

PHYSICOCHEMICAL PROPERTIES AND BIOFLOCCULATING EFFICIENCY OF POLAR RESIDUE OF PARTITIONED EXTRACT OF *TELFAIRIA OCCIDENTALIS* LEAF

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Abstract

Water contamination has become more pronounced as a result of human economic growth all over the world. This study investigated the physicochemical properties and bioflocculating activity of the polar residue of partitioned extract (PRPE) of *Telfairia occidentalis* leaf. The bioflocculating activity of the extract was evaluated for optimum dosage, pH, temperature, and metal ion using Kaolin clay suspension; the optimum activities obtained were used in wastewater treatment. The optimum flocculating activity of the PRPE was obtained at the dose of 1 mg/mL, pH of 4, the temperature of 50°C, and divalent cation (Ca^{2+}). The physicochemical characteristics of removal of turbidity, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS) and heavy metals were determined before and after treatment. Upon treatment of two different domestic wastewaters (DW1 & DW2), the physicochemical parameters were reduced except for nitrate and turbidity, which increased for DW2. The bioflocculant displayed the highest activity in DW1 with turbidity removal of 74%, TSS removal of 48%, nitrate removal of 24%, BOD and COD removal of 87.5% and 50% respectively. Heavy metals were also removed effectively in DW1 compared to DW2. The FTIR characterization revealed the presence of primary amine (N-H), cyclic alkene (C=C), phenol (O-H), sulfoxide (S=O) and, halo compound (C-Br) groups present in the bioflocculant which are essential as they promote the flocculation process. The findings from this study attest to the ability of PRPE of *Telfairia occidentalis* to act as a flocculant.

Keywords: Bioflocculant; Physicochemical; *Telfairia occidentalis*; Wastewater

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Introduction

Clean and safe water is of paramount importance to life; it is considered as one of the basic human rights according to the United Nations. It is also estimated that the daily requirement of an individual for clean and safe water is 20-50 Litres (National Academy of Sciences, 2016). The rapid economic growth of human enterprises has resulted in the increase of contamination of water by organic or inorganic contaminants, posing risks to both aquatic and human lives (Ahmed *et al.*, 2020).

Approaches such as the use of activated carbon, membrane filtration, ion exchange, and flocculation has been employed in the treatment of wastewater (Al-wasify *et al.*, 2017; Rachdi *et al.*, 2017), with coagulation/flocculation being the most applied approach (Ortiz-Oliveros and Flores-Espinosa, 2019).

Organic and inorganic flocculants such as aluminum have long been used in wastewater treatment but the usage is associated with low performance, non-biodegradability, and associated health risks (Das *et al.*, 2021). The use of micro-organism or plant-based flocculants (bioflocculants) have gained much attention because of their improved flocculating properties, nontoxic, biodegradable and eco-friendly nature (Kothari *et al.*, 2017; Shahadat *et al.*, 2017). According to Dao *et al.* (2016), a good bioflocculating agent contains large amounts of proteins, nucleic acids, glycoproteins, and polysaccharides. The flocculating mechanism of plant-based flocculants is their ability to produce both positive and negative charge compounds with high density at any pH condition allowing the neutralization of charges and formation of bridges; creating heavy flocs that would then sediment (Mukhtar *et al.*, 2015). Plant species such as Aloe vera (Irma *et al.*, 2015, Albizia gum (Afolabi and Adekanmi, 2017), malva nut gum (Ho *et al.*, 2014), Lepidium sativum (Allafchian *et al.*, 2019), Moringa oleifera (Zaid *et al.*, 2019), and Trigonella foenum-graecum (Jones and Bridgeman, 2019) have been reported to show promising results with respect to the treatment of biological, tannery, sewage effluents, textile and dye-containing wastewater, landfill leachate.

Telfairia occidentalis (Fluted pumpkin) is a popular and widely grown vegetable crop in the eastern, mid-western, and south-western regions of Nigeria. It is often referred to as “ugu”. The leaf is rich in minerals, antioxidants, vitamins and phytochemicals (Airaodion *et al.*, 2019).

The use of eco-friendly flocculants in wastewater treatment becomes pertinent as an alternative to the chemical flocculants in order to

prevent the aforementioned drawbacks, hence the need for this study to determine the potential of *T. occiendatalis* in flocculating wastewater.

