

PHYSICOCHEMICAL CHARACTERIZATION OF GREWIA SPECIES BIOPOLYMER FOR EFFECTIVE WATER TREATMENT

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Abstract: Synthetic coagulants and flocculants, including aluminium sulphate, iron chloride, polyacrylamide, and polyaluminium chloride, are widely utilized in water treatment processes due to their effectiveness. However, their use often leads to the production of metal-contaminated sludge and residual aluminium and iron in treated water, posing potential health risks such as Alzheimer's disease and certain cancers. In response to these concerns, alternative coagulation and flocculation agents derived from plant extracts have emerged as promising alternatives. Plants such as *Moringa oleifera*, *Hibiscus esculentus* (Okra), *Strychnos potatorum*, and *Bridelia Ferruginea* have demonstrated significant potential in serving as natural coagulants and flocculants, offering a sustainable and environmentally friendly approach to water treatment. This paper provides an overview of the effectiveness and potential health implications of synthetic coagulants and flocculants, as well as the emerging use of plant extracts as alternative treatment agents. By synthesizing existing research and empirical evidence, it highlights the advantages and limitations of both approaches and underscores the importance of further exploration and development of natural coagulation and flocculation agents for safe and sustainable water treatment practices.

Keywords: Coagulation-flocculation, Synthetic coagulants, Plant extracts, Water treatment, Health implications.

INTRODUCTION

Due to their effectiveness, synthetic coagulants/ flocculants such as aluminum sulphate, iron chloride, polyacrylamide, and polyaluminium chloride are most commonly used in the coagulation-flocculation unit operation (Okaiyeto et al., 2016; Dihang et al., 2008; Okuda et al., 2001). However, their use results in the production of large quantities of metal-contaminated sludge, and in addition, aluminum and iron residues in treated water has been linked to the development of certain diseases, as Alzheimer's and some cancers (Okaiyeto et al., 2016; Sotero-Santos et al., 2007; Ruden, 2004; Campbell, 2002). To overcome this issue, some plants extracts like *Moringa oleifera*, *Hibiscus esculentus* (Okra), *Strychnos potatorum* and *Bridelia Ferruginea* have shown great potentials to serve as coagulation/flocculation agents (Georgiadis et al., 2011; Pritchard et al., 2010; Kolawole et al., 2007). Plants extracts used as natural

coagulants/flocculants are macromolecules which could be extracted from different parts of plants

(barks, leaves, roots, among others) (Daza et al., 2016; Babu et al., 2013; Yang et al., 2011). The bark extracts of *Grewia* species plants are traditionally used as a clarifying agent for beverages, domestic effluents and surface water in the northern part of Cameroon. Given that the active compound of a biocoagulant/flocculant can be a protein, a polysaccharide or a combination of both, there is need to investigate the composition and properties of substances from biological origin having coagulation/flocculation properties as it shall provide information on the nature of these extracts in order to justify its application in water treatment (Miller et al., 2008).

There is not enough information on the nature of the molecules responsible for the coagulant/flocculant properties of *Grewia* spp. biopolymer, thus the extraction and partial purification (precipitation with absolute ethanol) of the crude extracts prior to its characterization was the main objective in this study.

