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Biodiesel Production via Direct Transesterification from *Cladophora* sp. Isolated from Basrah, Iraq

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ABSTRACT

Algae are emerging as a promising source for biodiesel production, with the potential to completely replace fossil fuels. Since two-thirds of the Earth's surface is covered by water, algae present a renewable option with significant global potential to meet future energy needs. The current study investigated biodiesel production from *Cladophora* sp. via direct transesterification. Oil was extracted from the algae to determine the oil yield. The study also explored algae pretreatment methods, including ultrasound and heat treatment (autoclaving), to enhance oil extraction. The results demonstrated that the ultrasound method was superior. The percentage of extracted oil was 26%, representing a 3.5% increase compared to direct extraction without pretreatment, which yielded 22.5%. The direct transesterification process was performed using an algae-to-methanol ratio of 8:1 (w/w). Concentrated sulfuric acid (H_2SO_4) at a 3% (w/w) ratio was used as a catalyst. The mixture was heated for 60 minutes at 50°C. The resulting biodiesel was characterized using GC-MS and FTIR. The biodiesel yield was calculated at 78%. Key properties, including density, viscosity, pour point, cloud point, and acidity value, were determined and compared with standard diesel properties according to ASTM specifications. The results indicated that *Cladophora* sp. is a valuable source for biodiesel production, offering a reasonable yield of biodiesel with properties that conform to ASTM standards.

INTRODUCTION

The unsustainable increase in the world population growth rate is driving up demand for natural resources such as food, energy, and water **(Aylan *et al.*, 2023)**. Due to the limited supply of fossil fuels, this population growth causes a decline in fossil fuel consumption. Moreover, the excessive use of fossil fuels, which are the primary contributors to environmental pollution and global warming due to the release of sulfur compounds, carbon dioxide, and other pollutants such as carbon monoxide (CO), nitrous oxide (NO), and volatile organic compounds (VOCs), is another consequence of global economic growth **(Khreebsh & Azeez, 2024)**. People's lives are at risk from these contaminants in a number of ways **(Rahpeyma & Raheb, 2019)**. The ongoing rise in energy demand, escalating political unrest, pollution damage, and warming temperatures have made it imperative to find alternatives to conventional energy sources like coal, oil, and natural gas. Furthermore, the depletion of conventional fuels has prompted scientists to look for alternate energy sources in order to protect the environment and the world economy **(Almansoory *et al.*, 2019; Ethaib *et al.*, 2020)**. Renewable energy sources are inexhaustible and are constantly being replenished, making them a reliable source of energy, especially during times of increased demand or when disruptions occur at fossil fuel power plants **(Castán Broto & Kirshner, 2020)**. Biofuels are the most significant renewable energy source. Biodiesel appears to be the most environmentally friendly of the various forms of biofuels due to its clean combustion, regeneration, and biodegradation properties **(Dong *et al.*, 2016)**. With only minor engine modifications, biodiesel can be utilized straight in diesel engines or blended with petroleum diesel fuel **(Gumus & Kasifoglu, 2010)**. Biodiesel is an alcoholic ester of a combination of fatty acids called FAMEs (fatty acid methyl esters) made from vegetable and animal fats **(Meng *et al.*, 2009)**. Algal oils, a viable renewable raw material for biodiesel production, can also be used to generate biodiesel **(Brennan & Owende, 2010)**.

