ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Short Communication

GCMS ANALYSIS FOR SEPARATION AND IDENTIFICATION OF BIOACTIVE COMPOUNDS FROM FRESHWATER CYANOBACTERIUM *NOSTOC FUSCESCENCE*

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ABSTRACT

The freshwater cyanobacteria *Nostoc fuscescence* were found to be a source of various active compounds. In the present study, the methanolic extract of *Nostoc fuscescence* was analyzed by GC-MS. The GCMS analysis reveals the presence of a total of 21 different bioactive compounds and these bioactive compounds were identified by comparing with their standards available on NIST. Based on GC-MS analysis, the compounds of *Nostoc fuscescence* are mainly fatty acids and fatty acid esters. The major compounds in methanolic extract of *Nostoc fuscescence* are Hexadecanoic acid, methyl ester (19.65%), Ethyl oleate (16.45%), Heptadecane (9.74%), Benzenepropanoic acid, 3,5-bis(1,1-dimethyl ethyl)-4-hydroxy-, methyl ester(6.41%). This is a preliminary study to get some idea about the different types of bioactive compounds present in this methanolic extract. Further work will be focused on studying the efficacy of these bioactive compounds.

Keywords: *Nostoc fuscescence,* GC-MS, Cyanobacteria, Bioactive compounds.

1. INTRODUCTION

Cyanobacteria (Blue-Green Algae) are an ancient, photosynthetic, gram-negative prokaryotes organism having a diverse type of habitats. To survive in extreme environmental sources most species of cyanobacteria are known to produce intracellular and extracellular bioactive compounds with different biological properties such as antifungal, antibacterial, and pesticidal etc. [1]. Due to different bioactive compounds cyanobacteria is used to produce these bioactive compounds at commercial level.

During the past few decades screening of cyanobacteria and algae for antibiotics, pharmacologically active compounds, and other bioactive compounds have received remarkable attention. The freshwater cyanobacterium *Fischerella ambigua* has antibacterial, antifungal and anticyanobacterial activity and can be used as a cell factory for the synthesis of antibacterial, antifungal, antitumor as well as other value-added compounds [2]. The freshwater algae *Rhizoclonium hieroglyphicum* contains secondary metabolites with higher medicinal activities which can be a significant source of important compounds used in pharmaceutical industries [3]. Over 2,400 bioactive compounds have been isolated and identified from a diverse group of algal communities and emergence concerns have been raised to promote structural and functional properties of the bioactive compounds described in the algal crude extract [4]. The majority of Nostoc and Anabaena Species produce more than 200 Secondary metabolites [5].

Identification of different bioactive compounds from the cyanobacteria by Gas Chromatography and Mass Spectrometry is a useful tool to know about the diversity of these bioactive compounds and GC-MS is used to separate the volatile and thermally stable substitutes in the sample. GC-MS is one of the techniques to identify the bioactive constituents of long-chain, branched-chain hydrocarbons, alcohols, acids, esters, etc [6]. The aim of the present study was to detect the bioactive compounds from the methanolic extract of *Nostoc fuscescence* with the help of GC-MS analysis.

2. MATERIAL AND METHODS

2.1. Collection of algal material:

The species of *Nostoc* were collected from adjoining areas of Satara, Maharashtra.

2.2. Algal extracts preparation

The freshly collected algae were washed and cleaned thoroughly in tap water to remove the sand and other unwanted debris and dried on blotting paper. Then clean algae were shed dried for 7-8 days until all the moisture

content was evaporated. Then dried algae were powdered by using a grinder and finally extracted with methanol at room temperature for 3 days. The extracted solution was filtered by Whatman filter paper and evaporated in a rotary evaporator under reduced pressure. The dried extract was kept in the refrigerator for further use [7].

