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Moringa oleifera seed extract-mediated flocculation as an efficient method for harvesting *Chlorella lewinii* biomass

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Abstract

The aim of this research was to investigate the potentials of harvesting *Chlorella lewinii* through flocculation by *Moringa oleifera* seed extract. Water, ethanol, and sodium chloride solution were used to extract flocculating agents from both whole and de-fatted *Moringa oleifera* seeds and their ability to flocculate *C. lewinii* cells were evaluated. The effects of extracting solvents, extract concentration, incubation period and culture age on flocculation efficiency were investigated. The lipid contents of the biomass harvested by flocculation using *M. oleifera* seed extract were compared with those harvested by centrifugation. The results showed that 1M sodium chloride solution was the most effective solvent for extracting *M. oleifera* active ingredient. The optimum extract concentration was 600±0.10 mg/L with approximately 60±0.46 % efficiency, while the optimum length of period for incubating a mixture of cell culture and seed extract was 80±0.26 min. Defatted *Moringa oleifera* seed extract was more efficient than whole seed extract with an efficiency of 50%. The percentage lipid content of biomass harvested by centrifuge and moringa extracts decreased in the following order: Centrifugation-(22.55%) > de-fatted seed extract (16.63%) > whole seed extract (14.42%). These results indicate that *M. oleifera* seed extract is a reliable method of harvesting microalgae biomass.

Keywords: ***Moringa oleifera*, *Chlorella lewinii*, flocculation, flocculation efficiency, seed extracts**

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INTRODUCTION

Human population keeps increasing with a corresponding increase in energy demand and environmental pollution (Krishnana *et al.,* 2021; Moodley and Trois, 2021). Majority of the energy used all over the world is derived from fossil fuels (Zhu *et al.,* 2018; Moodley and Trois, 2021). The over reliance on fossil fuels has been one of the major sources of greenhouse gas emission into the atmosphere and the consequential cause of global warming (Barbir *et al*., 1990; Hunt *et al.,* 2020). In order to reduce burden placed on fossil fuels and the amount of greenhouse gas generation, researches into alternative sources of energy have been ongoing for some decades now (Li and Loo, 2014). There has been evolution in generations of biofuels ranging from first to fourth generation based on their sources (Alalwan *et al.,* 2019). All these are in a bid to meet up with the need for renewable, sustainable and eco-friendly forms of energy.

Among the current forms of biofuel, liquid biofuels such as biodiesel and bioethanol are the most important and sought after, because they are required for the transportation of humans, goods and services and can also be converted to other forms of energy (Hernadez and Kafarov, 2009). Microalgae lipids are reliable third generation feedstock for biodiesel oil production. These are based on many positive attributes of microalgae which include high lipid contents (Ogbonna *et al.,* 2021; Roy and Mohanty 2019), high growth rate (Ogbonna and Nwoba, 2021; Roy and Mohanty, 2019), ability to utilize animal wastes (Ogbonna *et al.,* 2021) and wastewater (Nwoba *et al.,* 2020a, Vadiveloo *et al.,* 2019; Nwoba *et al.,* 2021; Zewdie and Ali, 2020) and waste gas (Kandimalla *et al.,* 2016; Hanifzadeh *et al.,* 2017; Zewdie and Ali, 2020) as medium for growth. Microalgae have the potentials to grow in environments that are not suitable for conventional agriculture (Nwoba *et al.,* 2020b; Vadiveloo *et al.,* 2019).

However, one of the difficulties in microalgae biomass utilization is the problem of harvesting (Ogbonna and Edeh, 2018; Ogbonna and Chioke, 2018; Ogbonna and Nwoba, 2021). The cost of harvesting microalgae biomass for use as feed stock for lipids and other bioactive compound extraction accounts for about 10 -15% of the total production cost (Ogbonna and Nwoba, 2021; Fasaei *et al.,* 2018). The conventional methods of harvesting microalgae are centrifugation (Naijai and Abu-Shamleh, 2020, Japar *et al.,* 2017), floatation (Laamanen *et al.,* 2016; Xu *et al.,* 2021), filtration (Kim *et al.,* 2015; Singh and Patidar, 2018) and even natural sedimentation. However, these methods are either very expensive or complex and they either require much energy or long period of time to accomplish. Several strategies have been reported as ways of reducing the cost of harvesting microalgae and these include harvesting by flocculation using flocculating agents. There are many commercially available flocculants among which are some inorganic salts like aluminum salts ($AlSO_4$, $AlCl_3$), and Zinc salts (Surendhiran and Vijay, 2013). However, these inorganic salts are not friendly to the ecosystem since a lot of residues that pollute the environments including water bodies are generated after their use (Sun *et al*., 2021). Some of the inorganic flocculants have negative impact on the harvested microalgae biomass and may have negative effect on their further processing. Other strategies such as adjusting the pH of microalgae culture medium to induce flocculation (Chen *et al.,* 2013) have been adapted but this strategy also has a negative effect either on the biomass or renders the effluent unrecyclable unless the pH is readjusted with consequent increase in the production cost. The use of self-flocculating strains of microalgae (Li *et al*., 2021) or bio-based flocculants such as microbial flocculants or flocculants of plant origin are safe and environmentally friendly methods (Ogbonna and Nwoba, 2021). Plant seeds and plant vegetative parts have been employed in harvesting microalgae (Ogbonna and Edeh, 2018; Ogbonna and Chioke, 2018; Mainho *et al.,* 2022).