In addition to growing more quickly than other traditional crops and producing more biomass, algae offer various benefits over traditional crops because of their basic cellular structure. They can grow in different environments without the need for agricultural land for their production, and this can solve the problem of competition between food and fuel. In addition, it can grow in different sources of water, such as fresh water, salt water, and wastewater **(Arun & Singh, 2012; Khan & Fu, 2020)**. Algae are divided into two groups based on the complexity of their cells: microalgae and macroalgae. Both macroalgae and microalgae are significant sources of biofuel, according to several study findings. However, producing biodiesel from microalgae presents a number of formidable obstacles. The production and harvesting of these crops are expensive. Because of this, it is not economically feasible to produce. Furthermore, using complicated systems like bioreactors to expose microalgae to contaminants and other elements raises the cost and complexity of production procedures. However, microalgae have been extensively researched as source materials for biodiesel because of their high oil output **(Saifullah *et al.*, 2014; Tripathi *et al.*, 2015)**. Macroalgae are so easy to grow and harvest, therefore they are less expensive for both parties **(Firemichael *et al.*, 2020)**. Additionally, macroalgae can efficiently and unaffectedly take nutrients (N, P) from effluent and industrial wastes **(Murphy *et al.*, 2013)**.

Because some lipids are attached to cell membranes, it can be challenging to extract lipids from algal cells. For effective lipid recovery, microalgae biomass must be pretreated before extraction in order to degrade cells and breach cell walls **(Rokicka *et***

***al*., 2021)**. This is because intracellular lipid release is inhibited by a robust and thick cell wall structure **(Jegathese & Farid, 2014)**. Breaking down the cell structure before extraction is beneficial to the process because it reduces extraction time, decreases solvent consumption, increases solvent penetration into the cell, and increases the release of cell content **(Rokicka *et al*., 2021)**. Several pretreatment methods effectively disrupt algal cells such as homogenization, bead milling, ultrasound, autoclaving, freezing, and osmotic shock **(Taher *et al*., 2011)**.

Biodiesel is produced either by indirect/conventional transesterification or by direct transesterification. Indirect transesterification processes convert fats into biodiesel. This is done using catalysts. After tearing the cells apart, the fat is extracted using a variety of solvents, including methanol, chloroform, and hexane. A homogeneous or heterogeneous acid, base, or biocatalyst is subsequently used to transform the extracted lipids into biodiesel **(Lee *et al*., 2022)**. Because it uses algal biomass, transesterification whether done *in situ* or directly is a cost-effective method that lowers the expenses of pretreatment and oil extraction **(Liu *et al*., 2018)**. The aim of the current study was to investigate the direct transesterification of the algae *Cladophora* sp. to produce biodiesel.

MATERIALS AND METHODS

1. Collect algae samples

The algae were collected directly from the aquatic environment in different aquatic areas (Shatt al-Arab District and Baghdad Street) in Basrah province, southern Iraq. They were placed in sterile plastic containers and then transported to the laboratory. The samples were washed with tap water to remove impurities, and then washed several times with distilled water to ensure cleanliness.

2. Diagnosis of algae

The algae species *Cladophora* sp. was phenotypically identified by preparing temporary glass slides and examining them under a light microscope to determine their morphological characteristics and identify them based on taxonomic references **(Prescott, 1975)**.

3. Algae pretreatments using ultrasound

Pretreatment of the algae was carried out according to **Suganya *et al.* (2014)** using ultrasound. Water was added to the dried algal biomass in a 1:3 (v/w) water-to-biomass ratio in a glass beaker. The mixture was exposed to ultrasound using an ultrasound probe at 24kHz, maintained at a constant temperature of 50 ± 1°C for 6 minutes. After the algal cell walls were disrupted, the biomass was dried in an oven at 60 ± 5°C **(Suganya *et al*.,**

2013). The treated algal biomass was then used to produce biodiesel via direct esterification.

4. Heat treatment using an autoclave

The algae biomass was subjected to heat treatment using an electric autoclave to increase the percentage of extracted oils, as described by **Surendhiran and Vijay (2014)**. A mixture of the algae biomass and distilled water, in a 1:3 (weight/volume) ratio, was prepared. The mixture was then transferred to the autoclave, where heat treatment was conducted at 121°C and 15 atm pressure for 30 minutes. Afterward, the treated biomass was dried in an electric oven at 60°C for 8 hours.

