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Characterization of Bioactive Compounds with Antioxidant Activity and Antimicrobial Activity from Freshwater Cyanobacteria

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Abstract

Cyanobacteria are one of the most promising candidates for production of alternative biomolecules that can be used for biotechnological applications. In the current study the methanolic extract from four cyanobacterial isolates were screened for their total phenolics, total flavonoids contents, antioxidant and antimicrobial activities. The highest total phenolic content (14.33±0.76 mg GAE/g DW) was detected in *Oscillatoria sancta* SN2 (MZ504750) extract while the highest flavonoid content (3.13±0.04 mgQE/g DW) was recorded for *Limnothrix planktonica* SN4 (MZ504752). All extracts were discovered to have antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the IC50 values ranged between 1.61 mg/ml to 5.68 mg/ml for *Limnothrix planktonica* SN3 (MZ504751) and *Oscillatoria sancta (SN2* MZ504750) respectively. The extracts were examined for antimicrobial activity and showed varying degrees of activity against various gram negative, gram positive bacteria and yeast. GC/MS analysis showed that all extracts contain numerous active biomolecules including 3Allyl-6-methoxyphenol, cis-Vaccenic acid, cis-13-Eicosenoic acid, Tetradecanoic acid "Myristic acid", Phytol, Palmitoleic acid, Palmitic acid methyl ester, n-Hexadecanoic acid "Palmitic acid", Linolenic acid methyl ester, Linolenic acid, and other compounds which exerts antimicrobial and antioxidant activities.

Keywords: Cyanobacterial extracts, Phenolic compounds, Flavonoids, Antimicrobial activity, Antioxidant activity, GC – MS.

1. Introduction

Cyanobacteria produce a wide range of primary and secondary metabolites which considered as a novel natural bioactive compounds that can be used for various industrial, pharmacological and biotechnological applications **[1],** these metabolites include proteins, lipids, poly unsaturated fatty acids, vitamins, pigments, polyketides, lipopeptides, alkaloids, terpenes, flavonoids and polyphenols **[2, 3].** Cyanobacterial secondary metabolites had been accumulated in their biomass and were reported to have potential biological activities including antimicrobial, antiviral, anticancer, antiprotozoal, antioxidant and anti-inflammatory activities **[4, 5, 6].** The oxidative stress resulted from the accumulation of reactive oxygen species (ROS) in cells and tissues has harmful effects on human body and plays an important role in the progression of several chronic diseases such as diabetes, cancer and metabolic disorders; these effects could be removed by antioxidant compounds which have the ability to reduce or completely prevent the damage caused to cells by free radicals **[7].** cyanobacterial organic extracts were observed to have antioxidant activity due to the presence of glutathione, ascorbic acid, phenolic and flavonoid compounds which have the ability to scavenge free radicals and protect the biological system from endogenous damage by oxidative stress **[8, 9, 10].**The increase of bacterial resistance to antibiotics gives great attentions to use prokaryotic microalgal extracts as promising sources which could be used as alternative antibiotics for treatment the infections with multi-drug resistant bacteria, these extracts had been reported to contain various bioactive compounds with

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antimicrobial activity and inhibit the growth of bacteria, fungi and viruses **[11, 12, 13, 2, 14].** *Oscillatoria redekei* and *Scytonema hofmanni* have been reported to produce antibacterial compounds **[15, 3].** Five cyanobacterial extracts were examined by **Ostensvik *et al*. [16]** for their antibacterial activity; they had discovered that the methanolic extracts have promising antibacterial activity against *Bacillus cereus* and *Bucillus subtilis*. The current study aimed to quantify the total phenolic, total flavonoid contents and to evaluate the antioxidant activity and antimicrobial activity of the methanolic extracts of four fresh water cyanobacterial isolates followed by the characterization of chemical composition of these extracts using GC/MS.

2. Experimental

2.1. Organisms and culture conditions.

Four cyanobacterial isolates were provided by Hydrobiology lab, inland water and lakes division, National institute of Oceanography and Fisheries (NIOF), Egypt. These isolates are *Merismopedia* sp. SN1 (MZ504749), *Oscillatoria sancta SN2* (MZ504750), *Limnothrix planktonica* SN3 (MZ504751), *Limnothrix planktonica* SN4 (MZ504752). They are fresh water cyanobacteria that have been isolated from Lake Nasser. The isolates were cultured in 500 ml flasks contain 300 ml BG11 medium, pH (7.1), and incubated at 25^0C,16:8 light dark cycles under 37 µmol m^{-2} s^{-1} photon flux density till exponential phase (about 3 weeks).

2.2. Free cell extracts preparation

Cyanbacterial biomass were harvested by centrifugation at 4000 rpm for 10 min, washed with distilled water and re-centrifuged then the supernatant decanted and the biomass were collected and dried at 50^0C till constant weight. One gram of each sample dry biomass were grounded to fine powder packed into a Soxhlet apparatus and extracted with100 ml absolute methanol at 60–65°C for 3–4 h. the mixtures were filtered through whatman filter paper and the filtrates were concentrated under reduced pressure, dried and weighed.

2.3. Total phenolic content

Total phenolic content was determined by the Folin–Ciocalteu method **[17]**, 100µl of sample's methanolic extract were added to 1ml of diluted Folin-Ciocalteau reagent (1:10 distilled water). After 4 min the mixture was neutralized by addition of 800 µl saturated sodium carbonate solution (75g/L) with shaking, the mixture was incubated for 2hrs at room temperature followed by measuring the absorbance at 765 nm. Total phenolic compounds calculated from linear regression equation obtained from Gallic acid standard curve and expressed as mg Gallic acid equivalent of 1 g dry weight (mg GAE/g DW).

2.4. Total flavonoid content.

Total flavonoid content was determined by aluminium chloride colorimetric method **[18, 19]**. Briefly, 100µl of sample's methanolic extract was mixed with 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 mol/L potassium acetate, 2.8 ml distilled water and incubated at room temperature for 30 min, the absorbance were measured at 415 nm against blank solution prepared from all reagent except for aluminium chloride was replaced with distilled water. Total flavonoid content was calculated from linear regression equation obtained from quercetin calibration curve and expressed as mg Quercetin equivalent of 1g dry weight (mg QE/g DW).

