

Functional characterization of Detarium microcarpum seed proteins and polysaccharides as sustainable coagulant precursors for wastewater treatment

Nwaneri Theodore U¹, Nnadikwe Johnson², Onwukwu Cajetan N³, Iheme Chigozie⁴
Akuchie J. Chukwuma⁵, Ogbennaya Jane Frances Chinwe⁶

Chemical Engineering Department, Imo state University, Owerri^{1,3}. Petroleum Engineering Department, Nnamdi Azikiwe University, Awka². Chemical Engineering Department, Dr B.U.Nzeribe State Polytechnic, Imo State Nigeria^{4,5,6}

Chemical coagulants pose health and environmental concerns, driving search for plant-based alternatives for wastewater treatment. *Detarium microcarpum* seed, an underutilized leguminous plant in Nigeria, has potential as a natural coagulant but remains uncharacterized. This study characterized the physical properties, proximate composition and functional groups of *Detarium microcarpum* seed powder to assess its suitability as a bio-coagulant. Seeds were processed into powder and analyzed for yield, apparent density and proximate composition (moisture, ash, crude protein, crude fat, crude fibre, carbohydrate) using standard AOAC methods. Functional groups were identified by Fourier Transform Infrared (FTIR) spectroscopy in the range 4000–400 cm⁻¹. Powder yield was 28.31% with apparent density of 0.235 g/mL. Proximate analysis showed carbohydrate 56.76%, crude protein 19.77%, crude fat 10.53%, moisture 7.25%, ash 3.48% and crude fibre 2.20%. FTIR revealed nine bands, notably broad O–H/N–H stretching at 3272.6 cm⁻¹, aliphatic C–H at 2914.8 cm⁻¹, C=O/amide at 1636.3 cm⁻¹, and C–O/C–O–C stretching at 1148.0 and 1043.7 cm⁻¹, indicating hydroxyl-rich polysaccharides and proteinaceous matter. The high carbohydrate and protein contents coupled with O–H, N–H, C=O and C–O functional groups confirm *D. microcarpum* seed possesses the chemical features required for charge neutralization and inter-particle bridging, supporting its potential as an eco-friendly bio-coagulant.

Keywords: Natural coagulant; *Detarium microcarpum*; characterization; FTIR; point of zero charge; sustainable water treatment

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Corresponding Author:

Nwaneri Theodore U
Chemical Engineering Department, Imo state University, Owerri

1. Introduction

Access to safe water remains a major development challenge in many low-income countries. Rapid population growth, unplanned urbanization and expansion of agro-processing activities have increased the volume of wastewater discharged without adequate treatment (Yin, 2010; Oladoja, 2015). In Nigeria, effluents from abattoirs, markets and food processing units are often released into drains and rivers with high turbidity, suspended solids and organic load, posing risks to public health and aquatic life. Coagulation-flocculation is widely applied as a primary treatment step for turbidity and colloid removal. Conventional coagulants such as aluminium sulphate and ferric chloride are effective, but their continuous use is constrained by high cost, large sludge generation, pH alteration and concerns over residual metals in treated water (Ndabigengesere et al., 1995; Obiora-Okafo et al., 2020). These limitations are more pronounced in decentralized and rural treatment systems where chemical supply is irregular. This has led to growing interest in plant-based natural coagulants, which are biodegradable, low-cost and locally available. The coagulation potential of *Moringa oleifera* seed has been well established, with active agents identified as

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low molecular weight cationic proteins that act through charge neutralization and bridging (Ndabigengesere et al., 1995; Okuda et al., 2001). Similar mechanisms have been reported for *Opuntia* spp. mucilage and common bean seed extract, where polysaccharides and proteins contribute to floc formation (Miller et al., 2008; Antov et al., 2012).

