

Physicochemical, Spectroscopic and Rheological Studies on *Eucalyptus Citriodora* (EC) Gum

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Abstract Analysis of physicochemical properties of *Eucalyptus citriodora* gum revealed that the gum is mildly acidic, brownish in colour, ionic and has the potentials to swell four times its original volume. GCMS analysis of the gum indicated the presence of some carboxylic acids, pyran-4-one, 1,3-dioxolane, benzofuran and 1,2-ethanediyl acetate while analysis of its FTIR spectrum revealed functional groups that are common to polysaccharides. Scanning electron micrograph of the gum also revealed the existence of particle aggregations with some internal bridges within the system. Rheological properties of the gum were found to be affected by pH, concentration, temperature and by the presence of some electrolytes (KCl, CaCl₂, AlCl₃ and urea). Average value of intrinsic viscosity deduced from Huggins and Kraemer plots was 3.51 dL/g while the sum of their constants was 0.36 (i.e. < 0.5 and suggested the absence of molecular association). Calculated value of the Power law constant was (b= 0.812) less than unity and pointed to a rod like conformation. From the Master's curve, the existence of dilute and concentrated regimes, (corresponding to $\eta_{sp0} \propto C^{1.04}$ (at $C > C^*$) and $\eta_{sp0} \propto C^{0.95}$ (at $C < C^*$) respectively) was established. *Eucalyptus citriodora* gum is a shear thinning, non-Newtonian polymer that is characterized by pseudoplastic behavior. The gum has some potentials for use as food additives and for other industrial applications.

Keywords: *Eucalyptus citriodora* gum, physicochemical parameters, GCMS, FTIR, SEM, rheology

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1. Introduction

Exudate gums are obtained as natural exudates of different tree species and exhibit unique properties in a wide variety of applications including pharmaceutical, food, adhesive, paper, textile and other industries [1]. For example, *Albizia zygia* and some *Albizia lebbek* gums are useful as natural emulsifiers for some foods, pharmaceutical and as a substitute for Arabic gum in the mining and metallurgical industries [2,3]. In the froth flotation of base and platinum group metal ores, guar gum is used as a depressant for naturally hydrophobic waste minerals such as talc. The role of polysaccharide is to render the metal hydrophilic and prevents its flotation, after it is been adsorbed on the gum surface. In the pharmaceutical industries, functional properties of Guar gum are of primary importance for controlling the release of drugs in the gastrointestinal tract, such as carrier for colon targeted drugs, for anti-cancer drugs in the treatment of colorectal cancer and for oral rehydration solutions in the treatment of cholera in adults [4]. Guar gum is also used in transdermal drug delivery systems, as a synthetic cervicle mucus and as a visco-supplementation agent in osteoarthritis treatment. *Detarium senegalense* and

Gmelin gums have been reported to be useful in pharmaceutical and food industries. They have the potential of controlling drug release and in modifying the texture of some food [5].

The various applications that can be derived from gums have been found to depend on their functional properties such as physicochemical, proximate, cationic content, phytochemical and rheological properties. For example, in spite of its favourable rheological composition, *Albizia lebbek* gum cannot be used as food additives because of its high content of tannins and high proportion of aluminum [2]. Pablyana *et al.* [4], 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.* [6] found that emulsifying properties of polysaccharides depends on its turbidity, Interfacial rheology of gums has also been found to be an essential parameter in predicting the emulsifying properties of gums [7]. *Fenugreek* gum is widely used as thickening, water holding, stabilizing and emulsifying agents in food industries because its galactose and manose content gives it a high viscosity in aqueous solution [8]. Similarly, Rinaudo [9] stated that gum exudates have wider industrial applications because of their excellent water holding capacity (which facilitate the production of gels or highly viscous solutions). Gums also have the

ability to enhance the stability of some emulsions and foams. However, these properties depend on the chemical structure of the gum, its polysaccharides content and solvent phase conformation (which is dependent on pH, temperature, ionic strength, acetyl and pyruvate contents of the gum) [10]. Higiro *et al.* (2006), also stated that Xanthan gum yields a strong interaction with galactomannans and that this property is harnessed in thickening of food, that requires gelling. Menon [11] also found that rheological properties of polymer solution is essential in determining their processability and hence their applications.

In view of the above, several gums have been studied but literature is scanty on functional characteristics of *Eucalyptus citriodora* (EC) gum hence the present study is aimed at investigating physicochemical, spectroscopic and rheological properties of EC gum.

2. Results and Discussions

2.1. Physicochemical Properties

Figure 1 shows photographs of crude and purified samples of EC gum while Table 1 presents physicochemical properties of EC gum. From the results obtained, EC gum is brownish in colour and exhibited characteristic odour of an orange. The gum is acidic and presents a sour taste. The yield of the gum after purification was 97.60 %, which is relatively high when compared to those of other gums. The gum has the potential of swelling to about four times its original capacity. The gum is soluble in cold and hot water, insoluble in acetone and chloroform but slightly soluble in ethanol. The measured conductivity (116 $\mu\text{s}/\text{cm}$) of the gum is comparable to those of ionic compounds. From the point of view of solubility and conductivity, EC gum is ionic. High conductivity and solubility in solvents with high dielectric constant are characteristics of ionic compounds [12]. The turbidity of the gum (303 NTU) was relatively high indicating that the gum may contain higher amount of insoluble substances and may be good as an emulsifying agent [4,6]. From Table 1, it is also evident that the total dissolved solid content of EC gum is 55 mg/L indicating that the gum has high concentration of insoluble substances, a character that may encourage its potential as a good emulsifying agent.



Purified sample



Crude sample

Figure 1. Photographs of crude and purified samples of EC gum

Table 1. Physicochemical parameters of EC gum

Parameter		Metals (ppm)	
Colour	brownish	Mn (ppm)	0.212
Odour	orange	Fe (ppm)	2.408
Taste	sour	Zn (ppm)	0.705
pH (29.2 °C)	3.91	Pb (ppm)	0.088
Percentage yield (% w/v)	97.60	Mg (ppm)	0.085
Swelling capacity	4	Cd (ppm)	0.001
Solubility in cold water	5.2	Ca (ppm)	8.742
Solubility in hot water	1.2	Cu (ppm)	0.039
Acetone	0.0	Ni (ppm)	0.124
Chloroform	0.0		
Ethanol	0.2		
TDS (mg/l)	54.6		
Conductivity (µs/cm)	116.3		
Salinity (0/00)	0.0		
Turbidity (FAU)	303		
λ_{max} (nm)	305		
Melting point (°C)	181-195		
Nitrogen (%)	0.69		
Protein (%)	4.29		
Ash content (%)	0.30		
Fat and Oil (%)	1.3		
Moisture content (%)	4.0		
Carbohydrate (%)	90.11		
Fibre content (%)	0.0		

Table 1 also presents proximate composition of EC gum. From the results obtained, excessive concentration of carbohydrate (90.11 %) and absence of dietary fibre were observed. This finding is consistent with those reported for most plant gums [13]. Generally, plant and seed gums are complex polysaccharides/carbohydrate polymers and are mostly used as thickening agent, foaming agent, film, emulsifier, stabilizer and drug delivery agent because of their high carbohydrate content. Concentrations of nitrogen and protein in the gum were 0.69 and 4.29 % respectively, which is comparable to those reported for *Ferula gumosa* gum (0.69 % ;[14]), *Albizia gums* (0.46 – 1.09 %; [3]). However, mean concentration of nitrogen in EC gum was found to be higher than those reported for gum Arabic (0.26 – 0.39 % ; [15]) and are also comparable to the range of values

reported for some species of *Acacia senegal* gum (0.21 to 0.35 %; [16,17,18]). The important of nitrogen (hence protein) in gums 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 (Youssef *et al.*, 2009). According to Pablyana *et al.* [4], 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.

