

## Phytochemical Components of Two Cyanobacterial Local Strains

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### Abstract

*Anabaena variabilis* was collected by isolation from saline soil at Elhamol city, Kafr Elsheikh guberanate, Egypt. *Spirulina platensis* was obtained from cyanobacteria Lab., Microbiology Dept., Sakha Agricultural Research Station, Egypt. GC/MS analysis showed that 37 and 7 bioactive compounds from *A. variabilis* and *S. platensis* were identified, respectively. Moreover, the area percentage of the same bioactive compound varied according to the type of cyanobacteria. The major phytochemical constituents in the methanol extract of *A. variabilis* are Pentadecanoic acid 29%, 12,15-Octadecadienoic acid methyl ester 10.26%, Hexadecanoic acid methyl ester (Z) 8.3%, Thieno [3,4-C] pyridine,1,3,4,7-tetrahydropyridine 5.86%. While, *S. platensis* methanol extract contain Hexadecanoic acid 29%, 9,12,15-Octadecadienoic acid methyl ester 24% and 9,12 Octadecatrienoic acid methyl ester, 24.36%.

**Keywords:** Cyanobacteria, GC/MS analysis, Phytochemicals, saline soil.

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### Introduction

Cyanobacteria are photosynthetic Gram-negative prokaryotes and can grow in a variety of aquatic and terrestrial environments (Harjinder *et al.* 2014). Cyanobacteria are known to be the spearhead organisms in major habitats and along with their diversity in structure and ability to supply a variety of compounds as pigments, vitamins, and enzymes (Seddek *et al.* 2019). Very few studies have isolated the cyanobacteria from El-Hamoul.

Maybe climate changes affect the structure and composition of cyanobacterial communities which may lead to a change in the phytochemical composition of cyanobacteria. There are possibility of using these strains in the pharmaceutical industry and agriculture. *Anabaena variabilis*, *Nostoc muscorum*, *Nostoc linckia*, *Oscillatoria acuminata*, *Oscillatoria amphigranulata* and *Spirulina platensis* were isolated from different regions of paddy fields in Nile Delta, Egypt and these isolates have different bioactive compounds (Gheda and Ismail 2020). The presence of secondary or primary metabolites of varieties bioactive

compounds within the plant and cyanobacteria recommended using them for phytopharmaceutical importance (**Prakash et al. 2011; Varsha and Meeta 2019**). Cyanobacteria used as antioxidants, anticancer, antiviral properties used in agriculture, industry, medicine, biotechnology, and pharmaceutical fields (**Patra et al. 2008**), and antimicrobial that can inhibit or kill pathogenic microorganisms. The mode of action of these antimicrobial compounds is modifications of the structure, function, or damage of the cytoplasmic membrane, destruction of enzymes, and suppression of protein synthesis (**Swain et al. 2017**). The activity of antimicrobial depends on the specie of alga and the type of used solvents (**Radhika et al. 2012**). Cyanobacteria produce natural products, which increase the ability to survive in a variety of environmental stress. Natural products are used in disease control for decades. Cyanobacteria have new drugs to treat incurable diseases (**Bethan and Carole 2018**).

Modern advances in biotechnology are geared towards increasing the production of desired products in cyanobacteria to be used for various industrial applications (**Abed et al. 2011; Rama-Murthy et al. 2012; Lau et al. 2015**). Cyanobacteria produce several biocidal metabolites, including antitumor activity (**Yadav et al. 2016; Camila et al. 2018**), toxins (**Agrawal, et al. 2006**), and enzyme inhibitors (**Skulberg, 2000**). These metabolites have many functions that can be helpful in pathogen control against predators, chemosensory, and photo-protection. These properties can be utilized in industrial biotechnology as nutraceuticals, cosmeceuticals, and pharmaceuticals, due to adaptation mechanisms of microorganisms against the action of antimicrobial drugs (**Elshouny et al. 2017**), which considered the main reason for continued research into antimicrobial compounds (**Al-Wathnani et al. 2012**). The present work aims to study the phytochemical components of two cyanobacterial strains isolated from soil and aquatic habitat, identify the chemical constituents for possible exploitation in industry and other application.