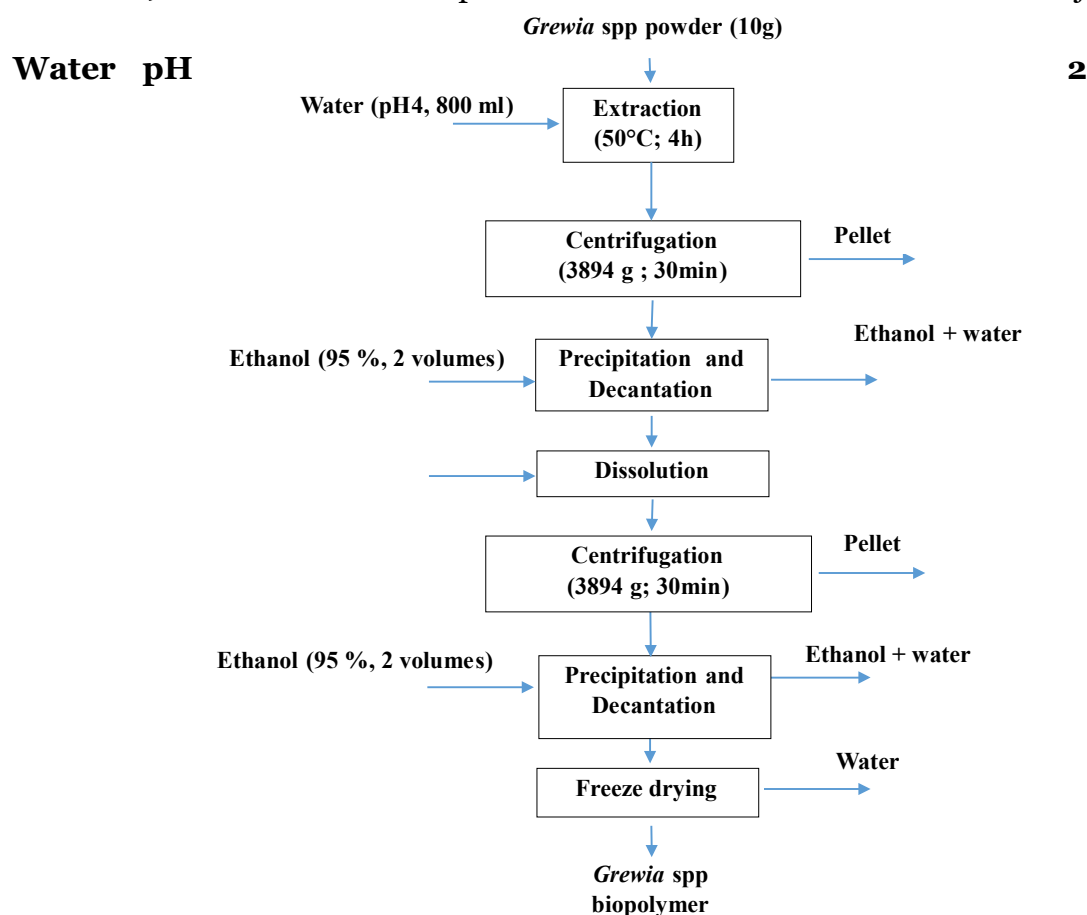


Figure 1. Extraction and purification process of *Grewia* spp. Biopolymer.

MATERIALS AND METHODS

Sampling and extraction of *Grewia* spp. biopolymer

Grewia spp. barks were collected from Mokolo (North region of Cameroon), transported to the laboratory, dried and stored at room temperature. Crude *Grewia* spp. biopolymer was extracted following the method of Somboonpanyakul et al. [(2006) with some modification. The dried and pulverized bark of *Grewia* spp. was dispersed in milli Q water (1:80 w/v, pH 4) at 50°C for 4 h. The

fibrous material from the dispersed mucilage is removed by straining through centrifugation at 3894 g for 20 min. Thereafter, the crude mucilage was precipitated with 2 volumes of 95% absolute ethanol and freeze-dried. The extraction process is as shown in Figure 1. The *Grewia* spp. biopolymer extraction yield was obtained using the following equation:

$$\text{yield} = \frac{m}{m_i} \times 100 \quad (1)$$

Where 'm' is the biopolymer mass after extraction and 'mi' the biopolymer mass before extraction.

Characterization of *Grewia* spp. biopolymer

Proximate composition of Grewia spp. biopolymer

Proximate composition (protein, lipids and ash contents) of *Grewia* spp. biopolymer was determined according to the methods of the AOAC (1975). Total sugar was determined by Anthrone sulfuric acid reaction. The difference between total and free sugar content of samples represented the polysaccharide content which was assimilated to gum content.

Determination of the viscosity of Grewia spp. biopolymer extract

Powder samples of *Grewia* were dissolved in milli Q water in order to obtain samples at various concentrations (0.005, 0.01, 0.015, and 0.02 g/dL). The viscosity of *Grewia* spp. biopolymer was measured with an Ostwald capillary viscometer. 15 ml of extracts was introduced into the viscometer which was immersed in a thermostatic water bath at 25°C. The efflux time of the biopolymer extract at various concentrations was measured (t) and that of water was also measured (t₀). The relative viscosity (η_{rel}) was calculated thus:

$$\eta_{rel} = \frac{t}{t_0} \quad (2)$$

Specific viscosity (η_{sp}) was also determined according to:

$$\eta_{sp} = \eta_{rel} - 1 \quad (3)$$

Reduced viscosity (η_{red}) is given by:

$$\eta_{red} = \frac{\eta_{rel}}{C} \quad (4)$$

where C is the concentration of *Grewia* spp. biopolymer solution in (g/dL).

Functional groups

The functional groups of the *Grewia* spp. biopolymer was determined by infrared spectroscopy. Infrared spectra of *Grewia* spp. biopolymer samples were recorded with a Fourier Transform Infrared Spectrometer (Perkin Elmer Spectrum GX). Approximately, 1 mg of sample was mixed with 25 mg KBr and both were ground together in an agate mortar. Thereafter, a pellet was prepared using a 5 tons caver type press. The spectra were recorded in the 4000 to 400 cm⁻¹ wavenumber range.

Powder X-ray diffraction

X-ray diffraction was carried out on a Panalytical Empyrean (p-Xrd) diffractometer, Netherland. The equipment used Cu target and a pixel 3D solid state X-ray detector. Powder samples were placed on a sample holder and inserted into the X-ray machine. Samples were analyzed over an angular range of 2 Theta: 5 - 89.9823° with a step size of 0.0130 with a scan step time of 18.8700 s. The X-ray source was Cu K-α₁ (1.54060 Å) and Cu K-α₂ (1.54443 Å).

Scanning electron microscopy with energy dispersive X-rays (SEM-EDX)

A pinch of *Grewia* spp. biopolymer was suspended in 100 μ L ethanol and agitated for proper dispersion of the biopolymer. This was closely followed by the addition of 900 μ L of MilliQ water followed by vortexing and sonication for 30 s. The sample was then placed on aluminium stubs and allowed to dry in desiccator overnight. SEM Images were collected with a JEOL, JSM-7100F Field Emission Scanning Electron Microscope (Model SM 71031SE2A) Japan.

