Smita Kavadikeri et al /J. Pharm. Sci. & Res. Vol. 12(12), 2020, 1481-1485

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Extraction and characterization of microalgal oil and Fucoxanthin from diatom

Smita Kavadikeri, Sumathra Manokaran and A.H. Manjunatha Reddy.
Department of Biotechnology, RV College of Engineering®, Mysore Road, Bangalore 560059, Karnataka, India

Abstract:

Diatoms are jewels of the plant kingdom. The diatoms represent a large and extraordinary ecologically flexible group of unicellular eukaryotic photosynthetic microalgae. Diatoms are the good source of both oil and carotenoids. Diatoms are rich in free fatty acids. High energy demand and inadequate fossil fuel led to inflated oil price and global warming. This energy crisis had drawn great attention to a renewable energy resource like algal biofuels. Microalgae are also enriched with the other Nutraceutical compounds with oil like astaxanthin, lutein and fucoxanthin. In the current study freshwater diatom was isolated from the fresh water lake and identified as Navicula sps. Navicula sps was cultured on different media shows good growth on chu #10 medium and the maximum crude oil was obtained by Sonication with hexane as a solvent 24.73% of dry mass. Compared to soxhlation and microwave assisted extraction Sonication resulted in a high percentage of oil yield. The residue from oil extracted is utilized to extract carotenoid pigment. NMR analysis confirms the presence of fucoxanthin as a carotenoid in the residual biomass, with high antioxidant potential and pharmaceutical importance. Antioxidant assay using DPPH showed 36 µg/ml. Thus, antioxidant assay confirms fucoxanthin as a potential antioxidant agent.

Key words: Microalgae, Fucoxanthin, NMR, DPPH

INTRODUCTION

The upswing of global interest in alternative renewable energy due to depleting fossil fuels, rise in the high energy crisis and alarming global warming drew great attention to microalgae as a sustainable energy source with bioactive compounds [1]. Liquid biofuels have emerged as the most attractive source of renewable energy compared with other sustainable energy sources [2]. The advantage of microalgae compared to other resources includes high biomass yield, good oil content, minimum land requirement, easy culture requirement and cost [3,4]. The biofuel obtained from algal oil is stable compared to vegetable oil which performs poorly in the low temperatures [5]. Diatoms fecundity more compared to the other microalgae; Si depletion can induce the plenty amount of oil production compared to other microalgae [6].

Microalgal lipids are classified into two groups according to their carbon number, Polyunsaturated fatty acid (PUFAs) useful for biofuel production and polyunsaturated fatty acids (PUFAs) with more than 20 carbon atoms were used as health food supplements like DHA and EPA. Diatoms are well known for essential ω3 fatty acids [7]. Diatoms, unlike other oil crops, grow extremely rapidly, and some can double their biomass within 5 hours to 24 hours. Diatom lipids have been suggested as a potential diesel fuel substitute with an emphasis on the neutral lipids due to their lower degree of unsaturation and their accumulation in algal cells at the end of growth stage. Their high productivity and the associated high lipid yields make them an attractive option for renewable energy [8]. Diatom can be used as a source of sustainable energy resource because the Geologists claim that much crude oil comes from diatom and can solve the productivity gap by getting 10-200 times more oil, compared to oilseed crops [9]. In addition, microalgae are also known to produce carotenoids with chlorophyll, which are responsible for photosynthetic metabolism. The carotenoids like lutein, fucoxanthin, astaxanthin and β-carotene have high market value due to their therapeutic property [10]. Fucoxanthin is a natural, bioactive beneficial epoxy carotenoid present in Bacillariophyta and Chrysophyta. The nutraceutical properties of fucoxanthin include non-toxicity, anti-inflammatory, anti-tumor, anti-obesity, anti-angiogenic, and neuroprotective effects due to its anti-oxidant nature [11]. Lifestyle diseases like obstructive pulmonary disease, type 2 diabetes, heart disease, metabolic syndrome, atherosclerosis, Alzheimer's disease and cancer are becoming very prevalent problems due to chronic oxidative stress. Led to exceeding demand for natural antioxidants, so fucoxanthin can be used as one of the antioxidants [12, 13]. Fucoxanthin is also used to cure metabolic syndromes without side effects [14,15].