## Materials and methods

### *Cyanobacterial cultivation*

*Anabaena variabilis* was collected by isolation from saline soil at Elhamol city, Kafr Elsheikh governerate, Egypt. *Spirulina platensis* was obtained from cyanobacteria Lab., Microbiology Dept., Sakha Agricultural Research Station, Egypt. Cyanobacterial species was purified and grown in 100 ml sterilized BG-11 nitrogen-free medium, pH 7.5, and incubated under illumination (5000 Lux) at  $28 \pm 2$  °C. After 20 days, the cyanobacterial strains were transferred to 6L transparent glass bottles, containing 4L BG-11 medium inoculated with 100 ml of pre-cultured strains. The flasks were aerated with an air pump at regular pressure.

### *Preparation of cyanobacterial methanol extracts*

The cyanobacterial biomass from the exponential growth phase was centrifuged at 10,000 rpm for 3 min. then filtered through filter paper (Wattman-4) and air-dried (**Starr et al. 1962**). For extraction, the dried cell mass of cyanobacteria was extracted by dissolving in methanol (1g/10 ml) and kept overnight for complete extraction. The supernatant was separated by filter with 0.5  $\mu$ m pore size, the dry residue was re-dissolved in dimethyl-sulfoxide (DMSO), and this is called the crude extract (100%). Then they were kept in fresh glass vials in the dark at 4°C till using for phytochemical screening by GC mass (**Lefort et al. 1988**).

### *Gas chromatography -Mass spectrophotometry*

Profile of metabolite has been developed as a new technology platform in biological samples that describes complex chemical matrices and identifies the compounds. In particular, GC/MS is a fast and precise tool commonly used in diagnostics, functional genomics, and screening (**Rohloff 2015**). The GC/MS is a direct and fast analytical method for the identification of cyanobacterial extracts components. Extracts of *Anabaena variabilis* and *Spirulina platensis* were performed using Trace GC-TSQ Quantum mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m x 0.25 mm x 0.25  $\mu$ m film thickness). The temp of the column oven was initially held at 50°C and then increased by 5°C /min to 200 °C hold for 2 min. Increased to the final temperature of 290°C by

30°C /min and hold for 2 min. The injector and MS transfer line temperatures were kept at 270, 260°C, respectively. The carrier gas was helium at a constant flow rate of 1 ml/min.

## Results and discussion

Physical and chemical characteristics, of the collected soil samples were presented in Table 1. There is a large variation between those samples in electric conductivity (E.C), which ranged from 7.75 to 15.93 dS.m<sup>-1</sup>. Soils were classified as saline soil. Saline soils have traditionally been classified as those in which the EC is higher than 4 dS m<sup>-1</sup>. The major problem with saline soils is the presence of soluble salts, primarily Cl<sup>-</sup>, SO<sub>4</sub><sup>2-</sup> (Richards, 1954). In saline soils, the concentration of soluble chloride ions is highest among anion concentrations. Following the chloride ion are sulfate ions, which are followed by bicarbonate ions (Cao et al. 2012). Sodium ion concentration is also increased with an increase in soil salinity (Weggler-Beaton et al. 2000).

**Table 1:** Physical and chemical characteristics of soil samples.

Variables	El-Hamoul city	
	Site 1	Site 2
Sand %	7.13	8.65
Silt %	32.43	34.95
Clay%	60.34	56.40
Texture	Clay	Clay
pH	7.93	7.87
EC, dS.m	15.93	7.75
Mg+2	34.43	23.92
Ca+2	33.50	21.44
Na+	90.50	30.60
K+	1.50	1.54
SO4-2	69.14	43.58
HCO3	4.69	3.12
Cl-	86.10	31.80

## Cyanobacterial species

The cyanobacterial species *Anabaena variabilis* and *Spirulina platensis* were described in Fig 2 and 3, respectively. These pictures were taken by the OPTICA microscope (Italy) fitted with a Canon Powershot G12 digital camera (El-Gamal et al. 2008).