2.5. Antioxidant activity

The free radical scavenging activity of cyanobacterial extracts was determined using 2,2diphenyl-1-picrylhydrazyl (DPPH) **[20, 21].** Briefly, 200 µl of each extract was mixed with 2.8 ml of freshly prepared 0.1 mM DPPH methanolic solution and kept in dark conditions at room temperature for 30 min; DPPH solution without test sample was used as control. The increase in antioxidant activity reflected by the increasing in discoloration of DPPH solution which determined by measuring the absorbance at 517 nm and the scavenging activity percentage was calculated using the formula:

*Scavenging activity (%) = [(A0–A1)/A0]*100*

Where A0 = Absorbance of control and A1 = Absorbance of test sample after 30 min

Serial dilutions of each extract were prepared and tested to calculate the IC50 value.

2.6. Antimicrobial activity

2.6.1 Preparation of microbial inoculum

The antimicrobial activity was determined against gram negative bacteria [*Aeromonas hydrophila*, *Salmonella typhi* ATCC-15566, *Escherichia coli* ATCC-25922, *Pseudomonas aeruginosa* PTCC-1074], Gram positive bacteria [*Staphylococcus aureus* ATCC-47077, *Staphylococcus epidermidis*, *Enterococcus faecalis* ATCC- 29212, *Bacillus cereus* ATCC-12228] and one fungi species [*Candida albicans* ATCC- 10231], all pathogen species were provided by Hydrobiology lab, National institute of Oceanography and fisheries (NIOF). All bacterial strains were cultured overnight in tryptic soy broth medium (TSB, Difco Laboratories, Detroit, USA) at 37^0C and the cell density adjusted at 10^8 cells/ml using 0.5 McFarland standard **[22],** while *Candida*

albicans was cultured in Potato dextrose broth medium at 37^0C for 48hr.

2.6.2 Antimicrobial assay

The antimicrobial activity of the cell free extract previously dissolved in DMSO (50mg/ml) was determined using agar well diffusion method **[23, 24, 25].** Briefly, Mueller–Hinton agar (Oxoid) plates were inoculated with 100µl of appropriate bacterial strains by spreading the inoculum over the entire agar surface, sterile cork borer was used to punch wells with 6 mm diameters, 50µl of each extract was introduced into the wells and the plates were incubated for 24 hr. at appropriate temperature suitable for the test microorganism. Anti-fungal activities were assessed using Sabouraud dextrose agar medium (Oxoid). All plates were examined for the presence of growth inhibition zones and the diameters of complete inhibition zones including the well diameters were measured. DMSO was introduced as negative control and Doxycycline (30mcg) was used as positive control.

2.7. Chemical composition (GC-MS) analysis

The chemical composition of cyanobacterial extracts was analyzed using Agilent 7000 series Quadruple Gas chromatography mass spectrometry (GC-MS) with electron impact ionization, in the central laboratory of national institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt. Helium was used as carrier gas and the instrument was operated according to protocol mentioned by Abd El-karim **[2].** The NIST MS spectral library and Agilent's Retention Time Locked (RTL) database were used to identify the components of the extracts; the closest matches with highest probability in the library were recorded.

2.7. Statistical analysis

Statistical analysis was carried out with XLSTAT 2019.1 software. All the results were calculated as mean ± standard deviation. One-way ANOVA was applied to test for significant differences at $P < 0.05$.

3. Results and Discussion

3.1. Total phenolic and Flavonoid compounds

Cyanobacteria are considered as one of the greatest biomass producers on the earth that produce large numbers of bioactive compounds **[26, 27, 28].** Phenolic compounds are one of the most important biologically active compounds produced by cyanobacteria and received a great attention for their potential antioxidant activity and health beneficial properties **[29]**. In the current study, *Oscillatoria sancta* SN2 isolate was the topmost isolate for its total phenolic compound (TPC) content (14.33± 0.76 mg GAE/g DW) followed by *Limnothrix planktonica* SN4 (11.75± 0.53 mg GAE/g DW), while the lowest TPC was determined in *Limnothrix planktonica* SN3 (6.28± 0.33 mg GAE/g DW), these results were higher than others reported for *Leptolyngbya* sp (7.44 mg GAE/g), *Phormidium* sp (6.16 mg GAE/g), *Scytonema* sp (3.2 mg GAE/g), *and Cyanosarcina* sp 2.36 mg GAE/g **[30]**. The total phenolic content for *Oscillatoria sancta* SN2 and *Limnothrix planktonica* SN4 were higher than reported for *Phormidium corium* (5.41 mg GAE/g), *Chroococcus turgidus* (7.94 mg GAE/g), *Nostoc commune* (8.19 mg GAE/g), *Oscillatoria sancta* (7.81 mg GAE/g) and *Spirulina major* (7.15 mg GAE/g), and similar to that recorded for *Lyngbya confervoides* (13.80 mg GAE/g), *Oscillatoria fremyii* (17.37 mg GAE/g), *Oscillatoria geminata* (16.33 mg GAE/g) and *Phormidium tenue* (9.22) mg GAE/g **[31].** *Oscillatoria sancta* SN2 isolate contains TPC complied with that of *Oscillatoria limosa* (14 - 16.3 mgGAE/gDW) studied by Sarmah and Rout [9]. Table 1. represented the results of total phenolic compounds and total flavonoids contents for cyanobacterial isolates. Cyanobacteria produce wide variety of flavonoid compounds which are belonging to poly-phenolic group and have beneficial effects on human health as they are potential therapeutic agents against a wide variety of diseases **[32].** The highest flavonoids content was determined in *Limnothrix planktonica* SN4 (3.13± 0.04 mg QE/g DW) followed by *Oscillatoria sancta* (SN2) (2.77± 0.01 mg QE/g DW), while the lowest quantity was measured in *Merismopedia* sp SN1 (MZ504749) (1.84± 0.07 mg QE/g DW). These results were consistent with the results recorded for *Oscillatoria sancta* (2.39 mg QE/g DW), *Nostoc commune* (2.48 mg QE/g DW), *Lyngbya confervoides* (3.98 mg QE/g DW) and *Spirulina major* (2.21 mg QE/g DW) **[31]**. although our results were higher than that of *Phormidium tenue* (1.44 mg QE/g DW), *and Phormidium corium* (0.74 mg QE/g DW) they were lower than *that of Oscillatoria fremyii* (4.5 mg QE/g.DW) and *Oscillatoria geminata* (4.41) mg QE/g DW **[31]**. The dry biomass of *Oscillatoria limosa* was recorded to have total flavonoids content slightly higher (4.4 and 3.8 mg QE/g.DW) than obtained from the present study **[9],** but the total flavonoids contents recorded by **Singh *et al*., [29] and El-Chaghaby et al., [33]** were lower than recorded from the current one.

