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BIOLOGICAL SCIENCES

Natural products from some soil cyanobacterial extracts with potent antimicrobial, antioxidant and cytotoxic activities

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Abstract: The ethyl acetate, n hexane and methanol extracts of six cyanobacterial species isolated from paddy fields in Egypt were assessed for their antimicrobial activity, using disc diffusion method. *Oscillatoria acuminata*, *Oscillatoria amphigranulata* and *Spirulina platensis* methanolic extracts showed the highest inhibition zones. Minimum inhibitory concentration of *O. amphigranulata* extract recorded lower values using agar streak dilution method. *O. acuminata* methanolic extract exhibited the highest antioxidant activity (6.58 and 34.60 % using DPPH (2, 2- diphenyl-1- picrylhydrazyl) and $ABTS^{*}$ (2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) methods, respectively, followed by *O. amphigranulata* then *S. platensis*. Similarly, *O. acuminata* methanolic extract showed very strong cytotoxicity activity against HepG2 and HCT-116 cell lines and strong activity with MCF-7 cell lines. *O. amphigranulata* extract showed strong cytotoxicity for HepG2 and HCT-116 as well as moderate cytotoxicity for MCF-7 cell line. Whereas, *S. platensis* extract exhibited moderate cytotoxicity for all cell lines. Results of gas chromatography/mass spectroscopy analysis pointed out that the potential activity of these cyanobacterial extracts might be attributed to a synergistic effect between their pronounced contents of fatty acids, alkaloids, phytol, hydrocarbons, phenolics and phthalates, especially fatty acids. We recommend cyanobacteria as a rich source of natural products with potent pharmacological and medical applications.

Key words: antimicrobial, Antioxidant, Cyanobacteria, Cytotoxicity, GC-MS, Natural products

INTRODUCTION

The increase in the rate of infection by antibiotics-resistant microorganisms alarms for exploring of natural sources of antimicrobial compounds (Pandy 2015). The search for cyanobacteria with antimicrobial activity has gained importance in recent years due to their richness in natural metabolites with medicinal and pharmacological uses (Singh et al. 2011). The cyanobacteria-derived bioactive compounds have been reported to exhibit antibacterial (Heidari et al. 2012, Malathi et al. 2014), antifungal (Soltani et al. 2005, Najdenski et al. 2013), antiviral activity, anticoagulant, antiinflammatory, antimalarial, antiprotozoal, antituberculosis and antitumor activities (Shanab et al. 2012, Varshney & Singh 2013, Pradhan et al. 2014, Abd El Sadek et al. 2017). Several active antimicrobial secondary metabolites were identified from cyanobacteria such as fatty acids (Gheda et al. 2013), acrylic acid, halogenated aliphatic compounds, terpenes, Sulphur containing heterocyclic compounds, carbohydrates and phenols (Plaza et al. 2010, Pandy 2015). In addition, cyanobacteria contained numerous biologically active molecules which have been known to exhibit antioxidant activity, such as polyunsatured fatty acids (PUFA),

phycobiliproteins, β-carotene, pro-vitamins and phenolic compounds (Hajimahmoodi et al. 2010, Shanab et al. 2012) which may act in combination and induce antimicrobial as well as cytotoxic activities (Bharat et al. 2013). Nowadays, natural antioxidants are in greater demand than synthetic ones due to their non- carcinogenicity, high stability and better compatibility (Rajishamol et al. 2016). On the same direction, several studies have shown that extracts from *Spirulina* sp., *Oscillatoria* spp., *Fischerella sp*. and many other cyanobacterial species could prevent or inhibit cancer in humans and have immune-promoting effects. Natural compounds with antitumor activities against colon CT-26 and lung 3LL cell lines from different extracts of 24 cyanobacterial strains were reported (Silva-Stenico et al. 2013). Dietary supplementation of *Spirulina platensis* was helpful in the prevention and treatment of pancreatic cancer (Konickova et al. 2014). On their reviews, Singh et al. 2011, Vijayakumar & Menakha 2015 stated several compounds such as borophycin, cryptophycin 1, symplostatin, dolastatin, coibamide A, apratoxin A, curacin A, largazole and tolyporphin which were derived from different algal and cyanobacterial species that have valid mechanisms against variety of cancer cell lines. As reported by Ahmed et al. 2018 crude extracts of cyanobacterium *Fischerella sp.* BS1-EG had anti-cancer and anti-diabetic activities.