Thermal properties of *Grewia* spp. biopolymer

Thermal degradation of the *Grewia* spp. biopolymer was studied on a Thermo-gravimetric Analyzer (NETZSCH, TG 209 F1 Libra, Germany). Approximately, 1 mg of sample was introduced into an aluminium oxide crucible and heated within a temperature range of 35 to 500°C at a rate of 5K/min under nitrogen atmosphere. Differential Scanning Calorimetry (DSC) (NETZSCH, DSC 204F1 Phoenix, Germany) was also used to study the thermal properties of the *Grewia* spp. biopolymer. Known weights of powder *Grewia* spp. biopolymer was loaded on an aluminium pan and the energy level of the samples was scanned in the range -20 to 500°C under nitrogen atmosphere with a temperature gradient of 5°C/min.

RESULTS AND DISCUSSION

Proximate composition of the *Grewia* spp. biopolymer

Results of the proximate composition of *Grewia* spp. biopolymer before and after purification are presented in Table 1. Chemical analysis shows that the purified biopolymer is composed approximately of 4% of protein, 42% of total sugar and 40% of gum. The sugars represent the main components, among which gums defined as polysaccharides are predominant. Our results are close to those obtained by Akdowa et al. (2014) and Saidou et al (2011). Results also show that pH does not have significant effect on the proximate composition of *Grewia* spp. biopolymer. The extraction and purification procedure used to isolate biopolymer appears efficient, since the most of proteins, lipids and the mineral present on crude biopolymer were removed after purification. This could lead to an improvement in the coagulation/ flocculation performance of *Grewia* spp. biopolymer. The proximate composition of *Grewia* spp. biopolymer is similar to that of Okra gum used for the coagulation of colloidal suspensions (Sengkhamparn et al., 2009).

***Grewia* spp. biopolymer extraction yield at different pH**

In order to study the influence of pH on the *Grewia* spp. biopolymer extraction yield, the extraction was carried within the pH range of 2 to 7, and the results are presented in Figure 2.

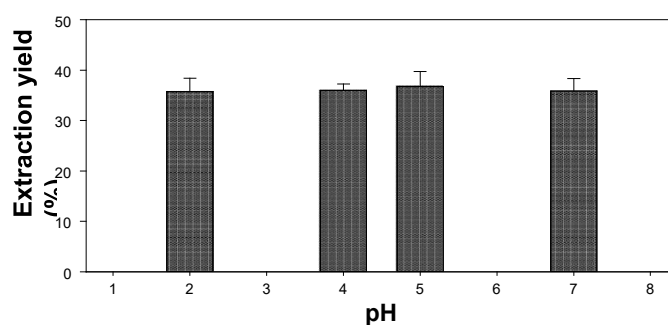
The extraction yields of *Grewia* spp. biopolymer at pH 2, 4, 5, and 7 calculated after freeze drying are 35.73 ± 0.67 , 36 ± 1.28 , 36.74 ± 1.60 , and $35.83 \pm 1.67\%$, respectively. We note that pH does not have significant influence on the extraction yield of *Grewia* spp. Akdowa et al. (2014) also showed during their work that pH does not have significant influence on the viscosity and extraction yield of *Grewia mollis* gum. The extraction yield obtained is close to those obtained by Nep et al. (2010).

Influence of pH and biopolymer concentration on the viscosity

Figure 3 shows the pH and biopolymer concentration effects of the *Grewia* spp. biopolymer viscosity. Results display the increase of the viscosity with the biopolymer concentration irrespective of the pH. The gradual increase of viscosity as the amount of polymer increases could be due to the fact that

Table 1. Proximate composition of the *Grewia* spp. Biopolymer.

Composition (g/100 g MS)	Crude powder	<i>Grewia</i> spp. biopolymer			
		pH 2	pH 4	pH 5	pH 7
Sugar content	53.02 ± 0.06	44.41 ± 0.23	43.41 ± 0.08	41.61 ± 0.1	39.25 ± 0.15
Gum content	52.79 ± 0.05	42.41 ± 0.15	43.36 ± 0.07	40.21 ± 0.2	37.41 ± 0.25
Protein content	8.58 ± 0.05	2.5 ± 0.07	3.66 ± 0.07	4.45 ± 0.5	5.70 ± 0.37
Lipid content	3.23 ± 0.08	-	-	-	-
Ash content	14.6 ± 1.25	5.3 ± 0.09	7.6 ± 0.08	7.9 ± 0.15	9 ± 0.95

**Figure 2.** Influence of pH on *Grewia* spp. biopolymer extraction yield.

Grewia spp. biopolymer displays a non-Newtonian flow behavior (Huang et al., 2019; Kamal et al., 2015; Jamal, 2012). That behavior is close to the work carried by Nep et al. (2010).

It is also noted that the pH has no significant effect on the viscosity. For the same biopolymer concentration, the viscosity of the *Grewia* spp. extract shows little change over the range of pH 2 to 7. This sharp reduction in viscosity may be due either to chemical degradation in acid milieu, or to a change in molecular conformation, which may or may not be reversible (Sorbie et al., 1992). Our results are in agreement with those obtained by Akdowa et al. (2014).