Table (1): Total phenolic compounds and total flavonoids constituents of cyanobacterial isolates.
- Values within the columns with different superscripts were significantly different (P < 0.05)

Isolate name	TPC. mg GAE/g DW.	T. Flavonoids mg QE/g DW.
Merismopedia sp SN1 (MZ504749)	7.16± 0.42[a]	1.84± 0.07[a]
Oscillatoria sancta SN2 (MZ504750)	14.33± 0.76[b]	2.77± 0.01[b]
Limnothrix planktonica SN3 (MZ504751)	6.28± 0.33[a]	2.30± 0.13[c]
Limnothrix planktonica SN4 (MZ504752)	11.75± 0.53[c]	3.13± 0.04[d]

3.2. Antioxidant activity.

The antioxidant activity of cell free extracts was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals that is widely used to evaluate free radical scavenging activity of natural products due to its stability, reproducibility and simplicity **[34],** higher scavenging activity reflects higher antioxidant activity **[35].** The uppermost free radicals scavenging activity was recorded for *Limnothrix planktonica* SN3 extract which inhibited 53.8% of DPPH activity at concentration 1.61 mg/ml, followed by *Limnothrix planktonica* SN4 and *Merismopedia* sp SN1 which scavenged more than 50% of DPPH activity (IC50) (60.39 % and 59.48%) at concentrations 1.81 and 1.91 mg/ml respectively. The lowest scavenging free radical activity were recorded for *Oscillatoria sancta* SN2 extract which reached its IC50 value at concentrations 5.68 mg/ml. The results of current study proved that the crude extract of the four cyanobacterial isolates have antioxidant activity and scavenged DPPH free radicals with concentration dependent assays (Fig.1) and this may be due to the presence of phenolic and flavonoid compounds in the extracts **[8]**. The IC50 values obtained from two *Limnothrix planktonica* isolates and *Merismopedia* sp SN1 (MZ504749) isolates in our study were relevant to that described for *Limnothrix* sp. (1.82 mg/ml), *Dichothrix* sp (1.72 mg/ml) and *Chroococcus* sp. (1.56 mg/ml) **[29]** but higher than *Limnothrix obliqueacuminata* (2.95 mg/ml), *Nostoc ellipsosporum* (8.91 mg/ml) and *Microcheate tenera* (4.28 mg/ml) **[29].** Furthermore, our results were higher than *Nostoc commune* and *Arthrospira platensis* which didn't scavenge DPPH free radicals even at high tested concentrations 40mg/ml **[36]** Cyanobacterial extracts had been reported to produce bioactive compounds with high antioxidant capacity **[13, 33, 37].**

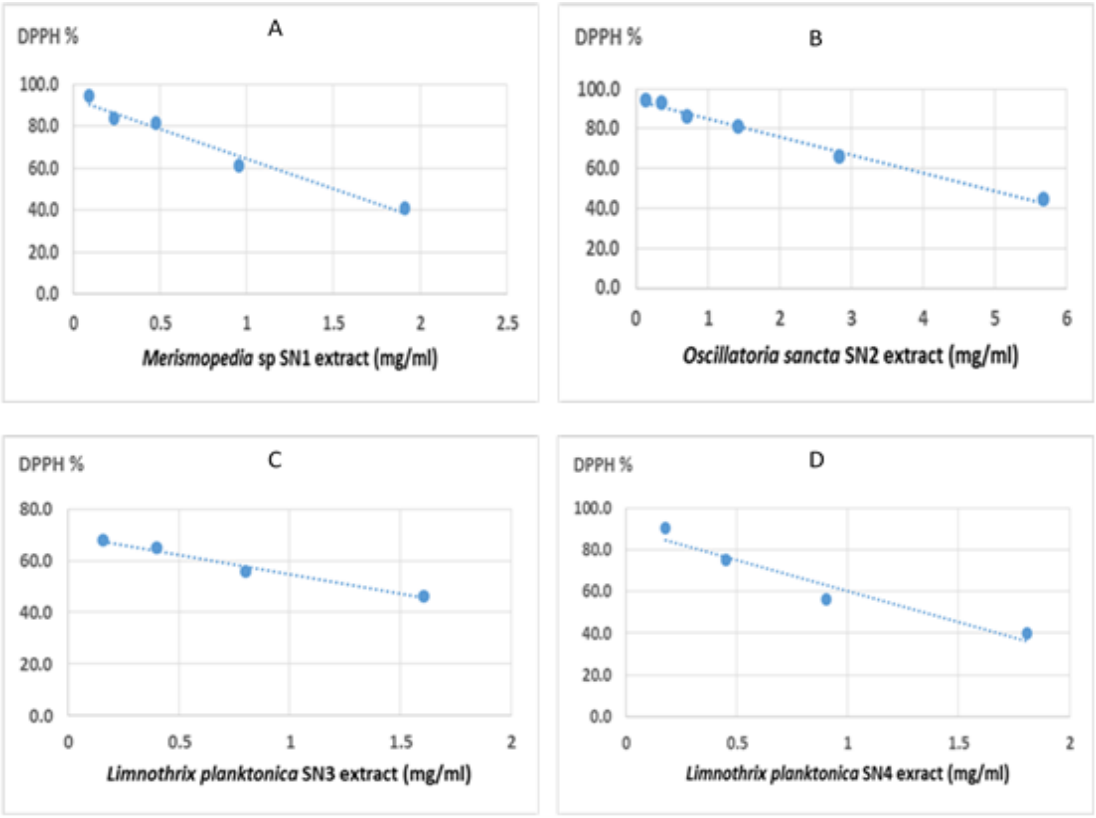

Fig.1. Free radical scavenging activity of different cyanobacterial crude extracts against DPPH