**Fig. 2** *Anabaena variabilis*

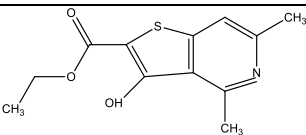
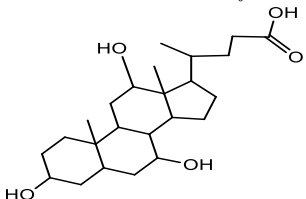


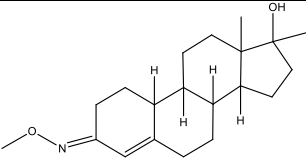
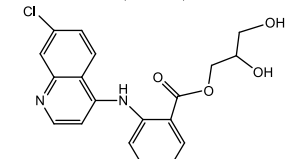
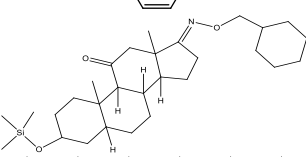
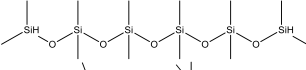
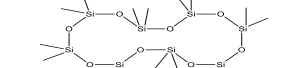
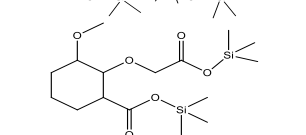
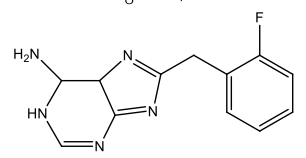


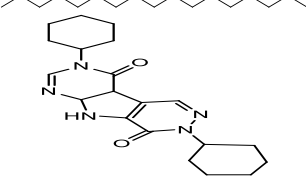
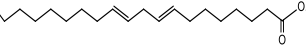
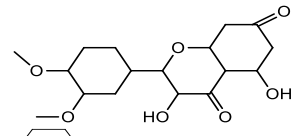
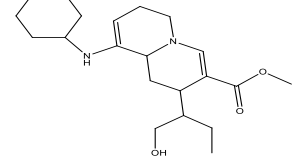
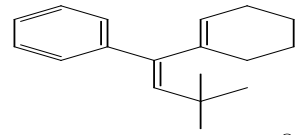
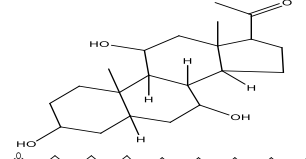
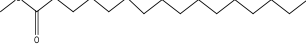
**Fig. 3** *Spirulina platensis*

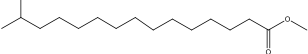
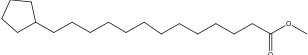
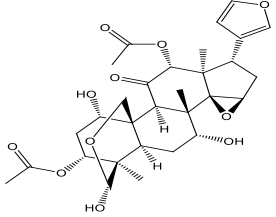
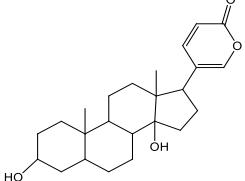
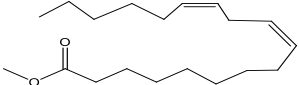
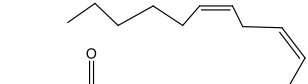
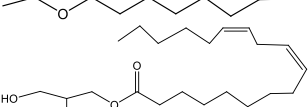
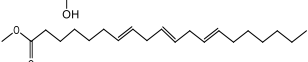
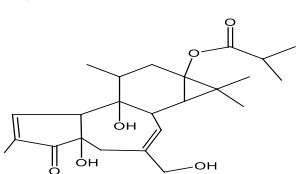

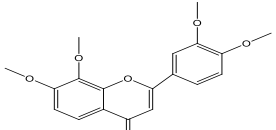
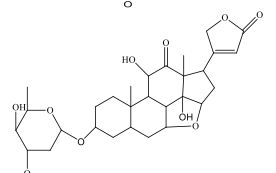
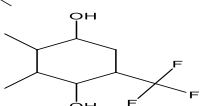