Diatoms which grow rapidly, have high fucoxanthin content, and perform robustly in controlled culture conditions. Fucoxanthin content is 100 times higher in diatom (0.224%-2.16% of dry weight). The low light intensities and nitrate enriched medium are shown to produce high fucoxanthin. Fucoxanthin-containing microalgae include the diatoms *Odontella aurita*, *Phaeodactylum tricornutum*, *Chaetoceros gracilis*, *Thalassiosira weissflogii*, and *Cyclotella meneghiniana*, *Emiliania huxleyi*, *Pavlova lutheri*, *Phaeocystis pouchetii*; and *Pelagococcus subviridis* [16, 17, 18].

MATERIALS AND METHODOLOGY

Culturing of the diatom: Water sample is collected from Kengeri lake (12.9165° N, 77.4878° E) and cultured on five different media like – Bold basal medium, Bacillariophyceae medium, Diatom medium, Chu fresh water medium and modified chu#10 medium. Shake flask and a closed pond system with aeration culturing methods are used. The flasks and media were sterilized in autoclave. Around 15 ml of collected samples inoculated. Diatom is isolated from other algae by capillary tube method [19]. The flasks were kept in the incubator shaker provided with an illuminator at 20°C and 100 rpm for 15 days. After 15days diatoms were observed in the binocular

microscope. The isolated species of Navicula were inoculated into fresh medium for further multiplication and used as inoculum for further studies. The diatom biomass is collected by centrifugation at 15, 000 rpm for 10 mins [20, 21]. Diatoms were identified in a compound microscope according to the method of Karthick et al., (2010).

The percentage extraction yield (%) was calculated according to the formula:

Yield (%) = weight of extract (g) /weight of dry biomass (g) × 100%.

Extraction of lipids from diatom biomass

Dried diatom biomass is ground and lipid was extracted by three different methods, sonication and soxhlation and microwave assisted extraction using different solvent like: N-hexane, Petroleum ether: methanol and chloroform: methanol and Sonication was carried out by the sample was suspended in 5 ml of hexane and subjected to sonication for 5 min at 31 Amp. The diatom biomass was subjected to soxhlation using hexane as solvent for 18hr [18] sonication with the hexane solvent was more cost effective. Chemical properties like diatom oil were evaluated and Thin layer chromatography analysis of diatom oil was carried out followed by Gas Chromatography -Mass Spectrometry analysis. GCMS analysis was carried out to find out the fatty acid composition of oil. The weight of oil extracted per gram of biomass was measured to determine the lipid content.

Extraction and identification of fucoxanthin

The remaining residue obtained after algal oil extracted was washed with distilled water followed by drying of residue. Purification of Fucoxanthin based on the optimal extraction conditions 25 g dried microalgae powder produced from 15-day culture of Navicala Sp. extracted with 25ml ethanol at 45 °C for 1 h in dark condition.

The extracts were filtered and then concentrated in a rotary evaporator (under vacuum conditions at 45 °C. The concentrated extracts were loaded onto a silica gel packed into a glass column (2 × 30 cm) and equilibrated with a mixture of n-hexane: acetone (6:4). All extracts were stored in −80 °C freezer at minimal light exposure.

Antioxidant activity:

The scavenging activity of DPPH radical was determined. In brief, 2 mL ethanolic extract of fucoxanthin (0.02–0.2 mg mL−1) was mixed with 2 mL of 0.16 mM DPPH solution prepared in ethanol. Ascorbic acid was taken as a positive control. The mixture was incubated for 30 min at room temperature in the dark. The absorbance was measured at 517 nm. The increased absorbance indicates an increased reducing power. The scavenging ability was calculated using the following formula [22].

