidES* (TA) AND *ANOGEISSUS* *LATIFOLIA* (AL) GUM

K. I. OMONIYI¹, P. O. AMEH^{2*} AND U. USMAN³

¹Department of Chemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

²Department of Chemistry, Nigeria Police Academy Wudil, Kano State Nigeria

³Department of Physical Sciences, College of Arts, Science and Remedial Studies, Kano, Nigeria.
[nocaseoche@yahoo.com]

ABSTRACT

The work reports the physicochemical properties of gum exudates from two Combretaceae tree species (*Terminalia avicennioides* (TA) and *Anogeissus latifolia* (AL)) determined by standard methods. The gum exudates exhibited differences in physicochemical qualities. The physicochemical properties of the gum were in the following ranges: moisture (11.3 - 11.5%); swelling capacity at 30°C (11 - 13%); pH (4.34 - 5.24); melting temperature (218- 242°C); total ash (3.30- 3.54%); nitrogen (0.70 - 0.71%); protein (4.38 - 4.46%) and ash content (0.53 - 0.60). The two gum samples did not contain tannin and fibre. The gum samples had high proportion of Ca and contained Ni, Pb and Cd at levels below the maximum permissible limits. The physicochemical parameters obtained for TA and AL compare well with those reported in previous studies on other gums. This signals the need for exploitation of the exudates for potential industrial applications.

Keywords: *Terminalia avicennioides*, *Anogeissus latifolia*, physicochemical properties, gums

INTRODUCTION

Plant gums are biopolymeric materials that are composed of complex heteropolysaccharides and proteinaceous material, in addition to some mineral elements (Amech *et al.*, 2012). They are capable of displaying colloidal properties in an appropriate solvent or swelling agent. Gums have been found to be useful as natural emulsifiers for food and pharmaceutical (Mhinzi, 2002). Other applications of plant gums are in the manufacture of adhesives for postage stamps and in the formulation of paints and inks; in lithography and textile industry as a sizing and finishing agent and more recently as corrosion inhibitors (Jafar *et al.*, 2007; Nep and Conway, 2010; Umoren *et al.*, 2008).

The various applications that can be derived from gums have been found to depend on their functional properties such as physicochemical, proximate, cationic content and phytochemical properties. For example, in spite of its favourable rheological composition, *Albizia lebbek* gum cannot be used as a food additive because of its high content of tannins and high proportion of aluminum (De Paula *et al.*, 2001). Pablyana *et al.* (2007) stated that the presence of protein in polysaccharides can induce an inflammatory response in tissues, which can inhibit the pharmacological uses of gums. Yadav *et al.* (2008) found that emulsifying properties of polysaccharides depends on its turbidity, interfacial rheology of gums has been found to be essential parameter in predicting emulsifying properties of gums (Elmanan *et al.*, 2008). *Fenugreek* gum is widely used as thickening, water holding, stabilizing and emulsifying agents in food industries because it is composed of galactose and manose and gives a high viscosity in aqueous solution (Youssef *et al.*, 2009). Similarly, Rinaudo (2001) stated that a wide industrial application of gum exudates is due to their water holding capacity to produce gels or highly viscous

solutions and ability to enhance the stability of emulsions and foams and that these properties depend on the chemical structure of gum exudate polysaccharides and their conformation in the solvent.

Due to the numerous applications of plant gums, several studies have been conducted on the physicochemical, rheological, proximate, cationic and polyelectrolyte compositions of some gums (Ahmed *et al.*, 2009; Okafor, 2001; Eddy *et al.*, 2013). However, there is dearth of detailed properties of *Terminalia avicennioides* (TA) and *Anogeissus latifolia* (AL) gum exudates.

The objective of the present study is to investigate and model the functional properties of *Terminalia avicennioides* (TA) and *Anogeissus latifolia* (AL) gum exudates in order to assess the suitable applications.