3.3. Antimicrobial activity of cyanobacterial extracts

The continuous increase in antimicrobial resistance is one of the most public health threats which make the antibiotics ineffective in treatment of microbial infections and increase the number of mortality all over the world. So, intensive efforts required to discover new potential antimicrobial agents **[38]**. Cyanobacterial extracts are promising sources for a new novel bioactive agent and were intensively investigated for their antimicrobial activity **[29, 39].** In the current study, four cyanobacterial crude extracts were investigated for their antimicrobial activity against gram negative bacteria, gram positive bacteria and Candida sp. the results showed that all extracts exhibited different degrees of antimicrobial activity against the tested microorganisms as showed in table (2). Of the four cyanobacterial extracts, extract from *Limnothrix planktonica* SN4 (MZ504752) showed the highest antibacterial activity against *Aeromonas hydrophila*, *Salmonella typhi* ATCC 15566 and *Bacillus cereus* ATCC- 12228, with inhibition zones of 13.3±1.2 mm, 13.7±1.2 mm and 15.3±1.2 mm, respectively. The extract of *Oscillatoria sancta* SN2 made maximum inhibition zones against *Ps. aeruginosa PTCC-1074 (*11.7±0.9 mm), *S. epidermidis* (11.7±1.2 mm) and *Enterococcus faecalis ATCC- 29212* (10.3±0.5 mm), while *Merismopedia* sp. SN1 extract gave highest inhibition zone against *S. aureus* ATCC-47077 (11.7±0.5 mm) and *Limnothrix planktonica* SN3 extract formed highest inhibition zone with diameter 15.0±1.6 mm against *Candida albicans* ATCC- 10231, however all extracts had no antibacterial effect against *E. Coli ATCC- 25922***.** The variation in results from extract to another illustrated that the antimicrobial activity depends on the type of cyanobactrial species and the tested organism **[40, 41].** Cyanobacterial extracts have been determined to contain various components with antimicrobial activity **[42, 2, 6].** Lipophilic extracts of *Phormidium* sp. biomass were detected to have antibacterial activity against *Escherichia coli* and *Salmonella typhi* **[43].** *Oscillatoria margaritifera* was reported to produce oscillapeptin compound which has antibacterial and cytotoxic activity **[1].** The antimicrobial activity of Cyanobacterial extracts was attributed to the presence of many compounds that characterized by their antibacterial and antifungal activity such as saturated and poly unsaturated fatty acids **[4].**

3.4. Chemical composition of Cyanobacterial extracts.

The results of GC/MS analysis of cyanobacterial methanolic extracts as shown in Tables (3-8) identified compounds of great importance and might be responsible for beneficial health activity. The major bioactive components detected in the methanolic extract of *Merismopedia* sp. SN1 isolate found to include 3-Allyl-6-methoxyphenol (0.55), phytol (8%), methyl palmitoleate (0.73%), 13-Docosenoic acid, (Z)- (Erucic acid) (1.08%), 17-Octadecynoic acid (0.46%), Palmitic acid methyl ester (4.63) and Palmitic acid (21.06%), while the extract of *Oscillatoria sancta* SN2 isolate was observed to contain bioactive compounds including 3-Allyl-2-methoxy-phenol (1.07%), cis-13-Eicosenoic acid (0.18%), Tetradecanoic acid "Myristic acid" (8.98%), Phytol (9.7%), Palmitoleic acid (0.68%), Palmitic acid methyl ester (2.95%), Palmitic acid (8.38%), Linolenic acid methyl ester (3.12%), Linolenic acid (8.75%) and Linoleic acid (1.32%). Also the major bioactive compounds in *Limnothrix planktonica* SN3 isolate extract were 3-Allyl-2-methoxyphenol (0.47%), methyl myristate (2.8%), Myristic acid (4.5%), Phytol (4.76%), Methyl palmitoleate (3.75%), Palmitoleic acid(4.41%), Palmitic acid, methyl ester (9.46%), Palmitic acid (14.73%), cis-Vaccenic acid (4.17%) and Stearic acid methyl ester (1.9%). Similarly, the methanolic extract of *Limnothrix planktonica* SN4 Isolate was discovered to have bioactive compounds including 3-Allyl-2-methoxy-phenol (0.6%), Myristic acid (4.91%), Pentadecylic acid (0.92%), Phytol (4.65%), Methyl palmitoleate (1.64%), Palmitoleic acid (18.07%), Palmitic acid methyl ester (2.87%), Palmitic acid (14.12%) and Methyl isostearate (1.05%).

Data recovered from GC/MS analysis illustrated that the biomass of our cyanobacterial isolates are promising precursors for extraction of bioactive compounds including phenolic compounds, saturated and unsaturated organic fatty acids which reported to have antioxidant, anticancer, antiviral, hypocholesterolemic and anti-inflammatory activity **[44, 45, 46, 6].** The extracts were determined to contain long chain fatty acids including hexadecenoic acid, cis-11-eicosenoic acid, α-linolenic acid and 12-octadecadienoic acid which have been reported to have antimicrobial and antioxidant activities **[47].**

The current study determined that the major bioactive compounds produced by cyanobacteria in amounts that could be used in pharmaceutical, cosmetics and food additives industries were Palmitic acid, Palmitoleic acid, Myristic acid, cis-Vaccenic acid, Phytol, Methyl isostearate, Linoleic acid, Linolenic acid and this results were consistent with several previous studies **[48, 44, 49, 50, 6].**Table (7) showed the major constituents detected in cyanobacterial methanolic extracts

and their bioactivity. The GC/MS analysis results explained the presence of antimicrobial and antioxidant activities for the extracts of cyanobacterial isolates and support their results