Among the plant seeds used for microalgae harvesting are seeds of *Moringa oleifera*. Moringa seeds have been confirmed to contain active flocculating agents used in various forms to purify water for drinking, treat wastewater from various sources and to harvest various species of microalgae for different applications. *Moringa oleifera* is a tropical and subtropical plant that grows very fast and produce plenty of seeds within a short period of time. The seeds are edible and biodegradable and are therefore safe for harvesting microalgae cells for various applications. Although the use of *Moringa oleifera* seeds to harvest different species of microalgae abound in literature, no one has reported on

harvesting *Chlorella lewinii* biomass using *M. oleifera* seeds as flocculating agent.

The aim of the present research was to evaluate the potentials of harvesting *Chlorella lewinii* biomass via *Moringa oleifera* seed extract induced flocculation.

MATERIALS AND METHODS

Microalga strain and culture media

Chlorella lewinii was obtained from the Department of Microbiology, University of Nigeria Nsukka. It was reactivated by sub-culturing in Blue - green (BG11) medium under continuous illumination at a light intensity of 150 $\mu molm^{-2}s^{-1}$ for seven days using a 32W cool white LED bulb (ASTRA NU-PARK, CHINA). The light intensity was determined by a lux meter (Lutron LX 101A, China). The BG 11 medium components were procured from WAKO Chemical industries LTD, Tokyo Japan. The medium was composed of the following (in gram per litre of distilled water): $NaNO_3$ 1.5, $K_2HPO_4.3H_2O$ 0.04, $MgSO_4.7H_2O$ 0.075, $CaCl_2.H_2O$ 0.036, Na_2CO_3 0.02, Citric acid 0.006, $C_6H_8O_7xFe^{3+}.y\ NH_3$ 0.006, Na_2 EDTA 0.001 and 1.0 ml trace metal element solution. The trace metal element solution was composed of (in g/L): H_3BO_3 2.860, $ZnSO_4.7H_2O$ 0.222, $MnCl_2 + 4H_2O$ 1.81, $CuSO_4.5H_2O$ 0.079, Na Mo $O_4.2H_20$ 0.390 and Co $(NO_3)_2.6H_2O$ 0.0494 (Leesing *et al*., 2011).

Preparation of *Moringa oleifera* seed powder and extraction of flocculating agent

Dry pods of *M. oleifera* were harvested from the Department of Plant Science and Biotechnology University of Nigeria Nsukka, Botanical Garden and the pods were shelled to release the seeds. The hulls enveloping the seeds were removed manually and only healthy seeds were selected using the methods of Katayon *et al. (*2006).

Prior to the preparation of seed extract, the seeds of *M. oleifera* were dried in an oven at 50°C for 48h to a constant weight. The dried *M. oleifera* seeds were ground using an electric blender and sieved through 400 µm stainless steel sieve to ensure uniform distribution of the fine powder. The seed powder was stored in an air tight brown reagent bottle to protect it from light (Hamid *et al*., 2016).

Preparation of defatted *Moringa oleifera* seeds

Oil extraction from *M. oleifera* seed powder (MSP) was done using Soxhlet extractor according to the method of Hadi and Salleh (2008) with some modifications. N-hexane (96%) was used to extract oil from *M. oleifera* seed powder using Soxhlet extractor. The n-hexane 100 ml was poured into a 250 ml capacity round bottom flask. The flask was put on a heating mantle and connected to a thimble equipped with a distillation tube connected to a condenser. Inside the Thimble 5 g of *M. oleifera* seed powder was added. The round bottom flask was heated to boil the n-hexane whose vapour travelled through the distillation tube to the condenser and then down to the thimble. The condensed vapour that entered the thimble wetted the moringa seed powder, dissolved and extracted the oil and the mixture was siphoned back to the round bottom flask. The vapour was circulated from the round bottom flask back and forth the thimble for five cycles until most of the oil in the powder was extracted. At the end of the cycle, the flask was dismantled from the other components. The defatted *M. oleifera* left in the thimble was collected and dried at 50°C in the oven for 48 h. The defatted seed powder was used for flocculant extraction.