Materials and Methods

Plant Materials

The leaf of *Telfairia occidentalis* was collected from Oja Oba market, Kwara State, Nigeria on 25th November, 2019, at approximately 7.30 am. The plant sample was authenticated at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, where a voucher specimen was deposited (UILH/002/2019/959).

Chemicals and Reagents

Kaolin clay, ethanol, chloroform, ethyl acetate, hexane and, petroleum ether were obtained from LobaChemie Pvt. Ltd., Mumbai, India. Ferric chloride, hydrochloric acid, sodium hydroxide, and butylated hydroxytoluene (Sasol, Sandton, South Africa). All other chemicals and reagents were of analytical grade obtained from the Department of Biochemistry Laboratory, University of Ilorin, Ilorin, Nigeria.

Wastewater

The domestic wastewaters were collected from Asa Dam Area and Oyun Area, Ilorin, Kwara State, Nigeria

Plant Extraction and Polarity Dependent Partitioning

The plant material was rinsed with tap water and air dried under shade till a constant weight was obtained. On complete drying, the plant material was pulverized uniformly with the use of a blender and was stored in an air tight container. The extraction was carried out by exhaustive extraction process; 10 g of the dried plant material was soaked in 200 mL of 50% ethanol for 24 hours in 25 different extraction jars, the mixture was then filtered. 100 mL of 50% ethanol was re-added to each jar, and then filtered. The process was repeated with 100 mL of 50% ethanol again. The filtrate was collected and dried. The dried extract was reconstituted by dissolving it in 200 mL of 50% ethanol. Polarity-dependent partitioning was done by adding 200 mL n-hexane to the mixture, it was mixed and allowed to separate, and the n-hexane layer was eluted. The process was repeated with petroleum ether, chloroform and ethyl acetate respectively. The resulting residue served as the

most polar fraction. It was dried and kept in an air tight container and stored in the freezer for further analysis.

Jar Test for Bioflocculant Activity

This test was carried out using the jar test machine (model number SSE-28/B).

Determination of Bioflocculant Dosage

Different concentrations (0.0625, 0.125, 0.25, 0.5, and 1.0 mg/mL) of the polar residue of *Telfairia occidentalis* were prepared, and their flocculating activities were measured against 4 g/L kaolin clay suspension. Exactly 3.0 mL of 1% (w/v) CaCl₂ was added to the different concentrations of the sample and mixed with 100 mL of kaolin clay suspension in 500 mL beakers. The solution was rapidly mixed at 160 rpm for 4 min, followed by gradual rotation at 50 rpm for another 4 min and sedimentation for 5 min. After sedimentation, 2 mL was gently withdrawn from the upper clarifying phase in order to measure the flocculating activity (Xiong *et al.*, 2001). The concentration dosage that gave the best flocculating activity was used for subsequent experiments.

Effect of Cations on Flocculating Activity

The effect of different cations on bioflocculant production was assessed by replacing CaCl₂ in the production medium with Na⁺, K⁺, Mg²⁺, Ba²⁺, and Fe³⁺ using the method of Kurane *et al.* (1994).

Effect of pH on Flocculating Activity

The effect of pH on the flocculating activity of the sample was assessed by adjusting the pH (PHS-3C desktop digital display) of the production medium using 0.1 M HCl and 0.1 M NaOH at the pH range of 3-12 (Xiong *et al.*, 2001).

Effect of Temperature on Flocculating Activity

Heat stability was evaluated by incubating the bioflocculant solutions in a water bath at a temperature range of 50, 60, 70, 80, 90, and 100°C for 25 min. Afterwards, the residual flocculating activity was determined using the protocol of Gong *et al.* (2008).

Application of bioflocculant in Wastewater Treatment

Exactly 3.0 ml of 1% (w/v) CaCl₂ was added to the optimum concentration of the bioflocculant and then mixed with 100 mL of the industrial wastewater sample in 1000 mL beaker. The solution was rapidly mixed at 160 rpm for 4 minutes, followed by gradual flocculation at 50 rpm for 4 minutes using jar test apparatus and then the treated sample was left to settle for 5 minutes. After

sedimentation, the supernatant was used for further physicochemical analyses. This procedure was conducted in triplicates. The turbidity, suspended solids, COD, BOD and nitrate were measured using spectrophotometer DR 3800 (Lee *et al.*, 2001).

The removal efficiencies were thereafter calculated as:

$$RE = \frac{A_o - A}{A_o} \times 100$$

Where A_o and A are the initial and final values obtained before and after treatment respectively.