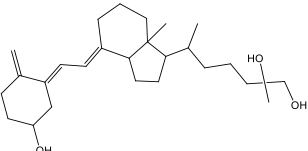
## GC/MS analysis of methanol extract

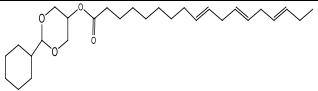
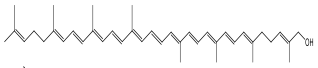
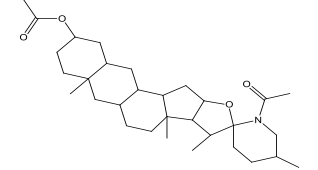
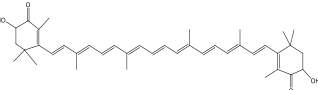
The present comparative study contributes valuable information on the bioactive compounds in *A. variabilis* and *S. platensis*, as summarized in Tables 2 and 3. Numerous bioactive compounds which belong to different classes as fatty acids, phenolic, antioxidants, alkaloids, flavonoids, and steroids were recorded.

**Table 2:** Methanolic extract components of *Anabaena variabilis*



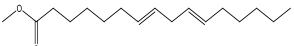
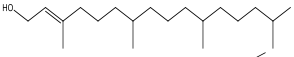
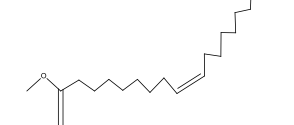
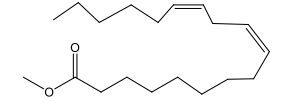
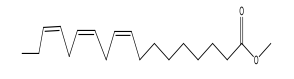
N	Compound Name	CSBC	RT	Area %	MF	MW
1	Thieno [3,4-c]pyridine, 1,3,4,7-tetraphenyl		4.06	5.20	C <sub>31</sub> H <sub>21</sub> NS	439
2	Cholan-24-oic acid, 3,7,12-trihydroxy-, (3à,5á,7à,12à)-		4.79	0.58	C <sub>24</sub> H <sub>40</sub> O <sub>5</sub>	408

3	19-Norethindrone, O-methyloxime		7.29	1.23	C <sub>21</sub> H <sub>29</sub> NO <sub>2</sub>	327
4	Glaufenin		9.92	0.92	C <sub>19</sub> H <sub>17</sub> ClN <sub>2</sub> O <sub>4</sub>	372
5	Androstane-11,17-dione,3- [(trimethylsilyl)oxy]-,17-[o- (phenylmethyl)oxime], (3à,5à)-		10.30	0.82	C <sub>29</sub> H <sub>43</sub> NO <sub>3</sub> Si	481
6	Hexasiloxane,,1,3,3,5,5,7,7,9,9,11,11- dodecamethyl-		12.37	0.90	C <sub>12</sub> H <sub>38</sub> O <sub>5</sub> Si <sub>6</sub>	430
7	Cyclooctasiloxane, hexadecamethyl- (Hexadecamethyl cyclooctasioxane)		12.87	0.93	C <sub>16</sub> H <sub>48</sub> O <sub>8</sub> Si <sub>8</sub>	592
8	Trimethylsilyl, 3-methyl- 4[(trimethylsilyl)oxy] Benzoate		14.60	0.89	C <sub>14</sub> H <sub>24</sub> O <sub>3</sub> Si <sub>2</sub>	296
9	1H-purin -6-amine, (2- fluorophenyl)methyl]		15.14	0.55	C <sub>12</sub> H <sub>10</sub> FN <sub>5</sub>	243
10	Nonadecane(AI3-36122)		16.68	5.69	C <sub>19</sub> H <sub>40</sub>	268
11	Heptadecane(AI3-36898)		17.23	4.85	C <sub>17</sub> H <sub>36</sub>	240
12	2,7-diphenyl-1,6- dioxopyridazino[4,52',3']pyrrolo[4',5'- d]pyridazine		18.51	0.77	C <sub>20</sub> H <sub>13</sub> N <sub>5</sub> O <sub>2</sub>	355
13	8,11-Eicosadienoic acid, methyl ester		19.21	1.27	C <sub>21</sub> H <sub>38</sub> O <sub>2</sub>	322
14	4H-1-benzopyran -4-one, 2-(3,4-di- methoxyphenyl)-3,5-dihydroxy-7- methoxy -(3',4',7-trimethylquercetin)		20.25	0.80	C <sub>18</sub> H <sub>16</sub> O <sub>7</sub>	344
15	18,19-Secoyohimban-19-oic acid, 6,17,20,21-tetrahydro-16- (hydroxymethyl)-, methyl ester, (15à,16E)-		20.34	0.71	C <sub>21</sub> H <sub>24</sub> N <sub>2</sub> O <sub>3</sub>	352
16	Benzene, 1,1'-(3,3-dimethyl-1- butenyldiene)bis-(1-Butene, 3,3- dimethyl-1,1-diphenyl)		20.47	0.48	C <sub>18</sub> H <sub>20</sub>	236
17	Allopregnane-3à,7à,11à-triol-20-one (3 ,7,11-Trihydroxypregnan-20-one)		21.03	0.77	C <sub>21</sub> H <sub>34</sub> O <sub>4</sub>	350
18	Hexadecanoic acid, methyl ester (Palmitic acid, methyl ester)		21.56	8.26	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270