DPPH radical scavenging activity (%) = [1 − (A1 − A2)/A0] ×100,

A0: absorbance of extract without fucoxanthin (distilled water),

A1: absorbance of sample with fucoxanthin

A2: absorbance of ethanolic extract of fucoxanthin solution (ethanol without DPPH)

RESULT AND DISCUSSION

Diatoms are unlikely to other oil crops which grow much faster and have a good amount of lipid present. Diatom are isolated from fresh water samples from the epilithic surface. Cobbles with light are used for the diatom collection. They are isolated based on Pasteur pipette method.

Culturing of Diatom:

The diatom was cultured in five different media *viz*., Bold basal media, chu fresh water, chu#10, Bacillariophyceae media and Diatom media. Amongst them, both chu fresh water and chu# 10 media have produced higher biomass of 4.920gm/100ml. Among all the culturing methods, the closed ponds with good aeration had given better results with incubation of 10-15 days. Factors like temperature, aeration, pH and surface area also contributes to the growth. Table 1 shows different medium and biomass yield. The diatom yield is comparatively high in ch#10 medium with closed pond with aeration. The Diatom medium and Bacillariophyceae medium shows average results. The crude lipid extracted is high in sonication method with hexane solvent. The samples collected from different water sources were also subjected to microscopic studies shown in Figure1. From the samples collected, one of the major diatom species is Navicula with the relative abundance of 34%. Navicula sps (Kingdom-Chromista, Phylum-Ochrophyta Class -Bacillariophyceae, Family-Naviculaceae, Genus- Navicula.

In this species the cells Frustules are generally linear in nature; lateral longitudinal ribs may be present in some forms. A characteristic feature of diatom cells is that they are encased within a unique cell wall made of silica (hydrated silicon dioxide) called frustules.

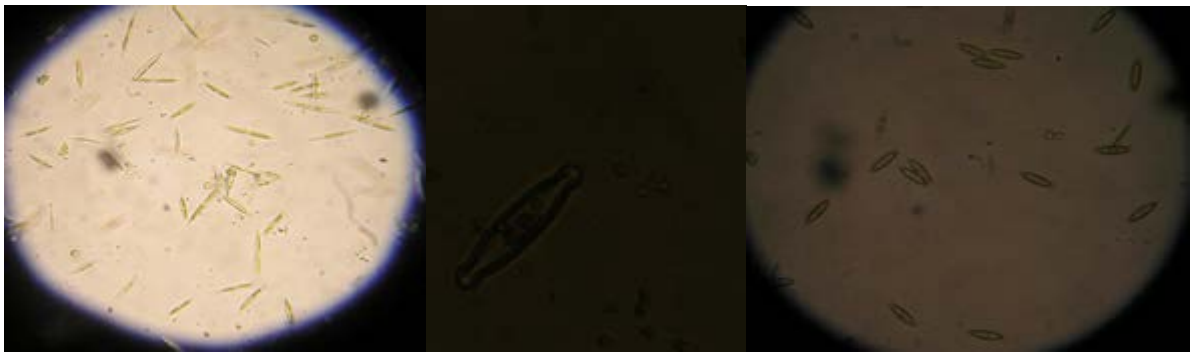

Figure:1 Binocular microscopic picture of Navicula sp.

Table 1. Biomass yield in various media and lipid obtained

Culturing media	Diatom biomass gm/100ml media	Crude lipid obtained by sonication(ml/gm)	Crude lipid obtained by soxhlation (ml/gm)	Crude lipid obtained by microwave assisted (ml/gm)
Bolds basal media	3.841	0.624	0.487	0.464
Chu freshwater medium	4.547	0.675	0.491	0.590
Ch#10 medium	4.920	1.217	0.696	0.582
Diatom medium	4.273	0.389	0.506	0.637
Bacillariophycea medium	4.049	0.697	0.613	0.661

Total lipid extraction and characterization:

The diatom biomass harvested by centrifugation was dried using a vacuum tray dryer. The algal oil is extracted by three different extraction methods such as Soxhlet, Sonication and the Microwave assisted method for extracting intracellular lipids from dried microalgal biomass. The amount of crude lipid obtained from different extraction was shown in Table 1. Sonication method with hexane solvent was found to be more effective compared to other methods. The yield percentage ranges from 12.08% to 24.73%. The Lowest yield obtained from Bold's basal media with microwave assisted technique -12.08%. The highest yield obtained with chu no#10 medium and sonication extraction methods with hexane as a solvent is 24.73%. Other extraction methods and culture conditions average results.