MATERIALS AND METHODS

2.1 Sample collection and description

The samples were obtained from two different *Acacia* tree species (*Terminalia avicennioides* (TA) and *Anogeissus latifolia* (AL)) found naturally in surrounding forests of Kanya village, Jigawa State. Samples were collected from the tree barks as dry nodules or lumps. Each bulk sample (1.0 kg) was obtained by combining the collections from randomly identified matured trees of same species. Samples were then kept in separate labelled containers.

2.2 Treatment of Exudate Gums

Modified method of Femi-Oyewo *et al.* (2004) was adopted for the treatment of the gums. The gums were hydrated in double strength chloroform (Sigma-Aldrich, Germany) water for 5 days with intermittent stirring to ensure complete dissolution and then, strained through a 75 µm sieve to obtain particulate free slurry which was allowed to sediment. Thereafter, the gums were

precipitated from the slurry using absolute ethanol (Sigma-Aldrich, Germany), this was filtered and defatted with di-ethyl ether. The precipitate was dried in an oven (BS size one, Gallenkamp, model; OV-160) at 40°C for 48 h. The dried flakes were then pulverized into powder using a blender and stored in an air-tight container.

2.3 Physicochemical Analysis

2.3.1 Determination of the solubility of the gums

The solubility of TA and AL was carried out in cold and hot distilled water, acetone, chloroform and ethanol. 10.0 mg sample of each gum exudates was added to 10 ml of each solvent and left overnight. 5 ml of the clear supernatants were put in small pre-weighed evaporating dishes and heated to dryness over a digital thermostatic water bath (Model. HHS, McDonald Scientific International). The weights of the dried residue with reference to the volume of the solutions were determined using a digital analytical balance (Model XP-300, Denver instrument, USA) and expressed as the percentage solubility of the gums in the solvents (Carter, 2005).

2.3.2 Moisture sorption studies

The method described by Josiah (1991) was adopted for moisture sorption studies. Two dried evaporating dishes were weighed using an analytical balance, following which 2.0 g of each of the gum samples was weighed into each evaporating dish. The final weight of each dish was noted, then each dish was placed over water in a desiccator for a period of 5 days, thereafter each was removed and transferred into another desiccator over activated silica gel (desiccant) for another 5 days. Each of the dish with its contents was weighed on a daily basis, and the amount of water adsorbed calculated.

2.3.3 Swelling property

The method reported by Ameh *et al.* (2012) was adopted for the determination of swelling property of the gums. A 5 g quantity each of the gum powder was placed in a 200 ml measuring cylinder and tapped 200 times. The volume (V_i) of the gum in the cylinder was recorded. Water was added to the mass to reach the 100 ml mark of the cylinder, the mixture was then left to stand for 24 h. The new volume of the gums in the cylinder was then recorded as (V_f). The swelling capacity (Φ) was calculated as the ratio of the final volume (V_f) to the initial (V_i). The measurement was repeated to obtain triplicate values.

2.3.4 Tannin content

A 0.1 cm³ aqueous FeCl₃ solution was added to 20 cm³ of 2% aqueous solution of the gum sample and the mixture was centrifuged. Absence of black precipitate or blackish colouration indicated the absence of tannin (FAO, 1998).

Determination of the pH of the gum exudate

The pH of a 2.0 g amount of each of the gum mucilage in 100 cm³ of distilled water was determined using a Jenway pH meter (Model 3505).

2.3.5 Determination of moisture content

The method described by AOAC (1990) was adopted. A clean crucible was dried to a constant weight in an air oven at 105°C, it was then cooled in a desiccator and weighed (W_1). Two grammes of the finely ground sample was accurately weighed into the previously labelled crucible and reweighed (W_2). The crucible containing the sample was dried in an oven at 60°C to a constant weight (W_3). The percentage was calculated thus:

$$\% \text{ Moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad 2$$

2.3.6 Determination of ash content

A porcelain crucible was dried in an oven at 100°C for 5 min, this was cooled in a desiccator and weighed (W_1). Two grammes of finely ground TA or AL sample was placed into the previously weighed porcelain crucible and reweighed (W_2). It was then transferred into a furnace which was then set at 550°C. The sample was left in the furnace for 8 h to ensure proper ashing (Chukwu and Nwankwo, 1991). The crucible containing the ash was then removed and cooled in a desiccator and weighed (W_3). The percentage ash content was calculated as:

$$\% \text{ Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times 100 \quad 3$$

