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Gas Chromatography- Mass Spectrometry Analysis and Antibacterial Activity of Fatty acid Mixture of *Spirulina platensis*

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Abstract
Spirulina platensis is a micro alga which contains many essential fatty acids, like gamma linolenic acid, stearic acid, myristic acid, linoleic acid, heptadecanoic acid etc. The composition of individual fatty acids had been found out through GC-MS analysis based on their retention time and molecular weight. Winterization procedure was used for the extraction of fatty acid mixture. Fatty acid mixture had been screened for antimicrobial activity using agar diffusion method against four bacterial species. The mixture showed comparable activity with the standard.
Keywords: Fatty acids, GC-MS, Winterization procedure, Antimicrobial activity.

INTRODUCTION

The Cyanobacteria represents a large group within the prokaryotic kingdom. They are the oldest oxygenic photosynthetic organisms known so far and they also serve as a rich source of novel bioactive metabolites, including many cytotoxic, antifungal and antiviral compounds [1]. *Spirulina platensis*, a blue green micro alga, has been used since ancient times as a source of food because of high protein and nutritional value [2]. It is a microscopic blue-green alga in the shape of a spiral coil, living both in sea and fresh water. *Spirulina* is the common name for human and animal food supplements produced primarily from two species of cyanobacteria: *Arthrospira platensis*, and *Arthrospira maxima.* Several studies have shown that *Spirulina* or its extracts are having several biological activities like anticancer, antiviral, antioxidant and antimicrobial activities [3]. *Spirulina platensis*, is a potential source of gamma linolenic acid, an essential polyunsaturated fatty acid of excellent economic interest [4]. Most cultivated Spirulina is produced in open-channel raceway ponds, with paddle-wheels used to agitate the water. The largest commercial producers of *Spirulina* are located in the United States, Thailand, India, Taiwan, China, Pakistan, Burma (a.k.a. Myanmar) and Chile [5].

Spirulina platensis may contain significant quantity of lipids with the composition similar to those of vegetable oils. Winterization is a process that involves chilling of the oil to allow the solid portion to crystallize and the subsequent filtration of the two phases. The term winterization was originally applied decades ago when seed oil was subjected to winter temperatures to accomplish the process of the removal of solids at selected temperatures. The methods generally used to separate individual fatty acids from the fatty acid mixture are based on their transesterification.

The present study was carried out to extract the fatty acid mixture from *Spirulina platensis* by winterization procedure, to determine its composition by GC-MS analysis and to screen its antimicrobial activity.

EXPERIMENTAL

Collection of plant:

Plant powder of *Spirulina Platensis* was collected from Antenna Research Foundation Pvt Limited, Madurai, Tamilnadu, India.

Extraction of Fatty acid mixture:

Plant powder was collected from the natural *Spirulina platensis*. Free fatty acids (FFA) were prepared by the saponification of *Spirulina platensis*. A mixture containing 1 g of plant powder and 2 ml of the hydroalcoholic solution was heated at 60°C with magnetic stirring at 550 rpm for 1 h. Then 0.40 ml of water and 4 ml of hexane were added, and the solution was stirred for 1 h. The upper phase containing unsaponificable matter was removed and discarded. Then, the pH of the lower layer was adjusted to 2.0 with the drop wise addition of hydrochloride solution. The FFAs formed were extracted by the addition of hexane and the hexane layer was separated. The FFA-containing upper phase was dried with anhydrous magnesium sulfate, and the solvent was evaporated in a vacuum rotary evaporator at 35°C.