Moisture content of EC gum (4 %) was relatively low indicating that the gum has low water absorbing or retaining capacities. Similarly, the lipid content of the gum was as low as 1.3 % indicating that the gum has low potential to turn rancid. The low ash content of the gum (0.30 %) also suggests that it has much organic content than inorganic constituents. Plant gums are polysaccharides containing various metal ions as neutralized cations. Therefore, concentrations of Mn, Fe, Zn, Mg, Ca, Cu, Ni, Na and K in EC gum were analysed using atomic absorption spectrophotometer and the results obtained, indicated high concentrations of essential metal ions (Ca = 8.74 %, Fe = 2.41 % and Zn = 0.75 %). Trace metals were present in trace concentrations (i.e, Mn = 0.212 %, Mg = 0.085; Cu = 0.039 %; Ni = 0.124 %) while toxic metals were found in concentrations that did not exceed their tolerance limit (i.e Pb = 0.088 and Cd = 0.001 %) [19]. Therefore, EC gum is may be a good source of mineral nutrition.

2.2. Spectroscopic Study

The GCMS spectrum of EC gum indicated the presence of 15 peaks (spectrum not shown). Chemical structures of compounds identified from each line on the spectrum are presented in Figure 2 while Table 2 presents the molar mass, retention time and percentage concentrations of the identified compounds. From the results obtained, line 1 to 4 revealed the presence of 1.13 %, 3.70 %, 4.93 % and 3.58 % of pyran-4-one, 1,3-dioxolane, benzofuran and 1,2-ethanediyl acetal while lines 5 to 14 revealed the presence of carboxylic acids at concentrations and retention time presented in Table 2.

Table 2. Analytical parameters deduced from GCMS spectrum of EC gum

Line no	IUPAC name	Molecular formula	Molar mass (g/mol)	Retention time (m)	Concentration (%)
1	Pyran-4-one	C ₆ H ₈ O ₄	144	8.367	1.13
2	1,3-dioxolane	C ₈ H ₁₀ O	122	9.058	3.70
3	Benzofuran	C ₈ H ₈ O	120	10.217	4.93
4	1,2-ethanediyl acetal	C ₂₁ H ₃₄ O ₂	318	13.975	3.58
5	Trans-cinnamic acid	C ₉ H ₈ O ₂	148	14.525	1.46
6	2-propenoic acid	C ₉ H ₈ O ₃	164	20.667	12.54
7	Hexadecanoic acid	C ₁₇ H ₃₄ O ₂	270	22.508	11.53
8	Pentadecanrcarboxylic acid	C ₁₆ H ₃₂ O ₂	256	23.417	14.13
9	9,12-octadecadienoic acid	C ₁₈ H ₃₄ O ₂	294	26.442	30.86
10	Elaidic acid	C ₁₉ H ₃₆ O ₂	296	26.642	5.12
11	Stearic acid	C ₁₉ H ₃₈ O ₂	298	27.258	11.03
12	9-hexadecenoic acid	C ₁₆ H ₃₀ O ₂	254	27.557	1.13
13	Hydrofol acid	C ₁₈ H ₃₆ O ₂	284	28.042	3.70
14	Ricinoleic acid	C ₁₉ H ₃₆ O ₃	312	29.858	4.93