Lipid profiling is carried out using TLC analysis. The visualization of lipid shows the presence of Triacylglecerol Lipid presence is confirmed by TLC shown in the figure 2.

Figure 2: TLC showing TAG.

Table 2: Chemical properties of crude oil

Analytical parameter	Values
Iodine Number	86.48
Saponification value	185.32
Acid value	38.15

Analytical parameter Values

Iodine Number 86.48 Saponification value 185.32 Acid value 38.15 chemical analysis of crude oil extracted from diatom biomass confirms the presence oil is described in the table 2.

Bio-oil obtained from diatom was given for Gas Chromatography. Fatty acid composition obtained from Gas Chromatography with a percentage is shown in the table 3.

Table 3: Fatty acid composition of algal oil.

Fatty Acid	Percentage	Fatty Acid	Percentage
Caprillic Acid	10.1	Linoleic Acid	3.92
Capric Acid	12.3	Linolenic Acid	0.55
Lauric Acid	10.4	Arachidonic Acid	1.9
Myristic Acid	8.5	Behenic Acid	0.48
Palmitic Acid	6.7	Oleic Acid	13.89
Stearic Acid	1.89	Lignoceric Acid	0.32

Fucoxanthin extraction and purification:

Residual Microalgae Pellets were suspended in ethanol for pigment extraction (ethanol:algae culture volume = 1:1; *v* / *v*). As noted in the literature using ethanol a good amount of fucoxanthin is extracted with the extraction yield being ethanol > acetone > ethyl acetate [23]. The pigment extract was separated by centrifugation at 4000× *g* . Carotenoid and other pigments presence is visualized using TLC Silica gel using hexane: acetone = 6:4 as the mobile phase. The pigment spots were detected, the yellow fraction is scraped from the plate, and then re-suspended in ethanol for further purification silica gel column chromatography is used. Purified pigment is identified and confirmed as fucoxanthin by NMR analysis.

NMR analysis of fucoxanthin

Extracted and purified compounds were submitted for the NMR analysis. The proton NMR data was collected on a 400MHz Varian instrument with one pulse sequence. The pulse width was set to 45 degree and recycle delay was set to 1 second and number transients to get a better signal to noise ratio was kept at 254. The acquisition data was set to 64k and processed with exponential decay function with line broadening of 0.3 Hz and processed to 64k data points. The spectrum was referenced to an external TMS peak which is set to 0ppm. The structure of the Fucoxanthin compound is given below with atoms naming. The same naming is shown in the NMR spectrum. Fucoxanthin observes significantly in the range of 450 to 540 nm.

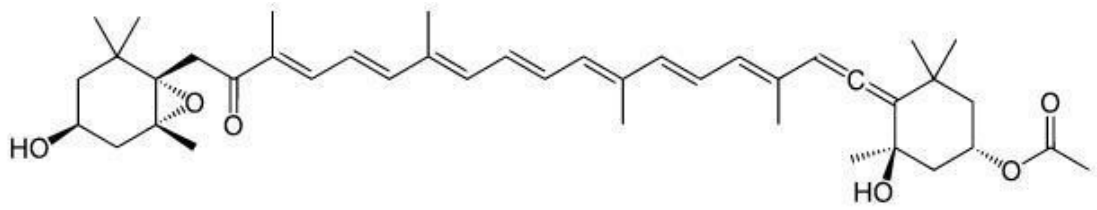

Figure 3: Structure of the Fucoxanthin.