Winterization:

FFA solution were prepared in thermally isolated vials (10%, 20% and 40% w/w) using either hexane as solvent. Each solution was stirred with a magnetic stirrer at 35°C under a nitrogen atmosphere until all the oil or FFAs were dissolved. The solutions were cooled to room temperature and then stored for 20 hr. at +4°C, -24°C or -70°C, respectively. The cooling rate was 0.07^0C/min. Thereafter, the samples were immediately centrifuged at the respective winterization temperature (excepting vials stored at -70°C, which were

centrifuged at -24°C) at 8000 rpm (6000×g) for 10 min in a Remi Cooling Centrifuge. Thus, the crystallized fraction (CF) was separated from the liquid fraction (LF) by decantation. The fatty acid content of the LF was determined by GLC. The CF consists mainly of higher melting point FFAs while the LF concentrates contained low melting point FFAs.

GC-MS Analysis:

The GC system coupled to Perkin Elmer Turbo Mass MS. Perkin Elmer™ 30m×0.25mm×0.25µm PE-1 methyl silicon column was used with helium as the carrier gas 1 ml/min. The oven program was kept at 60°C for 10 min, programmed to reach 180°C at arate of 5°C/min, and 1 ml injection (split 1:10) at 280°C were made. Mass spectra were recorded at 70eV. Mass range was m/z 40-250. The fatty acid mixture diluted with chloroform and then injected in column. The quantification of the component was performed on the basis of their GC peak areas on the column. The identification of separated free fatty acids was achieved through retention indices and mass spectrometry by comparing mass spectra of the unknown peaks with those stored in the Nist 98/Nbs 75K GCMS library.

***Antimicrobial activity*:**

The fatty acid mixture from *Spirulina platensis* was tested against four pathogenic microorganisms: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Salmonella typhi*. Initially the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 hours. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 hour old cultures (100µl, 10^{-4}) and spread evenly on the plate. After 20 minutes, the wells were filled with fatty acid mixture at different concentrations. The control wells with gentamycin were also prepared. All the plates were incubated at 37°C for 24 hours and the diameter of inhibition zone was noted [6]. The results are given in Table.2

RESULTS AND DISCUSSION

The GC chromatogram showed the presence of eight compounds with retention times at 10.79, 13.16, 16.43, 17.59, 20.38, 20.86, 21.22 and 22.77. Out of these eight compounds five are major and the other three are minor compounds. Stearic acid, gamma linolenic acid, linoleic acid, heptadecanoic acid and oleic acid were found to be major compounds.

Table 1. Composition of Fatty acid mixture

Retention Time (min)	Fatty acid
10.79	Stearic acid
13.16	Gamma Linolenic acid
16.43	Linoleic acid
21.22	Heptadecanoic acid
20.38	Oleic acid

The compound with retention time 10.79 may be said as stearic acid as it exhibited a peak at m/z 285.33 for the stearic acid moiety. The compound with retention time 13.16 may be said as gamma linolenic acid as it exhibited a peak at m/z 280.24 for the linolenic acid. The compound with retention time 16.43 may be said as linoleic acid as it exhibited a molecular ion peak at m/z 281.24. In addition to that it also exhibited peaks at m/z 59.90 for $[CH_2COOH]^+$ ion, m/z 72.86 for $[CH_2=CHCOOH]^+$, m/z 87.83 for $[CH_2=CH\text{-}CH_2COOH]^+$, m/z 100.82 for $[CH_2=CH\text{-}(CH_2)_2COOH]^+$ m/z 184.65 for $[CH_2\ CH=CH\text{-}(CH_2)_7COOH]^+$, m/z 212.61 for $[CH=CH\ CH_2\ CH=CH\text{-}(CH_2)_7COOH]^+$. The compound with retention time 21.22 may be identified as heptadecanoic acid and it exhibited a molecular ion peak at m/z 270.43.The compound with retention time 20.38 may be identified as oleic acid and it exhibited a pseudo molecular ion peak at m/z 283.36 for $[M+H]^+$ ion. Further it exhibited ionic peaks at m/z 212 for $[(CH_2)_3CH=CH\text{-}(CH_2)_2COOH]^+$and at m/z 230 for $[(CH_2)_5CH=CH\text{-}(CH_2)_2COOH]^+$. The compound with retention time 22.77 may be identified as stearic acid and it exhibited a pseudo molecular ion peak at m/z 285.33 for the stearic acid moiety.