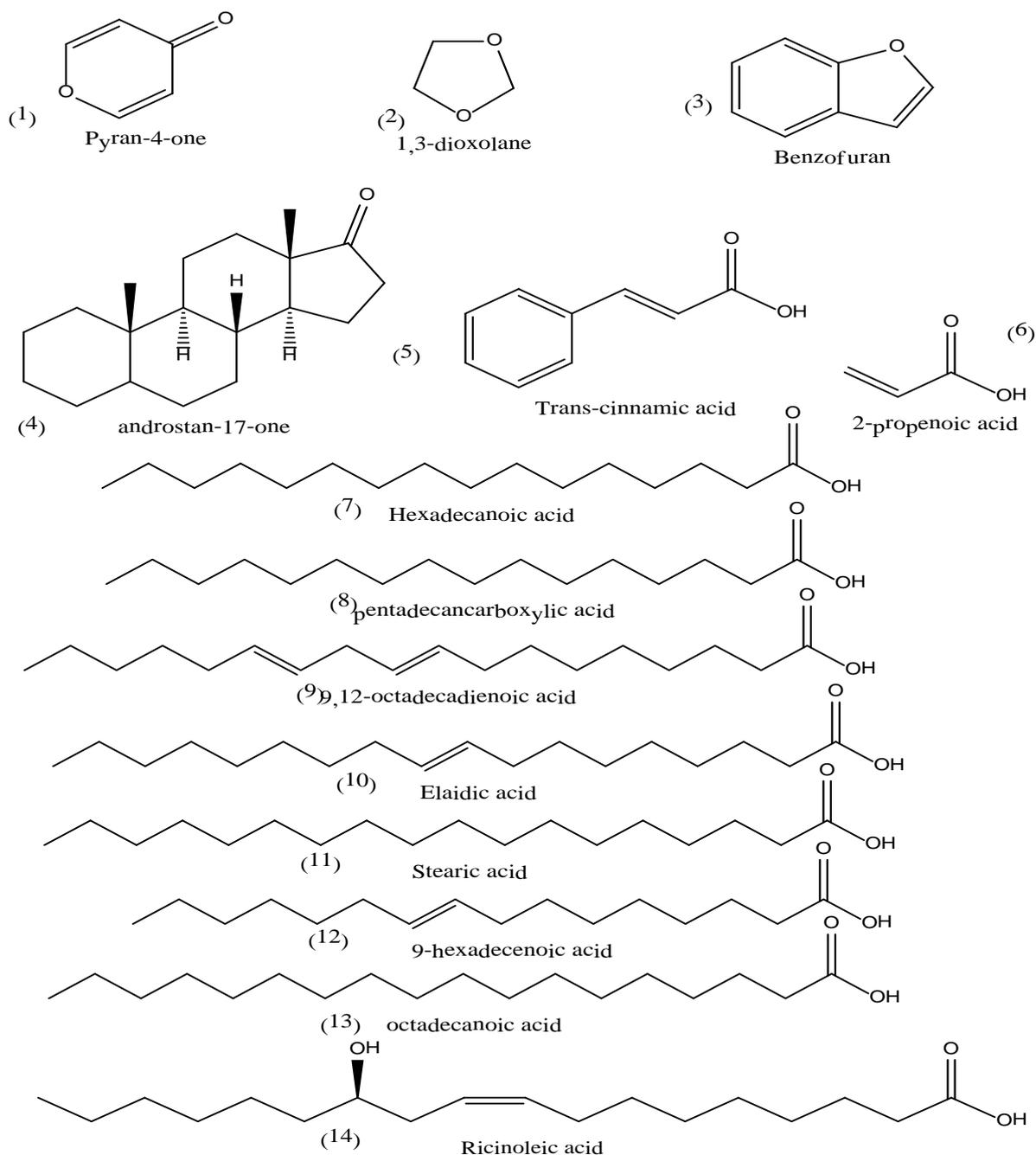


Figure 2. Chemical structures of compounds in EC gum

Figure 3 presents FTIR spectrum of EC gum while Table 3 presents peaks and frequencies of IR adsorption as well as associated functional groups. The spectrum displayed features, typical of polysaccharides. Thus OH stretch vibrations were found at 3492, 3379 and 3268 cm^{-1} respectively. The peak obtained at 2911 cm^{-1} is due to stretching modes of C-H bonds of methyl group (CH_3). According to Nep and Conway [20], natural gums usually contain fractions of sugar acid units which will usually impart a weakly anionic character to the gum macromolecule. C=O stretches due to acetyl groups were prominent at 1710 and 1245 cm^{-1} respectively. Absorption bands at 1615 and 1049 cm^{-1} are typical for carboxylate groups of the galacturonic acid residues [21]. Other functional groups identified were CH_2 twisting vibration at 1313 cm^{-1} , OH stretch due to carboxylic acid at 1448 cm^{-1} , NO asymmetric stretch at 1510 cm^{-1} and C-X stretch vibrations due to halides at 837, 749, 572 and 524 cm^{-1} .

Table 3. Peak, frequency and assignment of FTIR absorption by AO gum

Peak	Intensity	Area	Assignment
614	29.48	141.27	-C-H bend due to alkyne
696	30.58	74.37	-C-H bend due to alkyne
1069	22.83	115.87	C-O stretch
1141	23.14	64.89	C-O stretch
1277	38.55	49.27	OH bending
1402	25.31	111.43	OH bending
1631	31.75	108.81	C-O stretch
2911	23.70	295.16	CH stretch
3133	23.58	99.93	C-H stretch
3220	23.73	68.40	OH stretch
3333	24.50	110.52	OH stretch
3546	24.81	160.00	OH stretch

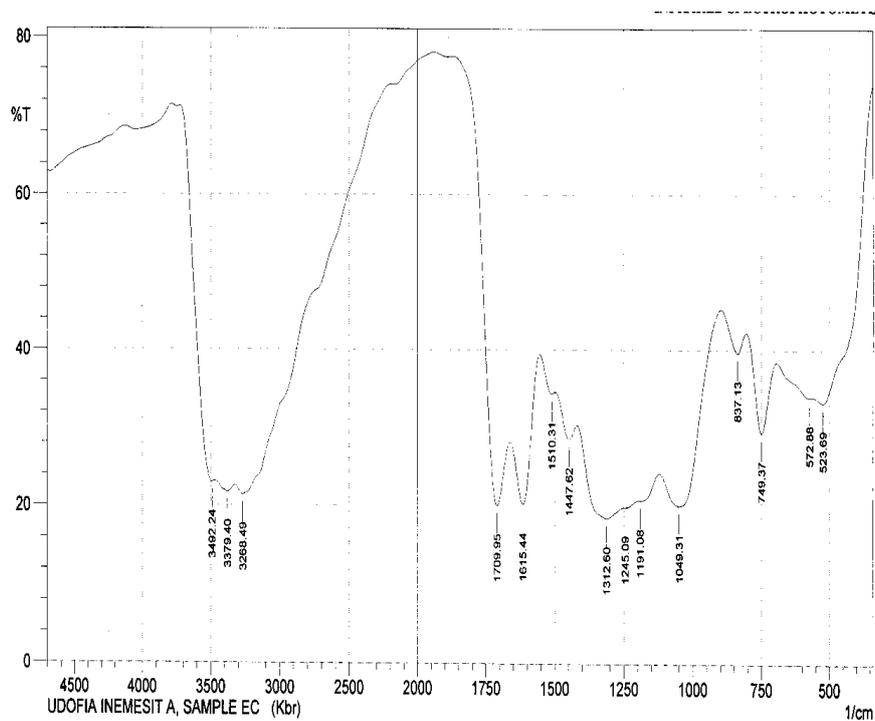


Figure 3. FTIR spectrum of EC gum

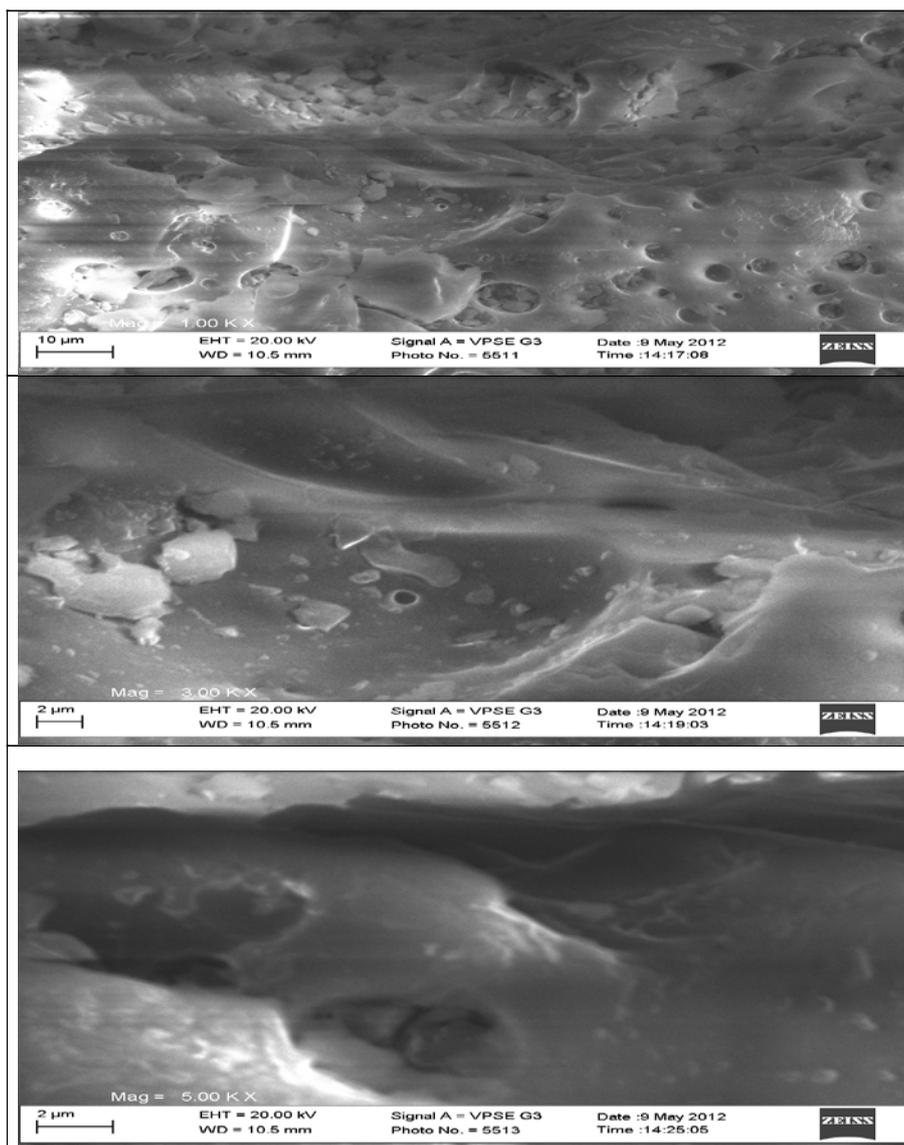


Figure 4. Scanning electron micrograph of EC gum at 1.00 KX magnification and scale bar of 2 µm