Preparation of whole and defatted *Moringa oleifera* seed extracts

Aqueous, ethanol and IM NaCl extracts of moringa seeds were prepared. Ten grams (10 g) of either whole or defatted *Moringa oleifera* seed powder was added to 50 ml of each solvent separately; (water, absolute ethanol or IM NaCl) solution in a 250 ml beaker and stirred on a magnetic stirrer for 60 min. The mixture was allowed to stand with intermittent stirring for 24h at room temperature (25°C±2). After 24h, the suspension was filtered using a sterile cheese cloth. Both the filtrate and the residues were dried in an oven at 50°C for 48 h or until a constant weight was attained (Hamid *et al*., 2014). The dry extract was stored in an airtight brown container for use in the flocculation experiment.

Microalga Flocculation with *Moringa oleifera* seed extracts

Comparison of the flocculation efficiency of the three solvent extracts of *Moringa oleifera* seed

One gram (1g) of the extract from each of the three solvents was dissolved in 10 ml of distilled water in 100 ml beaker placed on a magnetic stirrer with a magnetic bar and stirred for 20 min to homogenize properly (100 g/L stock solution). One milliliter (1ml) of each extract was added to 99 ml of either 14 or 21 days old *C. lewinii* culture and stirred with a Galllenkamp magnetic stirrer for 3 min at a high speed, to enable the microalgae mix homogeneously. This was followed by a slow stirring for another 1 min. Twenty milliliters (20 ml) of the mixture was dispensed into test tubes and allowed to stand for 2 h at room temperature for the formed flocs to sediment. During this incubation, 2 ml sample was withdrawn every 20 min from the center of each test tube at a depth of about 1/3 the height to measure the optical density (OD) at 680 nm using a UV- visible spectrophotometer (Shimazu Model UV-1200, Japan) (Okuda et al 1999)

The flocculation efficiency was then calculated as follows:

$$\text{Flocculation efficiency} = \frac{OD_{t0} - OD_t}{OD_{t0}}$$

where OD_{t0} is the optical density taken at time zero and OD_t is the optical density of the sample taken at time t. All the experiments were carried out in triplicates.

Effect of extract dosage (whole or defatted seed) on the flocculation efficiency

Different concentrations of NaCl extract from both whole and defatted seeds were prepared by dissolving 1g of the extract in 10ml of distilled water in 100ml beaker to make 100 g/L stock solution. Then 1000 mg/L, 800 mg/L, 600 mg/L, 400 mg/L or 200 mg/L equivalents of the extract were added to 90 ml of either 14- or 21-day old *C. lewinii* culture in a 250 ml beaker. Distilled water was used to make up the volume of extract solution to 10ml in all the beakers. The mixture was stirred with a Gallen Kamp magnetic stirrer for 3 min at a high speed. This was followed by a slow stirring for 1min. The test tubes were left to stand without disturbance during which 2 ml samples were carefully withdrawn from the center of each test tube at a depth of about $^1/_3$ the height to measure optical density (OD) at 680 nm using a UV- visible spectrophotometer (Shimazu Model UV-1200, Japan)

Harvesting *Chlorella lewinii* by centrifugation and flocculation using extract from whole or defatted moringa seed powder

Chlorella lewinii cells cultured for 14 or 21 days were harvested by either centrifuging at 10,000 rpm for 30 min or flocculation for 60 min using 800 mg/L of extract from defatted or whole moringa seed. After harvesting and drying the biomass at 50^{o}C for 48 h, lipid was extracted from the dry biomass following the methods of Dyer and Bligh (1959).

Statistical analysis

All the experiments were performed three times and the data obtained were subjected to one way analysis of variance (ANOVA). The results obtained were presented as means ± standard error at $P \leq 0.05$ level of significance. Where there was significant difference, the mean values were separated using least significant difference (LSD).

RESULTS AND DISCUSSION

Effects of extracting solvent on flocculation efficiency of *M. oleifera* seed extract

The results of the effects of extracting solvent on flocculation efficiency of the *M.oleifera* seed extract are shown in Fig. 1. The extract obtained using one Molar (1M) sodium chloride solution gave the highest flocculation efficiency among the three solvents used with a flocculation efficiency of 59.65 ± 0.46%. This was followed by ethanol extract with a flocculation efficiency of 41.59 ± 0.28%. The least flocculation efficiency of 31.28 ± 0.21 was obtained with aqueous extract. There was statistically significant difference in the flocculation efficiency obtained from the three solvents. The active flocculating agent in *M. oleifera* seed was reported to be protein (Okuda et al 1999) and this has shown that it is more soluble in salt solution than in aqueous solution. Thus, the highest flocculation efficiency obtained with sodium chloride extract among the three solvents tested. This result is in agreement with the work of Bouchareb *et al.* (2021) who used 1M sodium chloride solution to extract active coagulating agent from *M. oleifera* seeds for use in municipal wastewater treatment and reported 97% turbidity removal with 140 g/L of 1 M NaCl extract against 88% obtained with 170 mg/L of aqueous extract. Okuda *et al.* (1999) also reported that 1M sodium chloride solution was more efficient than distilled water in