Therefore, the development of natural, antimicrobial and antitumor products from cyanobacterial metabolites is a valuable trail. The wide distribution of cyanobacteria in the Egyptian environments such as in soil of the paddy fields supports this demand. In this study, six cyanobacterial species were isolated from paddy fields in Egypt. The antimicrobial and the antioxidant potentials of these cyanobacterial species were evaluated. The chemical composition of the extracts was identified by Gas Chromatography Mass Spectrometer (GC-MS) and their cytotoxicity effect against hepatocellular (HepG-2), mammary gland (MCF-7) and colorectal (HCT-116) cancer cell lines were investigated.

MATERIALS AND METHODS

Isolation of cyanobacteria and culture conditions

Soil samples were collected from different regions of paddy fields in Nile Delta region, Gharbia Government, Tanta, Egypt. Samples were cultured on BG11 medium (Rippka et al. 1979). Axenic cyanobacterial cultures were identified using morphological and taxonomical approaches according to (Desikachary 1959, Prescott 1962) and further confirmed by AlgaeBase (http://www.algaebase.org). Six species were identified as *Anabaena variabilis* (Kutz.), *Nostoc muscorum* (Agardh), *Nostoc linckia* (Bornet), *Oscillatoria acuminata* (Gomont), *Oscillatoria amphigranulata* (Goor) and *Spirulina platensis* (Gomont). Working cultures were established on BG-11 medium for all species while *Spirulina platensis* was grown on its specific medium (Zarrouk 1966). The incubation temperature was 25 ±2 °C at 80 $\mu Em^{-2}s^{-1}$ of illumination and 16hr light/8hr dark regime. The cultures were harvested at late exponential phase and the collected biomass was dried, weighted and used for further assays.

Collection of pathogenic microorganisms

Five gram +ve and gram –ve bacteria were used in this study as: *Staphylococcus aureus* TC-8325, *Bacillus subtilis* STR-168, *Pseudomonas aeuroginosa* AE-4091, *Esherichia coli* MG-1655 and *Klebsiella pneumoniae* HS-1286. The examined bacterial strains were identified by sequencing their 16S rRNA gene (SIGMA Scientific Service Company). The fungal strains were obtained from

the Microbial Culture Collection (MCC), Faculty of Science, Assiut University, Egypt. These strains were identified as *Candida albicans* SC-5314 and *Aspergillus flavus* GCA-3357 using 18S rRNA PCR gene sequencing.

Preparation of cyanobacterial extracts

The extractions were performed by soaking the dried cyanobacterial material (0.1: 2 w/v) in (85%) methanol, n hexane or ethyl acetate solvents. The extract mixture was sonicated at 30% amplitude, interval 5 sec, pulse 5 sec for 15min. The extract was then shaken on a rotary shaker at 120 rpm at 28° C for 48 hrs; and filtered using Whatman No 4 filter paper. The solvent was evaporated under reduced pressure up to dryness and the obtained residue (crude extract) was stored at -20° C in airtight bottles until used (Hajimahmoodi et al. 2010, Bharat et al. 2013).

Antimicrobial activity assay

The susceptibility of the tested pathogenic bacteria to various cyanobacterial extracts was assessed according to the Clinical and Laboratory Standards Institute (CLSI 2012) using the modified Kirby-Bauer disk diffusion method on Muller Hinton agar medium (Oxoid). Similarly, the anti-fungal activities were tested using Sabouraud dextrose agar medium (Oxoid). Each extract material was dissolved in DMSO solution (1 mg /ml). Previously sterilized filter paper discs soaked in the extract solutions were placed aseptically in the Petridishes containing agar media and previously seeded with the tested microorganisms (at a concentration of 10^6 cfu /ml). The Petridishes were incubated at 37 ° C and the inhibition zones were recorded after 24 h and 48 h of incubation for bacteria and fungi, respectively. Each treatment was replicated three times. Ampicillin (100 µg/ml) and fluconazole (100 µg/ml) were used as common standard for antibacterial and antifungal activity while DMSO (1%) was used as a negative control using the same procedure as above. The minimum inhibitory concentration (MIC) of cyanobacterial extracts was determined by agar streak dilution method (Hawkey & Lewis 1994). A stock solution of the extracts (100 µg/ml) in DMSO was prepared and graded quantities were tested against different pathogenic microorganisms. The MIC value was considered to be the lowest concentration of test extract exhibiting no visible growth of bacteria or fungi on the plate.