Scanning electron microscopy (SEM) gives image of a surface of a materials/specimens at a desired position and therefore displayed topographic/morphological picture with better resolution and depth of focus. SEM is often used in studying the morphology of polymer materials [22]. The scanning electron micrographs of *EC* gums at 1.00, 3.00 and 5.00 KX magnifications are shown in

Figure 4. At magnification of 1.00 KX, the micrograph revealed a smooth surface with irregularly spaced pores. The system has an irregular network but could likely provide favoured adsorption sites. At 3.00 and 5.00KX, the micrographs indicated the existence of particle aggregations with some internal bridges within the system

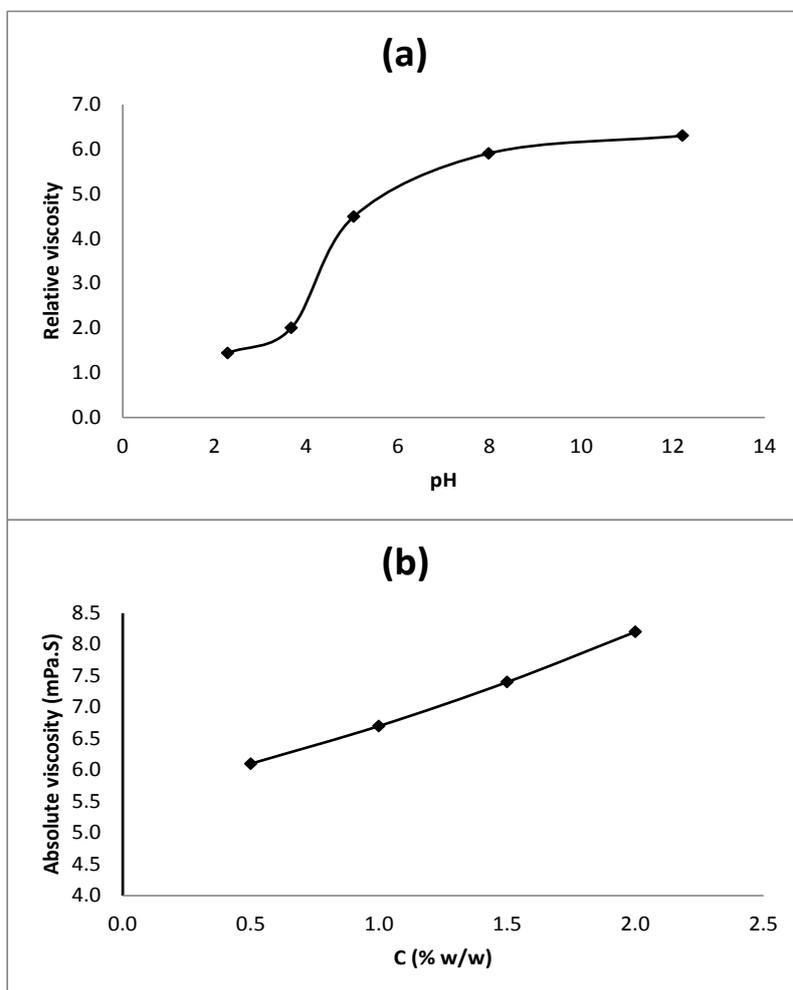


Figure 5. Variation of (a) relative viscosity with pH (b) absolute viscosity with concentration for *EC* gum

2.3. Rheological Study

2.3.1. Effect of Concentration and pH

Figure 5a shows the variation of viscosity with concentration for *EC* gum. The absolute viscosity of the gum is found to increase with increase in concentration indicating that there is an increase in internal resistant of the gum to shear or flow. This may be attributed to intermolecular friction exerted when layers of fluids attempt to slide over one another.

According to Newton law of friction, dynamic or absolute viscosity of a polymer can be expressed as follows [23],

$$\eta = \tau \frac{1}{dC/dy} \quad (1)$$

where τ is the shearing stress, dC/dy is the concentration gradient. From equation 1, it can be seen that at constant shear rate, the absolute viscosity of a viscous fluid is expected to increase with increase in concentration as

shown in Figure 5a. On the other hand, Figure 5b presents plots for the variation of specific viscosity with pH for *EC* gums. It is evident from the Figure that the specific viscosity of the gum tends to increase with increase in pH. The observed trend may be explained as follows. At low pH, the electrostatic repulsions between gum particles tend to increase indicating that the particles will present a decreasing association as a results of the formation of weak intermolecular forces (including hydrogen bond, Van der Waals and other weak forces). Therefore the viscosity of the gum will increase until a certain critical pH is approached. Above the critical pH (pH = 8), the dissociation of the particles may increase due to decrease in the strength of electrostatic repulsions.

2.3.2. Intrinsic Viscosity

In viscosity study, several terms relating solution and solvent viscosities can be defined as follows,

$$Relative\ viscosity : \eta_{rel} = \frac{\eta_{solution}}{\eta_{solvent}} \quad (2)$$

$$\text{Specific viscosity : } \eta_{sp} = \eta_{rel} - 1 \quad (3)$$

$$\text{Inherent viscosity : } \eta_{inh} = \frac{\ln \eta_{rel}}{C} \quad (4)$$

$$\text{Reduced viscosity : } \eta_{red} = \frac{\eta_{rel} - 1}{C} \quad (5)$$

where η_{sp} is the specific viscosity, η_{rel} is the relative viscosity, $\eta_{solution}$ is the viscosity of the solution, $\eta_{solvent}$ is the solvent viscosity and C is the concentration. The intrinsic viscosity $[\eta]$ is a measure of the inherent ability of a polymer to increase solution viscosity. The most appropriate relationship that relates intrinsic viscosity with the viscosity of a dilute polymer solution is a power series in concentration and can be written as follows;

$$\frac{\eta_{sp}}{C} = [\eta] + k_1 [\eta]^2 C + k_2 [\eta]^3 C^2 + k_3 [\eta]^4 C^3 + \dots \quad (6)$$

The expression, η_{sp}/C is the reduced viscosity and as $C \rightarrow 0$, it becomes the $[\eta]$ hence the Power series expressed in equation 5 becomes truncated to a linear approximation which is known as the Huggins equation [24]:

$$\eta_{red} = [\eta] + k_H [\eta]^2 C \quad (7)$$

where η_{red} is the reduced viscosity and k_H is the intrinsic viscosity. From equation 7, a plot of $\eta_{red} = f(C)$ should be linear with intercept and slope equal to $[\eta]$ and $k_H[\eta]^2$ respectively. Figure 6 presents Huggins plot for *EC* gum.