Determination of Physicochemical Parameters

Chemical Oxygen Demand (COD) Determination

Chemical oxygen demand (COD) determines the amount of oxygen required for the chemical oxidation of organic matter using a strong chemical oxidant, such as potassium dichromate, under reflux conditions. Exactly 2 mL from the test samples, domestic wastewater (DW 1 and 2) were taken and added to 1 mL of chemical oxygen demand (COD) digestion reagent inside a conical flask. This was then placed in the oven for an hour at 150°C, thereafter allowed to cool. After cooling, 2 drops of ferroin solution was added followed by titration with 0.1 M ammonium ferrous and then observed for orange colour (McCrary, 1966; WPCF, 1985 and AWWA, 2011).

Turbidity Determination

The turbidity of the test samples was measured using a UV-visible spectrophotometer (Model 371, Elico India Ltd) at 420 nm. Also, 1% transmittance of the test samples was measured as well (Lee *et al.*, 2001).

pH determination

The pH of the test samples (DW1 and DW2) was measured respectively, using pH meter (PHS - 3C desktop digital display) (Xiong *et al.*, 2010).

Nitrate Determination

Briefly, 1 mL of the test samples was taken respectively and 0.25 mL of 2 % copper sulphate was added including 1 mL of 0.1 M sulphanilamide (ETIM) and 0.5 mL of NED reagents. This was read using a Spectrophotometer at 540 nm (UV/Visible Spectrophotometer Model 371, Elico India Ltd).

Biochemical Oxygen Demand (BOD) Determination

The test samples were each measured for dissolved oxygen at day zero (DO₀) using BOD

probe thereafter kept in a dark room for five days and another reading for dissolved oxygen at day 5 (DO_5) were taken using the BOD instrument (Lee *et al.*, 2001). The percentage BOD removal efficiency was subsequently estimated using the equation:

$$\%BOD = \frac{DO_1 - DO_5}{DO_1} \times 100$$

Where; DO_1 = DO measured at day 1; DO_5 = DO measured at day 5

Total Suspended Solid (TSS) Determination

TSS level in the collected water samples was determined using gravimetric method. A homogeneous aliquot of water sample was filtered through a pre-weighed glass fiber filter. The filter was dried in the oven at a temperature of 105°C overnight. The filter paper was removed and allowed to cool to room temperature in a desiccator till a constant weight was achieved. The increase in mass of the dry filter paper was later recorded and used for calculating TSS.

TSS is calculated using the equation:

$$\text{Total suspended solids (mg L}^{-1}\text{)} = \frac{(A - B)}{\text{volume of sample (ml)}} \times 100$$

Where: A = weight of the filter after filtration (mg), B = weight of the filter before filtration (mg).

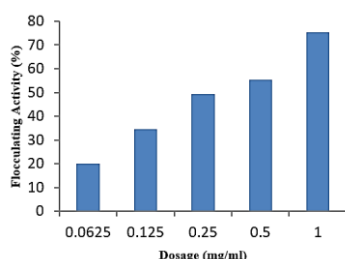


Figure 1. Effect of dosage on flocculating efficiency of the polar residue of partition extract of *Telfairia occidentalis* leaf on Kaolin clay. Values are expressed as means $\pm$ SEM of three determinations

Heavy Metal Analysis

Wastewater samples were digested by nitric acid, and the digest was analyzed using Atomic Absorption Spectrophotometry (AAS, Perkin-Elmer, Melville, NY, USA) according to the method described by Edokpayi *et al.* (2017).

Results and Discussion

Exhaustive extraction of the powdered dried sample with hydro-ethanol gave about 15.8 g and on polar partitioning, the resulting yield was weighed at 8.83 g. the results obtained from the jar test analysis to determine the optimum dosage, temperature, and pH on the bioflocculant are as shown in Figure 1, 2, and 3 respectively. It was observed that at the dosage range of 0.625 to 1.0 mg/mL, the PRPE of *Telfairia occidentalis* leaves displayed its highest flocculating activity of 75.39% at the highest dosage of 1 mg/mL. There was an increase in activity with increasing dosage concentration. The optimum flocculating activity of 87.7% was obtained at temperatures of 50°C and 60°C while the flocculating activity of 93.80% was achieved at acidic pH 4 of the medium.

Table 1 shows the impact of metal ions on flocculation efficiency of the bioflocculant from polar residue of the partitioned extract of *T. occidentalis* Leaf and the highest flocculating of 78.13% was observed when Fe^{3+} was used.