The intrinsic viscosity was determined graphically from the Huggins plot (Figure 4). From that figure the intrinsic viscosity obtained was 16.5 dL/g. Morris (1990), proposes a classification of the polymers size according to their intrinsic viscosities and indicates that the intrinsic viscosities between 5 and 25 dL/g correspond to the high molecular mass polymers. Therefore, based on our results, *Grewia* spp. biopolymer falls into this range and is considered as high molecular mass biopolymer, suggesting that it can favor adsorption and bridging effect during coagulation-flocculation, encourage the densely packed aggregate nature of floc, and thus enhance floc settling velocity (Li et al., 2006; Niu et al., 2013; Li et al., 2013). These observations are in agreement with our previous works (Kameni et al., 2019).

Functional groups of *Grewia* spp. biopolymer by FTIR

The FTIR spectrum of *Grewia* spp. biopolymer before and after purification is presented in Figure 5. Spectra exhibited the typical bands and peak characteristic of polysaccharides (Kameni et al., 2019).

There was no major difference between spectra obtained before (a) and after (b) purification. The broad band at 3406 cm^{-1} indicates the presence of hydroxyl (-OH) groups due to moisture but could also arise from the hydroxyl of sugar rings. The band at 2933 cm^{-1} indicates the presence of sugars (galactose, arabinose and rhamnose); also, the presence of alkane C-H stretch and aldehyde C-H stretch. The glucuronic acids have specific vibrations such as the band at 1428 cm^{-1} due to COOH group. The band at 1255 cm^{-1} represents C-O-H bend of CH_2OH . The peak obtained at 1734 cm^{-1} results from stretching mode C=O stretch (Filippov, 1992). The wave numbers between 800 and 1200 cm^{-1} represent the fingerprint region for carbohydrates. All these groups can serve as active sites for the attachment of colloidal particles. The FTIR spectrum of *Grewia* spp. biopolymer is similar to the one of Chitosan and Okra gum used for the aggregation of colloidal particles (Li et al., 2013; Freitas et al., 2015; Ani et al., 2012).

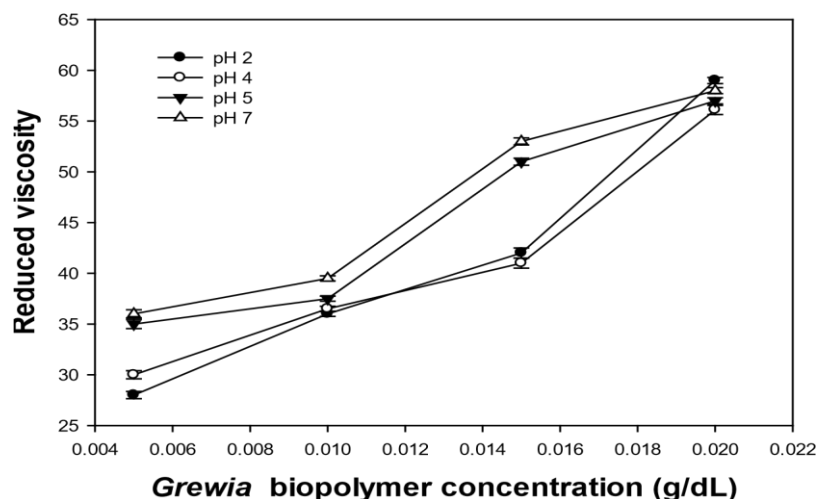


Figure 3. Influence of *Grewia* spp. biopolymer concentration on the viscosity of at different pH.

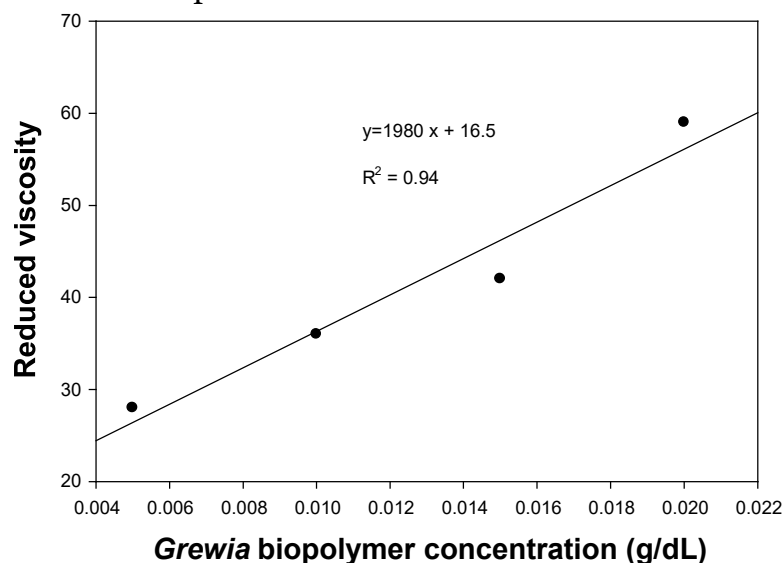


Figure 4. Huggins plot of *Grewia* spp. Biopolymer.