extracting active coagulant from *M. oleifera* seeds for use in synthetic wastewater treatment. Their results suggested that 1M sodium chloride solution was the best among other salt solutions tested (KCl, $NaNO_3$, KNO_3) with kaonite removal efficiency of 95% from low turbidity wastewater of 50 NTU against 78% removal obtained with distilled water extract. Texaira and Texaira (2017) also reported that sea water and sodium chloride solution which have high salt concentration were the best solvents for extracting active coagulating agent from *M. oleifera* seed for use in harvesting *Chlorella vulgaris*. Salt solution was proven to be more efficient in extracting the active coagulating agent from *M. oleifera* seed than distilled water. Madrona *et al.* (2010) also reported that 1M KCl extract of *M. oleifera* seed was a more efficient coagulant in the removal of colour and turbidity from wastewater than its distilled water extract counterpart. This is because the active coagulant is reported to be a high molecular weight cationic protein which is more soluble in high salt strength than in aqueous solution. The mechanism of salting which brings about protein-protein dissociation and increase in solubility was suggested by Madrona *et al.* (2010). Mainho *et al.* (2022) also reported that defatted *M. oleifera* seed coagulant extracted with salt solution exhibited a higher flocculation efficiency than aqueous extract on *Tetradesmus dimorphus*.

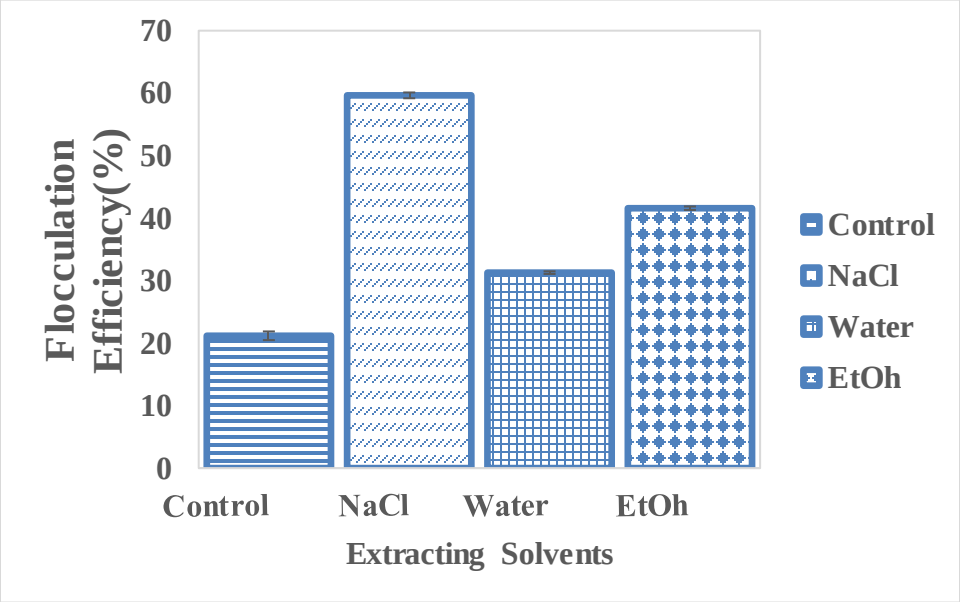

Figure 1: Effect of extracting solvents on flocculation efficiency of M*oringa oleifera* seed extract

Effects of extract concentration on flocculation efficiency

The results of the effect of *M. oleifera* seed extract concentration on the flocculation of *C. lewinii* cells are shown in Fig.2. The ability of *M. oleifera* seed extract to induce flocculation of *Chlorella lewinii* cells increased with increase in the extract concentration. However, from economic and environmental point of view, the lower the amount of extract used, the better, therefore using 800 mg/l of the extract to harvest 66.82 % was considered more economical than using 1000 mg/L to harvest 69.63 % of the microalga cells. Therefore, the optimum extract concentration for harvesting *C. lewinii* cells in this experiment was considered to be 800 mg/L. When the unit of biomass harvested per given mg of extract was calculated, it was noted that the higher the extract concentration used, the lower was the flocculation efficiency. For example, with 200 mg/L of the extract, 43.80 % of biomass was

flocculated but with 400 mg/L 50.20% was flocculated giving a unit flocculation efficiency of 0.219 % and 0.126 %/mg for the two extract concentrations respectively. This agrees with the findings of Ruiz-Marin, (2019) who harvested *Scenedesmus obliquus* from wastewater using extracts from *Moringa oleifera* seed after oil extraction. They reported their optimum extract dosage to be 380 mg/L using 5 % ammonium sulphate as the extracting solvent at pH8-9 and they obtained a flocculation efficiency of 80.33 %. Although, they obtained a higher concentration of harvested biomass than the present experiment, it might be because, they used a different extracting solvent with a different extraction method and the microalga species harvested was differed from the present study. The flocculation efficiency obtained in the present work was lower than previous report where 1g/L of *M. oleifera* seed powder was used to harvest 70 % of *Chlorella variabilis* biomass. The difference in the flocculation efficiency might be as a result of some experimental variables including microalga species, their concentration and the form of *M. oleifera* seed. Furthermore, in the present study there was no pH adjustment to avoid other external effects on the harvested microalga biomass and this might be the reason for low flocculation efficiency.