19	Pentadecanoic acid, 14-methyl-,methyl ester		21.62	13.97	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270
20	Cyclopropanoethanoic acid, 2-octyl-,methyl ester		21.89	1.32	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296
21	Toosendanin		23.12	1.32	C <sub>30</sub> H <sub>38</sub> O <sub>11</sub>	574
22	Bufa-20,22-dienolide, 3,14-dihydroxy-, (3á,5á)-5á-Bufa-20,22-dienolide, 3á,14-dihydroxy		23.41	0.67	C <sub>24</sub> H <sub>34</sub> O <sub>4</sub>	386
23	12,15-Octadecadienoic acid, methyl ester		24.41	10.23	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294
24	Linoleic acid ethyl ester (Ethyl linoleate)		24.86	0.40	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	308
25	9,12-octadecadienoic acid (Z,Z)-, 2,3dihydroxypropyl ester ( Linolein, 2-mono-)		25.56	2.04	C <sub>21</sub> H <sub>38</sub> O <sub>4</sub>	354
26	7,10,13-Eicosatrienoic acid, methyl ester		25.97	1.80	C <sub>21</sub> H <sub>36</sub> O <sub>2</sub>	320
27	12-desoxyphorbol-13-isobutyrat		26.09	1.28	C <sub>24</sub> H <sub>34</sub> O <sub>6</sub>	418
28	Cyclopropanoethanoic acid,2[(2pentylcyclopropyl)methyl]-, methyl ester		26.22	0.59	C <sub>21</sub> H <sub>38</sub> O <sub>2</sub>	322
29	7,8,3',4'-Tetramethoxyflavone		27.54	0.45	C <sub>19</sub> H <sub>18</sub> O <sub>6</sub>	342
30	Sarreroside		28.95	1.32	C <sub>30</sub> H <sub>42</sub> O <sub>10</sub>	562
31	Phen-1,4-diol, 2,3-dimethyl-5 trifluoromethyl-		30.74	1.23	C <sub>9</sub> H <sub>9</sub> F <sub>3</sub> O <sub>2</sub>	206
32	Oxiraneoctanoic acid,3-octyl-, methyl-ester,trans (methyl 8-(3-octyl-2 oxranyl)octanoate)		31.05	0.59	C <sub>19</sub> H <sub>36</sub> O <sub>3</sub>	312
33	9,10-Secocholesta-5,7,10(19)-triene-,25,26-triol		31.86	0.63	C <sub>27</sub> H <sub>44</sub> O <sub>3</sub>	416