Different concentrations of fatty acid mixture (25,50,100,200.400 and 800 µg/ml) were tested against the Gram- positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, the Gram negative bacteria *Salmonella typhimurium*, *Escherichia coli*. The primary screening was done by agar diffusion method. The investigation of antibacterial screening data revealed that the fatty acid mixture was found to have moderate to good bacterial inhibition. Among the organisms tested the mixture showed significant antibacterial activity against *Salmonella typhimurium.*Also the mixture showed comparable activity against other organisms tested.

Table.2. *In-vitro* antibacterial activities of fatty acid mixture

Compound	Conc. µg/ml	Zone of inhibition in mm			
		B. subtilis	*S. aureus*	*E. coli*	*S. Typhi*
Sample	25	0.6	1.2	1.5	0.1
	50	1.4	1.8	2	1
	100	1.2	1.9	2.5	1.5
	200	1.7	2.3	2.6	2
	400	2.4	2.5	3	2.3
	800	2.5	3.5	3.2	2.4
	25	0.8	1.3	0.8	0.2
	50	1	1.8	2.1	1.3
Gentamycin	100	1.5	2.1	2.7	1.6
	200	1.9	2.5	2.9	2.1
	400	2.2	2.7	3.2	2.5
	800	2.5	3.4	3.4	2.7

The fatty acid mixture has been seperated from *S.platensis* by various methods like supercritical fluid extraction, argentated silica gel chromatography [7, 8]. In the present study the winterization procedure is adopted for the separation of fatty acid mixture from *S.platensis*.Owing to the alarm of new infectious diseases, due to the continuos failure of chemotherapeutic agents, there is always need for antimicrobial agents from medicinal plants [9]. The methanolic extract of S.platensis was found to have antimicrobial activities [10]. In the present study, the results of testing the

fatty acid mixture isolated from *S.platensis* against four bacterial species might be due to the synergistic effect of all long chain fatty acids. Also the enzyme Fab I inhibition might be the cause for the antibacterial activity showed by the fatty acid mixture. FabI is an enoyl-ACPreductase which catalyzes the final and rate-limiting step of the chain elongation process of the type II FAS. Since there is a lack of an overall sequence homolog with the corresponding one of humans, FabI has been identified as a target for antibacterial drug development.

In conclusion, this study demonstrates that the mixture of long chain fatty acids exhibits considerable antibacterial activity.

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REFERENCES

1. Patterson G M L, Larsen L K and Moore R E *Journal of appl phycol* .1994, **6,151**-157.
2. Dillon J C, Phuc A P and Dubacq J P *World rev nutr diet*, 1997, **77**, 32-46.
3. Mendiola J, Jiame L, Santoyo S, Reglero G, Cifuentes A, Ibanez E and Senorans, F.J *Food chem* , 2007, **102**, 1357-1367.
4. Sajilata M G, Singhal R S and Kamat M Y *Food chem*,2008, **109**,580-586.
5. Threlfall E J, Fisher I S T, Ward L, Tschape H and Gernersmidt P *Microb drug resist*, 1999, **5**,195-199.
6. Walker R D, Antimicrobial susceptibility testing and interpretation of results. *In:* Antimicrobial Therapy in Veterinary Medicine, Prescott JF,Baggot JD, Walker RD, ed. Ames, IA, Iowa state University press, pp12-26.
7. Guil G J L, Campra M P, El Hassan B *Process biochem*, 2000, **36**, 341-354.
8. Mendes R L, Alberto D R, Ana P P, Miguel T C, Antonia F P and Jose P C *Chem eng*, 2005, **105**,147-152.
9. Ujjwal N, Ruchi S, Rashmik M and Raju K C *Current res bacterial*, 2008, **1(1)**, 1-10.
10. Juan C L M, Pablo C M and Jose L G G *J.biosci.bioeng*, 2003, **5**,294-29