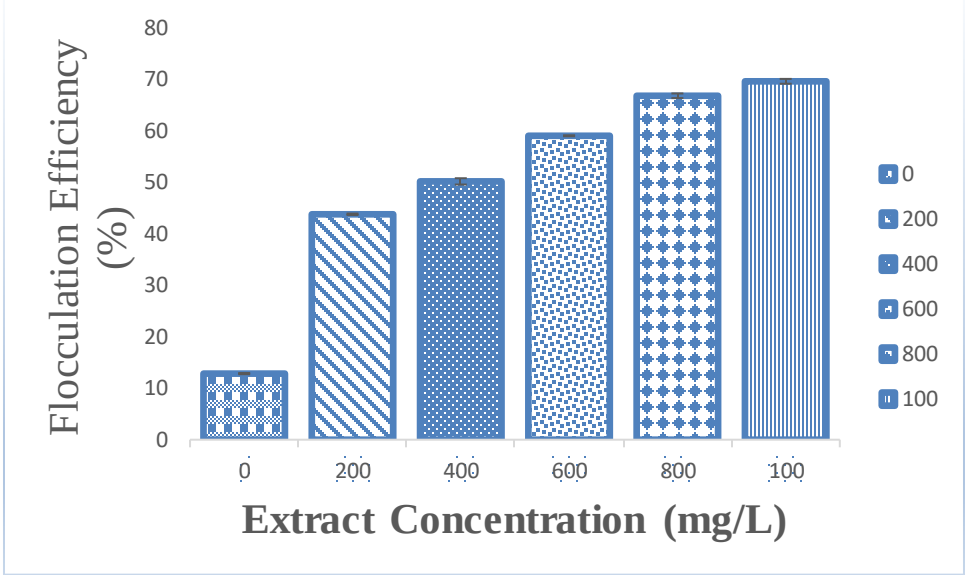

Figure 2. Effects of extract concentration on flocculation efficiency of *Moringa oleifera* seed extract

Effects of period of incubating microalga culture with *Moringa oleifera* seed extract on flocculation efficiency

The results of the period of incubating *C. lewinii* with *M. oleifera* seed extract is shown in Fig. 3. The flocculation efficiency increased with increase in the incubation period. However, the unit of biomass harvested per unit time decreased with increase in the incubation period. Nonetheless, there was no significant difference in the flocculation efficiencies among the samples incubated between 80 minutes and 120 minutes. After 60 mins incubation 47.76 ± 0.45 % of the microalga biomass was flocculated and it increased to 57.1 % ±0.26 % at 80 min. It was observed that the longer, the incubation period the less was the flocculation efficiency obtained per unit time. The flocculation efficiency reported in this work was generally low and this might be because there was no pH adjustment during the sedimentation experiment. However, Xu *et al*. (2021) reported about 40 % flocculation after 60 min incubation of *Chlorella vulgaris* culture with protein extract of wallnut even with pH adjustment. The optimum period to incubate a mixture of flocculant and the suspended particle

to be flocculated is very necessary to save costs and resources. Different researchers reported different incubation periods of flocculating agents with the particle suspension intended to sediment. The time varied depending on the efficiency of the flocculating agent used and the experimental conditions. This result agrees with the findings of Bisht and Lal (2019) who reported that at a prolonged sedimentation time of the flocculation assay of a bacterial flocculant (BF-VB2) with kaolin and dye suspension, the flocculation activity was reduced. Although some authors have reported very short incubation period for various bioflocculants used in wastewater treatment, their report was on purified and characterized flocculants. Bisht and Lal (2019) reported an optimum flocculation activity after 10 min incubation during treatment of wastewater with a bacterial bio-flocculant BF-VB2 produced by their isolate of *Bacillus* sp. TERI - VB2. Endut *et al.* (2016) reported 20min incubation period when *M. oleifera* seed powder and protein powder were compared with alum in harvesting *Chlorella* sp. biomass from culture broth. They reported microalga biomass recovery efficiency of 97 % with *M. oleifera* seed powder dosage between 10 - 50 mg/L in 20 min. It has been reported by previous researchers, that microalgae flocculation efficiency or wastewater treatment efficiency of flocculating agents are species specific (Ogbonna and Nwoba, 2021).

Niemi and Gentili (2021) reported that 60 mg/ L of chitosan reduced the OD of *C. vulgaris* cultured in wastewater by 93 % within a period of 6 days and the reduction reached up to 99 % when 100 mg/L of Chitosan was applied. They reported that OD reduction in *Scenedesmus obliquus* cultured in the same municipal wastewater for 6 days was only 25 % with 60 mg/L of Chitosan and only 40 % when chitosan concentration was increased to 100 mg/L. They reported that chitosan could only reduce the OD by 37 % for microalgae cultured for 12 days in the same municipal wastewater.