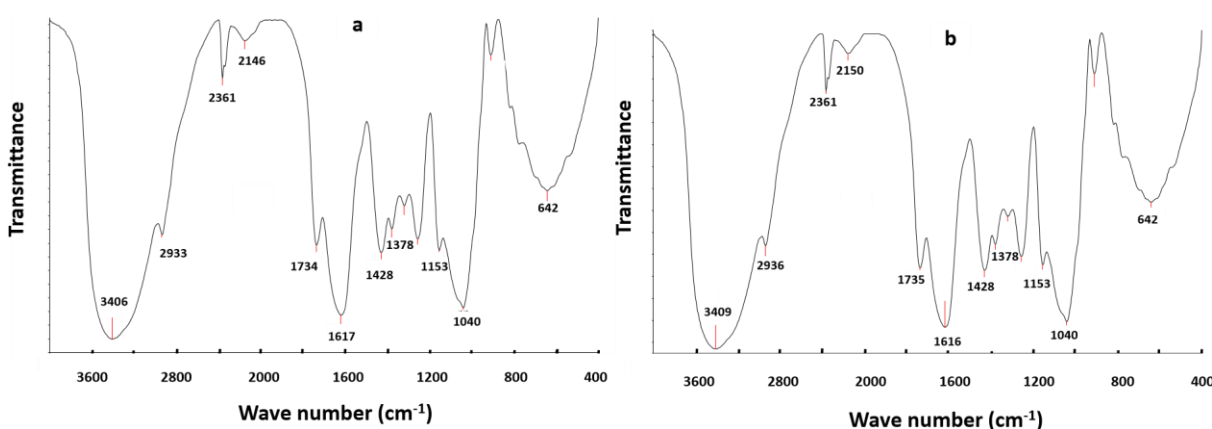


Figure 5. FTIR spectrum of *Grewia* spp. biopolymer before (a) and after purification (b).

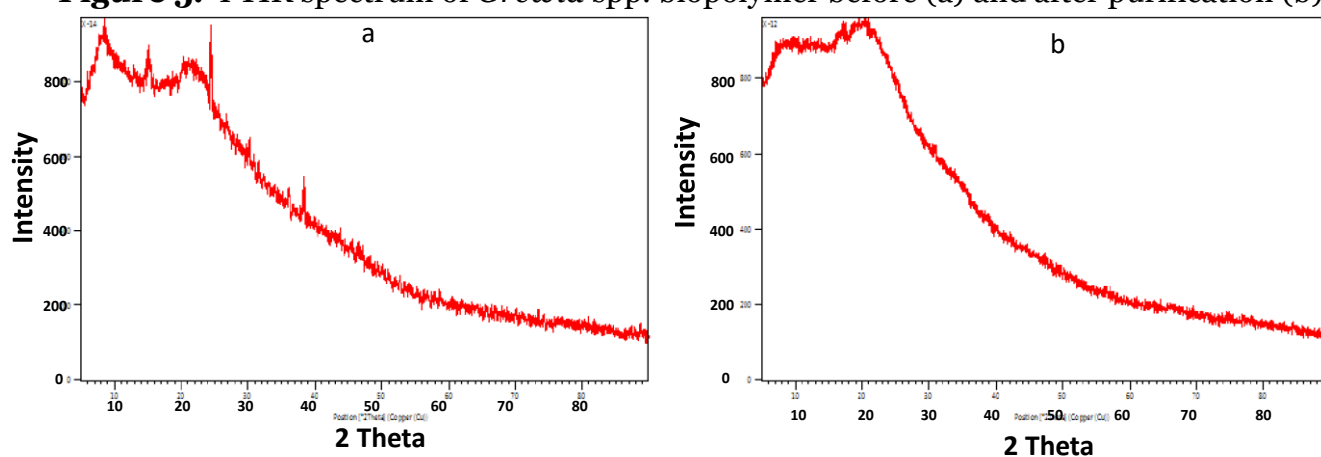


Figure 6. X-Ray pattern of *Grewia* spp. biopolymer before (a) and after purification (b).

***Grewia* spp. biopolymer crystalline structure**

XRD analysis was applied to detect the crystalline structure of the *Grewia* spp. biopolymer (Figure 6). The XRD pattern shows that *Grewia* spp. biopolymer before purification (a) is consisted of amorphous and crystalline structure. It shows peaks at 2 theta within the range of 8 to 41°C. After purification, the XRD pattern shows zero peak, which suggests that purified *Grewia* spp. biopolymer is consisted of amorphous structure. The XRD graph of *Grewia* spp. biopolymer is comparable to the one of Okra gum used for the destabilization of colloidal suspension (Freitas et al., 2015).

Scanning electron microscopy (SEM) pattern of *Grewia* spp. biopolymer

SEM images of biopolymer before (a, b) and after (c, d) purification are represented in Figure 7 at different magnifications. In both case the microphotographs are indicative of an amorphous material. The particles are mostly seen as aggregates of irregular shapes. SEM and XRD analyses confirm that *Grewia* spp. biopolymer is an amorphous material.

Electron dispersive spectroscopy pattern of *Grewia* spp. biopolymer

EDX was carried out to detect the elements present at the surface of a particular area of *Grewia* spp. biopolymer. The EDX spectrum of biopolymer before (a) and after purification (b) is presented in Figure 8. Result shows that some elements present on the surface of crude biopolymer disappeared after purification. We can observe that elements like Mn and Sr present on the surface of crude

biopolymer are absent after purification. This suggests that the purification process used is efficient to reduce the metal content of biopolymer. The major elements present in *Grewia* spp. biopolymer are K, Ca, Cl, C, Mg and O.