5. Extracting oil from algae

The extraction process was carried out using the Soxhlet apparatus, according to **Ahmad *et al.* (2023)**. Forty grams of algae were charged to the thimble filter paper. Then, it was transferred to the extraction column. Three hundred ml of n-Hexane was supplied to the round bottom flask. Continuous extraction was applied at 60°C. After completing the process, the solvent was evaporated using a rotary evaporator at 40°C to yield the product as oil. The extracted algal oil was kept in clean, sterilized bottles.

6. Estimate the percentage of algal oil

The percentage of oils produced from algae was estimated according to **Subramanian *et al.* (2015)** using equation (1) as follows:

Oil produced % = Weight of oil produced (g) / Dry weight of algae (g) X100 (1)

$$\% \text{ oil Yield} = \frac{W1}{W2} \times 100 \qquad (1)$$

W_1 = weight of oil extracted (g)

W_2 = weight of the dried algae (g)

7. Production of biodiesel by direct esterification

Biodiesel was produced from *Cladophora* sp. using the direct esterification method. The implementation was carried out in conjunction with the extraction of oils from algae **(Chamola *et al.*, 2019)**. The algae pretreated using ultrasonication were mixed with methanol at a ratio of 8:1 (w/w). Then, concentrated sulfuric acid (H_2SO_4) was added at a ratio of 3% (w/w). The mixture was heated for 60min at 50°C. To maintain atmospheric pressure within the reaction and avoid loss of solvent through evaporation, the system was equipped with a condenser. After the end of the experimental period, the mixture was centrifuged at 5000 rpm for 10min to remove algal residue. The liquid mixture was transferred to a separating funnel to isolate the biodiesel layer by adding regular hexane as a solvent. At 55°C, a 30% (v/v) solution of distilled water was used to wash the biodiesel (top layer). After the solvent evaporated, any remaining water and solvent residues were removed by heating the biodiesel to 100°C for 15 minutes. The biodiesel yield was then determined based on the percentage of algal oil content in the biomass. Using the equation below, the yield was calculated as a percentage (2) **(De Jesus *et al.*, 2020)**:

Biodiesel % = (biodiesel mass (g) / algae mass (g)) × oil content in algae (%) × 100% (2)

8. Determine the properties of biodiesel

Chemical analysis of biodiesel was done with FTIR spectroscopy techniques (Gasco-4200/Germany) and GC-Mass Agilent 5977 A MSD techniques (USA). Fuel properties such as density (ASTM D1480), kinematic viscosity (ASTM D445), fog point (ASTM D2500), spill point (ASTM D97), and acidity value (ASTM D664) were determined and compared with the standard properties of diesel according to ASTM.

RESULTS AND DISCUSSION

1. Phenotypic diagnosis of algae

After examining the algae samples under an optical microscope, it was determined that, based on their shape, they belong to the green algae genus *Cladophora* (Fig. 1). Cladophora is a filamentous alga with true branching, often occurring bilaterally or alternately. It is commonly found in ponds, lakes, and streams, where it grows attached to rocks and mud or floats on the water's surface. The cell walls are thick, and the reticuloplasm fills most of the cell, with amyloid centers distributed throughout. The cells are cylindrical, with a width ranging from 20 micrometers.

