Result and Discussion

Isolation and Authentication

The microscopic images of the isolated microalgae were shown in the Figure-2. It was observed to be filamentous and cell division occurs near septum and apical cap (folded structure near the septum); cell body cylindrical, laterally straight or wavy or swell at one end of the cell body in some species; chloroplast reticulated with many pyrenoids. Therefore, the isolated microalgae was identified to be *Oedogonium tyrolicum*.

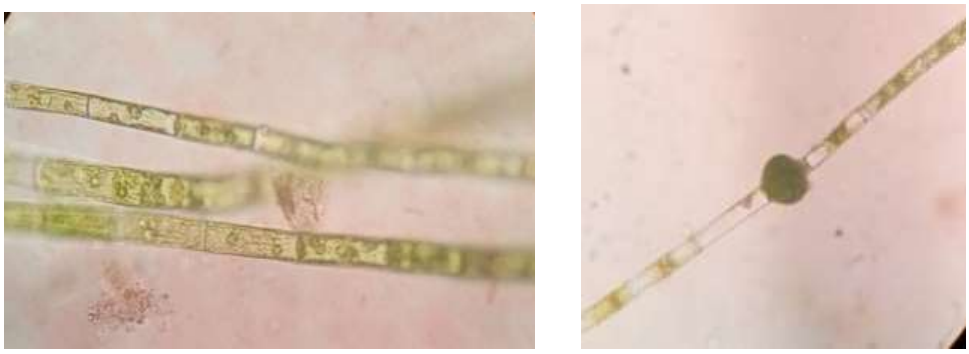

Figure-2: Microscopic observation of isolated microalgae

Microalgae Harvesting

The microalgae cells were harvested using a Beckman Avanti J-251 high speed centrifuge (Beckman Coulter, chaska, MN, USA) at 8000 rpm for 10 min. The samples were then transferred to pre-weighed Petri dishes. In order to determine the dry weight of the algal sample, the resulting biomasses were freeze dried. The dry weight of microalgae (Mdw) in milligrams was found to be 1.2gm for 100ml (Figure-3).

Figure-3: Biomass extraction

Extraction of lipid

Lipid Extraction

The Folch method for lipid extraction was used in this work. The solution volume was 20 times greater than the volume of biomass in order to obtain an adequate volume to continue the experimental analysis. The mixture was then shaken for 20 min using a Bioline BL4600 orbital shaker at 150 rpm. The solution containing chloroform-methanol and lipid was separated from the biomass by gravity filtration using Machery-Nagel 615MN filter papers.

This solution was collected in centrifuge tubes and then the lipid was rinsed in the test tube by chloroform to minimize lipid losses. These tubes were re-filled by volume of Milli-Q water equal to 20% of the total volume of the sample. To separate the chloroform-methanol phase, the samples were centrifuged at 3000 rpm for 10 min. The upper phase was discarded by siphoning using a Pasture pipette and the lower phase containing lipids was evaporated under vacuum in a rotary evaporator. The samples were dried and left at the room temperature for one day for further drying, then weighed to determine the lipid content (Figure-4 and 5).