2.3.7 Determination of nitrogen and crude protein

The nitrogen content of the gum exudates was determined using a semi-micro Kjeldahl method (AOAC, 1990). Gum sample (0.5 g) was accurately weighed and placed on a filter paper. The sides of the filter paper were folded around the sample and was introduced into a 100 cm³ Kjeldahl's flask. Three grammes (3.0 g) of catalyst (100 g of K₂SO₄, 10 g of CuSO₄.5H₂O and 1 g of selenium) was added into the flask followed by the addition of 20 ml of concentrated sulphuric acid (98%). The flask was placed on the digestion heater of the Kjeldahl's apparatus and heated slowly until frothing ceased. The digestion was then continued under strong heating with occasional turning of the flask, for about 30 minutes so that the solution was cleared and all its carbon content was oxidized.

After the digestion, the heater was turned off and the flask was allowed to cool until fuming ceased. Before the contents of the flask solidified, 50 cm³ of cold distilled water was added carefully while the flask was cooled under running water. The volume was then made to 100 cm³ in a volumetric flask with distilled water. This solution (5 ml) was transferred into the Markham distillation apparatus, followed by the addition of 5 cm³ of 40% sodium hydroxide solution. The cup of the apparatus was rinsed down with distilled water. Ten millilitres (10 ml) of 4% boric acid solution was measured into a titration flask and few drops of mixed indicator of methyl red and bromo cresol green were added. The titration flask was then placed under the condenser, the end of which was dipping into the boric acid solution. The contents of the Markham apparatus were then heated by passing steam through it and the

distillate was collected in the titration flask until the volume was about 50 ml. The contents of the flask were titrated against standard hydrochloric acid (0.01 M) solution from a burette. The end point was a sharp change of colour from blue to red. The volume of 0.01 M hydrochloric acid used was noted.

A blank experiment using filter paper without sample was also carried out. The volume of 0.01 M hydrochloric acid used for the blank was subtracted from that used for the actual experiments to obtain the volume of 0.01M of hydrochloric acid used against the titrable nitrogen compound. The percentages of nitrogen and crude protein (AOAC, 1986) in the various gum samples were calculated using the equation 4 and 5

$$\% N_2 = \frac{14 \times M \times V(V_2 - V_{31})}{W \times V_1 \times 1000} \times 100 \quad 4$$

$$\% \text{ Crude protein} = \% \text{ Nitrogen (N}_2) \times 6.25 \quad 5$$

2.3.8 Determination of crude fibre

A 2.0 g finely ground TA or AL sample was weighed into a round bottomed flask. Then 100 ml of 0.25M tetraoxosulphate (VI) acid solution was added and the mixture was boiled under reflux for 30 min. The hot solution was quickly filtered under suction. The insoluble matter was washed several times with hot water until it was acid free. And then quantitatively transferred into the flask, then 100 ml of hot 0.3M sodium hydroxide solution was added and the mixture was boiled again under reflux for 30 min and was quickly filtered under suction. The soluble residue was washed with boiling water until it was base free. It was then dried to constant weight in an oven at 100°C, and allowed to cool in a desiccator and weighed (C₁). The weighed sample (C₁) was then incinerated in a muffle furnace at 500°C for 2 h, this was cooled in a desiccator and weighed (C₂) (AOAC, 1990). Calculation:

The loss in weight on incineration = C₁-C₂. The calculation was carried out thus:

$$\% \text{ Crude fibre} = \frac{C_1 - C_2}{\text{Weight of original sample}} \times 100 \quad 6$$

All the measurements were taken in triplicates.

3.0 RESULTS AND DISCUSSION

3.1 Physicochemical parameters

Table 1 presents some of the physicochemical parameters of TA and AL gum exudates. The results obtained revealed that the colour of AL and TA are yellowish and milky yellowish respectively with no odour. AL and TA gums exhibited bland and sour tastes respectively. The yields of the purified gums were relatively high, with mean 88.94% for AL and 92.60 % for TA. AL gum exhibited better swelling property than TA gum.