2.3. Gas Chromatography-Mass Spectrometry Analysis

GC-MS analysis of methanolic extract was carried out for the identification of different bioactive compounds. GC-MS analysis was performed on a Shimadzu (USA) MS QP-2010 with nonpolar 30 M SH-Rxi-5Sil MS capillary column, full scan mode, injector mode-split, (split ratio 25). The injection temperature was 250°C, GC-MS interface temperature was 250°C and ion source temperature was 200°C. The injection volume was 1µl. Mass spectra were detected at 0.7eV. Temperature programming was set as follows: Column temperature was started from 60°C and linearly increased by 10°C/min to 250 °C. Helium was employed as a carrier gas, at a pressure of 60 KPa; flow rate was 1ml/min. Total GC running time was 43 min.

2.4. Identification of compounds

The GC-MS chromatogram of methanolic extract of *Nostoc fuscescence* showed 21 compounds and they have been identified by comparison of the mass spectrum of the unknown component with the mass spectrum of the known components stored in the National Institute of Standard and Technology (NIST) library to ascertain its Name, Molecular weight, Molecular formula, and Structure.

3. RESULTS AND DISCUSSION

3.1. Bioactive compounds identified by GC-MS

The bioactive compounds present in the methanolic extract of *Nostoc fuscescence* were identified by GC-MS analysis. The 21 compounds are listed with their retention time (RT), Molecular formula, and peak area (%) in Table 1. Hexadecanoic acid, methyl ester (19.65%), Ethyl Oleate(16.45%), Heptadecane (9.74%) and Benzenepropanoic acid, 3,5-bis(1,1-dimethyl ethyl)-4-hydroxy-, methyl ester(6.41%) were the major compounds in the extract.

Table 1: Bioactive compounds identified in the methanolic extract by GC-MS

Sr. No.	RT	Name of the compound	Molecular formula	Area %
1.	7.253	Undecane, 2,3-dimethyl-	$C_{13}H_{28}$	0.53
2.	7.571	Dodecane	$C_{12}H_{26}$	4.39
3.	9.310	Tetradecane, 2-methyl-	$C_{15}H_{32}$	1.03
4.	11.145	Pentadecane, 8-hexyl-	$C_{21}H_{44}$	1.59
5.	11.411	Heptadecane	$C_{17}H_{36}$	4.04
6.	12.346	n-Pentadecanol	$C_{15}H_{32}O$	1.58
7.	12.419	Heptadecane	$C_{17}H_{36}$	9.74
8.	13.269	Heptadecane, 3-methyl-	$C_{18}H_{38}$	2.11
9.	13.660	Heptadecane	$C_{17}H_{36}$	2.05
10.	14.211	Neophytadiene	$C_{20}H_{38}$	3.80
11.	15.393	Methyl hexadec-9-enoate	$C_{17}H_{32}O_2$	3.07
12.	15.781	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	19.65
13.	15.992	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	$C_{18}H_{28}O_3$	6.41
14.	16.524	Dibutyl phthalate	$C_{16}H_{22}O_4$	3.33
15.	19.677	Methyl 10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$	3.53
16.	19.846	Ethyl Oleate	$C_{20}H_{38}O_2$	16.45
17.	20.564	Methyl stearate	$C_{19}H_{38}O_2$	4.19
18.	32.626	Triacontane, 1-iodo-	$C_{30}H_{61}I$	1.35
19.	33.638	Phthalic acid, di(2-propylpentyl) ester	$C_{24}H_{38}O_4$	4.70
20.	36.124	Eicosane	$C_{20}H_{42}$	2.34
21.	39.633	Dotriacontane	$C_{32}H_{66}$	4.13