Detarium microcarpum Guill. & Perr., a leguminous tree native to the savanna belt of West Africa, is an underutilized species with promising coagulant potential (Kalinganire et al., 2008). The seed is traditionally used as a soup thickener, indicating high polysaccharide content, and proximate studies have reported crude protein of 26–37%, carbohydrate above 50%, and appreciable fibre and minerals (Alatise et al., 2021; Emiri and Chukwu, 2017; Oluchi, 2018). These constituents are relevant to coagulation, as proteins provide cationic sites for colloid destabilization while polysaccharides promote inter-particle bridging (Oladoja, 2015; Obiora-Okafo et al., 2022).

Fourier transform infrared (FTIR) spectroscopy is a useful tool for identifying the functional groups responsible for coagulant activity, such as hydroxyl, carboxyl, amino and carbonyl groups (Coates, 2000). Despite its abundance and nutritional documentation, *D. microcarpum* has received limited attention as a coagulant source, with existing literature focused mainly on food, dye and medicinal applications (Zakari et al., 2018; Sanusi, 2020). A systematic linkage between its proximate composition and surface chemistry is therefore needed to support its development as a sustainable alternative to chemical coagulants.

Aim of the study

This study aims to functionally characterize *Detarium microcarpum* seed proteins and polysaccharides as sustainable coagulant precursors for wastewater treatment through proximate analysis and FTIR spectral characterization.

Objectives

To determine the proximate composition (moisture, ash, crude protein, crude fat, crude fibre and carbohydrate) of *Detarium microcarpum* seed To identify the surface functional groups in *Detarium microcarpum* seed relevant to coagulation using FTIR spectroscopy. To evaluate the protein-polysaccharide functional attributes that support the use of *Detarium microcarpum* seed as a sustainable natural coagulant for wastewater treatment.

2. Materials and Methods

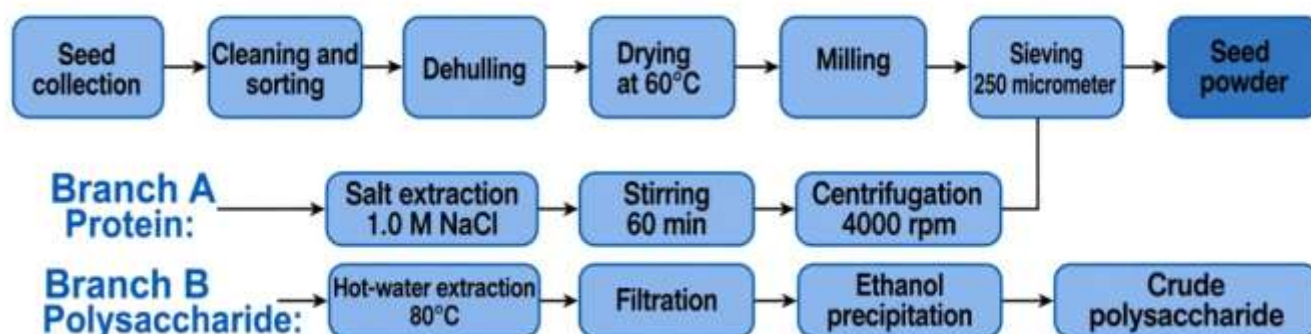
Sample collection and authentication

Mature *Detarium microcarpum* fruits were harvested from [LGA] (Latitude, Longitude) during the peak fruiting season. The plant material was identified and authenticated at the Herbarium of [Department of Plant Biology, University Name], where a voucher specimen was deposited with voucher number [XXX]. Healthy, undamaged fruits were selected, washed with tap water to remove surface contaminants, and transported in jute bags to the laboratory.[Locality][State][Nigeria][X][Month][Year]

Sample preparation

Seeds were manually separated from the pulp, rinsed three times with distilled water to remove residual mucilage, and drained. The seeds were first sun-dried for 72 h and further oven-dried (Memmert UN55, Germany) at 60 °C to constant weight. The dried seeds were dehulled manually, milled using a laboratory hammer mill (Thomas-Wiley Model 4, USA), and sieved through a 0.5 mm stainless steel sieve to obtain homogeneous powder.