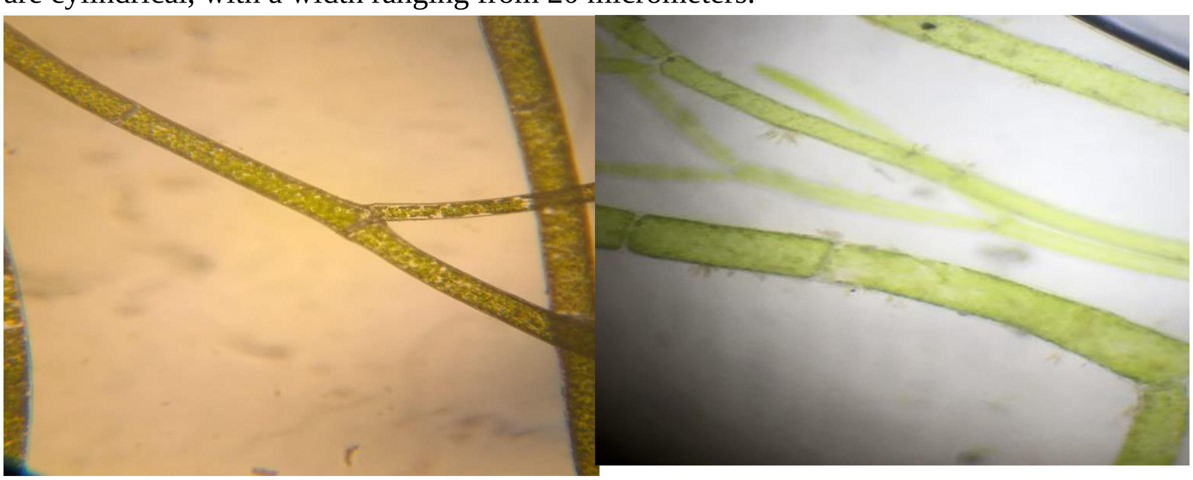

Fig. 1. The vegetative phase of the algae *Cladophora* sp. 40X

2. Oil productivity

Oil was extracted from *Cladophora* sp., with the direct extraction method yielding 22.5% oil without any pretreatment. Ultrasound treatment resulted in a 26% oil extraction rate, while autoclave heating recovered 25% of the oil. This demonstrates the superiority of the ultrasound method as a pretreatment, which increased the oil extraction by 3.5% compared to direct extraction without pretreatment **(Rokicka *et al.*, 2021)**. Ultrasound is the most promising technique for breaking down algae cells due to the solid nature of the algae's cell walls. This makes it essential to use an efficient pretreatment technique to extract lipids and ensure sustainable biodiesel production **(Mubarak *et al.*, 2016)**.

3. Oil analysis using GC-Mass

The findings from the GC-Mass analysis of Cladophora sp., as shown in Table (1) and Fig. (2), indicate the presence of seven fatty acids: palmitic acid (C16:0), α-linolenic acid (C18:3), myristic acid (C14:0), palmitoleic acid (C16:1), linoleic acid (C18:2), stearic acid (C18:0), and arachidonic acid (C20:4). The total percentage of fatty acids in the algae oil was 48.48%.

Table 1. Results of GC-Mass analysis of *Cladophora* sp.

NO.	fatty acids	Chemical formula	The number of strings	Molar mass (g/mol)	Fatty acids %
1	Palmitic acid	$C_{16}H_{32}O_2$	C16:0	256.42	25.21
2	α- Linolenic acid	$C_{18}H_{30}O_2$	C18:3	278.43	12.17
3	Myristic acid	$C_{14}H_{28}O_2$	C14:0	228.37	3.07
4	Palmitoleic acid	$C_{16}H_{30}O_2$	C16:1	254.41	2.92
5	Linoleic acid	$C_{18}H_{32}O_2$	C18:2	280.4	2.87
6	Stearic acid	$C_{18}H_{36}O_2$	C18:0	284.48	1.15
7	Arachidonic acid	$C_{20}H_{32}O_2$	C20:4	304.5	1.09
					48.48