Table (2): Antimicrobial activity of cyanobacterial extracts against pathogenic microorganisms (Inhibition zone measured in mm)

Isolate name	*A. hydrophila*	*S. typhi*	*E. coli*	*P. aeruginosa*	*S. aureus*
***Merismopedia*sp SN1 (MZ504749)**	**9.3±0.5**	**NZ**	**NZ**	**10.3±0.5**	**11.7±0.5**
***Oscillatoria sancta* SN2 (MZ504750)**	**12.0±0.8**	**11.3±1.2**	**NZ**	**11.7±0.9**	**9.3±1.2**
***Limnothrixplanktonica*SN3 (MZ504751)**	**10.7±0.5**	**11.0±1.4**	**NZ**	**11.7±0.5**	**10.7±0.5**
***Limnothrixplanktonica*SN4 (MZ504752)**	**13.3±1.2**	**13.7±1.2**	**NZ**	**NZ**	**9.7±1.2**
Doxycycline 30 mcg (Control)	**9±0.5**	**14±1.2**	**17±0.8**	**8±0.5**	**20±1.4**

Isolate name	*S. epidermidis*	*E. faecalis*	*B. cereus*	*C. albicans*
***Merismopedia*sp SN1 (MZ504749)**	**9.7±1.2**	**NZ**	**NZ**	**10.7±1.2**
***Oscillatoria sancta* SN2 (MZ504750)**	**11.7±1.2**	**10.3±0.5**	**9.7±0.9**	**9.3±1.2**
***Limnothrixplanktonica*SN3 (MZ504751)**	**8.3±0.5**	**8.7±1.2**	**12.3±1.7**	**15.0±1.6**
***Limnothrixplanktonica*SN4 (MZ504752)**	**9.7±1.2**	**8.3±0.5**	**15.3±1.2**	**13.3±1.2**
Doxycycline 30 mcg (Control)	**13±0.5**	**13±0.8**	**11±1.4**	**17±1.2**

NZ= No zone

Table (3). GC/MS profile of *Merismopedia* sp SN1 (MZ504749) isolate.

NO.	Phytochemical compound	Rt	M. formula	M. wt.	Area %
1	Semicarbazide, 1-(4-tert-butyl-phenoxy)-acetyl-4-phenyl-3-thia-	5.66	$C_{19}H_{23}N_3O_2S$	357	0.55
2	3-Allyl-6-methoxyphenol	6.33	$C_{10}H_{12}O_2$	164	0.65
3	9-Octadecene, 1,1'-[1,2-ethanediylbis(oxy)]bis-, (Z,Z)-	6.48	$C_{38}H_{74}O_2$	562	0.28
4	2-cis-9-Octadecenyloxyethanol	9.98	$C_{20}H_{40}O_2$	312	0.57
5	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-	12.58	$C_{19}H_{36}O_3$	312	0.21
6	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	13.68	$C_{25}H_{42}O_2$	374	0.41
7	3,7,11,15-Tetramethyl-2-hexadecen-1-ol(phytol)	14.48	$C_{20}H_{40}O$	296	6.0
8	13-Docosenoic acid, (Z)- (Erucic acid)	14.59	$C_{22}H_{42}O_2$	338	1.08
9	17-Octadecynoic acid	14.85	$C_{18}H_{32}O_2$	280	0.46
10	3,7,11,15-Tetramethyl-2-hexadecen-1-ol(Phytol)	15.12	$C_{20}H_{40}O$	296	2.45
11	9-Hexadecenoic acid, methyl ester, (Z)- (Methyl palmitoleate)	15.45	$C_{17}H_{32}O_2$	268	0.73
12	Hexadecanoic acid, methyl ester (Palmitic acid, methyl ester)	15.73	$C_{17}H_{34}O_2$	270	4.63
13	9-Hexadecenoic acid	16.01	$C_{16}H_{30}O_2$	254	1.52
14	n-Hexadecanoic acid(Palmitic acid)	16.29	$C_{16}H_{32}O_2$	256	21.06
15	10-Octadecenoic acid, methyl ester	17.8	$C_{19}H_{36}O_2$	296	0.64
16	11-Octadecenoic acid, methyl ester	17.86	$C_{19}H_{36}O_2$	296	0.73
17	Ethyl iso-allocholate	17.94	$C_{26}H_{44}O_5$	436	0.7
18	Heptadecanoic acid, 16-methyl-, methyl ester (Methyl isostearate)	18.07	$C_{19}H_{38}O_2$	298	1.23
19	cis-13-Octadecenoic acid	18.29	$C_{18}H_{34}O_2$	282	2.19
20	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (Palmitin1,2-di-)	18.48	$C_{35}H_{68}O_5$	568	0.64
21	Phthalic acid, di(2-propylpentyl) ester	23.38	$C_{24}H_{38}O_4$	390	0.01