Figure-4: Flask containing biomass in methanol:chloroform

Figure-5: Trans-esterification

Charetterization of Biodiesel

UV-Spectroscopy Analysis

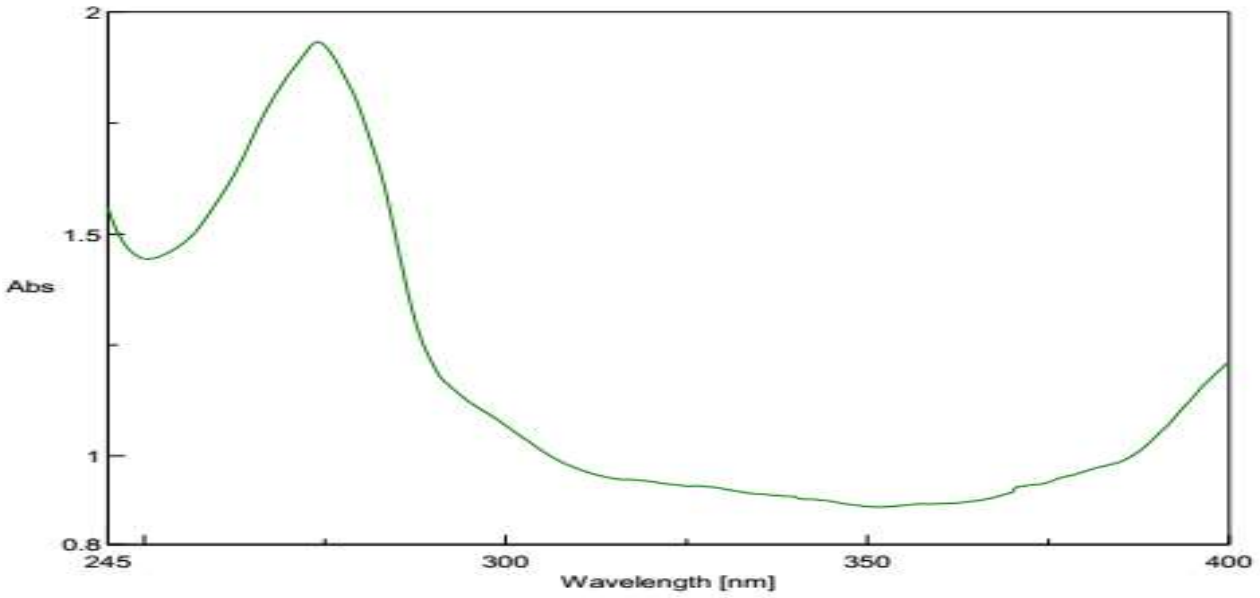

Figure-6: UV-Spectroscopy Analysis

The extracted and transesterified biofuel was studied under UV spectrophotometer at different wavelength such as 245nm, 300nm, 350nm and 400 nm. The absorbance was high in the wavelength of 275 nm indicating the presence of FAME (Figure-6).

FTIR Analysis

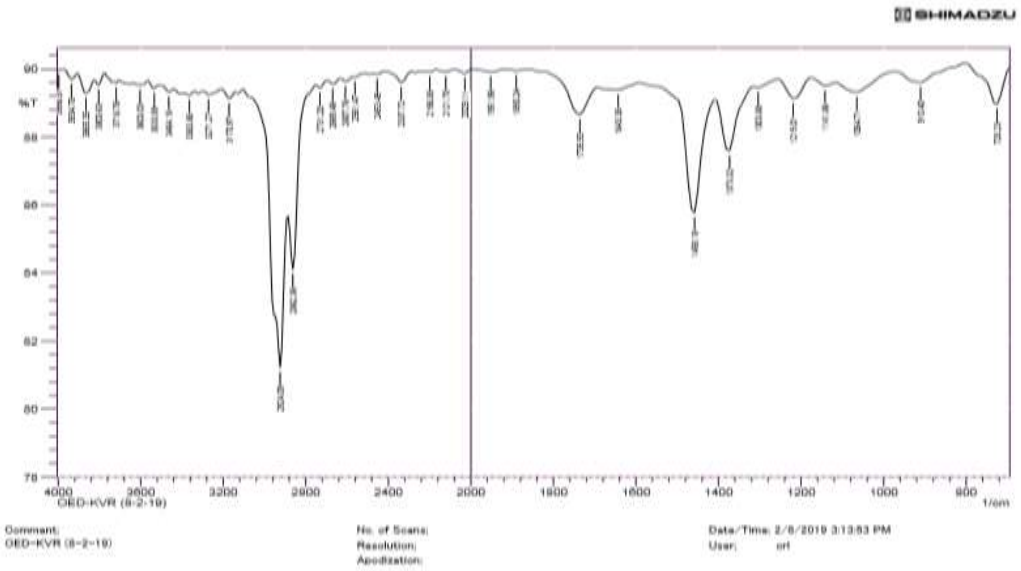

Figure-7: FTIR Analysis

Table-1: Functional groups presence in the biofuel

S.NO	Peak cm^{-1}	Functional group
1	3271.27	Carboxylic acid
2	3170.91	Carboxylic acid
3	1735.93	lipids
4	1458.18	Alkane (Methyl group)
5	1373.32	lipids