The pH of AL and TA gums at room temperature was 5.24 and 4.34 respectively. TA and AL were soluble in

water but insoluble in acetone, chloroform and ethanol. This also indicates that the gums are ionic. The solubility of the gums in water was found to increase with increase in temperature. The observed increase in solubility with temperature indicate that the heat given off in dissolving the gum is less than the heat required to break the gum apart. The net dissolution reaction is endothermic. Therefore, the addition of more heat facilitated the dissolution of the gum by providing energy to break bonds within the gums.

Table 1: Physicochemical parameters of AL and TA gums

Parameters	<i>Anogeissus latifolia</i> (AL)	<i>Terminalia avicennioides</i> (TA)
Colour	yellowish	yellowish
Odour	odourless	odourless
Taste	bland	Sour
pH (29.2 °C)	5.24±0.2	4.34±0.1
Percentage yield (% w/v)	88.94±1.3	92.60±1.5
Swelling capacity	13±0.8	11±0.6
Solubility in cold water	8.8±2.2	7.1±1.8
Solubility in hot water	9.3±0.8	7.8±0.6
Acetone	0.0	0.0
Chloroform	0.0	0.0
Ethanol	0.0	0.0
Total dissolved solute (mg/l)	153.3±5.2	148.3±3.8
Conductivity (µs/cm)	327.5±3.2	413.2±2.8
Salinity (0/00)	0.0	0.0
Turbidity (FAU)	110±0.2	135±0.5
Wavelength of maximum absorption (nm)	310	300
Melting point (°C)	218-220	220-242
Nitrogen (%)	0.70±0.0	0.71±0.02
Protein (%)	4.38±0.1	4.46±0.2
Ash content (%)	0.53±0.0	0.60±0.1
Fat and Oil (%)	3.80±0.1	2.90±0.2
Moisture content (%)	11.5±0.3	11.3±0.1
Carbohydrate (%)	89.02±0.2	83.26±0.3
Fibre content (%)	0.0	0.0
Tanin content	0.0	0.0

The measured conductivities of the gums were relatively high and comparable with those of ionic compounds (Rouxel, 2011). Also they were found to exhibit low salinity values indicating that the conductivity of the gums may not be primarily due to the presence of chloride ions but due to movement of charges within the colloidal system (Rouxel, 2011).

Turbidity is related to light scattering according to equation 7 (Dror et al., 2006),

$$\frac{I_t}{I_0} = \exp[-d] \quad 7$$

where I_t and I_0 are the intensity of the transmitted and incident light beam, l is the path length of the sample and τ is the turbidity. Taking logarithm of both sides of equation 7 yields equation 8, which can be compared with the Beer-Lambert law according to equation 9

$$2.303 \log \frac{I_t}{I_0} = -\tau l \quad 8$$

$$A = \varepsilon Cl = \log \frac{I_t}{I_0} = \frac{-\tau l}{2.303} \quad 9$$

where A is the absorbance of the system, ε is the molar absorption coefficient, which is defined as the specific absorption coefficient associated with a concentration of 1 mol/L and a path length of 1 cm. From equation 9, it can be seen that since the path length, l is constant, the turbidity of the studied gums is related to the concentration of the active species in the gum (i.e, C) and to the absorbance of the system. The turbidity for AL and TA was 110 and 135 FAU respectively. The values obtained for the gums indicate that TA has a higher ability to scatter light than AL. According to Pablyana et al. (2007), increasing values of turbidity implies higher amount of insoluble contents in the polysaccharides while Yadav et al. (2008) also related turbidity as a property that increases with increasing emulsifying property of a polymer.

The moisture content was $11.5 \pm 0.3\%$ and $11.3 \pm 0.1\%$ for AL and TA respectively and compares favourably with the minimum standards ($\leq 15\%$) for good quality gum, according to European specification (E-414). The value is low in all cases because of the resinous nature of the exudates but reflects the affinity of a material for moisture. High moisture sorption is a disadvantage when moisture sensitive active ingredients are to be formulated (Femi-Oyewo et al., 2004).