The plot reveals excellent degree of linearity for the plot ($R^2 = 0.9996$), which confirm the application of Huggins model. The intrinsic viscosity calculated from the intercept of the plot was found to be 4.31 dL/g while the k_H value was 0.13. Theoretically, the k_H should lies between 0.3 and 0.8 in a theta solvent. According to Ma and Pawlik (2007), a polymer exhibits a higher value of k_H in a poor solvent than in a good solvent because polymer-polymer interaction is favourable. However, calculated value of the k_H for *EC* gum is relatively lower than the expected range hence water is a theta solvent for *EC* gum. It has also been found that intrinsic viscosity can also be obtained from the Kraemer equation by extrapolation to zero concentration [25]:

$$\frac{\ln \eta_{rel}}{C} = [\eta] + k_K [\eta]^2 C \quad (8)$$

where k_K is the Kraemer constant. Kraemer's plot for *EC* gum is also shown in Figure 6. The intrinsic viscosity calculated from the Kraemer's plot ($[\eta] = 2.72$ dL/g) was lower than the value obtained from the Huggins plot. The degree of linearity of the plots ($R^2 = 0.9980$) was also excellent and the Kraemer constant ($k_K = 0.23$) was relatively higher than the k_H . However, the sum of Kraemer and Huggins constants was 0.36 which is below the range (i.e $0.5 \pm 10\%$) of values expected for gums that are characterized by the absence of molecular association. This suggests the existence of molecular association between the molecules of *EC* gum.

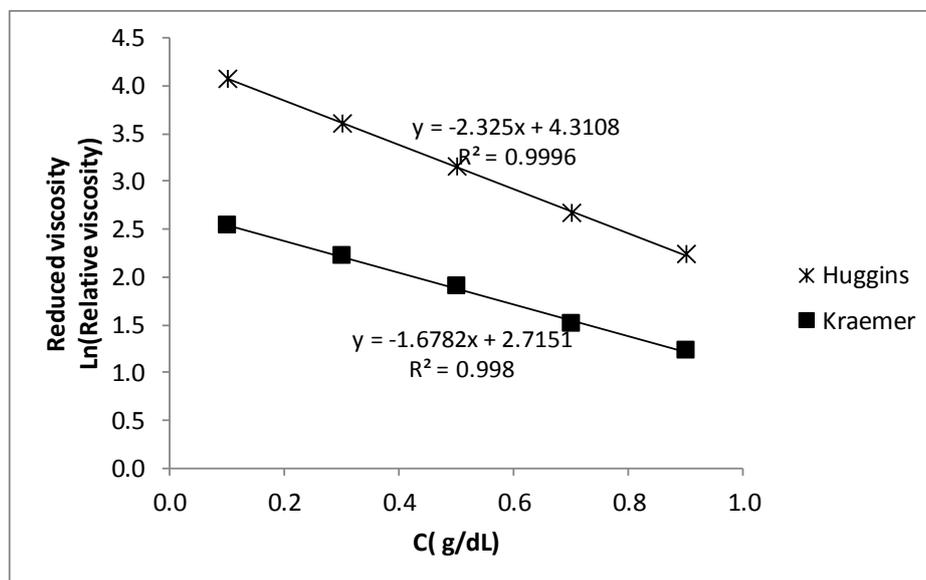


Figure 6. Huggins and Kraemer plots for *EC* gum

2.3.3. Molecular Conformation and Coil Overlap Parameter

The Power law model (equation 8) is useful for studying molecular conformation as well as the level of interactions between molecules in a solution of a polymer [26]

$$\eta_{sp} = aC^b \quad (8)$$

Simplification of equation 8 yielded equation 9 and from equation 9, a plot of $\ln(\eta_{sp})$ versus C was found to be

linear with slope and intercept equal to b and $\ln(a)$ respectively (plot, not shown).

$$\ln \eta_{sp} = \ln a + b \ln C \quad (9)$$

The results obtained indicated an excellent correlation ($R^2 = 0.9329$) while calculated 'b' value was 0.812 for *EC* gum. The value of 'b' is significant in rheological studies because it is an index for predicting the conformation of polysaccharides [26]. Since 'b' value greater than unity is associated with random coil conformation and 'b' value less than unity is associated with rod like conformation,

the conformation of *EC* gum is more rod/coil like than random like [27].

It has been found that in dilute solution, the individual polymer coils are separated from each other and are free to move independently but with increasing concentrations, the coil starts to overlap and interpenetrate one another. According to Morris *et al.* [28], the transition from dilute solution to concentrated solutions is usually accompanied by pronounced change in concentration dependence of solution viscosity and the corresponding concentration is called critical or coil overlap concentration (C^*). The concentration dependence of the specific viscosity (at zero shear rate) on the coil overlap parameter, $C[\eta]$ for *EC* gum (plot not shown) was found to displayed two regimes indicating the existence of molecular entanglement. The first and second regimes were observed at slope values of 1.04 and 0.95 respectively. This indicate that when $C > C^*$, $\eta_{sp0} \propto C^{1.04}$ while at $C < C^*$, $\eta_{sp0} \propto C^{0.95}$. Hence, $C^*[\eta] \sim 3.37$ and the critical concentration for coil overlap is 1.12 g/dL.

2.3.4. Effect of Temperature

The effect of temperature on the viscosity of *EC* gum was studied between a temperature range of 303 and 343 K. A decrease of viscosity was observed and there was no degradation or conformational transitions during heating. Since there was no conformational transition, the Arrhenius-Frenkel-Eyring equation was applied to calculate the activation energy of flow using equation 10 [29]

$$\eta = A \exp \left[\frac{E_F}{RT} \right] \quad (10)$$

where η is the absolute viscosity of *EC* gum, B is the pre-exponential constant, E_F is the activation energy of flow, R is the gas constant and T is the absolute temperature. From the logarithm of equation 10, equation 11 was developed and used to plot $\ln(\eta_{sp}) = f(1/T)$ as shown in Figure 7. The plots exhibited good correlation coefficient ($R^2 = 0.8723$) while calculated value of E_F was 16.67 kJ/mol. This value is within the range of values reported for some plant gums including *Albizia lebbek* gum (15.9

to 17.2 kJ/mol, [2]), Arabic gum (15 kJ/mol, [30]), *A. occidentale* gum (16.2 kJ/mol, [31]) and *A. macrocarpa* gum (16.8 kJ/mol, [32]). Generally, higher value of E_F indicates that the polymer solution is less sensitive to change in temperature and vice versa. Low E_F also indicates the existence of few inter- and intra interaction between polysaccharide chains in the studied concentration range, which is the case for the studied gum. The E_F values for polymers strongly bonded by inter and intra-molecular interaction has been found to in the range of 27 kJ/mol and above [33].

Thermodynamic parameters of viscous flow were also calculated using the Frenkel-Eyring equation [23], which can be written as follows:

$$\ln \left(\frac{\eta}{T} \right) = \left(\ln A - \frac{\Delta S_V}{R} \right) + \frac{\Delta H_V}{RT} \quad (11)$$

where A is the pre-exponential factor, R is the universal gas constant, T is the absolute temperature, ΔS_V and ΔH_V are the entropy and enthalpy changes of viscous

flow. From equation 11, a plot of $\ln \left(\frac{\eta}{T} \right)$ versus $1/T$ is expected to be linear with slope and intercept equal to $\frac{\Delta H_V}{RT}$ and $\left(\ln A - \frac{\Delta S_V}{R} \right)$ respectively. Figure 7 also

presents the Transition state plot for *EC* gum. Calculated values of ΔS_V and ΔH_V were -55.77 and 14.09 kJ/mol. The negative and positive values obtained for ΔS_V and ΔH_V respectively, suggest that the attainment of transition state of viscous flow is accompanied by bond breaking. The entropy change of activation from the initial to the transition state at a given composition is significant during an activated viscous flow process; indicating that this process may be entropy-controlled [34]. Negative values of ΔS_V is associated with uncoiling orientation of the polymer molecules. Thus, the system becomes more ordered in the course of flow. On the other hand, ΔH_V is related to the amount of energy needed by the gum molecules to jump from one equilibrium position to another (i.e the potential energy barrier) [35].

