Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2019.0503.59**

FATTY ACID AND METABOLOME PROFILING OF FRESHWATER MICROALGAE *TETRADESMUS ACUMINATUS* A POTENTIAL FEEDSTOCK FOR BIODIESEL PRODUCTION

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ABSTRACT: Microalgae has emerged as a potential feedstock for the production of biodiesel compared to other vegetative sources primarily because of their high lipid content and rapid growth also from its ability to grow in wastewaters. Biodiesel is an alternative fuel similar to conventional or 'fossil' diesel which can be produced from these fatty acids and lipids via transesterification. This study aims at identification of fatty acids for biodiesel and other metabolites commercially significant. *Tetradesmus acuminatus* was is isolated from fresh water lakes of Dharwad and identified by consulting algal monographs. GC-MS analysis indicated more presence of lipids, unsaturated and saturated fatty acids. Hexane, methanol and chloroform fractions showed the presence of hydrocarbons ranging from C_{15}-C_{28}. *Tetradesmus* indicated its potential for production of oil. GC-MS also revealed the presence of other phyto-compounds which may find its application in neutraceutical, pharmaceutical and cosmetic industries.

KEYWORDS: *Tetradesmus acuminatus*, GC-MS, Fatty acid profiling.

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1. INTRODUCTION

The world has been confronted with energy crisis due to depletion of finite resources of fossil fuel, so the consciousness of producing cleaner fuel technology is increasing globally. Biodiesel is the monoalkyl esters of long-chain fatty acids derived from renewable feedstock's and identified as one of the notable options for at least complementing conventional fuels [1]. In view of environmental

considerations, biodiesel is considered as 'carbon neutral' because all the carbon dioxide (CO_2) released during consumption had been sequestered from the atmosphere. Its production from renewable biological sources such as vegetable oils and fats has been reviewed widely [2]. Initially biodiesel was produced from edible oils [3, 4, 5]; however, it poses competition with food and arable land and has been a concern for researchers. Fatty acid methyl esters (FAME) are derived from the fatty acids/ lipids after trans-esterification process and are used as the biodiesel. It forms the basis for the production of biodiesel from any vegetative source. The fossil diesel, besides causing extensive damage to the environment, is now widely recognized as unsustainable because India meets nearly 75–80% of its total petroleum requirements through imports and expected to exceed to 90% by 2030[6]. The country faces problems in regard to the fuel requirement for increased transportation demand [7]. The increasing import of fuel has necessitated the search for other liquid fuels as an alternative to diesel, which is being used in large quantities in transport of industrial and agricultural sector [1]. So, securing an adequate sustainable energy supply could be a challenge and can only be fulfilled by the inception of biodiesel. Algae are currently considered to be one of the most promising alternative sources of non-edible oils for biodiesel as current research efforts have shown that algae are exceedingly fast growing and rich in oil (oil content in microalgae can exceed 80% by weight of dry biomass) [8] than the best oil crop. Biodiesel from algae has been reviewed [8, 9]. Hence an attempt has been made to isolate a microalgae potential feedstock for the production of biodiesel. *Tetradesmus acuminatus* is an example of colonial green algae which constitute oils as membrane components, storage products, metabolites and source of energy. This study mainly focuses on the identification of lipids, fatty acids and secondary metabolites present in *Tetradesmus acuminatus* through GC-MS.

2. MATERIALS AND METHODS

2.1 Isolation and identification

Isolation and enumeration of algae was done as per the methods described by Welch [11], Hosmani and Bharathi [12] and Adoni [13]. Identification was done by the standard algal monographs. The algal cells were observed under bright field microscope (Karl Zeiss). After identification and selection, a single cell was picked up using a micro pipette with 0.5 mm mouth diameter and 400mm in length and transferred to fresh autoclaved 100ml of BG-11 medium and incubated at 25±2 ^{0}C temperature with1.2±0.2 lux light intensity and 16:8 light dark cycle for two weeks to obtain pure cultures. Wet biomass was obtained by centrifugation. Algae samples were cleaned by removing epiphytes necrotic parts. Then the samples were rinsed with sterile water to remove any associated debris. Pure cultures were maintained by continuous sub culturing in BG-11 broth media and CHU-13 agar slants.