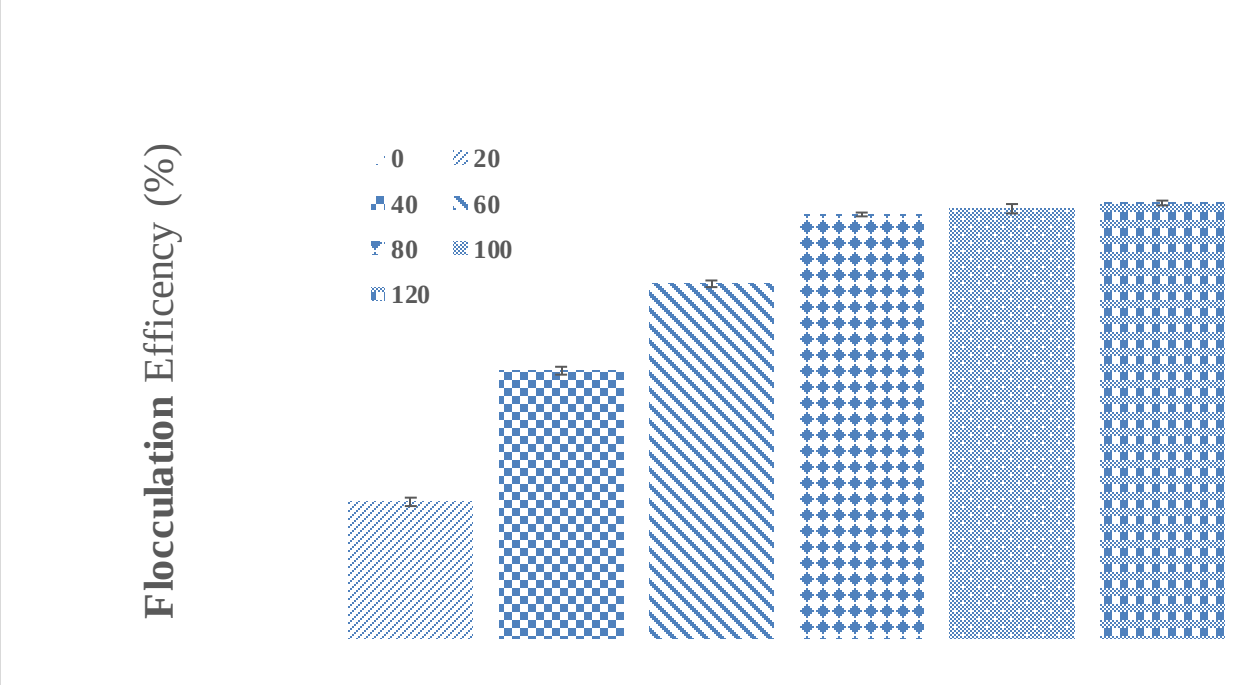

Figure 3: Effect of incubation period on flocculation efficiency

Comparison of the flocculation efficiency of extract of defatted and whole Moringa seed

Moringa oleifera seeds contain high oil content that ranges between 30-40% w/w (Garcia- Fayos *et al.,* 2016). *Moringa oleifera* seed oil has several applications including usage as biodiesel feedstock. It is therefore good to extract the oil before coagulant extraction to reduce the bulk of the coagulant Bouchareb *et al.* (2021). Furthermore, the oil content may interfere with the activity of the coagulating agent in *M.oleifera*

seed when used for microalgae harvesting or water treatment. It is therefore necessary to extract the oil before extracting the active flocculating agent. It is also imperative to compare the flocculation efficiency of extracts from defatted and whole Moringa seed for economic reasons. In this study, extracts from both whole and defatted *M.oleifera* seed were used to harvest *Chlorella lewinii* biomass. The results obtained showed a significant difference in microalga flocculation efficiency of *M.oleifera* extract with 48.823%±0.08 and 42.32% ± 0.10 for defatted and whole seed extract respectively. This was in agreement with the results obtained by Mainho *et al.* (2022) who compared the ability of extract from whole seed (integral seed) and residue left after oil extraction on flocculation of *Tetradesmus dimorphus* and they reported 98% flocculation efficiency using salt extract.