FTIR analysis showed presence of several functional groups present in the biofuel. Figure-7 and Table-1 shows the functional group analysis of biofuel. Presence of carboxylic acid and alkane with methyl group confirms the tested sample is biodiesel.

Gas Chromatography - Mass Spectroscopy Analysis

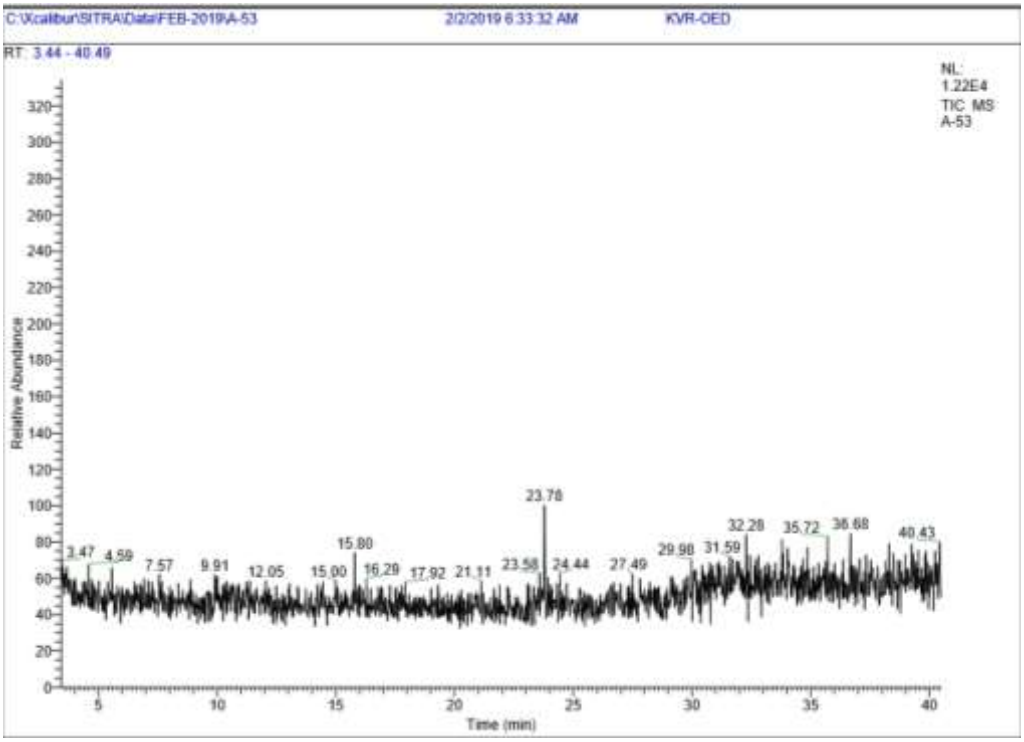

Figure-8: GC-MS Analysis

The biofuel was subjected to GC-MS analysis for identification of compounds present in the sample. Chromatogram shows the peaks of different compounds presence in the biodiesel (Figure-8). Totally 21 esters are present in this sample and approximately 69.54% of FAME in the biofuel. They are shown in the Table-2.