2.2 Cultivation and Mass production

Tetradesmus acuminatus was grown in two different culture media viz. BG-11 and NPK. The cultures were grown autotrophically in the batch culture of 1000 ml Erlenmeyer flasks and were kept on the rotary shaker at the rate of 140 rpm at 28 ^{0} C. Large scale cultivation of alga was done in 20 L capacity indigenously made photobioreactor. The photobioreactor is made up of high density polyethylene (40 cm height, 26 cm diameter). The agitation in the culture medium was carried out by sparging filtered air (using 0.2 lm syringe filter) from the bottom using silicon tubing. The photobioreactor was kept under the indirect sun light at an approximate light intensity of 8000 lux at a temperature of 28°C. Biomass was filtered by muslin cloth, dried and weighed.

2.3 Fatty acid and metabolome profiling of Hexane, Chloroform and Methanol extracts of *Tetradesmus acuminatus* by GC-MS

In this study, the hexane, chloroform and methanol extracts were subjected for the GC-MS analysis.GCMS-QP2010S model was used in the analysis that employs fused silica column. The components were separated using helium as a carrier gas at a constant flow of 1 ml/min. 1 µl sample extract was injected into the instrument. The initial temperature was set at 100 °C, whereas the injector temperature was set at 25 ^{0}C and throughout the process temperature flow was set at the speed of increasing 10 °C/min. The actual separation was observed at 24th minute, for which final temperature was adjusted to 28 ^{0}C and run for 5 min[14].

3. RESULTS AND DISCUSSION

In the present study, classical morphology based methods was used for the identification of *T. acuminatus* which was isolated from Navalur Lake of Dharwad district, Karnataka, India. The cells were arranged in chain of four cells suspended in creek water. Lipids as important storage compounds of microalgae are mostly synthesized during the stationary phase of growth [10, 16, 17]. During early stages of growth, green algae produce relatively large amounts of polar lipids and polyunsaturated C_{16} and C_{18} fatty acids. During the stationary phase of growth, the dominant lipids produce neutral and consisted primarily of saturated 18:1 and 16:0 fatty acids. The percentage of saturated fatty acids increases with days of growth forming a potent feedstock for biodiesel production [18].

3.1Isolation and identification

Tetradesmus is a unicellular green microalgae, which exists in both fresh and marine water [15]. *Tetradesmus acuminatus* was confirmed using algal monographs when observed under light microscope it revealed small chains of four cells (Fig.1). It has a spike like appearance and may be found floating in the creek water due to the oil content in them.

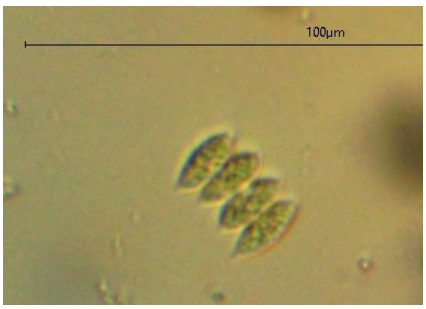

Figure 1. Microscopical view of *Tetradesmus acuminatus.*

3.2 Growth and harvest of *Tetradesmus acuminatus*

The dry biomass content of *Tetradesmus acuminatus* recovered from the BG11 media was 11g/20 liter. The biomass was allowed to naturally dry in the sunlight and later used for further analysis.