Process flow for *Detarium microcarpum* seed powder preparation and extraction



The powder was sealed in airtight low-density polyethylene bags and stored at 4 °C until analysis. Percentage yield was determined as:

$$\text{Yield (\%)} = (W_f / W_o) \times 100 \quad (1)$$

where W_f is mass of final dried seed powder (g) and W_o is mass of initial cleaned seeds (g).

Chemicals and reagents

All chemicals were of analytical grade and used without further purification: petroleum ether (40–60 °C, Sigma-Aldrich), concentrated H_2SO_4 , NaOH , H_3BO_3 , HCl , NaCl , KCl , KBr (FTIR grade), and absolute ethanol (BDH Chemicals, UK). Distilled water was used throughout.

Proximate composition

Proximate analysis was performed in triplicate following AOAC (1990) official methods. Moisture content (method 925.10) was determined by drying 5 g sample at 105 °C to constant weight. Ash (method 923.03) was determined by incineration of 5 g sample in a muffle furnace (Carbolite AAF 1100, UK) at 550 °C for 6 h. Crude protein (method 979.09) was determined by micro-Kjeldahl (Gerhardt Vapodest 200, Germany); nitrogen was multiplied by 6.25. Crude fat (method 920.39) was determined by Soxhlet extraction with petroleum ether for 8 h. Crude fibre (method 962.09) was determined by sequential digestion with 1.25% (w/v) H_2SO_4 and 1.25% (w/v) NaOH , followed by oven-drying and ashing. Carbohydrate was calculated by difference.

$$\text{Carbohydrate (\%)} = 100 - [\text{moisture} + \text{ash} + \text{protein} + \text{fat} + \text{fibre}] \quad (2)$$

Extraction of protein and polysaccharide fractions

Salt-soluble protein extraction.

Water-soluble coagulant protein was extracted following Okuda et al. (2001) with slight modification. Ten grams of seed powder was defatted with petroleum ether, then stirred in 100 mL of 1.0 M NaCl solution for 60 min at 25 °C using a magnetic stirrer. The suspension was filtered through Whatman No. 1 filter paper and centrifuged at 4000 rpm for 20 min. The supernatant (crude protein extract) was collected and protein content determined by Kjeldahl. Extraction yield was expressed as percentage of initial powder weight after freeze-drying.

Crude polysaccharide (mucilage) extraction

Polysaccharide was extracted by hot-water method. Twenty grams of defatted powder was dispersed in 400 mL distilled water, heated at 80 °C for 2 h with continuous stirring, and filtered through muslin cloth. The filtrate was precipitated with three volumes of 95% ethanol, kept at 4 °C overnight, and centrifuged at

4500 rpm for 15 min. The precipitate was washed with ethanol, oven-dried at 45 °C to constant weight, and weighed as crude polysaccharide yield.

Physicochemical characterization

Apparent density.

Ten grams of seed powder was gently transferred into a 100 mL graduated cylinder containing 50 mL distilled water. After 5 min equilibration, the displaced volume was recorded. Apparent density (g/mL) = mass / displaced volume. Triplicate determinations were made.

pH and point of zero charge (pHpzc)

Sample pH was measured by dispersing 1 g powder in 100 mL distilled water (1% w/v) and reading with a calibrated pH meter (Hanna HI 2211, USA). The pHpzc was determined by the salt addition method: 0.1 g sample was added to 50 mL of 0.01 M NaCl whose initial pH (pHi) was adjusted from 2 to 12 using 0.1 M HCl/NaOH. After 48 h equilibration, final pH (pHf) was measured. pHpzc was obtained from the plot of ΔpH (pHf – pHi) versus pHi.

FTIR spectroscopy.

FTIR analysis was performed on the dried seed powder before coagulation experiments using a Shimadzu FTIR-8400S spectrophotometer (Japan). The sample was ground with FTIR-grade KBr at 1:100 ratio and compressed into pellets. Spectra were recorded in the mid-infrared region (4000–400 cm^{-1}) at 4 cm^{-1} resolution with 32 scans. Bands were assigned to functional groups according to Coates (2000). The spectrum is presented as Fig. 1.