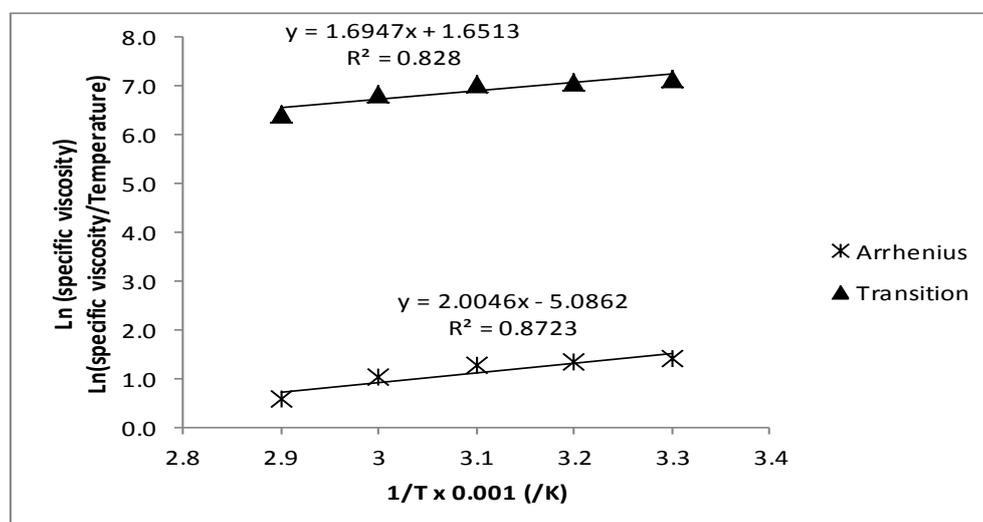


Figure 7. Arrhenius and Transition state plots for *EC* gum

2.3.5. Effect of Electrolytes

Like all other exudate gums, *EC* gum is acid polysaccharides containing various metal ions (including, Mn, Fe, Zn, Mg, Ca, Cu, Ni, Na and K) as neutralized cations. Since this gum will behave like polyelectrolyte, then solution viscosity of the gum will be affected by the addition of other electrolytes. In this study, effects of KCl, CaCl₂, AlCl₃ and urea on the viscosity of *EC* gum were investigated. Addition of these electrolytes was found to reduce the viscosity of the gum as shown in Figure 8. The trend in the decrease corresponded to decreasing charge of the ions (i.e, Al³⁺>Ca²⁺>K⁺). The decrease in viscosity can be attributed to the existence of fewer intermolecular interactions due to the screening of charges and contraction of the macromolecule in the presence of counter ion [2]. From literature, it is evident that aluminum has a tendency to establish strong interaction with macromolecules through intermolecular crosslinking effect. On the other hand, the strength of intermolecular crosslinking effect is lower in Ca²⁺ than in aluminum

while sodium is not a cross linking agent.. Ahmad *et al.* [35] found that in dilute solution, the turbidity of dilute polyelectrolyte increases with increase in the charge of the ions. Therefore, the variation of the viscosity of *EC* gum with increasing charge can also be attributed to their effect on turbidity. It is significant to state that the affinity between the gum and counter ions depends on the charge/ionic ratio. Generally, ion with higher charge will have a stronger affinity for the molecular chain of the gum. The charge to ionic radius ratio of K⁺, Ca²⁺ and Al³⁺ are 0.66, 1.75 and 4.41 respectively hence the expected order for the interaction should be Al³⁺> Ca²⁺>K⁺, which is in agreement with our findings. Urea was also seen to decrease the viscosity of *EC* gum because it has the ability to trap some of the gum's molecules into its clathrates, which is an organic guest that are held in channels formed by interpenetrating helices of hydrogen-bonded molecules. The withdrawal of some organic molecules by urea guest may lower polymer-polymer interaction and will result in a decrease in viscosity as observed in the study.

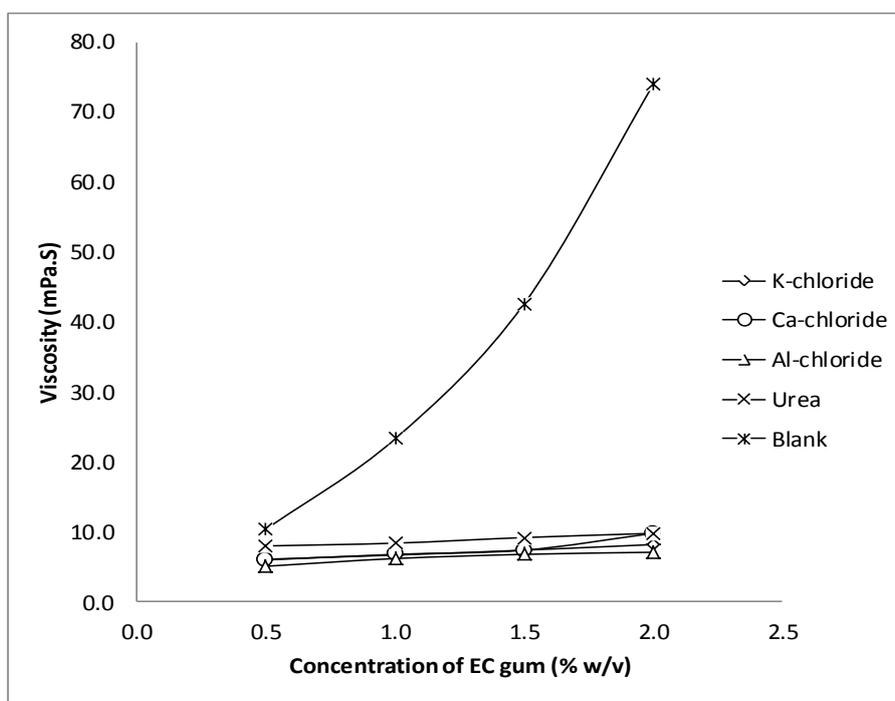


Figure 8. Variation of viscosity of *EC* gum with concentration in the presence of 0.1 M of various electrolytes (KCl, CaCl₂, AlCl₃ and urea)

Ionic strength is a very important factor in studying the rheology of polymer solutions. Ionic strength affects the thickness of the electrical double layer around the charged interfaces as well as modifying the conformation of the polymer attached to the surface (i.e spatial extension). The effect of ionic strength on the viscosity of *EC* gum was studied by monitoring the effect of various concentrations of the electrolytes on the apparent viscosity of 1% (w/v). The results obtained (plots not shown) revealed that the apparent viscosity of *EC* gum decreases with increase in the logarithm of the salt concentration, which may be attributed to shielding and cross linking effects. The cross linking effect seems to prevail more in the presence of Al³⁺, while the contribution of shielding effect led to chain contraction in Ca²⁺. As stated earlier, shielding and cross linking effect is associated with the ratio of charge to ionic charge of the metal. In Al³⁺, the ratio is highest therefore

cross linking effect dominates but K⁺ having the least ratio displayed strong shielding effect. Sanin [36], found that an increase in the ionic strength of a polymer solution will lead to a decrease in viscosity because as more ions are added to the system, the viscosity starts to give less respond by changing minimally. Consequently, the electrical double layer is compressed at high ionic strengths. Particles and flocs may assume closer localities with each other and the surface polymer may also assume a coiled conformation rather than a stretch conformation.