3.3 Fatty acid profiling from Hexane, Chloroform and Methanol extracts of *Tetradesmus acuminatus.*

The results pertaining to GC-MS analysis revealed a number of fatty acids. The GC-MS study of major peaks revealed the presence of straight chain and branched hydrocarbons ranging from C_{10}-C_{27}. Long chain hydrocarbons (>C20) were mostly present in this strain in comparison to long chain hydrocarbons (<C19).The hexane extract showed the presence of 6 hydrocarbon compounds including cyclooctacosane, 2-pentadecanone-6,10,14,trimethyl, behenic alcohol, 9-hexadecanoic acid, 10-octadecanoic acid and hexadecenoic acid.Methanol extract showed 3 compounds oxiranetetradecyl, Z,Z-8,10-hexadecadien-1-ol and pentadeonoic acid. Similarly chloroform extract showed the presence of 3 fatty acid compounds, 12-methyl-E,E-2,13, pentadecanoic acid and 12-methyl-E,E-2,13-octadecadien-1-ol These compounds were identified through mass spectra generated by mass spectrometry connected to GC (Table 1). The microalgae during the growth and under different stress conditions accumulate lipids which are integral components of thylakoid membranes and fundamental parts for the structural and functional integrity of the photosynthetic apparatus and galactolipids are the predominant lipids of photosynthetic membranes [19]. The lipid productivity in microalgae is the parameter which ultimately determines the rate of oil production. It has been suggested as the most appropriate kinetic parameter for the comparison of species for biodiesel production [20].

Table 1: Identified hydrocarbons by GC-MS in *Tetradesmusacuminatus*

Retention time	Identified hydrocarbons	Molecular formula	Molecular weight	Peak area	Solvent
14.388	Cyclooctacosane	$C_{28}H_{56}$	392	4.05	Hexane (Fig. 2)
17.472	2-pentadeconone-6,10,14,trimethyl	$C_{18}H_{36}O$	268	11.05	
18.573	Behenic alcohol	$C_{22}H_{46}O$	326	9.44	
18.471	9-hexadecenoic acid	$C_{16}H_{13}O_2$	254	7.24	
20.125	10-octadecenoic acid	$C_{19}H_{36}O_2$	296	2.12	
20.934	Hexadecenoic acid	$C_{16}H_{32}O_2$	256	7.86	
17.43	12-methyl-E,E-2,13-octa decadiene-1-ol	$C_{19}H_{36}O$	280	32.1	Chloroform (Fig. 3)
19.169	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	10.98	
19.328	12-methyl-E,E-2,13-octa decadiene-1-ol	$C_{19}H_{36}O$	280	3.33	
16.858	Oxiranetetradecyl	$C_{16}H_{32}O$	240	4.44	Methanol (Fig. 4)
18.558	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	11.31	
17.467	Z,Z-8,10-hexadecadien-1-ol	$C_{16}H_{30}O$	238	21.28	

The results pertaining to GC-MS analysis revealed a number of compounds, majority of the detected compounds were fatty acids. The hexane extract showed the presence of 6 compounds including cyclooctacosane, pentadecanone, hexadecanoic acid, heptacasonal, octadecanoic acid. Similarly chloroform extract showed 5 compounds including docosanol, pentadeccanoic acid, 12-methyl octadecadienol etc. These compounds were detected through mass spectra generated by mass spectrometry. The various compounds present in the extracts of *Tetradesmus acuminatus* were detected by the GC-MS. In methanol extract, totally 6 different compounds were detected with different retention time peak in chromatogram. Pentanoic acid ($C_{16}H_{30}O_4$) has the highest retention time of 19.173 and has wide applications in the field of pharmaceuticals. But has least retention time of 12, Methyl E,E, 2, 13, octadecadien-1-ol is 13.331. Totally 5 compounds were characterized with different retention time with base peaks. The compound with highest retention time is pentane acid ($C_{16}H_{30}O_4$) that is 23.608 used as marker in the butterfat. 1, Docosanol ($C_{22}H_{46}O$) with detected with retention time of 13.3. Hexane has 14 compounds with respective high and low retention time. Compound eicosana 2, methyl ($C_{21}H_{44}$) with retention time of 23.672 and low retention time of 14.388 compound cyclooctacosane ($C_{28}H_{56}$) which acts as catalyst.