Statistical analysis

All experiments were conducted in triplicate and results expressed as mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) was performed using SPSS version 23.0 (IBM Corp., USA). Significant means were separated by Duncan's multiple range test at $p < 0.05$.

3. Results and Discussion

Physical properties and proximate composition

The physical properties and proximate composition of *Detarium microcarpum* seed powder are summarized in Table 1 and illustrated in Fig. 1.

Table 1. Proximate composition and physical properties of *D. microcarpum* seed powder

Parameter	<i>D. microcarpum</i> seed	Unit
Yield	28.31	%
Apparent density	0.235	g/mL
Moisture	7.25	%
Ash	3.48	%
Crude protein	19.77	%
Crude fat	10.53	%
Crude fibre	2.20	%
Carbohydrate	56.76	%

Proximate composition of Detarium microcarpum seed powder

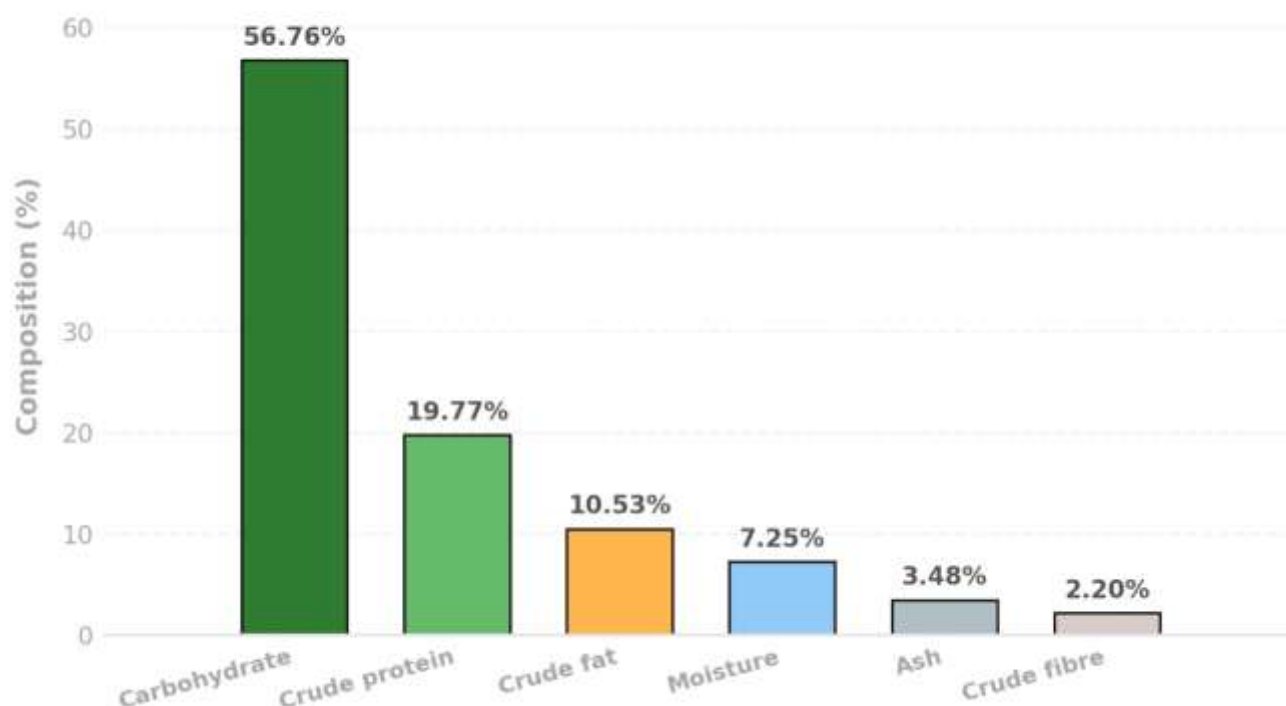


Fig. 1. Proximate composition of Detarium microcarpum seed powder.