Table-2: FAMEs present in the biofuel

S. No	COMPOUNDS NAME	AREA %	CMPD %
1	Cyclohexane, (1-hexadecylheptadecyl)-(CAS)	1.76	2.53
2	15(1)-Hydroxypurpurin-7 Lactone Dimethyl Ester	3.41	4.9
3	1-Octene, 2-methyl- (CAS)	1.91	2.74
4	3-[4-(2-Methoxy-ethoxymethoxy)-phenyl]-acrylic acid	2.36	3.39
5	2,2'-DIOXOSPIRILLOXANTHIN	1.6	2.3
6	TRIDEUTERIOMETHYL 10-EPOXY-7-ETHYL-3,11-DIMETHYLTRIDECA-2, 6-DIENOATE	6.38	9.17
7	2-(5-CHLORO-2-METHOXYPHENYL)PYRROLIDI NE	7.18	10.32
8	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	1.74	2.5
9	Dodecanoic acid, ethenyl ester (CAS)	3.05	4.38
10	Docosanoic acid, 8,9,13-trihydroxy-, methyl ester (CAS)	1.71	2.45
11	Dodecanoic acid, tetradecyl ester	2.80	4.02
12	3-Methoxymethyl-1-trimethylsilylhept-1-yn-3-ol	5.82	8.32
13	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	1.96	2.81
14	Docosane (CAS)	2.81	4.04
15	Dodecanoic acid, but-3-enyl ester	2.79	4.01
16	Dodecanoyl chloride (CAS)	3.37	4.84
17	4-Nitrophenyl laurate	2.71	3.89
18	Dodecanoyl chloride (CAS)	4.01	5.76
19	1,3,5-Triazin-2(1H)-one, 4,6-bis(ethylamino)- (CAS)	4.47	6.42
20	1,3-O-Benzylidene glyceryl-2-myristate	2.39	3.43
21	Lauric anhydride	5.82	8.32

Discussion:

The Algae are very huge and diverse autotrophic organisms, ranging from unicellular to multicellular forms. They affect water properties such as water colour, odour, taste, and the chemical composition, which may cause potential hazards for human and animal health. They are highly sensitive to the changes in their environment. Shift in algal species and population can be used to identify the environmental changes and the status of nutrient content. Algae are very good biological indications for water pollution assessment. Therefore, they have

long been used to assess the quality of waters in lakes, ponds, reservoirs, rivers, and so on. However, identification of algae at their taxonomy level and the application in environmental assessment is a difficult process. Several studies reported the conventional identification of algae by using microscopy images, which is easy in the laboratory level (Anto *et al*., 2020).

Nutrient-rich waters can contain many thousands of planktonic (floating/suspended) algae cells per ml of water. A small vial of water was taken from near the surface provides plenty of material for microscope analysis. A vial (5 ml) sample was collected from Sulur Lake, Coimbatore and algae was serially diluted and plated on BG-11 agar medium. The growth of the culture was measured using two different methods. The first method was using a Neubauer haemocytometer and light microscopy to measure the cell density. The UV/Vis spectrophotometer was used to monitor the growth curve by measuring the optical density (OD) of the culture at 515nm. This wavelength was selected based on preliminary tests for measuring the maximum absorbance at wide range of wavelengths. The OD is a simple and efficient method for measuring the growth curve (Chen and Lee, 2019).

The Folch method for lipid extraction was used in this work. Each sample of dried microalgae was mixed with a solution of (2:1) chloroform and methanol (analytical grade, Ajax Chemicals, Victoria, Australia). Widjaja *et al.* used chloroform and methanol (2:1, *v*/*v*) to extract the oil from microalgae. Biodiesel production from microalgae can be done using several well-known industrial processes, the most common of which is base catalyzed transesterification with alcohol. The transesterification is the reversible reaction of fat or oil (which is composed of triglyceride) with an alcohol to form fatty acid alkyl ester and glycerol. Stoichio-metrically, the reaction requires a 3:1 molar alcohol to oil ratio, but excess alcohol is (usually methyl alcohol is used) added to drive the equilibrium toward the product side. The reaction occurs stepwise: triglycerides are first converted to diglycerides, then to monoglycerides and finally to glycerol. Transesterfication can be catalyzed by acids, alkalis and lipase enzymes. However enzyme catalysts are rarely used as they are less effective. The alkali-catalyzed transesterification is about 4000 times faster than the acid catalyzed reaction. Consequently, alkalis such as sodium and potassium hydroxide are commonly used as commercial catalysts at a concentration of about 1% by weight of oil (Behera *et al*., 2015).