2.4. Steady Shear Properties

In studying steady shear properties of a polymer solution, parameters of interest are shear stress, shear rate, speed of rotation and viscosity. Base on the variation of viscosity with shear rate or shear stress with shear rate,

polymer solution can be classified as Newtonian or non Newtonian. Newtonian fluids are fluids which have a constant viscosity indicating that a plot of shear stress versus shear rate for Newtonian fluid should be linear [37-38]. Deviations from Newtonian systems have been attributed to several factors and it varies from one polymer to another polymer systems.

Fig. 9 shows plots for the variation of (a) shear stress with shear rate (b) speed of rotation with viscosity and (c) speed of rotation with torque for EC gum

speed of rotation with torque. The shape of the plot in Figure 9a indicated that EC gum is non Newtonian and displayed characteristics shear thinning property which is typical for a pseudoplastic flow behavior. Figure 9b revealed a linear plot which is typical for non Newtonian behavior [39]. From Figure 9a, the yield stress (i.e the amount of force needed to be applied to the gum before it can flow) was estimated through the intercepts.

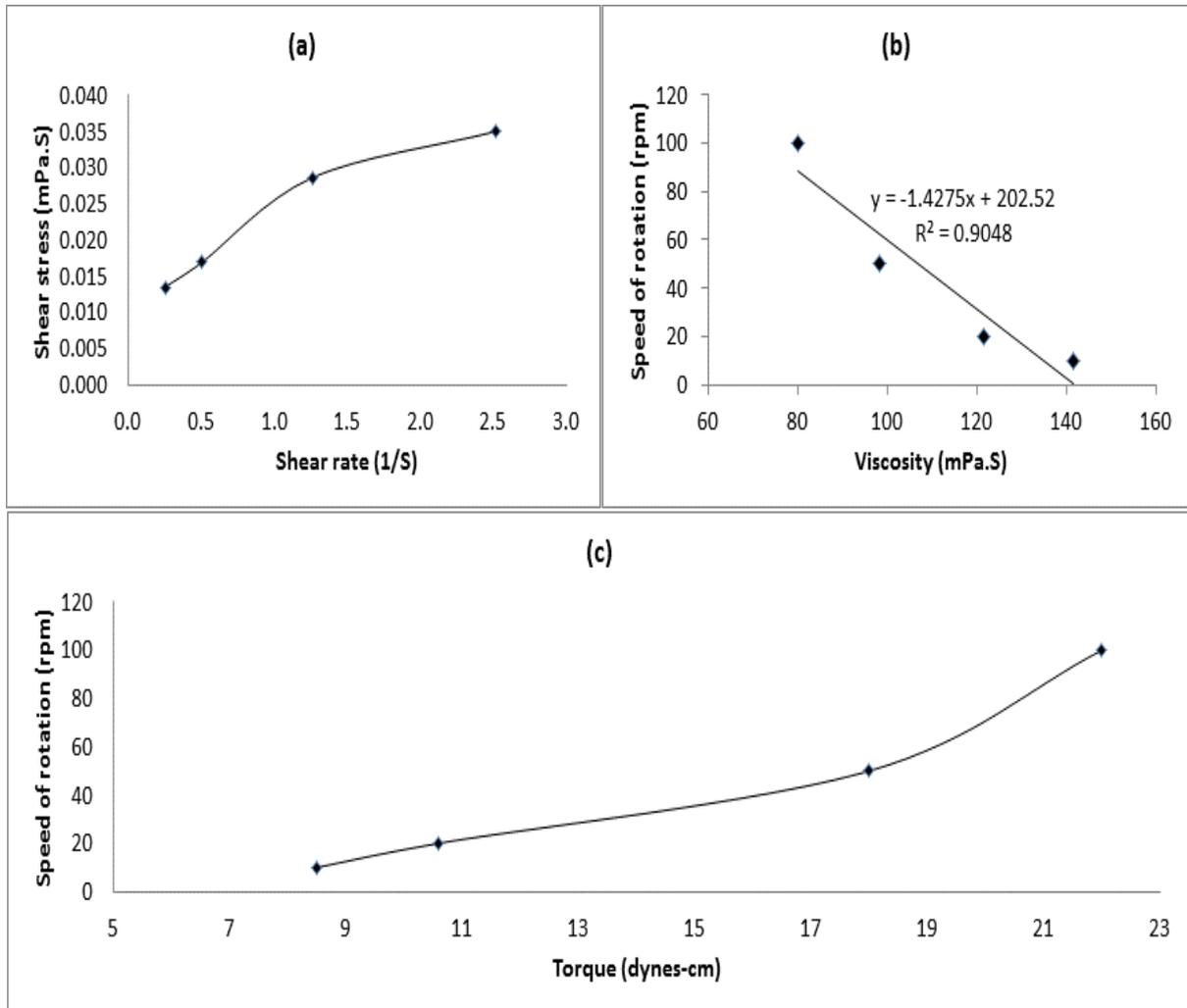


Figure 9. Variation of (a) shear stress with shear rate (b) speed of rotation with viscosity and (c) speed of rotation with torque for EC gum

Literature revealed that the angle formed by the straight line against the y-axis is an index for predicting the flow behavior of a gum [39]. The relationship between this angle is given by the power law index, thus, Power law index, $N = \tan(\text{the angle between the plot line and y-axis})$. If the angle is less than 45 degrees, the fluid is pseudoplastic and a dilatant if it is greater than 45 degrees. Values of yield stress, $N(\theta)$ and θ deduced from the plot were 32.37° and 138.87 mPaS respectively. Therefore, EC gum exhibited a shear thinning property with a pseudoplastic non Newtonian behavior. Triantafillopoulos [40] stated that non Newtonian fluid can also be characterized by considering the pattern of variation of rotation speed with torque as shown in Figure 9c. The pattern observed in this case, also confirmed that EC gum is a shear thinning non Newtonian gum with a pseudoplastic behavior [41].

In pseudoplastic non-Newtonian flow phenomenon, viscosity decreases with shear rate because both secondary and primary bonds are being broken isothermally and reversibly [42]. Also clusters and aggregates of particles originally present in suspension are destroyed by shear. Specific mechanisms responsible for this kind of rheological behavior are:

- (i) structure-breaking due to hydrodynamic effects where the rate of particle disassociation is greater than the rate of their association;
- (ii) favorable orientation of macromolecules or dispersed asymmetric particles in the flow field;
- (iii) diminution or removal of the absorbed film (solvation layer) surrounding the particles due to hydrodynamic effects.

The degree of shear-thinning depends on the structural state of the dispersion prior to shearing and on the

composition of the dispersing phase. Consequently, hydrocolloids are excellent shear-thinning agents.