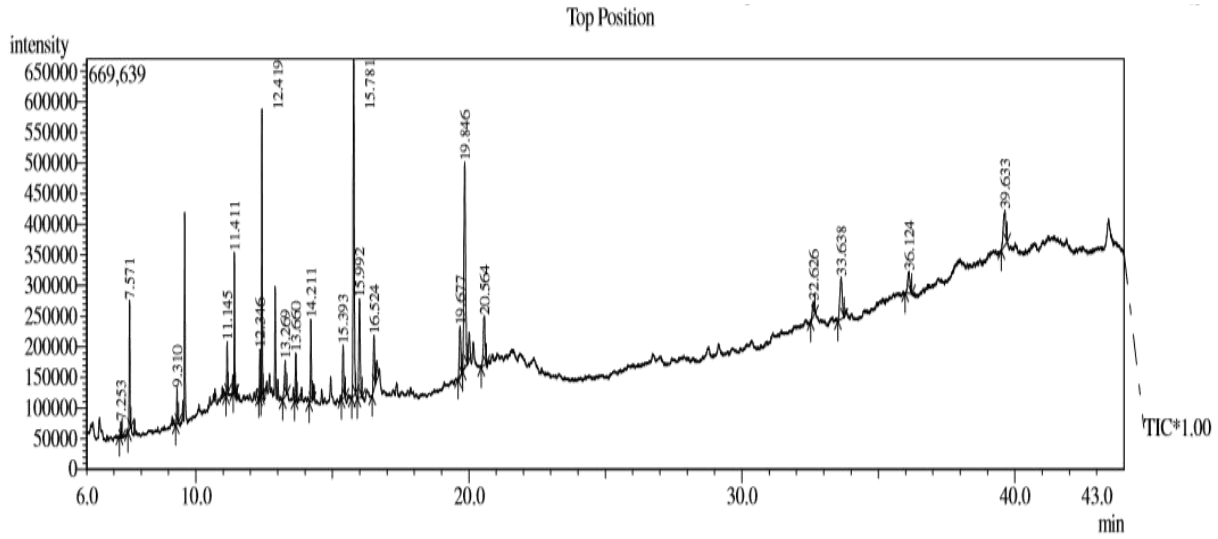

Fig. 1: GC-MS chromatogram of methanolic extract of *Nostoc Fuscescence*

3.2. Bioactivity of Major compounds found in *Nostoc fuscescence*

GCMS analysis successfully identified 21 different compounds from which four major compounds are found identical. Based on the previous work and literature bioactivity of these four major compounds are described as following.

3.2.1. Hexadecanoic acid, methyl ester

It is a fatty acid ester and also known as methyl palmitate, methyl hexadecanoic acid. Methyl palmitate has a pesticidal activity and it has been suggested as an insect repellent and can inhibit the development of larvae of *Gryllodes sigillatus* [8, 9, 10]. Methyl palmitate seems safe for vertebrates, as it is widely used in food, pharma-ceuticals, cosmetic and industrial applications [11]. Methyl palmitate isolated from the green walnut husk has a miticidal effect and they have suggested methyl palmitate as a promising botanical miticide [12].

3.2.2. Ethyl Oleate

It is a fatty acid ethyl ester and it has a highly favorable safety profile and is well tolerated. They are used as potent drug delivery vehicles due to their thermodynamic stability, apical clarity and ease of preparation without requiring a large input of energy [13].

3.2.3. Heptadecane

Heptadecane is an alkane hydrocarbon. It is a volatile compound, consists of antioxidant property, and reduces age-related oxidative stress and it is an important anti-inflammatory compound with potential therapeutic applications [14].

3.2.4. Benzenepropanoic acid, 3, 5-bis (1, 1-dimethylethyl)-4-hydroxy, methyl ester

It has antifungal and antioxidant activity [15]. This is the first report on the GC-MS analysis of a methanolic extract of *Nostoc fuscescence*, worldwide. From the result of this study is the methanolic extract is found to be rich in several bioactive and industrially important compounds. The obtained results in this study suggested that the cyanobacterium *Nostoc fuscescence* has biologically active compounds and can be used in the production of natural pharmaceutical substances.

4. CONCLUSION

GCMS analysis showed that there were four major compounds identified in *Nostoc fuscescence* different percentages of abundance. From this study, we can conclude that *Nostoc fuscescence* has pesticidal, anti-inflammatory, antioxidant, and antifungal activity hence, this methanolic extract can be a promising biopesticide and could be auspices candidate for pharmaceutical industries and agricultural applications.

5. ACKNOWLEDGEMENTS

Authors would like to appreciate Babasaheb Ambedkar Research and Training Institute, Pune for financial support, and grateful to Department of Zoology, Yashvantrav Chavan Institute of Science Satara for scientific and technical support.

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