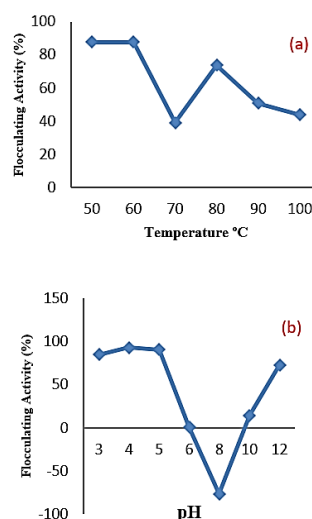


Figure 2. Effect of (a) temperature (b) pH on flocculating efficiency of the polar residue of partition extract of *Telfairia occidentalis* leaf on Kaolin clay. Values are expressed as means $\pm$ SEM of three determinations.

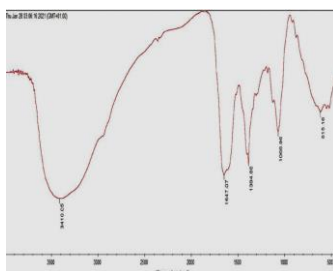


Figure 3. Effect of dosage on flocculating efficiency of the polar residue of partition extract of *Telfairia occidentalis* leaf on Kaolin clay. Values are expressed as means $\pm$ SEM of three determinations

Table 1. The efficiency of bioflocculant from polar residue of the partitioned extract of *Telfairia occidentalis* Leaf in the presence of various cations

Cations	Flocculating Efficiency (%)
Na ⁺	57.23 $\pm$ 0.01
K ⁺	-14.84 $\pm$ 0.02
Ca ²⁺	75.39 $\pm$ 0.01
Mg ²⁺	38.36 $\pm$ 0.02
Fe ³⁺	78.13 $\pm$ 0.00
Al ³⁺	36.23 $\pm$ 0.00

Values are expressed as means $\pm$ SEM of three determinations.

Table 2. The efficiency of bioflocculant extracted from *Telfairia occidentalis* leaves on Kaolin Clay suspension compared to DW1, and DW2 in the presence of Ca²⁺

Parameters	Efficiency (%)
Kaolin clay	75.39
Domestic wastewater 1	44.40
Domestic wastewater 2	67.57

Table 3. Physicochemical characteristics of domestic wastewater 1 and 2 (DW1 and DW2) before and after treatment with the polar residue of the partitioned extract of *Telfairia occidentalis*

Parameters	DW1		% Removal Efficiency	DW2		% Removal Efficiency	FME Discharge Standard	WHO Discharge Standard
	BT	AT		BT	AT			
pH	9.10	-	-	8.50	-	-	6.5-8.5	6.5-8.5
Turbidity(NTU)	12.55	3.24	74.18	3.35	3.45	-	5.00	5.00
TSS (mgL ⁻¹)	0.06	0.04	33.33	0.15	0.08	48.12	0.25	NS
Nitrate (mgL ⁻¹)	6.76	0.51	92.46	3.90	5.94	-	9.10	30.00
COD (mgL ⁻¹)	200.00	100.00	50.00	220.00	126.00	42.73	30.00	80.00
BOD (mgL ⁻¹)	160.00	20.00	87.50	140.00	90.00	35.71	3.00	40.00

NTU = Nephelometric turbidity units; COD = Chemical oxygen demand; BOD = Biological oxygen demand; TSS = Total Suspended solids; BT = Before treatment; AT = After treatment

Table 4. Performance of PRPE *Telfairia occidentalis* leaf in the removal of heavy metals in the domestic wastewater (DW1 and DW2)

Parameters	DW1		% Removal Efficiency	DW2		% Removal Efficiency	FME Discharge Standard
	BT	AT		BT	AT		
Copper	0.025	0.00	100	0.02	0.025	-	0.010
Chromium	0.03	0.00	100	0.08	0.115	-	0.500
Lead	0.1	0.00	100	0.1	0.00	100	0.100

DW1 = Domestic waste water 1; BT = Before Treatment; DW2 = Domestic wastewater 2; AT = After Treatment

Table 5. FTIR spectrum table of polar residue of the partitioned extract *Telfairia occidentalis*