Phytochemical analysis of cyanobacterial extracts

The cyanobacterial extracts were analyzed for the presence of secondary metabolites such as tannins, phenolics, flavonoids, saponins, terpenoids and sterols according to the standard phytochemical methods (Edeoga et al. 2005). Total phenolic content was estimated as Gallic acid (GA) equivalent per gram extract dry weight (Taga et al. 1984).

Antioxidant activity of cyanobacterial extracts

DPPH *assay*

Antioxidant activities of cyanobacterial extracts were assayed by the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging method (Blois 1958). Cyanobacterial samples were dissolved in methanol and the methanolic DPPH served as controls. Ascorbic acid was used as a reference and the percentage of DPPH–decolorization was calculated as:

$$\text{Free radical scavenging \%} = (Ac - As) / Ac \times 100$$

Where: Ac = Absorbance of control and As = Absorbance of sample.

ABTS$^+$ assay

ABTS$^+$ assay was performed by modified method of (Paixão et al. 2007)**.** ABTS$^+$ solution (3ml) was added to 3, 15 and 30 µl of each cyanobacterial methanolic extract to prepare 1, 5 and 10 ppm final concentration, respectively. The absorbance was measured at 415 nm using ascorbic acid as a positive control and ABTS$^+$ solution as negative control. Percentage of inhibition was measured as (% inhibition) according to the previous formula.

Cytotoxicity (MTT) assay of cyanobacterial extracts

The cell lines hepatocellular carcinoma (HepG-2), mammary gland (MCF-7) and colorectal carcinoma (HCT-116) were used to determine the inhibitory effects of the cyanobacterial extracts using the MTT assay (Skehan et al. 1990). This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in the viable cancer cells. Doxorubicin was used as a standard anticancer drug for comparison (Fadda et al. 2012). The relative percentage of cell viability was calculated as:

(A_{570} of treated samples/A_{570} of untreated sample) X 100.

Where: A is the absorbance at 570.

Gas chromatography-Mass spectrometry analysis

GC-MS analysis of the active cyanobacterial extracts was carried out as per standard procedure using Perkin Elmer: Clarus 580/560 S model system. Identification of metabolites in the extracts was recognized by comparison of retention time and fragmentation pattern with mass spectra in the NIST spectral database library software. Relative area value of each constituent (as a percentage of total volatile composition) were directly obtained from total ion current (TIC) and expressed as peak area normalization.

Statistical analysis

The results were expressed as mean±standard deviation of three replicates. Data was statistically analyzed using ANOVA (SPSS version 19) software**.**

RESULTS AND DISCUSSION

Antimicrobial activity of the cyanobacterial extracts

Antimicrobial activity of the cyanobacterial extracts against tested bacterial and fungal species were presented in Table I. All extracts exhibited different degrees of antimicrobial activity unrelatedly to the tested pathogen. Generally, the methanolic extracts showed the highest antimicrobial activity followed by ethyl acetate and then hexane extracts. Methanolic extracts of *A. variabilis* showed broad activity with the highest inhibition zone recorded against *B. subtilis* STR-168 (19 mm) while all *A. variabilis* extracts showed no activity against the two tested fungal species. Similarly, methanolic extracts of *N. muscorum* recorded the highest inhibition zones against *A. flavus* GCA-3357 (17mm) and *B. subtilis* STR-168 (16mm). Regarding *N. linckia* methanolic extract, significant inhibition zones were recorded only for *B. subtilis* STR-168, *K. pneumoniae* HS-1286, *C. albicans* SC-5314 and *A. flavus* GCA-3357 (17, 10, 17 and 22 mm), respectively, with a noticeable antifungal activity. Hexane and ethyl acetate extracts of *N. linckia* showed comparable activities against the tested bacterial pathogens. The highest inhibition zones were recorded for *A. flavus* GCA-3357 followed by *C. albicans* SC-5314 hexane and ethyl acetate extracts of *N. linckia*, respectively.