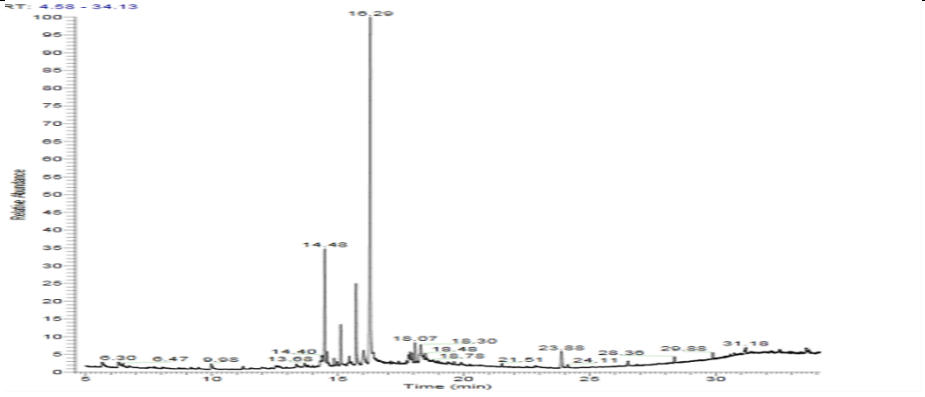

Fig.2. *Merismopedia* sp SN1 (MZ504749) GC/MS chromatogram

Table (4). GC/MS profile of *Oscillatoria sancta* SN2 (MZ504750) isolate

NO.	Phytochemical compound	Rt	M. formula	M. wt.	Area %
1	2-Bromomethyl-3,4,5,6-tetramethoxytetrahydropyran	5.86	$C_{10}H_{19}BrO_5$	298	0.56
2	3-Allyl-2-methoxyphenol	6.24	$C_{10}H_{12}O_2$	164	1.07
3	2-Methylhexadecan-1-ol	6.44	$C_{17}H_{36}O$	256	0.27
4	cis-13-Eicosenoic acid	9.45	$C_{20}H_{38}O_2$	310	0.18
5	2-Bromooctadecanal	11.7	$C_{18}H_{35}BrO$	346	0.23
6	2-Hexyldodecan-1-ol	11.97	$C_{18}H_{38}O$	270	2.11
7	Tetradecanoic acid, methyl ester(Methyl Myristate)	12.51	$C_{15}H_{30}O_2$	242	1.94
8	Tetradecanoic acid (Myristic acid)	13.42	$C_{14}H_{28}O_2$	228	8.98
9	2-Methylhexadecan-1-ol	14.4	$C_{17}H_{36}O$	256	0.55
10	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl(phytol)	14.48	$C_{20}H_{40}O$	296	6.45
11	3,7,11,15-Tetramethylhexadecyl acetate	14.58	C22H44O2	340	0.98
12	3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol)	15.12	$C_{20}H_{40}O$	296	2.53
13	Hexadecanoic acid, methyl ester (Palmitic acid, methyl ester)	15.72	$C_{17}H_{34}O_2$	270	2.95
14	cis-9-Hexadecenoic acid (Palmitoleic acid)	16.09	$C_{16}H_{30}O_2$	254	0.68
15	n-Hexadecanoic acid(Palmitic acid)	16.29	$C_{16}H_{32}O_2$	256	8.38
16	8,11-Octadecadienoic acid, methyl ester	17.73	$C_{19}H_{34}O_2$	294	0.88
17	9,12,15-Octadecatrienoic acid, methyl ester,(Linolenic acid, methyl ester)	17.81	C19H32O2	292	3.12
18	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl- (Phytol)	17.94	$C_{20}H_{40}O$	296	0.7
19	Heptadecanoic acid, 16-methyl-, methyl ester (Methyl isostearate)	18.06	$C_{19}H_{38}O_2$	298	0.74
20	cis-9,cis-12-Octadecadienoic acid	18.2	$C_{18}H_{32}O_2$	280	1.32
21	9,12,15-Octadecatrienoic acid, (Linolenic acid)	18.29	$C_{18}H_{30}O_2$	278	8.75
22	9-Octadecenoic acid, 1,2,3-propanetriyl ester	18.47	$C_{57}H_{104}O_6$	884	0.34
23	à-D-Galactopyranose, 6-O-(trimethylsilyl)-, cyclic 1,2:3,4-bis(butylboronate)	18.85	$C_{17}H_{34}B_2O_6Si$	384	0.01
24	Phthalic acid, di(2-propylpentyl) ester	23.86	$C_{24}H_{38}O_4$	390	1.55
25	3,11,17,20,21-Pentamethoxypregnane	38.14	$C_{26}H_{46}O_5$	438	8.54
26	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester,	38.82	$C_{27}H_{52}O_4Si_2$	496	1.27
27	1-Monolinoleoylglycerol trimethylsilyl ether	47.13	$C_{27}H_{54}O_4Si_2$	498	1.59

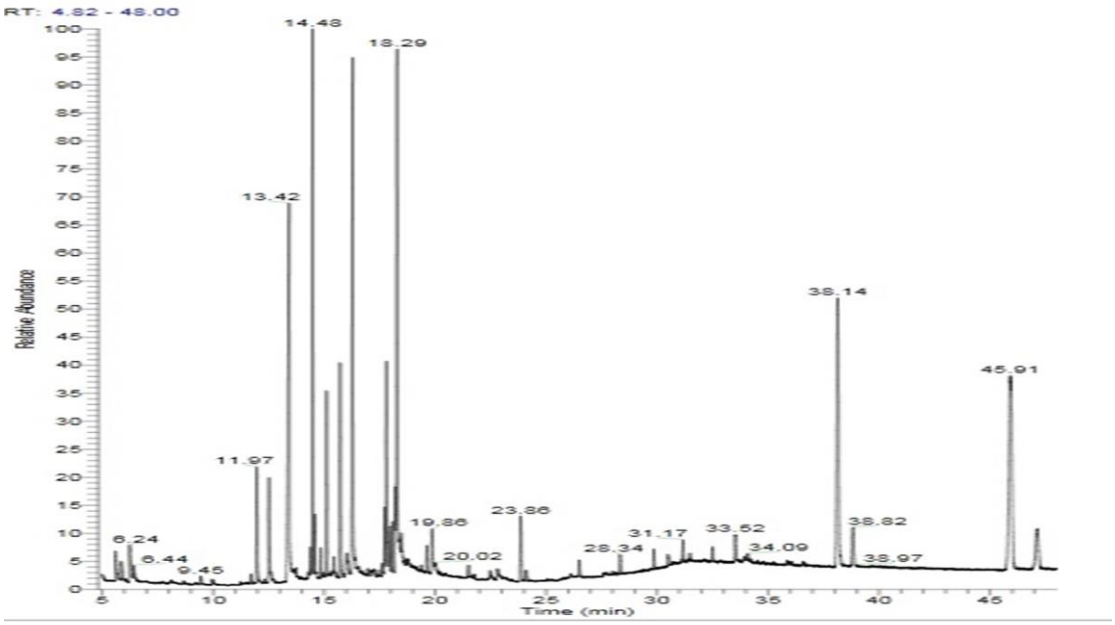

Fig.3. *Oscillatoria sancta* SN2(MZ504750) GC/MS chromatogram

Table (5). GC/MS profile of *Limnothrix planktonica* SN3 (MZ504751) isolate.