Frequency Range (cm ⁻¹)	Absorption (cm ⁻¹)	Appearance	Group	Compound class
3500	3410.05	Medium	N-H stretching	Primary amine
1648-1638	1647.07	Strong	C=C stretching	Cyclic alkene
1390-1310	1384.88	Medium	O-H bending	Phenol
1070-1030	1066.96	Strong	S=O stretching	Sulfoxide
690-515	615.16	Strong	C-Br stretching	Halo compound

The waste waters were better flocculated with the PRPE of *Telfairia occidentalis* leaf when compared with kaolin clay (laboratory standard), as shown in Table 2. The physicochemical parameters such as turbidity, nitrate, COD and BOD analyzed were reduced upon treatment of the domestic wastewater (DW1 and DW2) except for nitrate and turbidity that increased for DW2 as shown in Table 3.

Heavy Metal Analysis

Table 4 shows the heavy metals present in the domestic wastewaters before and after treatment. The heavy metals present were more efficiently removed in DW 1 than in DW 2.

Fourier - Transform Infrared Spectrophotometry Characterization of PRPE of *Telfairia occidentalis* leaves

Infrared spectrophotometry was used to analyze the purity of the bioflocculant produced from *Telfairia occidentalis* leaves, as illustrated in Figure 3 and Table 5. Clear absorption peaks were observed at 3410.05, 1647.07, 1384.88, 1066.96, and 615.16 cm^{-1} .

Discussion

There is a need for the determination of the efficient dosage for the flocculating activity of a bioflocculant; adequate dosage brings about efficient flocculating activity (Ntombela *et al.*, 2019), while overdosing could lead to Re-stabilization of the flocs (Hassan *et al.*, 2009). The jar test showed optimum dosage activity at 1.0 mg/mL (75.39%). There was a constant increase in flocculating activity from the lowest concentration (20%) to 1.0 mg/mL (75.39%), this is it agrees with the findings of Agunbiade *et al.* (2017) who reported that the bioflocculant produced by *Athrobacter hemicola* has best (optimum) flocculating activity of 89% at 0.8 mg/mL.

The bioflocculant from *Telfairia occidentalis* showed its highest flocculating activity of 87.7% at a temperature of 50°C. Further increase in temperature to 60°C resulted to similar flocculating activity of 87.7%, however, as the temperature was raised further there was a decline in flocculation efficiency of the bioflocculant. This agrees with the report of Liu *et al.* (2010), where the bioflocculant produced by *K. pneumonia* flocculated well in the range of 4-50°C, with the highest flocculating activity of 97.5% while the flocculating activity decreased when the temperature exceeded 60°C. This indicated that the bioflocculant could exhibit its great application potential in treating mild water temperatures (Luo *et al.*, 2014).

The optimal flocculating activity of 93% was observed at pH 4, and these study findings agree with those reported by Yim *et al.* (2007), where bioflocculant produced by their test organism attained its maximum flocculating activity at pH 3. Metal ions play a stimulatory mechanism in flocculating activity, thereby neutralizing and stabilizing the residual charge of functional groups on the flocculant, and the addition of metal ions results in the increase in ionic strength of the suspended particles in a solution, thereby reducing Coulomb forces of the suspended solids (Ntombela *et al.*, 2019). The tested metal ions were successful in stimulating the flocculating activity of the bioflocculant, as they showed flocculating activity of over 50% on Kaolin clay suspension, with the greatest stimulation by Fe^{3+} which resulted in the optimum flocculating activity of 78.13% followed by Ca^{2+} with flocculating activity of 75.39%. This is in line with the report of Ntombela *et al.* (2019), where the bioflocculant produced by Consortium *Bacillus safensis* and *Bacillus* sp. were stimulated best by Ca^{2+} , Ba^{2+} , and Fe^{3+} .

Turbidity is a measure of the degree to which the water loses its transparency due to the presence of suspended particles, therefore affecting the quality of water. Total suspended solids are the amount of solids that can settle in the water. The more total suspended solids in the water, the murkier it seems and the higher the turbidity (Aniyikaiye *et al.*, 2019). The turbidity values for the raw domestic wastewaters (1 and 2) were found to be 12.550 and 3.345 mg/L, respectively. However, after treatment with the partitioned extract of *Telfairia occidentalis* leaf turbidity of domestic wastewater, one was significantly reduced to 3.241 mg/L with a percentage reduction efficiency of 74.16%, while that of the domestic wastewater two was found to be 3.45 mg/L with no removal efficiency. The TSS values for the raw domestic wastewater (1 and 2) were found to be 0.055 and 0.145 mg/L, respectively. TSS removal efficiencies of 36.36% and 48.28% were recorded for wastewaters (1 and 2) respectively. This result agrees with that of Agunbiade *et al.* (2017) where bioflocculant produced by *Athrobacter hemicola* has turbidity and suspended solids removal rates of 81.3% and 55.7% respectively. Also, the bioflocculant MBFA9 produced by *B. mucilaginosus* had an intense flocculating activity for suspended solids with a removal rate of 85.5% (Deng *et al.*, 2003). This shows that the bioflocculant was very efficient in reducing the level of turbidity in wastewater one but not 2. The result also showed that the bioflocculant efficiently reduced the TSS level in both wastewaters. The removal was sufficient as they both complied with the Nigeria limitation guidelines