The nitrogen content of AL and TA gums were 0.70 and 0.71 % respectively. This gave protein values of 4.38 and 4.46 and 4.29 % respectively. Nitrogen and amino acid contents of gums are useful parameters for distinguishing gums of different species. Karamalla et al. (1998) stated that the range of value expected for the nitrogen content of purified gum arabic is 0.26–0.39%, this is lower than the range (0.49–0.71 %) obtained in this study. In *Acacia senegal* gum concentration of nitrogen was reported to be in the range 0.21 to 0.35 % (Elmanan et al., 2008; Lelon et al., 2010; Siddig et al., 2005). The importance of nitrogen (hence protein) in gum cannot be overemphasized. The immune responses, which are important in providing evidence for the safety of food additives, are customarily accredited to the proteinaceous component of food (Akinhanmi et al., 2008; Youssef et al., 2009). According to Pablyana et al. (2007), the presence of protein in polysaccharide can induce inflammatory response to tissue and the response may have a vital role to play in its pharmacological applications.

The ash content of plant materials is an index of total organic or inorganic contents. Low ash content indicates that the sample is rich in organic content and

vice versa. From the results obtained, the ash content of AL and TA were 0.53 ± 0.0 and 0.60 ± 0.1 respectively. These values falls within the 4 % maximum limit reported by FAO (1998) for food and pharmaceutical quality gum arabic. This values obtained also implies that the studied gums are richer in organic content. However, in order to account for the inorganic composition of the gums, analysis was carried out to quantify their metallic composition.

3.2 Cationic composition

Elemental compositions of the exudates are presented in Table 2. The exudates contain Ca, K, Mn, Na and Mg ions. These metals may occur in the form of cation in the resin soap present in the exudates. The concentrations of K, Mn, Na, Ca and Mg ions obtained were found to be higher than those reported for gums such as *Anogeissus leiocarpus* gum (Eddy et al, 2012), Barijeh gum (Milani et al., 2007), *Khaya grandifolia* gum (Yusuf et al., 2006), *Acacia Senegal* gum (Mhinzi, 2003), and *Anacardium occidentale* gum (Zakaria et al., 1996).

However the values obtained were found to be lower than those for the gums of *Anogeissus leiocarpus* gum (Ahmed et al., 2009), cashew gum (Gyedu-Akoto et al., 2008) and gum Tragacanth (Balaghi et al., 2010). Calcium is found to be of high proportion owing to the fact that it is the most abundant element in plant (Mhinzi, 2002). Concentrations of some heavy metals including Ni, Pb and Cd were below the maximum permissible limits of 10 0.2 and 0.03 ppm respectively (FAO, 1988) hence the studied gums do not contain toxic concentrations of heavy metals. Since the studied gums is richer in essential minerals than those of heavy metals, they may be a good source of mineral nutrition suitable for use as pharmaceutical excipients and as food additives (Ahmed et al., 2009).

It is known however, that the amount of element is not fixed for exudates gum but vary depending upon the soil type, husbandry of the bush, age of the parent tree, amount of rainfall received, time of exudation and type of exudation. Also, the heavier the pruning condition, the higher the elemental composition (Mhinzi, 2003).

Table 2: Elemental composition of the gum exudates

Cations	Concentration (ppm)	
	AL gum	TA gum
Na	23.30	21.53
K	0.61	1.01
Zn	1.33	0.21
Al	0.51	1.43
Pb	0.65	1.01
Mg	5.52	11.40
Cd	0.001	0.004
Ca	59.21	51.21
Cu	0.04	0.04
Ni	0.18	0.08
Fe	0.04	0.33
Mn	0.08	0.05

4.0 CONCLUSION

The study indicates that AL and TA possesses good physico-chemical properties and high levels of essential minerals such as calcium, magnesium and iron. The protein and ash contents of the gums are found in comparatively small amount. The results of this study support the gums suitability for industrial application, especially in areas where commercial gum arabic is traditionally used.

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