NO.	Phytochemical compound	Rt	M. formula	M. wt.	Area %
1	3-Allyl-2-methoxyphenol	6.26	$C_{10}H_{12}O_2$	164	0.47
2	2-Bromooctadecanal	8.03	$C_{18}H_{35}BrO$	346	0.25
3	1,1-Bis(dodecyloxy)hexadecane	9.46	$C_{40}H_{82}O_2$	594	0.14
4	Tetradecanoic acid, methyl ester(Methyl Myristate)	12.52	$C_{15}H_{30}O_2$	242	2.8
5	E-9-Tetradecenoic acid	13.16	$C_{14}H_{26}O_2$	226	1.47
6	Tetradecanoic acid (Myristic acid)	13.39	C14H28O2	228	4.5
7	Pentadecanoic acid (Pentadecylic acid)	14.36	$C_{15}H_{30}O_2$	242	0.68
8	3,7,11,15-Tetramethyl-2-hexadecen-1-ol(Phytol)	14.48	$C_{20}H_{40}O$	296	1.4
9	cis-13-Eicosenoic acid	14.58	$C_{20}H_{38}O_2$	310	0.47
10	3,7,11,15-Tetramethyl-2-hexadecen-1-ol(Phytol)	15.12	$C_{20}H_{40}O$	296	0.52
11	9-Hexadecenoic acid, methyl ester (Methyl palmitoleate)	15.44	$C_{17}H_{32}O_2$	268	3.75
12	Hexadecanoic acid, methyl ester (Palmitic acid, methyl ester)	15.72	$C_{17}H_{34}O_2$	270	9.46
13	cis-9-Hexadecenoic acid (Palmitoleic acid)	16.03	$C_{16}H_{30}O_2$	254	4.41
14	n-Hexadecanoic acid(Palmitic acid)	16.3	$C_{16}H_{32}O_2$	256	14.73
15	7,10-Octadecadienoic acid, methyl ester	17.74	$C_{19}H_{34}O_2$	294	0.27
16	trans-13-Octadecenoic acid, methyl ester	17.85	C19H36O2	296	4.56
17	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl- (Phytol)	17.94	$C_{20}H_{40}O$	296	2.84
18	Octadecanoic acid, methyl ester (Stearic acid, methyl ester)	18.06	$C_{19}H_{38}O_2$	298	1.9
19	11-Octadecenoic acid (cis-Vaccenic acid)	18.29	$C_{18}H_{34}O_2$	282	4.17
20	9-Octadecenoic acid, 1,2,3-propanetriyl ester	18.47	$C_{57}H_{104}O_6$	884	0.27
21	cis-10-Nonadecenoic acid, methyl ester	18.96	$C_{20}H_{38}O_2$	310	0.79
22	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester	36.43	$C_{27}H_{52}O_4Si_2$	496	5.58
23	ether Methyl ((24-oxo-3,7,12-tris[(trimethylsilyl)oxy]cholan-24-yl) amino) acetate	38.12	$C_{36}H_{69}NO_6Si_3$	695	0.77

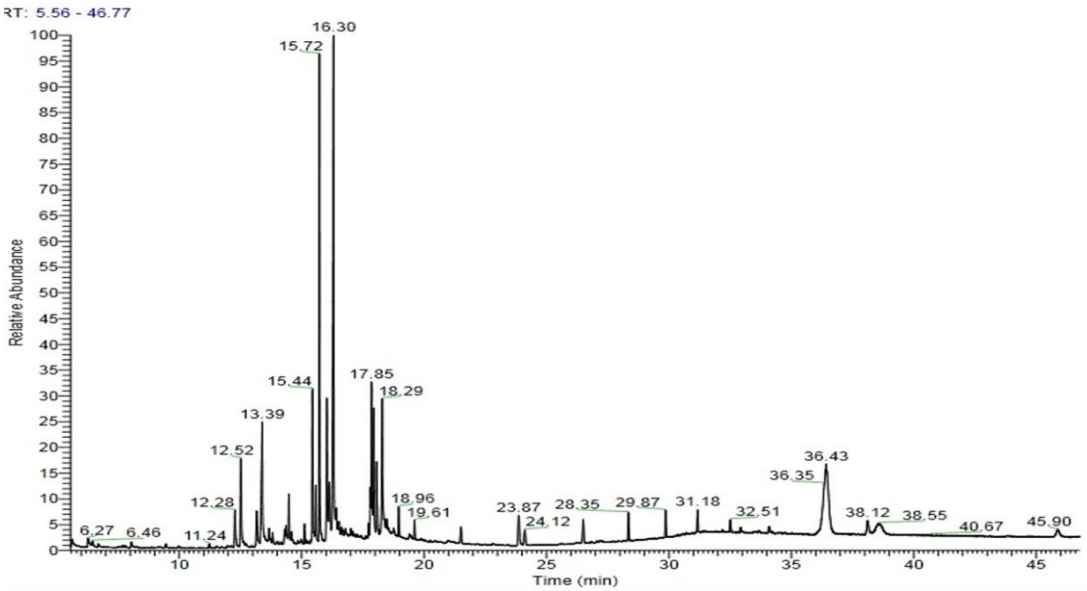

Fig.4. *Limnothrix planktonica* SN3 (MZ504751) GC/MS chromatogram

Table (6). GC/MS profile of *Limnothrix planktonica* SN4 (MZ504752) isolate.