3. Conclusion

From the results and findings of the study, the following conclusions were made,

1. *EC* contains carboxylic acids between lines 5 and 14. These included 1.46 % trans-cinnamic acid, 12.54 % 2-propenoic acid, 11.53 % hexadecanoic acid, 14.13 % penta decanoic carboxylic acid, 30.86 % 9,12-octadecadienoic acid, 5.12 % elaidic acid, 11.03 % stearic acid, 1.13 % 9-hexadecenoic acid, 3.70 % hydrofol acid and 4.93 % ricinoleic acid. However 1.13 % pyran-4-one, 3.70 % 1,3-dioxolane, 4.93 % benzofuran and 3.58 % 1,2-ethanediyl acetal.
2. *EC* gum is rich in some nutrient. Proximate composition of the gum indicated that carbohydrate is the major components while others were found in relatively low concentrations. Also, calcium was the most abundant metal. Trace metals were found in trace concentrations while the concentrations of heavy metals were very low.
3. Functional groups identified in the gum were found to be closely related to those of other polysaccharides and strongly reflected its chemical constituents.
4. The viscosity of the gum is affected by temperature, concentration, electrolytes and pH.
5. Calculated values of activation energy and thermodynamic parameters of flow favoured the existence of few inter and intramolecular interactions between the gum molecules.
6. *EC* gum is a non Newtonian, shear thinning polymer with characteristic pseudoplastic behavior.

4. Experimental Section

4.1. Collection and Purification of Samples

Crude samples of *EC* gum were obtained as exudates from *Eucalyptus citriodora* tree in Zaria, Kaduna state, Nigeria. Injury was sustained on the stem of the tree and the gum that exudated from the stem was collected. The procedure adapted for the purification of the gum was as follows; The gum was dried in an oven at 40 °C for 2 hours and grounded with a blender. It was hydrated twice (using an aqueous solution containing 30 % of chloroform) for five days. Within the hydration period, stirring was done in order to ensure complete dissolution of the gum. After five days, the processed gum was strained through a 75 µm sieve and the particulate free slurry obtained, was allowed to sediment, followed by precipitation with absolute ethanol, filtration and addition of diethyl ether to remove the fat. The final precipitate obtained was re-dried at 40 °C for 48 hours. The dried flakes were pulverized using a blender and stored in an air tight container. Figure 1 presents photographs of crude and purified samples of *EC* gum.

4.2. Physiochemical Analysis

In order to characterize the gums, it was subjected to the following physiochemical tests.

4.2.1. Determination of Percentage Yield of the Purified Gums

In order to determine the percentage yield of the gum, crude and purified samples of the gum were weighed and the percentage yield was calculated with reference to the weight of the crude gum.

4.2.2. Determination of Percentage Moisture Sorption

In order to determine the water sorption capacity of the gum, dried evaporating dishes were weighed and 2.0 g of the gum sample was weighed into the dish. The final weight of the dishes was noted and placed over water in desiccators. After 5 days, the dish was transferred to another desiccator over activated silica gel (desiccant) for another 5 days. The percentage sorption was calculated by difference in weight.

4.2.3. Determination of Solubility

The solubility of the gum was determined in cold and hot distilled water, acetone, chloroform and ethanol. 1.0 g of the sample was added to 50 ml of each of the solvents and left overnight. 25 ml of the clear supernatants were taken in small pre-weighed evaporating dishes and heated to dryness over a digital thermostatic water bath. The weights of the residue with reference to the volume of the solutions were determined using a digital top loading balance (Model.XP-3000) and expressed as the percentage solubility of the gums in the solvents.

4.2.4. Determination of Concentration of Metals

Concentrations of metals were determined using Perkin Elmer atomic absorption spectrophotometer. Calibration curves for each of the metals were prepared and the concentration of the metal was in the analyte was estimated by extrapolation.

4.2.5. Determination of Nitrogen and Protein Content

The nitrogen content of the gum was determined using the Kjeldahl method while the protein content of the gum was estimated by multiplying the nitrogen content by a conversion factor of 6.25.

4.2.6. Determination of pH

This was done by shaking a 1% w/v dispersion of the sample in distilled and deionized water (pH = 6.98) for 5 minutes and the pH was read from a pre-calibrated Oaklon pH meter (Model 1100), after inserting the probe of the meter into the sample.

4.2.7. Determination of Refractive Index of the Gums

In the determination of refractive index of the gum, Eloptron refractometer was used. The refractive index was read directly from the refractometer's output screen.

4.2.8. Determination of Moisture Content

In the determination of the moisture content of the sample, the oven drying method was used as described in the AOAC [43] method.

4.2.9. Determination of Ash Content

The ash content of the sample was determined using the method recommended by AOAC [43].

4.2.10. Determination of Crude Lipid Content and Crude Fibre

The lipid and fibre contents of the gum were determined using the methods recommended by AOAC [43].

4.2.11. Determination of Carbohydrate

The total carbohydrate content was determined by difference. The sum of the percentage moisture, ash, crude lipid, crude protein, and crude fibre was subtracted from 100. The total carbohydrate in the sample was calculated using the following equation,

$$\% \text{ Total carbohydrate} = 100 - \left(\begin{array}{l} \text{Moisture} + \text{Ash} \\ + \text{Fat} + \text{Protein} + \text{Fibre} \end{array} \right) \%$$

4.2.12. Determination of Salinity, Turbidity and Wavelength of Maximum Absorption

The salinity and turbidity of the gum were determined using salinity and turbidity meters respectively. Wavelength of maximum absorption was determined using spectrophotometer.

4.3. GC-MS Analysis

GCMS analysis was carried out on a GC clarus 500 Perkin Elmer system which has a AOC-20i Autosampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS). The instrument employed the following conditions: a column Elite-1 fused silica capillary column (30 × 0.25 mm ID × 1 μm df, composed of 100 % Dimethyl poly dioxane), operating in electron impact mode at 70 eV. The carrier gas (99.9 % helium) was injected at a constant flow rate of 1 ml/min and an injection volume of 0.5 μL was employed (split ratio of 10:1) while the injector and the ion-source temperatures were 250 °C and 280 °C respectively. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10 °C/min, to 200 °C, then 5 °C/min to 280 °C, ending with a 9 min isothermal at 280 °C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da were also used. The total GC running time was 36 minutes.

4.3.1. Interpretation of GC-MS Spectrum

Interpretation of the mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST), which has more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. Concentrations of the identified compounds were determined through area and height normalizations.

4.3.2. Viscosity Measurements

The viscosity of EC gum solution was determined in distilled water using a Cannon Ubbelohde capillary viscometer (Cannon Instruments, model I-71) which was immersed in a precision water bath maintained at specified temperature. The apparent viscosity of the mucilage was measured using a digital Brookfield DV I prime

viscometer while shear rate was measured using Schott Iberica, S.A 18549 rotational viscometer.

4.3.3. Scanning Electron Microscopy

The morphological features of the gums were studied with a JSM-5600 LV scanning electron microscope (SEM) of JEOL, Tokyo, Japan. The dried sample was mounted on a metal stub and sputtered with gold in order to make the sample conductive, and the images were taken at an accelerating voltage of 10 kV.

4.4. FTIR Analysis

FTIR analysis of EC gum was carried out using Scimadzu FTIR-8400S Fourier transform infra-red spectrophotometer. The sample was prepared in KBr and the analysis was carried out by scanning the sample through a wave number range of 400 to 4000 cm⁻¹.

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