This result also agrees with that of Skaf *et al*. (2021) who used extract from defatted and whole *M. oleifera* seed to reduce the turbidity of Kaolin and TiO_2 suspensions and reported that extract from defatted seed exhibited higher flocculation (turbidity clearing) efficiency than the extract from whole moringa seed. Camacho *et al.* (2018) also reported that defatted *M.oleifera* seed powder was more efficient than whole seed powder in removing chlorophyll and turbidity from wastewater with efficiencies of 97.4 % and 84 % respectively using an optimal dosage of 50 mg/L. Kusumawati *et al.* (2020) reported that sodium chloride extract of defatted *Moringa oleifera* seed with low protein content was very efficient in turbidity removal from wastewater with removal efficiency of 76.15 %. The whole seed powder with high protein content exhibited very low turbidity removal efficiency of 30.35 %. Garcia-Fayos et al. (2016) compared the effects of extracting solvents and method of extraction on the flocculation activity of the obtained extract. They reported that defatted extract had a higher flocculation efficiency of 88 % against 30 % obtained from non-defatted seed. Mauti *et al*. (2016) also obtained higher reduction in turbidity of river water treated with the same dosage of defatted *M.oleifera* seed powder than from its whole powder counterpart. They reported a residual turbidity of 3. 27±0.45 with 150 mg/L of defatted against 4.93±0.31 for whole seed powder. It has been reported that defatting the *M. oleifera* seed increases the polyelectrolyte content which is the active coagulating ingredient of the seed.

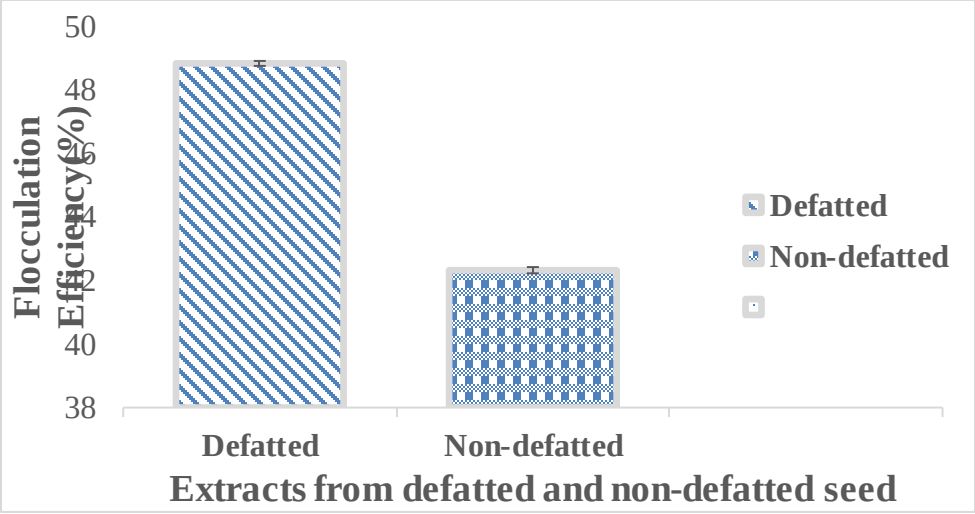

Figure 4: Comparison of the flocculation efficiency of extract from defatted and non - defatted *M. oleifera* seed

Effects of culture age on flocculation

Various microalgae strains and species grow at different rates. It was also reported that microalgae cultures tend to sediment easily as the culture gets older due to secretion of some polymeric compounds that induce sedimentation by attaching on the negative charges on microalgae cell thereby causing charge cancellation that destabilize the cells and force

them to settle to the bottom of the culture vessels. The results of the effect of age of harvesting *Chlorella lewinii* on flocculation efficiency is shown in Fig 5. There was a significant difference $P < 0.05$ in the flocculation efficiency of *M. oleifera* on *C. lewinii* culture harvested at 21 and 14 days. This result is contrary to the findings of Niemi and Gentili (2021) who reported higher flocculation efficiency of an organic flocculant (chitosan) on *Scenedesmus obliquus* cultured for 6 days than on a 12 days old culture. It implies that the flocculation efficiency of different flocculating agents on different species of microalgae cultured for different lengths of time vary. One cannot say categorically that older or younger cells flocculate faster or slower. The flocculation efficiency is microalgae species and flocculant specific and the concentration of the flocculating agent also has a role to play.

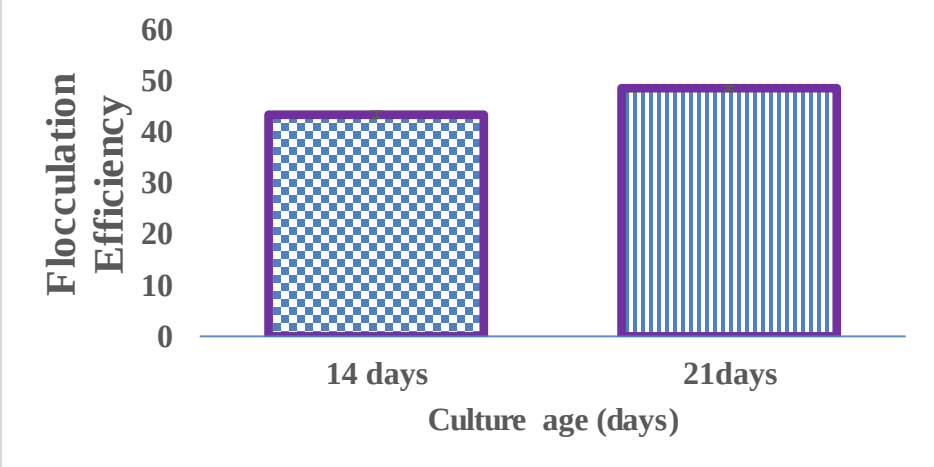

Figure 5: Effect of culture age on flocculation efficiency of *M. oleifera* seed extract