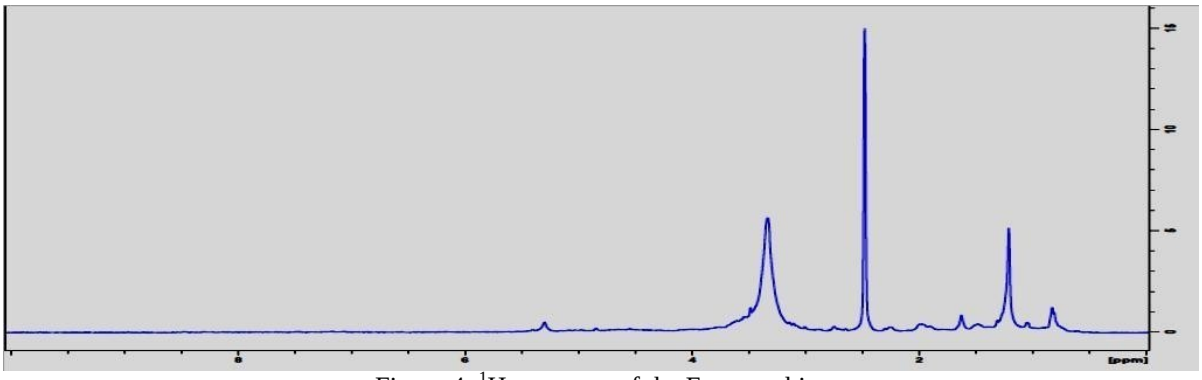

Figure 4: ^{1}H spectrum of the Fucoxanthin.

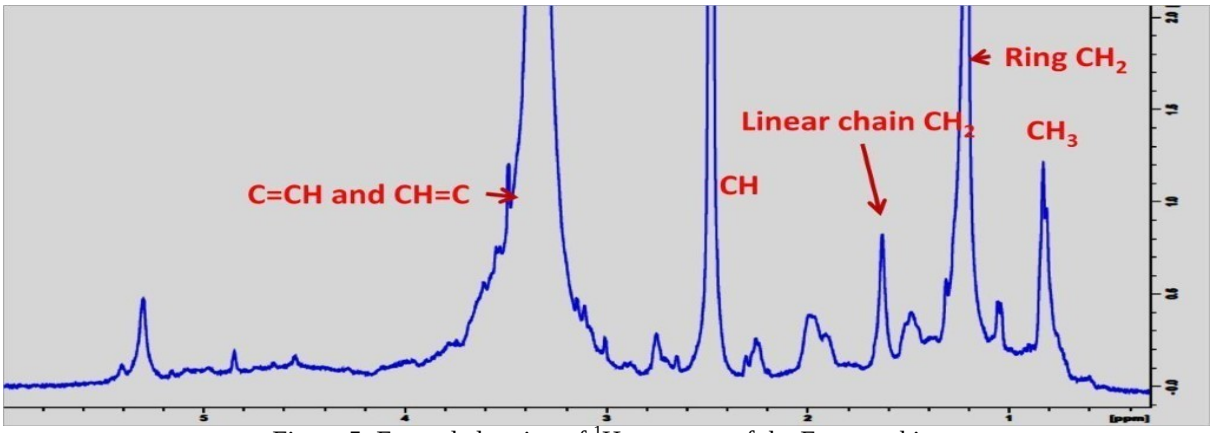

Figure 5: Expanded region of ^{1}H spectrum of the Fucoxanthin.

Antioxidant activity

To evaluate the antioxidant capacity of fucoxanthin purified from Navicula sps, DPPH based radical scavenging assays was carried out. Ascorbic acid was used as a positive control. Reducing power of fucoxanthin increased in a concentration-dependent manner. The DPPH radical scavenging activity was linearly dependent on the fucoxanthin concentration. The % scavenging activity of standard and sample are shown linearly in the graph in Figure 6.The IC 50 value the extract is 36 µg/ml.