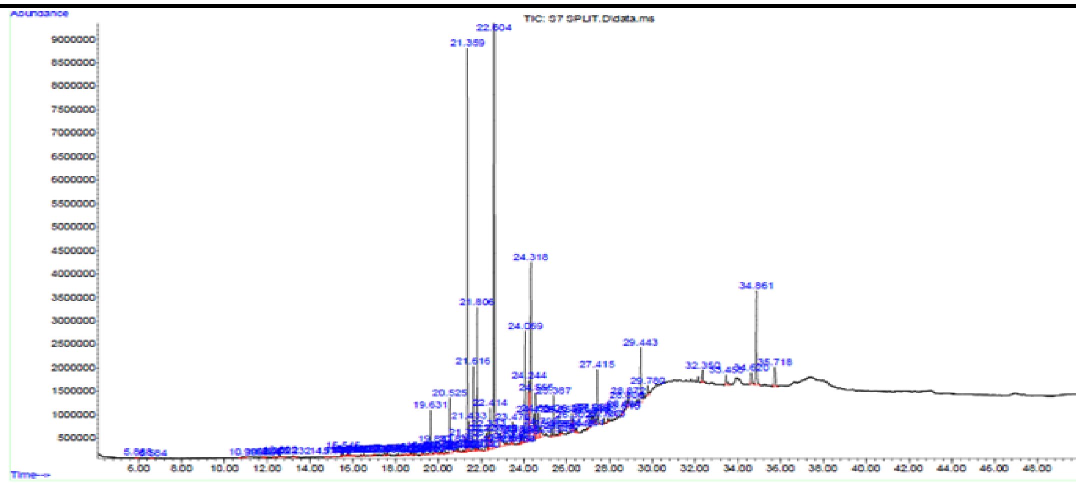

Fig. 2. Absorption spectrum of *Cladophora sp.* using GC-Mass technology

The results of the FTIR analysis of oil produced from Cladophora sp., as shown in Table (2) and Fig. (3), reveal peaks at 3748cm^{-1}, indicating the presence of the N-H stretch **(Sanches *et al.*, 2015)**. The vibration signals of the asymmetrical and symmetrical methylene groups (CH_2) at 2919cm^{-1} and 2853cm^{-1} suggest the presence of fatty acids in the algae oil **(Scarsini *et al.*, 2021; Algotiml *et al.*, 2022)**. The C-O stretching vibrations of carboxylic, ester, and ether groups appear in the range of 1108-1041cm^{-1} **(Sharma *et al.*, 2019)**. A strong and broad peak at 892cm^{-1} corresponds to the presence of the C-O-C amino bond, indicating the presence of amides **(Margariti, 2019)**.

Table 2. FTIR spectrum of oil produced from *Cladophora* sp.

Species	Frequency cm^{-1}	Bonds	Functional groups
Cladophora sp.	3748(m)	N-H stretch	1°,2°amines, amides
	2919(m)	CH_2 asymmetric stretching	Fatty acids
	2853(m)	CH_2 symmetric stretching	Fatty acids
	1108(m)	C-O Stretch	Carboxylic acids,ester,ethers
	1041(m)	C-O Stretch	Carboxylic acids,ester,ethers
	892(s,b)	N-H wag	1° , 2° amines