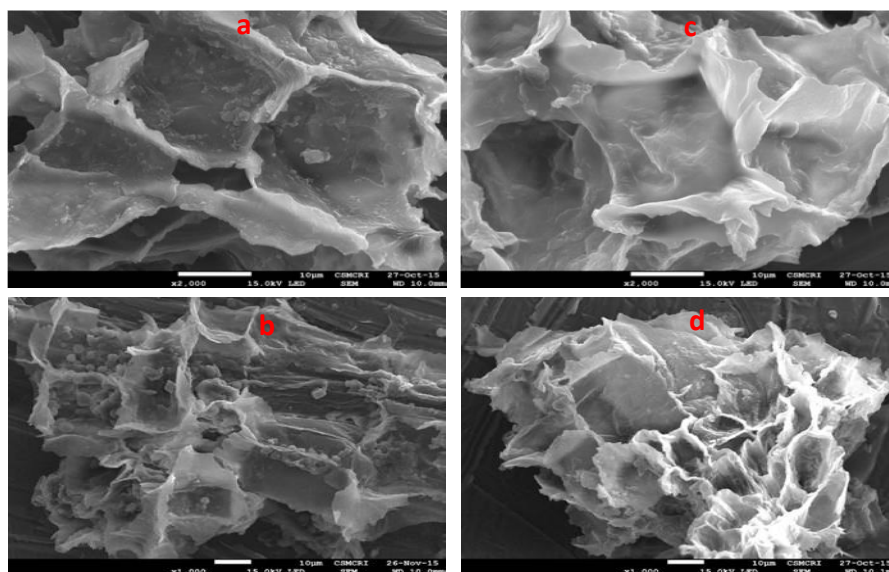


Figure 7. SEM images of *Grewia* spp. biopolymer before (a, b) and after purification (c, d).

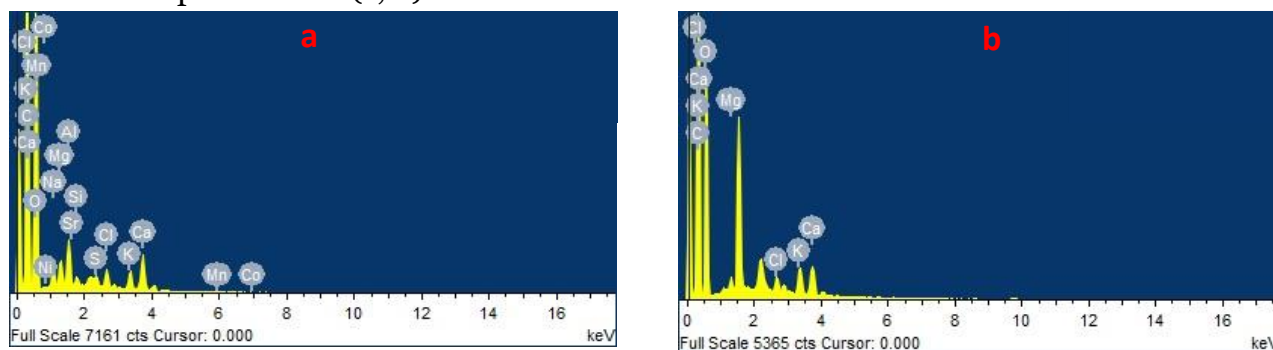


Figure 8. Energy dispersive spectroscopy pattern of *Grewia* spp. biopolymer before (a) and after purification (b).

Thermal behavior of *Grewia* spp. biopolymer

Thermal stability of the polymer is an important property that could make the material fit for industrial applications where material is thermally processed. Thermal stability analysis of polymer material is helpful in the selection of materials with the best properties for specific used. Thermal analysis of *Grewia* spp. biopolymer samples were carried out with Differential Scanning Calorimetry (DSC) and Thermal Gravimetric Analysis (TGA).

Differential scanning calorimetry

The results of DSC analysis of *Grewia* spp. biopolymer are presented in Figure 9. The analysis reveals that the glass transition temperature is around 140.3°C. Consequently, *Grewia* spp. biopolymer occurs in a glassy state at room temperature (Nurul et al., 2014). *Grewia* spp. biopolymer is preferably stored at or below room temperature in a dry environment to reduce occurrence of any chemical changes to

the structure of molecules, thus conserving its quality and functionality. The major intense peak recorded in the DSC graph is an endothermic transition. This may be due to moisture desorption. All those observations are close to results obtained by Nep et al. (2010) and Nurul et al. (2014).