Effect of harvesting method on percentage lipid content of *C. lewinii*

The results of the effects of harvesting method on the lipid content of *Chlorella lewinii* is shown on Figure 6. Microalga biomass harvested by centrifugation gave the highest lipid content of 24.4 ± 0.20 and 22.55 ± 0.32 for biomass harvested after 21 and 14 days respectively. This was followed by the one harvested by extract of defatted *M.oleifera* seed. This result was in agreement with the results obtained by Borges *et al.* (2016) who reported that *Nannochloropsis oculata* biomass harvested by centrifugation gave the highest lipid content of 45.4 ±0.8% against 4.40 ± 0.1 obtained from biomass harvested by flocculation with NaOH. Borges *et al.* (2016), further explained that though NaOH was effective in concentration of *N. oculata* biomass by flocculation it interfered with the lipid extraction process thereby giving low percentage lipid content and even affected the polyunsaturated fatty acid content of the biomass. Yasin et al. (2019) also compared the lipid contents obtained from *Chlorella vulgaris* biomass harvested by centrifugation, immobilization and coagulation. They reported that the highest oil content of 50.42% was obtained from biomass harvested by centrifugation. They further reported that biomass harvested by centrifugation also yielded the highest fatty acid methyl ester than the one harvested by the other two methods.

CONCLUSION

This work has shown that *Moringa oleifera* seed is a cheap and easily accessible source of bio-flocculant for harvesting microalgae biomass for various applications. Sea water is recommended as a cheap solvent for extraction of *Moringa oleifera* active flocculant. In addition to microalgae, *Moringa oleifera* seed extract can also be used to harvest other microorganisms for various applications.

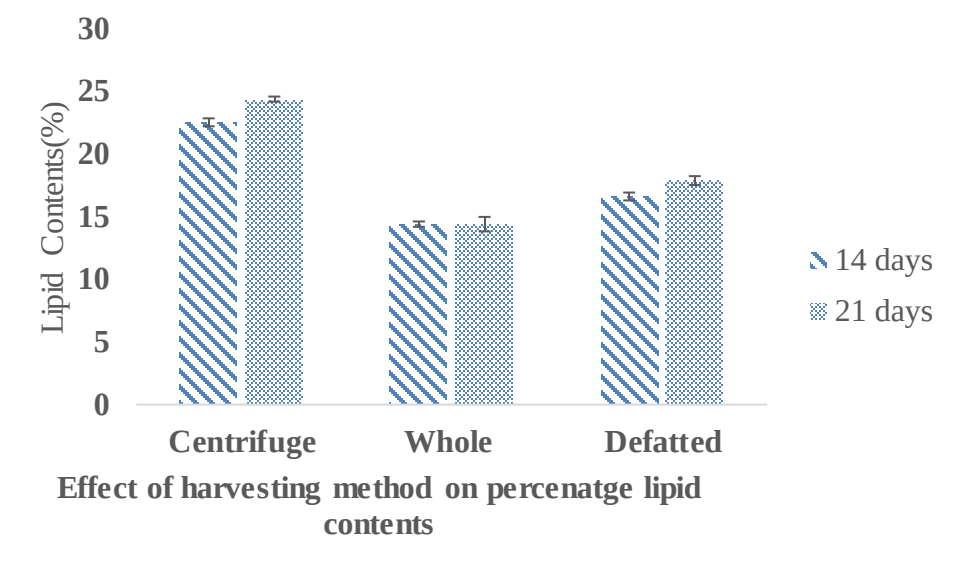

Figure 6: Effects of harvesting methods on the percentage lipid contents of *Chlorella lewinii biomass*

In the present study, the fatty acid profile of the lipids extracted from *Chlorella lewinii* biomass harvested with *Moringa oleifera* seed extract was not determined. Furthermore, the flocculation efficiency of sodium chloride extract of *Moringa oleifera* seed was not compared with that of the seed powder while the effects of sodium chloride concentration on *Moringa oleifera* active ingredient extraction efficiency were not checked. It is important to investigate these in order to further expand the use of *Moringa oleifera* seed as a bio-flocculant.

Conflict of interests.

The authors have no conflict of interest to declare.

Author contributions

OCN designed the experiments while ICF carried out the laboratory experiments under the guidance and supervision of OCN. OCN and ICF analyzed the experimental data. ICF wrote the first draft of the manuscript while OCN edited it to improve its overall quality. Both authors read and approved the final draft of the manuscript.

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