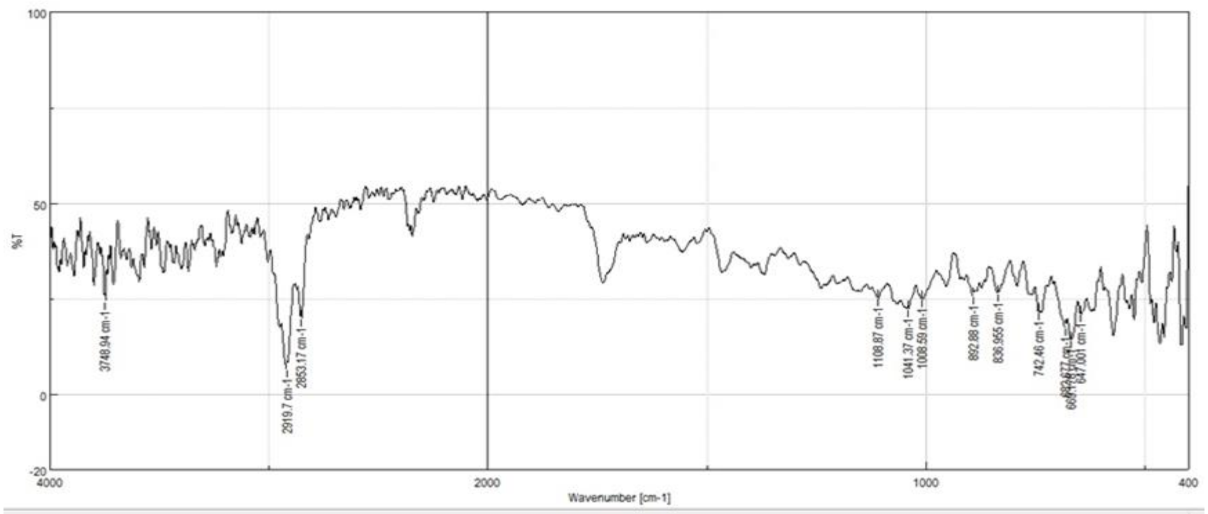

Fig. 3. FTIR spectrum of oil produced from *Cladophora* sp.

4. Biodiesel productivity by direct esterification

Biodiesel was produced from the algae *Cladophora* sp. using the direct transesterification method. The biodiesel yield was 78%, as calculated using equation (2). The produced biodiesel was then analyzed using GC-Mass and FTIR.

5. GC-Mass analysis of biodiesel produced

The results of the GC-Mass analysis of biodiesel produced from *Cladophora* sp. using the direct esterification method revealed nine types of fatty acid methyl esters. The highest percentage was attributed to palmitic acid methyl ester, which accounted for 28.74%, and the total percentage of all fatty acid methyl esters was 66.25% (Table 3 & Fig. 4).

Table 3. GC-Mass analysis of biodiesel produced from *Cladophora* sp. direct transesterification method

No.	Name of fatty acid methyl esters	Chemical formula	Molar mass (g/mol)	FAMEs%
1	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	28.74
2	Oleic acid, methyl ester	$C_{19}H_{36}O_2$	296.5	14.06
3	Myristic acid, methyl ester	$C_{15}H_{30}O_2$	242.4	8.76
4	Palmitoleic acid, methyl ester	$C_{17}H_{32}O_2$	268.4	5.42
5	Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	294.5	5.42
6	Eicosapentaenoic acid, methyl ester	$C_{21}H_{32}O_2$	316.5	1.64
7	Stearic acid, methyl ester	$C_{19}H_{38}O_2$	298.5	0.85
8	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256.4	0.72
9	7,10- Hexadecadienoic acid methyl ester	$C_{17}H_{30}O_2$	266.4	0.64
				66.25