Carbohydrate (56.76%) was the dominant fraction, followed by crude protein (19.77%) and crude fat (10.53%), while ash (3.48%), moisture (7.25%) and crude fibre (2.20%) were low. The high carbohydrate content is consistent with the mucilaginous nature of *Detarium* spp. and suggests abundant polysaccharide for bridging flocs. The protein content of 19.77% is comparable to *Moringa oleifera* seed (19–25%), where cationic proteins drive charge neutralization. Moisture below 10% indicates good storage stability, and the low apparent density (0.235 g/mL) suggests a porous powder favourable for rapid dissolution.

FTIR functional group characterization

Functional group analysis of the seed powder revealed characteristic absorption bands summarized in Table 2.

Table 2. FTIR band assignments for *D. microcarpum* seed powder

Observed band (cm ⁻¹)	Interpretation	Probable contribution
3272.6	Broad O–H / possible N–H stretching	Hydroxyl-containing carbohydrates, phenolics, absorbed moisture and protein N–H
2914.8	Aliphatic C–H stretching	Lipid and other aliphatic organic constituents
2120.9	Weak band; assignment not definitive	Minor/combination/background contribution possible; not used for a specific structural claim
1636.3	C=O/amide-associated and/or H–O–H bending contribution	Protein/amide environment and/or absorbed water
1438.8	C–H bending / possible COO ⁻ contribution	Aliphatic or oxygenated organic constituents

Observed band (cm ⁻¹)	Interpretation	Probable contribution
1401.5	C–H bending / fingerprint-region vibration	Organic matrix
1148.0	C–O / C–O–C stretching	Carbohydrates/polysaccharides/oxygenated compounds
1043.7	C–O / C–O–C stretching	Carbohydrates/polysaccharides/oxygenated compounds
928.1	Fingerprint-region deformation/skeletal vibration	Complex carbohydrate/organic matrix; assignment not unique

The broad band at 3272.6 cm⁻¹ indicates overlapping O–H and N–H stretching, confirming hydroxyl-rich polysaccharides and proteinaceous matter with hydrogen-bonding capacity. The band at 1636.3 cm⁻¹ corresponds to amide I C=O, supporting the proximate protein result. Strong bands at 1148.0 and 1043.7 cm⁻¹ are diagnostic of glycosidic C–O–C linkages, corroborating the high carbohydrate (56.76%) content. These O–H, N–H, C=O and C–O groups provide active sites for electrostatic interaction, hydrogen bonding and polymer bridging with colloidal particles, supporting a dual charge neutralization and bridging mechanism.

4. Conclusion

This study successfully characterized *Detarium microcarpum* seed powder for bio-coagulant application. The powder exhibited a yield of 28.31% and low apparent density (0.235 g/mL), favourable for dissolution. Proximate composition was dominated by carbohydrate (56.76%) and crude protein (19.77%), with moderate crude fat (10.53%) and low moisture (7.25%), ash (3.48%) and fibre (2.20%). FTIR analysis confirmed the presence of key coagulating functional groups: hydroxyl/amine (3272.6 cm⁻¹), aliphatic (2914.8 cm⁻¹), amide carbonyl (1636.3 cm⁻¹) and polysaccharide C–O–C (1148.0, 1043.7 cm⁻¹). These findings demonstrate that *D. microcarpum* seed contains polysaccharide and protein matrices with hydrogen-bonding and charge-neutralizing capacity, validating its potential as a sustainable alternative to chemical coagulants.

Recommendations

1. Jar-test studies should be conducted to evaluate turbidity, TSS, BOD and COD removal efficiency at varying dosages, pH and settling times.
2. The active coagulating agents (proteins/polysaccharides) should be extracted, purified and further characterized to optimize performance.
3. Comparative performance and cost-benefit analysis against alum and *Moringa oleifera* seed should be undertaken.
4. Toxicological assessment of treated water should be performed to ensure safety for domestic use.
5. Scale-up trials on industrial and municipal wastewater are recommended to validate field applicability.

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