Table I. Antimicrobial activity of cyanobacterial extracts using different solvents (mean± SD).

Cyanobacteria species	Diameter of inhibition zones (mm)							
	Solvent	*E. coli* MG-1655	*P. aeruginosa* AE-4091	*K. pneumoniae* HS-1286	*S. aureus* TC-8325	*B. subtilis* STR-168	*C. albicans* SC-5314	*A. flavus* GCA-3357
A. variabilis	Ethyl acetate	12^{aA}± 0.3	12^{aA}± 0.1	10^{bA}± 0.3	12^{aA}± 0.5	13^{cA}± 0.3	NA	NA
	Hexane	11^{aA}±0.02	NA	9^{bA} ± 0.05	11^{aA} ± 0.1	12^{cA}±0.2	NA	NA
	Methanol	NA	14^{aB} ± 0.04	10^{bA} ± 0.03	NA	19^{cC}± 0.2	NA	NA
N. muscorum	Ethyl acetate	11^{aA}±0.01	NA	9^{bA} ± 0.1	NA	11^{aA}±0.2	11^{aA}±0.3	NA
	Hexane	NA	13^{aA} ± 0.4	NA	NA	12^{bA}±0.2	12^{bA}± 0.2	15^{cB}± 0.3
	Methanol	12^{aA} ± 0.2	14^{bB} ± 0.1	10^{cA} ± 0.4	15^{dB} ± 0.3	16^{dB}±0.3	13^{bA}± 0.1	17^{eB} ± 0.4
N. linckia	Ethyl acetate	11^{aA}±0.04	12^{bA} ± 0.03	11^{aA} ± 0.2	11^{aA} ± 0.4	12^{bA}±0.1	13^{cA}±0.4	15^{dB}±0.2
	Hexane	13^{aA}±0.06	NA	12^{bB}± 0.04	12^{bB}± 0.2	14^{cB}± 0.6	15^{dB}±0.5	17^{eB} ±0.4
	Methanol	NA	NA	10^{aA} ± 0.03	NA	17^{bB}± 0.3	17^{bB}± 0.5	22^{cD} ± 0.4
O. acuminata	Ethyl acetate	NA	13^{aA} ± 0.01	13^{aA} ± 0.02	15^{bB}±0.04	17^{cB}± 0.3	12^{dA}± 0.1	20^{eC} ±0.3
	Hexane	NA	12^{aA} ± 0.01	12^{aA} ± 0.03	13^{bA}±0.04	15^{cB}±0.6	NA	18^{dC}± 0.3
	Methanol	14^{aB} ± 0.3	17^{bB} ± 0.02	12^{cA} ± 0.04	20^{dC} ± 0.1	22^{eD}±0.2	15^{fB} ± 0.3	26^{gE} ± 0.3
O. amphigranulata	Ethyl acetate	14^{aB}±0.06	17^{bC} ± 0.1	13^{cA} ± 0.03	21^{dC}±0.05	21^{dC}±0.03	20^{dC}±0.3	27^{eE}±0.1
	Hexane	NA	16^{aC} ± 0.2	15^{bB} ± 0.05	18^{cC}±0.01	19^{dC} ±0.4	21^{eC}±0.2	25^{fD}± 0.4
	Methanol	18^{aC}±0.05	21^{bC}± 0.03	20^{bC} ± 0.02	25^{cD}±0.02	27^{dE}±0.06	25^{cD}±0.3	31^{eF}±0.2
S. platensis	Ethyl acetate	13^{aA}± 0.1	12^{bA}± 0.3	13^{aA} ± 0.2	15^{cB} ± 0.3	19^{dC} ± 0.2	13^{aA}± 0.1	14^{eB} ± 0.2
	Hexane	12^{aA}±0.01	NA	10^{bA}± 0.01	16^{cB}± 0. 1	17^{dB} ± 0.4	NA	14^{eB} ± 0.1
	Methanol	16^{aB} ± 0.3	17^{bB} ± 0.2	19^{cC} ± 0.3	23^{dD}± 0.4	24^{eD} ± 0.1	21^{fC}±0.3	27^{gE} ± 0.1
Ampicillin		26^{aE}±0.3	29^{bE} ± 0.1	28^{cE} ± 0.2	29^{aE}±0.4	30^{dF}±0.3	--	--
Fluconazole		--	--		--	--	28^{aE}±0.4	32^{bF}± 0.6