set by the Federal Environmental and Protection Agency (FEPA) standard of 25 mg/L (Federal Ministry of Environment, 2011).

Nitrates are essential in water pollution because they are effective nutrient sources for algae (organic nitrogen is converted to ammonia, then ammonia is oxidized to nitrate) (Rachna and Disha, 2016). Excessive presence of phosphate, nitrates, and potassium causes algal blooms, resulting in the death of aquatic organisms (Rachna and Disha, 2016). After treatment, the reduction in the nitrate level indicated that the bioflocculant from the polar residue of *Telfairia occidentalis* leaves partition extract was more effective in wastewater one than in wastewater 2.

Chemical oxygen demand measures the equivalent amount of oxygen required to completely oxidize both biodegradable and non-degradable organic and inorganic matter (Ogbu *et al.*, 2016). The COD values, 200 (DW1) and 220 (DW2), obtained before treatment, were significantly reduced by 50% and 42.73%, respectively, in both wastewaters. The five-day biological oxygen demand (BOD5) is the most widely used parameter in the evaluation of organic pollution of surface waters. It is the amount of dissolved oxygen taken up by aerobic microorganisms to degrade oxidizable organic matter present in streams measured over a period of 5 days (Ogbu *et al.*, 2016). Higher concentrations of BOD in water lead to higher oxygen depletion in water bodies that are necessary for the survival of aquatic organisms (Aniyikaiye *et al.*, 2019). The bioflocculant showed removal efficiency of about 87.5% and 35.7% for BOD from DW1 and DW2, respectively after treatment with the bioflocculant produced by PRPE of *T. occidentalis*. Luo *et al.* (2014) reported on bio flocculant MBF-6 produced by *K. pneumonia* YZ-6 isolated from human saliva with the ability to flocculate several wastewaters from the textile, dairy, brewery, and sugar industries. The maximum flocculating efficiency observed with wastewater from the sugar industry showed the highest reduction of COD (77.8%) and BOD (80.7%). The COD and BOD values obtained after treatment were satisfactory and within FEPA and WHO permissible standards for effluent discharges to surface water, and *Telfairia occidentalis* was very efficient and can be employed as a BOD reduction agent (Federal Ministry of Environment, 2011; WHO, 2011).

Metal ions in industrial effluents tend to accumulate and assimilate into the food chain, posing a risk to aquatic life and humans (Okaiyeto *et al.*, 2016). The reduction of the heavy metals analyzed for both wastewater 1 and 2 after treatment was within the limits set by the FME (Federal Ministry of Environment, 2011).

The spectrogram for the PRPE of *Telfairia occidentalis* indicated the presence of amine, phenol, and alkene groups in the molecular chain of the bioflocculant, which are constituent of bioflocculants. According to Lee *et al.* (2014), flocculation is a direct flocculation process that is more effective than conventional flocculation as a result of the high charge density of amphoteric plant-derived flocculants. Due to the dual occurrence of anionic and cationic active compounds, biopolymers are capable of bridging at any pH condition (Mukhtar *et al.*, 2015).

Conclusion

The bioflocculant produced through the green method was displayed as an excellent flocculating agent against Kaolin clay, and the domestic wastewater samples were also tested. The optimum working conditions of the bioflocculant were 1 mg/mL dosage size with flocculating activity of 75.39%. The bioflocculant flocculated well at pH 4 with the flocculating activity of 93.80%, and the optimal flocculating activity was 87.70% at a temperature of 60°C in the presence of Ca^{2+} and the understudied physicochemical parameters of the domestic wastewaters. The observed bioflocculating efficiency could be attributed to the various functional groups found in the polar partitioned residue of the extract.

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