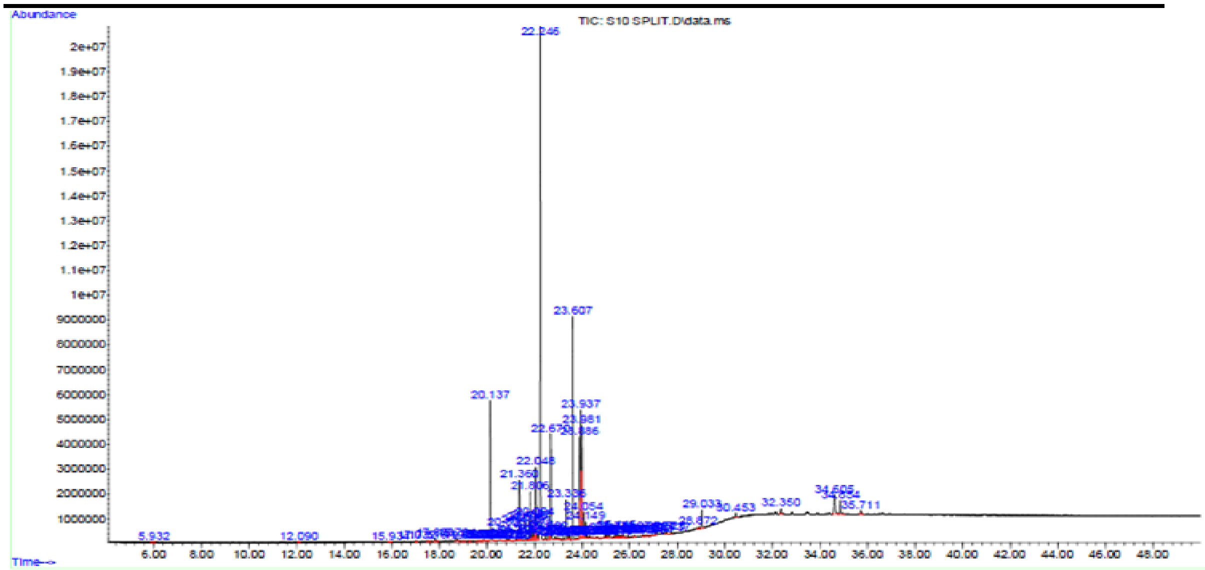

Fig. 4. The absorption spectrum of biodiesel produced from the alga *Cladophora* sp. using GC-Mass technology

The FTIR examination of the biodiesel produced by *Cladophora* sp. revealed peaks at 3427cm^{-1}, which suggests the presence of the hydrogen bond O-H stretch and consequently the presence of alcohols or phenols (Table 4 & Fig. 5) **(Cocean *et al.*, 2020)**. At 2924cm^{-1}, there is evidence of asymmetric and symmetrical CH_2 bonds, indicating the presence of alkanes, specifically the methyl group in the fatty acid methyl esters **(Maity *et al.*, 2014)**. The peak at 2361cm^{-1} corresponds to the asymmetric O-H stretch, suggesting the presence of a free OH group. The peak at 2086cm^{-1} indicates the presence of the C=N bond, which is characteristic of nitrite groups **(Najjar & Bridge, 2018)**. The peak at 1636cm^{-1} signals the presence of the C=O bond, confirming the ester group **(Sharma *et al.*, 2015)**. The peak at 1459cm^{-1} indicates the C-H bond, pointing to the asymmetric and symmetrical CH_3 group **(Lawer-Yolar *et al.*, 2021)**. Additionally, the peaks at 1740 and 1168cm^{-1} represent the C=O and C-O bonds, respectively, confirming the ester group's presence and validating the success of the esterification process and the formation of fatty acid methyl esters **(Arik *et al.*, 2022; Martínez Gil *et al.*, 2022)**.

Table 4. FTIR analysis results for biodiesel produced from *Cladophora* sp.

FAMEs	Frequency cm^{-1}	Bonds	Functional groups
FAMEs from *Chladophora sp.*	3427 (s,b)	O-H stretch	Alcohols, phenols
	2924 (m)	CH_2 asymmetric stretching	Alkenes
	2361 (m)	Asymmetric O-H stretching	Free OH groups
	2086 (W)	C=N stretch	Nitriles
	1740 (m)	C=O Stretch	Esters
	1636 (m)	C=O stretch	Esters
	1459 (m)	C-H bend	Alkanes
	1168 (m)	C-O stretch	Esters