NA, no activity recorded. Difference in superscript letters in each row or column indicate significance ($p \leq 0.05$).

The majority of *O. acuminata* extracts exhibited considerable antifungal and antibacterial effects. Among which *O. acuminata* methanolic extracts recorded the highest inhibition zones against *A. flavus* GCA-3357 (26 mm), *B. subtilis* STR-168 (22 mm) and *S. aureus* STR-168 (20mm). In addition, the ethyl acetate of *O. acuminata* showed antifungal activity of 20 mm for *A. flavus* GCA-3357. Among all tested cyanobacterial extracts, the highest antifungal and antibacterial effects were recorded for *O. ampigranulata* different extracts (except for *E. coli* MG-1655 with the hexane extract). The highest inhibition zones were recorded by the methanolic extracts of *O. ampigranulata* as 31, 27 and 25mm against *A. flavus* GCA-3357, *B. subtilis* STR-168, *S. aureus* STR-168 and *C. albicans* SC-5314, respectively. Similarly, ethyl acetate and hexane extracts displayed significant antifungal inhibition zones of 27 and 25 mm, respectively against *A. flavus* GCA-3357 strain. For *S. platensis*, the methanolic extract presented promising antimicrobial inhibition zone diameters of 27, 24, 23, 21 and 19 mm against *A. flavus* GCA-3357, *B. subtilis* STR-168, *S. aureus* STR-168, *C. albicans* SC-5314 and *K. pneumoniae* HS-1286 pathogens, respectively (Table I). These results indicated that the antimicrobial activity of the extracts depended mainly on the type of cyanobacterial species, the used solvent and the tested pathogen. The

same conclusion was reported by (Soltani et al. 2005, Malathi et al. 2014, Abo-State et al. 2015). The recorded values were promising, although of significant difference than the used controls Ampicillin and Fluconazole (Table I). Evidently, methanolic extracts of *O. amphigranulata*, *O. acuminata* and *S. platensis,* respectively exhibited the best potent antimicrobial activities. Gram +ve *B. subtilis* STR-168, *S. aureus* STR-168 and *A. flavus* GCA-3357 fungus were the most affected pathogens followed by *C. albicans* SC-5314 (Table I).

Cyanobacteria has been famed with living in diverse types of environments and under fluctuated growth conditions they produce different categories of primary and secondary metabolites to adopt with these environments and as a defense system to enable their survival (Heidari et al. 2012, Pandy 2015, Abd El-Karim 2016). Several metabolites as pigments, carbohydrates, polyphenols, fatty acids, lipids, hydrocarbons and some other cellular compound were endorsed with antimicrobial activity (Abu-Ghannam & Rajauria 2013, Pradhan et al. 2014).