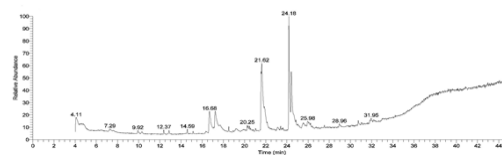
34	9,12,15-octadecatrienoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl-ester		31.95	1.50	C <sub>28</sub> H <sub>40</sub> O <sub>4</sub>	440
35	Lycoxanthin (psi.,psi.-Caroten-16-ol)		39.41	0.43	C <sub>40</sub> H <sub>56</sub> O	552
36	spirosolan-3-ol, 28-acetyl-,acetate (ester),(3á,5à,22á,25S)-28-acetylspirosolan -3-yl acetat		39.49	0.32	C <sub>31</sub> H <sub>49</sub> NO <sub>4</sub>	499
37	Astaxanthin (á,á-Carotene-4,4'-dione, 3,3'-dihydroxy(3S,3'S))		39.63	0.86	C <sub>40</sub> H <sub>52</sub> O <sub>4</sub>	596

**Table 3:** Methanolic extract components of *Spirulina platensis*

N	Compound Name	CSBC	RT	Area %	M F	MW
1	Docasane		16.63	0.66	C <sub>22</sub> H <sub>46</sub>	310
2	Hexadecanoic acid, methyl ester (Palmitic acid, methyl ester)		21.49	29.26	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270
3	7,10-Hexadecadienoic acid, methyl ester		21.68	8.29	C <sub>17</sub> H <sub>30</sub> O <sub>2</sub>	266
4	Phytol		23.94	4.28	C <sub>20</sub> H <sub>40</sub> O	296
5	9-Octadecenoic acid, methyl ester,(E)-Oleic acid, methyl ester		24.14	20.22	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	296
6	9,12-Octadecadienoic acid (Z,Z)-, methyl ester ( Linoleic acid, methyl ester)		24.36	34.40	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294
7	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (Linolenic acid, methyl ester)		24.75	2.89	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>	292

The major constituents of *Anabaena variabilis* methanolic extract, with its retention time (RT), concentration (area %), the chemical structure of bioactive components (CSBC), molecular formula (MF), and molecular weight (MW) are presented in Table (2) and Figure 4. Thirty seven bioactive compounds were identified. The most dominant compound was Pentadecanoic acid, 14-methyl-,methyl ester which represents (13.97%) , followed by 12,15-Octadecadienoic acid, methyl ester which represent 10.23% , followed by Hexadecanoic acid, methyl ester (Palmitic acid, methyl ester) acts 8.26 % , also Nonadecane (AI3-36122) represent as a percentage 5.69 % , thieno [3,4-c]pyridine, 1,3,4,7-tetraphenyl represents as 5.20% , and Heptadecane(AI3-36898) act as a

percentage 4.85%, followed by 9,12-octadecadienoic acid (Z,Z)-, 2,3dihydroxypropyl ester ( Linolein, 2-mono-) represents as 2.04%.

**Fig. 4** GC/MS chromatogram of *Anabaena variabilis*

The major constituents of *Spirulina platensis* methanolic extract, with its, retention time (RT), concentration (area %), chemical structure of bioactive components (CSBC), molecular formula (MF), and molecular weight

(MW) are presented in Table 3 and Figure 5. *S. platensis* has seven bioactive compounds. The most dominant percentage was ,12-Octadecadienoic acid (Z,Z)-, methyl ester (Linoleic acid, methyl ester) with percentage composition of 30.3 % was the major compound followed by Hexadecanoic acid, methyl ester (Palmitic acid, methyl ester) which represents as 29.26 % followed by 9-Octadecenoic acid, methyl ester,(E) - Oleic acid, methyl ester as 20.22%. The other compounds are [7, 10-hexadecadienoic acid-methyl ester], [Phytol], [9, 12, 15 Octadecatrienoic acid-methyl ester], and [Docosane] which represent as percentage 8.29%, 4.28%, 2.89 %, and 0.66%, respectively.