Alkali-catalyzed transesterification is carried out at approximately 60°C under atmospheric pressure, as methanol boils off at 65°C at atmospheric pressure. Under these conditions, reaction takes about 90 min to complete. A higher temperature can be used in combination with higher pressure. Methanol and oil do not mix; hence the reaction mixture

contains two liquid phases. Other alcohols can be used, but methanol is the least expensive. To prevent yield loss due to saponification reactions (soap formation), the oil and alcohol must be dry and the oil should have a minimum of free fatty acids. Biodiesel is recovered by repeated washing with water to remove glycerol and methanol. This process of biodiesel production is found to be most efficient and least corrosive of all the processes as the reaction rate is reasonably high even at a low temperature of 60°C (Stephenson *et al*., 2011).

Fatty acid profiles of microalgal lipids are different among different algal species. In general, microalgal lipids contain fatty acids that possess two unique signatures of relatively longer chain length and high unsaturation. Microalgae have a common FA chain length from C12 to C22, which is a crossover with typical vegetable oil FA ranges of C14 to C20 and polyunsaturated FA range of C20 to C22. Lipid composition of *S. limacinum* presents most of the fatty acids (C14, C16 and C18) in typical vegetable oils but with a higher portion of polyunsaturated fatty acids. C16:0 is the most abundant saturated FA in *S. limacinum* lipids, and C22:6 are the highest among the polyunsaturated fatty acids. FA profile can directly affect derived fuel properties. The longer chain length and higher saturation of a FA leads to a higher viscosity of the corresponding FAME. Therefore, the combination of the long chain and the level of unsaturation in FAs would decide the viscosity of a microalgal biodiesel (Chen *et al*., 2019).

However, the unsaturated FA is a concern due to their susceptibility to oxidation. Besides, microalgal lipids used in this study contain even more polyunsaturated fatty acids (approximately 50%) than those found in typical vegetable oils (typically in the range of 15%-35%), thus the algal biodiesel made from such lipids tends to be oxidatively unstable. Fortunately, cold flow and oxidative stability are two interacting factors controlled by the chain length and the level of unsaturation of FAs. Long-chain saturated FAs cause poor clod flow property of corresponding biodiesel but promise a decent oxidative stability property. Polyunsaturated FAs are more reactive than saturated FAs due to the carbon-carbon double bonds that are easily to open up and react with alcohols. In contrast, a good portion of microalgal lipids is polyunsaturated which counter balances the poor cold flow property caused by the longer chain Fas (Shuba and Kifle, 2018). FTIR analysis showed the functional groups present in the synthesized biodiesel, Further GC-MS analysis showed the presence of various FAME compounds which are responsible for the biofuel production.

Conclusion

Oedogonium tyrolicum were isolated from Sulur Lake. The isolated species were characterized by morphological identification. Then it was cultivated in higher level for

extraction of fatty acids. 16% of fatty acid were obtained from *Oscillatoria tyrolicum*. Trans esterification was done for the extracted fatty acids. Characterization of the biodiesel was done by UV Spectroscopy, FTIR and GCMS. In UV Spectroscopy the peak was observed at 275nm which refers the presence of FAME in biodiesel. Three functional groups corresponding to Carboxylic acid, Alkane (Methyl group) and Ester were identified using FT-IR analysis and there were totally 21 type of mono esters present in biodiesel. Through the GCMS 69.54% of FAME compounds present in the biodiesel. These results show the *Oedogonium tyrolicum*, was wonderful source for biodiesel production.

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