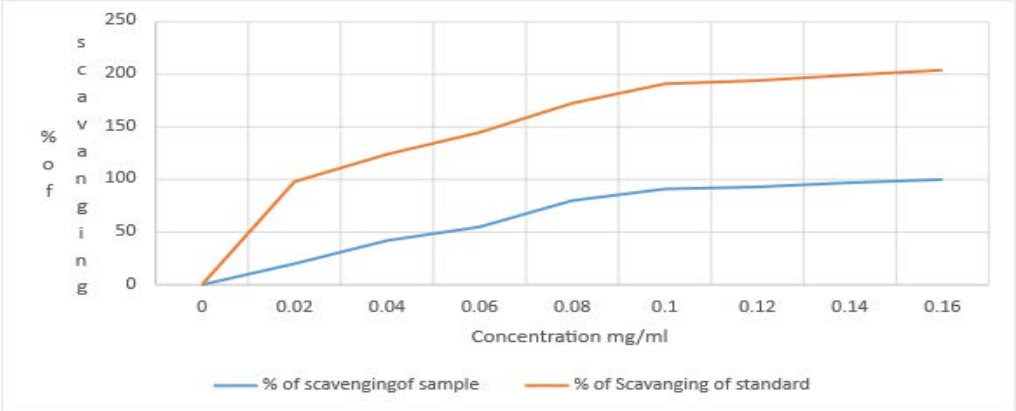

Figure 6: Antioxidant activity of fucoxanthin extract using DPPH radical scavenging.

CONCLUSION:

Diatom are reliable sources of oil and carotenoid, Because of quick biomass yield and good amount of lipid than the other crop plant. They need less landscape and reduce the climatic change by carbon sequestration and phytoremediation. Navicula is a one of the epilithic diatoms that yields better biomass in Chu freshwater medium with little alteration Navicula Sps has oil yield at around 25%. Despite these benefits, microalgae pose many challenges, including low lipid yield under limiting growth conditions and slow growth in high lipid content strain. The residue obtained after oil extracted used for carotenoid extraction Fucoxanthin is an important carotenoid with pharmaceutical and nutraceuticals potential. NMR spectra confirmed that the purified band contained all-trans-fucoxanthin as the major compound. The present study can infer that, a single diatom can be used for multiple benefits. Diatom is having wide scope for sustainability. Hence diatoms community is one of the hopes for the dwindling of world oil reservoir with bioactive compounds with nutraceutical importance.

REFERENCES:

1. Behera S, Singh, R., Arora, R., Sharma, N. K., Shukla, M., and Kumar, S. Scope of algae as third generation biofuels. Front. Bioenergy and Biotechnology.2015, 2:90.
2. Shuvashish Behera, Richa Singh, Richa Arora, Nilesh Kumar Sharma, Madhulika Shukla and Sachin Kumar. Scope of Algae as Third Generation Biofuels-Frontier Bioengineering and Biotechnology, 2014, 2-90.
3. Krzysztof Biernat. Introductory Chapter: Prospective Biofuels-intechopen.2018, 78663.
4. Kalpesh K. Sharma, Holger Schuhmann and Peer M. Schenk, High Lipid Induction in Microalgae for Biodiesel Production, Energies 2012, 5, 1532-1553.
5. Gammone, M.A and D'Orazio, N. Anti-obesity activity of the Marine carotenoid Fucoxanthin. Marine Drugs, 2015, 13,2196-2214.
6. Mark Hildebrand, Aubrey K Davis, Sarah R Smith, Jesse C Traller & Raffaela Abbriano, The place of diatoms in the biofuels industry-Biofuels,2012, 3(2),221–240.
7. Zulu N N,Zienkiewicz K,Volheyde K, Feussner I. Current trend to comprehend lipid metabolism in diatom. Progress in lipid Research, 2018, 70. 10-15.
8. Ramachandra, T.V., Mahapatra, D.M. and Karthick, B.Milking diatoms for sustainable energy: Biochemistry engineering versus gasoline-secreting diatoms solar panels. Ind. Eng. Chem. Res. 2009, 48(19), 8769-8788.
9. Samantray, S., Aakanksha, B., Supriya, G. and Ramachandra, T.V. Diversity of lipids in algae. Lake. 2010. 1-9.
10. Xiao-Man Sun, Lu-Jing Ren1, Quan-Yu Zhao, Xiao-Jun Ji and He Huang. Microalgae for the production of lipid and carotenoids: a review with focus on stress regulation and adaptation Biotechnology for Biofuels, 2018.11: 272.
11. Marilena DiValentin, ElenaMeneghin, LauraOrian, Antonino Polimeno, Claudia Büchel, Enrico Sal vadori, Christopher W.M. Kay, Donatella Carbonera, Triplet–triplet energy transfer in fucoxanthin-chlorophyll protein from diatom *Cyclotella meneghiniana* : Insights into the structure of the complex. Biochimica et Biophysica Acta (BBA)-Bioenergetics, 2013, 182(10), 1226-1234.
12. Maeda, H., Hosokawa, M., Sashima, T., Miyashita, K. Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK-Ay mice. Journal Agriculture and Food Chemistry, 2007, 7701–7706.
13. Pangestuti, R.; Kim, S.K. Biological activities and health benefit effects of natural pigments derived from marine algae. Journal of Functional Food, 2011, 3, 255–266.
14. Ihsanullah, Sumaira Shah, Muhammad Ayaz, Iftikhar Ahmed, Murad Ali, Naveed Ahmad and Irshad Ahmad, Production of Biodiesel from Algae- Journal of pure and applied microbiology, 2015, 9(1), 79-85.
15. Katam, G., A., V. , K., M. and Warkhade, G. Review on algae for biodiesel fuel production, its characteristics comparison with others and their impact on performance, combustion and emissions of diesel engine, *World Journal of Engineering* , 2017, 14,127-138.
16. Arthiya Kawea-ai,Ampin Kuntiya, Sang Mookim, Anticholinesterase and Antioxidant Activities of Fucoxanthin Purified from the Microalgae Phaeodactylum tricornutum-Natural product Communications, 2013, 8(10): 1381-6.
17. Dale D,McClurea AudreyLuiza, Blandine Gerbergeoffrey W.Bartona John M.Kavanagha. An investigation into the effect of culture conditions on fucoxanthin production using the marine microalgae *Phaeodactylum tricornutum*- Journal of Biotechnology, 2017. 241, 175-183.
18. Prabin Kumar Sharma, Manlisa Saharia, Richa Srivstava, Sajeev Kumar, Lingaraj Sahoo. Tailoring Microalgae for Efficient Biofuel Production, Frontiers in Marine Science. 2018, 5, 1-19.
19. Debenest T, Silvestre J, Coste M, Delmas F, Pinelli E. A New Cell Primo-culture Method for Freshwater Benthic Diatoms Communities, Journal of Applied Phycology, 2009, 21, 65-73.
20. Sanjay K. R, Nagendra Prasad M. N., Anupama S, Yashaswi B. R. and Deepak B. Isolation of diatom Navicula cryptocephala and characterization of oil extracted for biodiesel production, African Journal of Environmental Science and Technology, 2013, 7(1), 41-48.
21. Sangeetha, R.K, Bhaskar, N, Divakar, S. and Baskaran. Bioavailability and metabolism of fucoxanthin in rats: structural characterization of metabolites by LC-MS (APCI). Molecular and Cellular Biochemistry, 2010, 333(1-2), 299-331.
22. Xiao-Man Sun, Lu-Jing Ren1, Quan-Yu Zhao, Xiao-Jun Ji and He Huang. Microalgae for the production of lipid and carotenoids: a review with focus on stress regulation and adaptation Biotechnology for Biofuels, 2018. 11: 272.
23. Kim S.M, Jung Y.J, Kwon O.N, Cha K.H, Um B.H, Chung D, Pan C.H. A potential commercial source of fucoxanthin extracted from the microalga *Phaeodactylum tricornutum*, Applied Biochemistry. Biotechnology, 2012, 166, 1843–185.