NO.	Phytochemical compound	Rt	M. formula	M. wt.	Area %
1	3-Allyl-2-methoxyphenol	6.24	$C_{10}H_{12}O_2$	164	0.6
2	Tetradecanoic acid, methyl ester(Methyl Myristate)	12.52	$C_{15}H_{30}O_2$	242	0.31
3	Tetradecanoic acid(Myristic acid)	13.4	$C_{14}H_{28}O_2$	228	4.91
4	Pentadecanoic acid (Pentadecylic acid)	14.37	$C_{15}H_{30}O_2$	242	0.92
5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol(Phytol)	14.48	$C_{20}H_{40}O$	296	2.77
6	1-Hexadecanol, 2-methyl-	14.58	$C_{17}H_{36}O$	256	0.4
7	3,7,11,15-Tetramethyl-2-hexadecen-1-ol(Phytol)	15.12	$C_{20}H_{40}O$	296	1.14
8	9-Hexadecenoic acid, methyl ester, (Z)- (Methyl palmitoleate)	15.45	$C_{17}H_{32}O_2$	268	1.64
9	Hexadecanoic acid, methyl ester (Palmitic acid, methyl ester)	15.72	$C_{17}H_{34}O_2$	270	2.87
10	cis-9-Hexadecenoic acid (Palmitoleic acid)	16.09	$C_{16}H_{30}O_2$	254	18.07
11	n-Hexadecanoic acid(Palmitic acid)	16.32	$C_{16}H_{32}O_2$	256	14.12
12	10-Octadecenoic acid, methyl ester	17.86	$C_{19}H_{36}O_2$	296	0.81
13	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (Phytol)	17.94	$C_{20}H_{40}O$	296	0.74
14	Heptadecanoic acid, 16-methyl-, methyl ester (Methyl isostearate)	18.07	$C_{19}H_{38}O_2$	298	1.05
15	cis-13-Octadecenoic acid	18.29	$C_{18}H_{34}O_2$	282	4.15
16	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (Palmitin,1,2-di-)	19.88	$C_{35}H_{68}O_5$	568	0.54

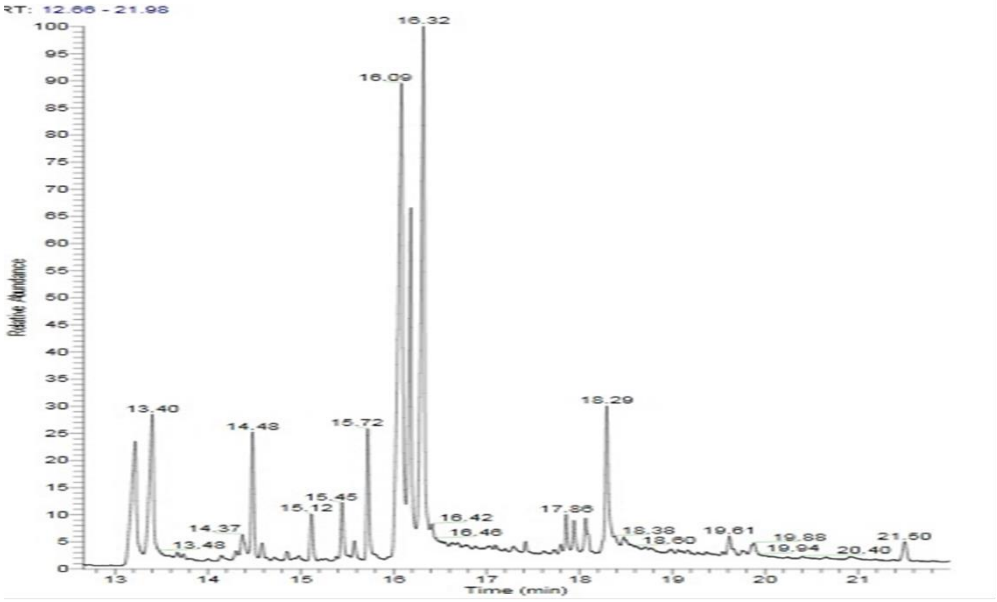

Fig.5. *Limnothrix planktonica* SN4 (MZ504752) GC/MS chromatogram

Table (7). The major constituents of the cyanobacterial extracts and their bioactivity.

NO.	Phytochemical compound	Nature	bioactivity	references
1	3-Allyl-6-methoxyphenol	Phenolic compound	Anaesthetic, Antihistaminic, anti-inflammatory, antimicrobial and antioxidant activities	[51, 52]
2	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	alcohol	Antimicrobial and Anti-inflammatory	[53]
3	17-Octadecynoic acid (17-ODYA)	fatty acid alkyne	inhibit the metabolism of arachidonic acid by cytochrome P450 in renal cortical microsomes of rats	[54]
4	Tetradecanoic acid (Myristic acid)	long-chain saturated fatty acid	impacts positively on cardiovascular health, immunomodulatory functions, Cosmetics	[55, 56]
5	cis-9-Hexadecenoic acid (Palmitoleic acid)	Mono unsaturated fatty acid,	anti-inflammatory, reduce risk of certain heart diseases	[57, 58]
6	9,12,15-Octadecatrienoic acid, (Linolenic acid)	omega-3 fatty acids	decrease the risk of cardiovascular diseases, immunomodulatory functions anti-inflammatory, anticancer, antimicrobial and antioxidant	[59, 60, 61]
7	Phytol	acyclic diterpene alcohol	Precursor for Vitamins E and K, Cytotoxic, Antimicrobial, Anti-inflammatory Anticancer and Diuretic	[62, 63, 64]

8	cis-9,cis-12-Octadecadienoic acid (Linoleic acid)	polyunsaturated essential fatty acid	Antioxidant	[65]
9	Pentadecanoic acid (Pentadecylic acid)	Odd-Chain saturated Fatty Acid	anticancer	[66]
10	11-Octadecenoic acid (cis-Vaccenic acid)	omega-7 fatty acid	antibacterial activity, hypolipidemic	[67, 68]
11	n-Hexadecanoic acid (Palmitic acid)	saturated long-chain fatty acid	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, antimicrobial activity and Flavor	[60, 69, 63, 70]
12	Phthalic acid	Organic acid	Antimicrobial activity	[71]

4. Conclusion

The current study concerned with the production of bioactive compounds from four cyanobacterial isolates, the results showed that all cyanobacterial extracts were detected to contain various mounts phenolic and flavonoid compounds. Also all extracts have been proved to exert free radical scavenging activity against DPPH and exhibited different degrees of antibacterial and antifungal activities; these results were supported by the data obtained from GC/MS analysis which determined the presence of many bioactive components in the biomass of the isolates. These results complied with many other studies about the importance of cyanobacterial species as a novel source for bioactive compounds that can be used in beneficial health and industrial applications.

Conflicts of interest

There are no conflicts to declare.

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