3.4 Identification of Secondary metabolites

GC-MS analysis of the three solvents hexane, chloroform and methanol revealed a number of secondary metabolites which have commercial applications and have been discussed in (Table 2). Recent studies have revealed that the microalgae are the reservoirs of bioactive natural substances and many metabolites isolated from these microorganisms have shown pharmacological applications benefitting mankind [21]. They accumulate several secondary metabolites ranging from pigments, phenolic compounds to vitamins which are high value products that may find their applications in industries [22, 23]. As many as 50,000 species of microalgae have been estimated that live in diverse habitats, subjected to extreme and harsh conditions, they must rapidly adapt to new environmental conditions for the survival. Due to this reason they produce an array of secondary metabolites which may not be found in other organisms [24]. The cultivation of microalgae provide several advantages as they can be grown in wastewaters with minimum nutrient requirements reducing the expenses [25]. Many microalgae are also grown in a bioreactor which enables us to control the growth conditions and stress induction [26, 27].A number of microalgae have been cultivated to obtain biologically important compounds such as the *Spirulina*rich in vitamins B1, B2, B12, and E (especially vitamin B12) [28], *Chlorella sps* for β-1,3 glucan [29] and *Dunaliella* for the production of β-carotene [30].

Table 2. Secondary metabolites identified in different extracts of *Tetradesmusacuminatus*

Sl. No.	Compound name	Mol. formula	Mol. Wt	Retention time	Peak area	Compound nature	Uses	Solvent
1	Octadocane-1(ethyeny-loxy)	$C_{20}H_{40}O$	296	16.851	5.72	-	Not reported	Hexane (Fig.2)
2	Phytol	$C_{20}H_{40}O$	296	19.146	8.69	Acyclic diterpene alcohol	Precursors for manufacture synthetic form of vit E_1& K_1	
3	Pentadecanoic acid-14-methyl,methyl ester	$C_{17}H_{34}O_2$	270	21.074	0.85	Ester	Drug for dermatocological disorders & disorders of cardiovascular System	

4	2-pentadecanone-6,10,14,-trimethyl	$C_{18}H_{36}O$	268	21.865	5.03	Polymer	Used as antineoplastic agent &drug for specific purpose such as eyesand hair.	
5	Octadecanol	$C_{18}H_{36}O$	268	22.682	2.88	Fatty acohol	Anti asthamatic	
6	Eicosane-2-methyl	$C_{21}H_{44}$	296	23.672	29.31	Alkane	Petrochemical industry	
7	12-docosanol	$C_{22}H_{46}O$	326	13.334	16.50	Saturated fatty alcohol	Emollient, emulsifier & used in food and drug administration	Chloroform (Fig. 3)
8	Pentane acid,2,4-trimethyl,3-carboxyconin	$C_{16}H_{30}O_4$	286	23.608	43.63	Ester	Drug for dermatocological disorders & disorders of cardiovascular system	
9	12-methyl, E, E2, 13, octadecadien-1-ol	$C_{19}H_{36}O$	280	13.331	21.27	Fatty alcohol	Used in food & drug administration	Methanol (Fig. 4)
10	Oxianeundecanoic acid-3-pentyl methyl ester	$C_{19}H_{36}O_3$	312	18.743	3.79	Esters	Antiacne	
11	Pentanoicacid-2,2,4-trimethyl,3-carboxyisopro	$C_{16}H_{30}O_4$	286	19.173	37.91	Polymer	Antiviral	