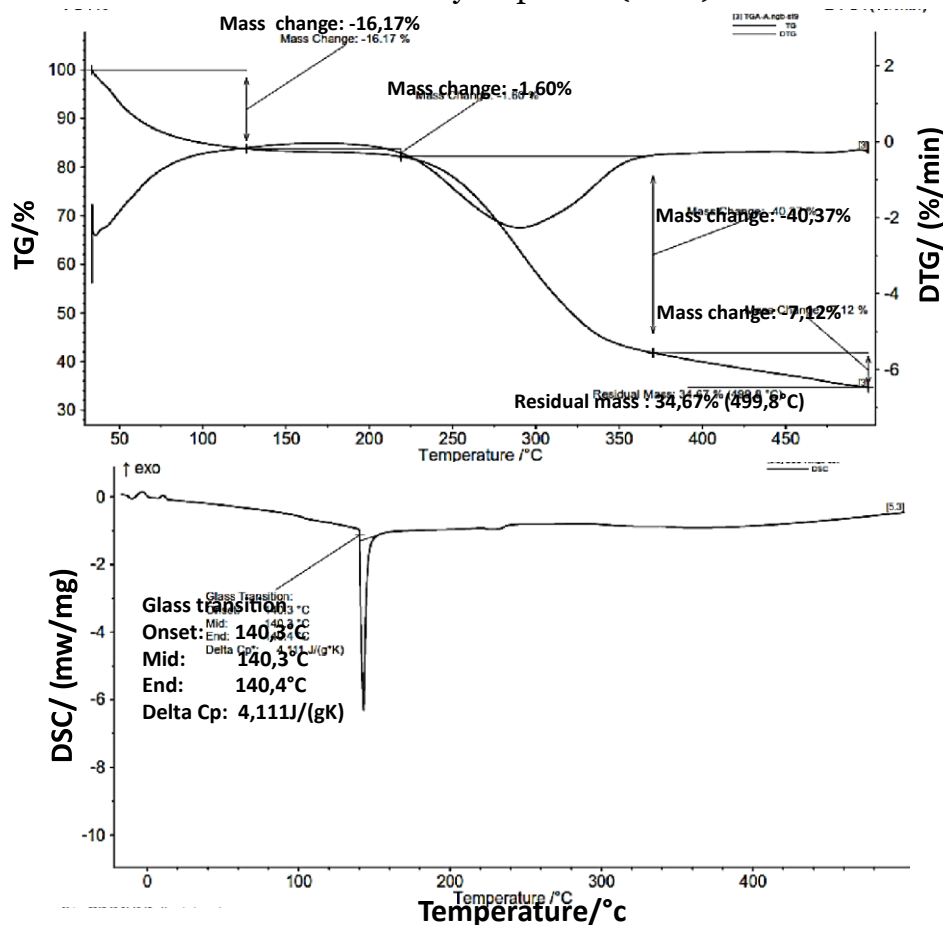


Figure 9. Differential Scanning Calorimetry graph of *Grewia* spp. biopolymer

Figure 10. Thermogravimetric analysis of *Grewia* spp. Biopolymer.

Thermogravimetric analysis

The results of TGA analysis of *Grewia* spp. biopolymer are displayed in shown Figure 10. The first mass loss taking place between 30 and 125°C may be attributed to the loss of adsorbed and structural water of biopolymer (Bothara et al., 2012), or due to desorption of moisture as hydrogen bound water to the biopolymer structure. This resulted in a weight loss of about 16.17%. The second weight loss event, with an onset of over 290°C, can be attributed to thermal decomposition of the biopolymer.

From the results, we can conclude that *Grewia* spp. is a thermal stable biopolymer. \

Conclusion

The extracted biopolymer was purified and characterized and the results revealed that both the crude extract and the ethanol precipitate were rich in polysaccharide type polymers. The results also revealed that the purification process led to a reduction in the lipid, protein and metal contents of crude *Grewia* spp. biopolymer. Physicochemical characterization showed that *Grewia* spp. biopolymer has a fairly

similar physicochemical composition to those of some biopolymers used as coagulation-flocculation agents principally the Okra gum. The use of *Grewia* spp. biopolymer in water treatment as a natural coagulant/flocculant may be an alternative with numerous advantages over chemical agents, mainly biodegradability, low toxicity and low residual sludge production. These coagulants are safe to human health and the environment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Akdowa EP, Njintang-Yanou N, Boudjeko T, Gaiani C, Scher J, Woguia AL, Mbofung CMF (2014). Optimization of variables for aqueous extraction of gum from *Grewia mollis* powder. Hindawi Publishing Corporation Journal of Polymers, 2014, 10 p.
- Ani JU, Nnaji NJ, Okoye COB, Onukwuli OD (2012). The coagulation performance of Okra mucilage in an industrial effluent by turbidimetry. International Journal of Chemical Science 10(3):12931308.
- Association of Official Analytical Chemists (AOAC) (1975). Methods of analysis of the Association of Official Analytical Chemists. 10th ed.
- AOAC, Washington, DC.
- Babu RP, O'Connor K, Seeram R (2013). Current progress on biobased polymers and their future trends. Progress in Biomaterials 2(1):1-16.
- Bothara SB, Singh S (2012). Thermal studies on natural polysaccharide. Asian Pacific Journal of Tropical Biomedicine 2(2):S1031-S1035.
- Campbell A (2002). The potential role of aluminum in Alzheimer's disease. Nephrology Dialysis Transplantation 17:17-20.
- Daza R, Barajas-Solano AF, Epalza JM (2016). Evaluation of the efficiency of bio-polymers derived from desertic plants as flocculation agents. Chemical Engineering Transactions 49:361-366.
- Dihang D, Aimar P, Kayem J, Ndi S (2008). Coagulation and Flocculation of Laterite suspensions with low levels of aluminium chloride and polyacrylamides. Chemical Engineering and Processing: Process Intensification 47(9-10):1509-1519.
- Filippov MP (1992). Practical infrared spectroscopy of pectic substances. Food Hydrocolloids 6(1):115-142.
- Freitas TKFS, Oliveira VM, de Souza MTF, Geraldino HCL, Fávaro SL, Garcia JC, Almeida VC (2015). Optimization of coagulationflocculation process for treatment of industrial textile Wastewater

using okra (*A. esculentus*) mucilage as natural coagulant. *Industrial Crops and Products* 76:538-544.