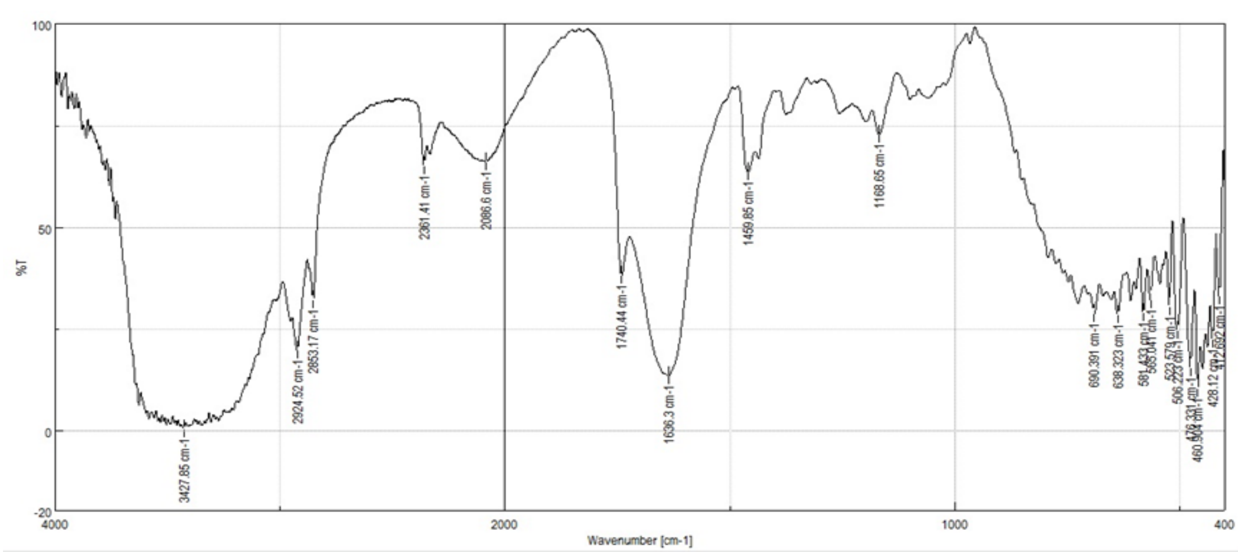

Fig. 5. The infrared absorption spectrum of biodiesel produced from *Cladophora* sp. direct transesterification method

6. Characterization of *Cladophora* sp. biodiesel

The characteristics of biodiesel produced from the alga Cladophora sp. via direct transesterification were compared with ASTM standard properties, as shown in Table (5). The density of the biodiesel was 0.836g/ cm^3, which is similar to the density of biodiesel produced from *Oedogonium nodulosum* (0.839g/ cm^3), as reported by **Chaib *et al*. (2019)**. The kinematic viscosity of the prepared biodiesel was 5.20mm^2/ s, closely matching the results of **Ahmad *et al.* (2013)**, where the kinematic viscosity of biodiesel from *Chlorella vulgaris* was 5.2mm²/ s. Similarly, biodiesel produced from Rhizoclonium hieroglyphicum had a kinematic viscosity of 5mm²/ s, while a biodiesel mixture had a slightly lower viscosity of 4.8mm²/ s. The cloud point of the biodiesel produced from *Cladophora* sp. was 4°C, consistent with the cloud point of biodiesel produced from date pit oil, as reported by **Jamil *et al*. (2016)**. The pour point of the biodiesel was -2°C, which is comparable to the pour point of biodiesel produced from sunflower oil in a study by **Al-Tabbakh *et al.* (2016)** and from brown algae using a nanocatalyst derived from animal bones, as reported by **Obaid *et al.* (2016)**. The acid number of the biodiesel produced from Cladophora sp. was 0.36mg KOH/ g, identical to the acidity value of biodiesel produced from brown algae oil, as noted by **Obaid *et al.* (2016)**.

Table 5. Characteristics of biodiesel produced from *Cladophora* sp. and compare them with ASTM standard properties.

Properties	*Cladophora* sp.	Standard	ASTM method
Density (g/cm^3) @15°C	0.836	0.86-0.9	D1480
Kinematic viscosity mm^2/s) (@40°C	5.20	1.9-6	D445
Cloud point (°C)	4	-3 to + 12	D2500
Pour point (°C)	-2	-15 to +10	D97
Acid value (mg KOH/ g)	0.36	< 0.8	D664
Diesel ratio %	78		

CONCLUSION

Cladophora sp., a type of macroalgae, presents a promising option for biodiesel production due to its numerous advantages, including economic benefits. The potential for producing a significant amount of biodiesel is enhanced by the increased biomass and oil concentration. Algae pretreatment proves to be an effective method for improving oil extraction. In this study, the direct transesterification method demonstrated its efficiency and cost-effectiveness in producing biodiesel, positioning it as a sustainable and eco-friendly fuel alternative.

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