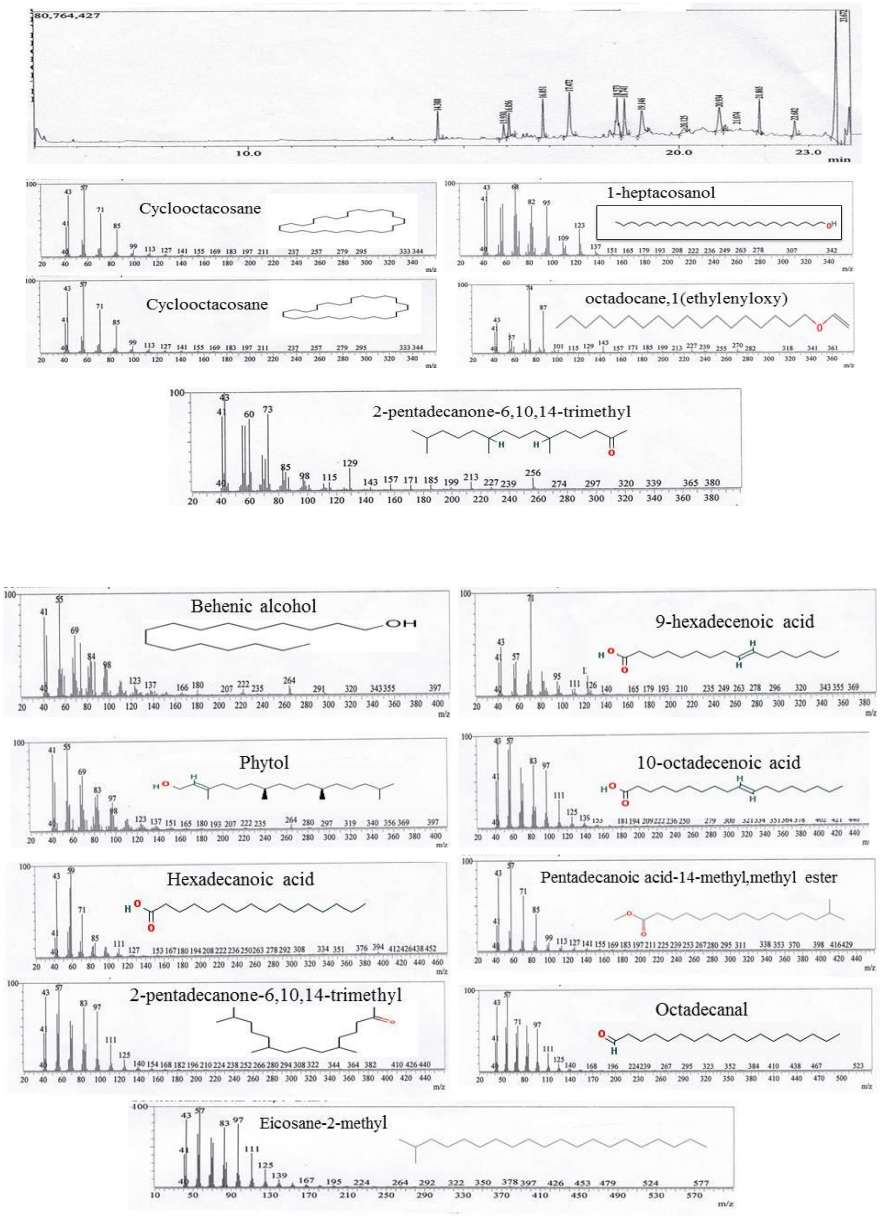

Figure 2. GC-MS analysis of Hexane extract from *Tetradesmus acuminatus.*

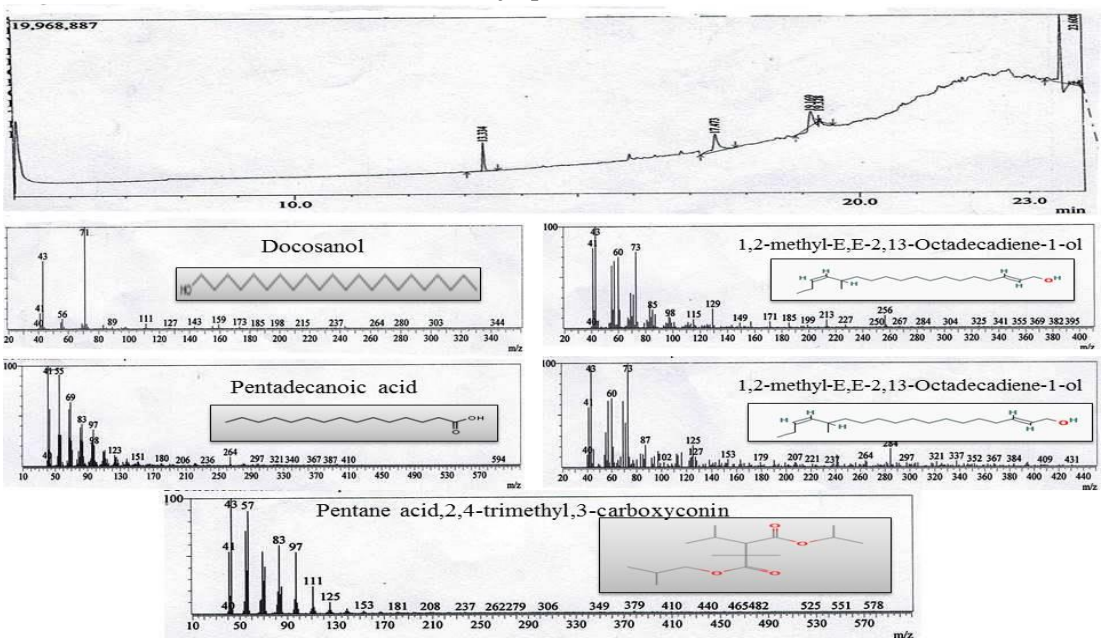

Figure 3. GC-MS analysis of Chloroform extract from *Tetradesmus acuminatus.*

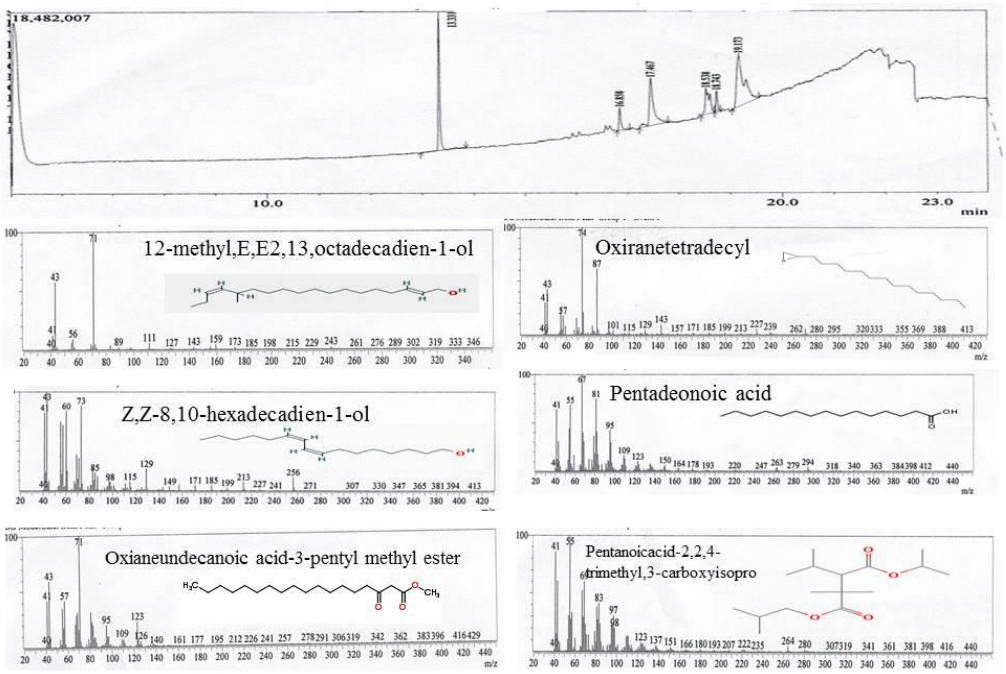

Figure 4. GC-MS analysis Methanol extract of *Tetradesmus acuminatus.*

4. CONCLUSION

In the present study, a fresh water microalgae *Tetradesmus acuminatus* was isolated, identified and mass cultured to obtain biomass. The dried algal biomass was subjected for sequential solvent extraction of hexane, chloroform and methanol. The study reveals a broad spectrum of compounds identified from GC-MS. Majority of compounds being fatty acids indicating that this can be a potential source for biodiesel production. Apart from the fatty acids, the solvent extracts in GC-MS revealed a range of secondary metabolites which can be exploited commercially for therapeutic uses.

ACKNOWLEDGEMENT

The authors would like to thank the Department of Biotechnology and Microbiology, Karnatak University Dharwad and USIC (University Scientific Instrumentation Centre).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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