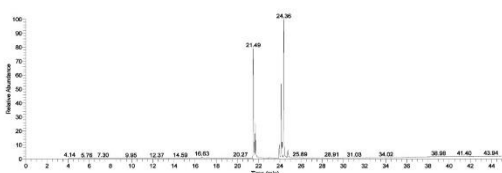


Fig. 5 GC/MS chromatogram of *Spirulina platensis*

As summarized in Tables 2 and 3, the two analyzed cyanobacterial species have variations in number, type, the arrangement of predominance, and concentration area for each bioactive component. Some strains of cyanobacteria have a high source of bioactive secondary metabolites, which can have therapeutic, industrial, and agricultural importance (Gupta *et al.* 2013; Rimsha *et al.* 2014).

Cyanobacteria and algae have various compounds like alkaloids, carbohydrates, flavonoids, pigments, phenols, steroids, and vitamins. (They have applications in many fields as agriculture, pharmaceuticals, biotechnology, and industrial fields. Michalak *et al.* 2016; Guiheneuf *et al.* 2016).

In the present study, Octadecane, Heptadecane, 9, 12-Octadecadienoic acid, Linoleic acid methyl ester, Oleic acid methyl ester were among the identified compounds. Octadecanoic acid methyl ester and 9,12,15 Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z,Z,Z) (Linolenic acid ester) reported to have activities as antimicrobial, antibacterial, antioxidant, anticancer anti-inflammatory, anti-arthritis, hypocholesterolemic, and cancer preventive (Lee *et al.* 2007; Mishra and Shree 2007; Wu *et al.* 2011).

## Conclusions

The present work indicated that, *Anabaena variabilis* as predominant soil cyanobacterial species, produced 37 bioactive secondary metabolites. While, *Spirulina platensis* as aquatic cyanobacteria produced only seven phytochemical products. Cyanobacteria extracts have potential bioactive compounds. The total bioactive products, their predominance, and concentration area depend on the specie of Cyanophyta. This work also indicated that the bioactive compounds of *A. variabilis* and *S. platensis* need further research to ascertain their biological properties.

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## عنوان البحث: المركبات الكيميائية لسالتين من السيانوبكتريا المحلية

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### الملخص العربي

تم تجميع طحلب الـ *Anabaena variabilis* من التربة الزراعية بكفر الشيخ حيث تمت دراسة الظروف البيئية والصفات الفيزيوكيميائية لهذه التربة الزراعية. أما طحلب الـ *Spirulina platensis* فتم الحصول عليه من معمل الميكروبيولوجي والذي تم تنميته بالظروف المعملية المثالية. وبدراسة المكونات الطبيعية لكلا الطحلبين باستخدام تقنية الـ GC/MS أظهرت الدراسة التأثير الكبير للعوامل البيئية على إنتاجية الطحلب للمنتجات الطبيعية كما و نوعا. حيث تم التعرف على ٣٧ مركب في طحلب الـ *Anabaena variabilis* والذي تعرض لظروف بيئية طبيعية داخل التربة الزراعية بكفر الشيخ. بينما طحلب الـ *Spirulina platensis* والذي تم تنميته في المعمل تم التعرف على ٧ مركبات فقط. و مما هو جدير بالذكر أن المركبات السائدة التي تم التعرف عليها في مستخلص *Anabaena variabilis* كانت Pentadecanoic acid 29%, 12,15-Octadecadienoic acid methyl ester 10.26%, Hexadecenoic acid methyl ester (Z) 8.3%, Thieno > [3,4-C] pyridine,1,3,4,7-tetraphenyl 5.86% والتي تختلف عن المركبات السائدة في مستخلص الـ *Spirulina platensis* وكانت كالاتي Hexadecanoic acid 29%, 9,12,15-Octadecadienoic acid methyl ester 24% and 9,12 Octadecatrienoic acid methyl ester, 24.36%.. وبناء على ما سبق ممكن القول أن المنتجات الطبيعية لمستخلص سيانوبكتريا التربة يختلف كما و نوعا و سيادة عن تلك المنتجات لطحالب السيانوبكتريا المعملية.