Georgiadis N, Ritzoulis C, Sioura G, Kornezou P, Vasiliadou C, Tsiptsias C (2011). Contribution of okra extracts to the stability and rheology of oil-in-water emulsions. *Food Hydrocolloids* 25(5):991-999.

Huang B, Li X, Fu C, Wang Y, Cheng H (2019). Study rheological behavior of polymer solution in different-medium-injection-tools. *Polymers* 11(319):14.

Jamal A (2012). Rheological behavior of polyacrylamide/Laponite nanoparticle suspensions in electrolyte media. *Journal of Macromolecular Science, Part B: Physics* 51(6):1139-1147.

Kamal SP, Nasser TM (2015). Experimental study on the rheological properties of polymer solutions and solid suspensions. *International Journal of Current Engineering and Technology* 5(2):8.

Kameni NMB, Ndi KS, Kofa GP, Kayem GJ (2019). Coagulation and Sedimentation of Concentrated Laterite Suspensions: Comparison of Hydrolyzing Salts in Presence of *Grewia* spp. *Biopolymer. Hindawi Journal of Chemistry* 2019, Article ID 1431694, 9 p.

Kolawole OM, Oguntoye SO, Agbede O, Olayemi AB (2007). Studies on the efficacy of *Bridelia ferruginea* benth bark extract for domestic wastewater treatment. *Bulletin of the Chemical Society Ethiopia* 21(2):505-211.

Li J, Jiao S, Zhong L, Pan J, Ma Q (2013). Optimizing coagulation and flocculation process for kaolinite suspension with chitosan. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 428:100110.

Li T, Zhu Z, Wang D, Yao C, Tang H (2006). Characterization of floc size, strength and structure under various coagulation mechanisms. *Powder Technology* 168(2):104-110.

Miller SM, Fugate EJ, Craver VO, Smith JA, Zimmerman JB (2008). Toward Understanding the Efficacy and Mechanism of *Opuntia* spp. as a Natural Coagulant for Potential Application in Water Treatment.

Environmental Science and Technology 42(12):4274-4279.

Morris ER (1990). Industrial hydrocolloids. In: Bloor D. M. and WyaJones E. (ed): *The structure, Dynamics and Equilibrium properties of colloidal systems*, Kluwer Academic Publishers pp. 449-470.

Nep EI, Conway BR (2010). Characterization of *Grewia* gum, a potential pharmaceutical excipient *Journal of Excipients and Food Chemicals* 1(1):30-40.

- Niu M, Zhang W, Wang D, Chen Y, Chen R (2013). Correlation of physicochemical properties and sludge dewaterability under chemical conditioning using inorganic coagulants. *Bioresource Technology* 144:337-343.
- Nurul DZ, Mohamed IN, Kadivar A (2014). The use of Hibiscus esculentus (Okra) gum in sustaining the release of propranolol hydrochloride in a solid oral dosage form. Hindawi Publishing Corporation BioMed Research International, 2014, Article ID 735891, 8 p.
- Okaiyeto K, Nwodo UU, Okoli SA, Mabinya LV, Okoh AI (2016). Implications for public health demands alternatives to inorganic and synthetic flocculants: bioflocculants as important candidates. *Microbiology Open* 5:177-211.
- Okuda T, Baes A U, Nishijima W, Okada M (2001). Coagulation mechanism of salt solution extracted active component in Moringa Oleifera seeds. *Water Research*, 35 (3):830-834.
- Pritchard M, Craven T, Mkandawire T, Edmondson AS, O'Neill JG (2010). A study of the parameters affecting the effectiveness of moringa oleifera in drinking water purification. *Physics and Chemistry of the Earth* 35(13-14):791-797.
- Ruden C (2004). Acrylamide and cancer risk—expert risk assessments and the public debate. *Food and Chemical Toxicology* 42:335-349.
- Saidou C, Tchatchueng JB, Ndjouenkeu R, Roux DCD (2011). Extraction and Partial Characterisation of Hydrocolloid Gums from Some African Legumes. *International Journal of Food Engineering* 7(3):10. Available at: <http://www.bepress.com/ijfe/vol7/iss3/art15>.
- Sengkhampan N, Verhoef R, Schols HA, Sajjaanantakul T, Voragen AGJ (2009). Characterisation of cell wall polysaccharides from okra (*Abelmoschus esculentus* (L.) Moench). *Carbohydrate Research* 344(14):1824-1832.
- Somboonpanyakul P, Wang Q, Cui W, Barbut S, Jantawat P (2006). Malva nut gum. (Part I): extraction and physicochemical characterization. *Carbohydrate Polymers* 64(2):247-253.
- Sorbie K S, Huang Y (1992). The effect of pH on the flow behavior of Xanthan solution through porous media. *Journal of Colloid and Interface Science* 149(2):303-313.
- Sotero-Santos RB, Rocha O, Povinelli J (2007). Toxicity of ferric chloride sludge to aquatic organisms. *Chemosphere* 68:628-636.
- Yang JS, Xie YJ, He W (2011). Research progress on chemical modification of alginate: a review. *Carbohydrate Polymers* 84(1):33- 39.