Cyanobacterial species such as *Spirulina platensis*, *Nostoc commune, N. muscorum, N. piscinale, Scytonema hofmanni, Oscillatoria anguistisima* and *Calothrix parietina, Tolypothrix tenuis* and *Anabaena variabilis,* among others, have been accredited as antimicrobial producing species against human pathogens in *in vitro* studies (Soltani et al. 2005, Plaza et al. 2010, Gheda et al. 2013, Abo-State et al. 2015). The resistance of G-ve bacteria to antibiotics was a common notice among many previous studies, and that was also found in our results (Table I). This may be due to the complex lipopolysaccharides present in the G-ve bacteria cell wall, which hinders active compounds penetration. However, variation in inhibition zones using methanol, ethyl acetate and hexane might be ascribed to the difference in the active metabolites composition dissolved in these extracts (Bharat et al. 2013, Najdenski et al. 2013, Rajishamol et al. 2016). For this, the most active extracts of *O. amphigranulata, S. platensis* and *O. acuminata,* in methanol were selected to test their antioxidant and cytotoxic activities on HepG-2, HCT-116 and MCF-7 cell lines. On the same direction, the MIC showed fluctuated values with methanolic extracts of different tested cyanobacteria. However, the values compared well as potent antifungal agents against *C. albicans* SC-5314 (1.17; 4.68 µg/ml) and *A. flavus* GCA-3357 (0.78 µg/ml; 1.56 µg/ml) for *O. amphigranulata* and *S. platensis* methanolic extracts, respectively. Lower MIC values were also recorded for *O. amphigranulata* extract against *E. coli* MG-1655 (4.68 µg/ml), *P. aeruginosa* AE-4091 (3.13 µg/ml), K. *pneumoniae* HS-1286 and *B. subtilis* STR-168 (2.34 µg/ml). These values were nonsignificant to those recorded for Ampicillin (except for *S. aureus* STR-168) and Fluconazole standard antibiotics implying feasible antibacterial and antifungal activities for this extract (Table II).

Phytochemical analysis of cyanobacterial extracts

The results shown in Table III represented the qualitative phytochemical composition of the active methanolic extracts for the three tested cyanobacteria species. Obviously, the extracts were containing active secondary metabolites for which antimicrobial activity have been already established in many studies (Shanab et al. 2012, Silva-Stenico et al. 2013, Abd El-Karim 2016). *O. acuminata* extract was rich in the phenols content (32.63±1.3 mg GA/g dry wt.) and in the presence of glycosides while flavonoids, alkaloids and saponins were apparent in all the tested extracts. As reported by (Mujeeb et al. 2014), these bioactive components exert diverse

mechanisms for their antimicrobial action. Flavonoids, as a natural phenol, may interact with soluble and extracellular proteins of microbial cells. They are synthesized by plants in response to microbial infection and have been proved as an effective antimicrobial agent. Terpenoids also can cause dissolution of microorganism cell walls through weakening the membranes. Saponins can cause proteins and enzymes outflow from the cells. Alkaloids inhibit nucleic acid synthesis and attenuate microbial cells pathogenicity and virulence gene mechanisms (Cushnie et al. 2014). The antimicrobial action of phenols is due to alteration of microbial cell membrane permeability, loss of internal macromolecules, cellular integrity and eventual cell death (Abu-Ghannam & Rajauria 2013, Namvar et al. 2014, Rajishamol et al. 2016). Glycosides also have been reported to effect on *S. aureus* and *C. albicans* pathogens (Bilková et al. 2015).

Antioxidant activity of cyanobacterial extracts

As shown in Table IV, *O. acuminata* methanol extract exhibited the highest antioxidant activity (6.58 and 34.60 %) with both methods, respectively, followed by *O. amphigranulata* and then *S. platensis*. Since, *O. acuminata* contained phenolic compounds (32.63 mg Gallic acid/g dry wt.) as well as flavonoids, saponins, alkaloids and

Table II. The minimum inhibition concentration (MIC µg/ml) of cyanobacterial methanolic extracts against different tested pathogens.

Compound	*E. coli* MG-1655	*P. aeruginosa* AE-4091	*K. pneumoniae* HS-1286	*S. aureus* TC-8325	*B. subtilis* STR-168	*C. albicans* SC-5314	*A. flavus* GCA-3357
S. platensis	12.5^{aA}±1.5	6.25^{bA} ±1.3	9.37^{cA}±0.5	9.37^{cA}±0.5	12.5^{aA}±1.5	4.68^{bA}±0.7	1.56^{dA}±0.2
O. acuminata	25.0^{aB}±1.5	9.37^{bB}±0.5	6.25^{bB}±1.3	37.5^{cB}±2.5	18.75^{dB}±1.1	6.25^{bA}±1.3	3.13^{eB}±0.3
O. amphigranulata	4.68^{aC}±0.7	3.13^{aC}±0.3	2.34^{bC}±1.0	3.13^{aC}±0.3	2.34^{bC}±1.0	1.17^{cB}±0.2	0.78^{dC}±0.5
Ampicillin	1.56^{aC}±0.2	2.34^{bC}±1.0	1.17^{aC}±0.2	0.78^{cD}±0.5	1.17^{aC}±0.2	----	----
Fluconazole	----	----	----	----	----	1.17^{aB}±0.2	0.58^{bC}±1.0

Difference in superscript letters in each row or column indicate significance at $p \leq 0.05$ level.

Table III. Qualitative phytochemicals screening of cyanobacterial species methanolic extracts.

Phytochemical compounds/ Test	*S. platensis*	*O. acuminata*	*O. amphigranulata*
Phenolic compounds, ferric chloride test	+	++	+
Flavonoid, alkaline reagent test	+	+	+
Terpenoids, Salkowski's test	+	___	+
Tannins, ferric chloride test	___	___	___
Saponins, Froth test	++	+	++
Alkaloids, Wagner's test	++	++	++
Steroids, Liebermann-Burchard test	___	___	___
Glycosides, Keller-Kiliani's test	___	+	___
Total Phenolics content (mgGA/g dry wt)	18.385±0.3	32.63±1.3	15.696±0.5

*** + = Presence of constituents; – = Absence of constituents.**

glycosides (Table III), it may reacted synergistically leading to the elevated antioxidant activity of this species. Many previous studies (Shanab et al. 2012, Rajishamol et al. 2016, Abd El Sadek et al. 2017) proposed synergetic action between secondary metabolites, especially phenolic compounds, flavonoids, polyunsaturated fatty acids, pigments and polysaccharides to be responsible for the antioxidant activity. Phenolic components are one of the most abundant classes of phytochemicals in algae and have potential antioxidant ability through scavenging singlet oxygen, superoxide and hydroxyl radicals, metal chelating activity, electron or hydrogen donation ability and also stabilizing lipid peroxidation. Furthermore, microalgae are famed with the ability to face oxidative stresses through stimulating their enzymatic and non-enzymatic antioxidants as a defense system. The contribution of these antioxidant products as chemo-preventive and tumors growth controlling agents were already established (Wang et al. 2010, Varshney & Singh 2013, Konickova et al. 2014, Ahmed et al. 2017). However, the phenomenon of synergistic effects of biological extracts was frequently crucial since sometimes this activity was lost when purified fractions were made. The advantage remains that bacterial resistance to synergistic drug formulations, like those of crude extracts, is often slower than for a single drug component (Cos et al. 2006). This justifies the feasibility of exploring crude extracts from cyanobacterial source for more biological activities than using isolated compounds.

Viability (Cytotoxicity) test of cyanobacterial extracts

The cytotoxicity assay was used to examine the cyanobacterial extracts as antitumor agent (Fadda et al. 2012)**.** The crude extracts of all tested cyanobacteria showed significant increased inhibition of cell viability with increasing concentration (Fig. 1). *O. acuminata* methanol extract recorded 90.7, 91.2 and 85.2 % with HepG2, HCT-116 and MCF-7, respectively at concentration of 100µg/ml. One the same trend, *O. amphigranulata* recorded cytotoxicity percentage of 81.7, 83.8 and 74.2%; while *S. platensis* revealed relative cell viability inhibition of 64.9, 73.6 and 67.3 % with the same cell lines, respectively. The results suggested that active compounds in the extracts interacted with cancer cell lines associated receptors or cancer cell special molecules and triggered some mechanisms that cause cancer cell death (Wang et al. 2010). In the present study, IC_{50} value (the concentration that caused 50% loss of the cell monolayer) of *O. acuminata* extract was of insignificant difference at $p \leq 0.05$ than that of Doxorubicin drug recording very strong cytotoxicity activity (8.91, 8.43 µg/ml) for HepG2, HCT-116, respectively; and strong toxic activity (11.16 µg/ml) for MCF-7 cell line (Table V). Similarly, *O. amphigranulata* methanol extract recorded strong IC_{50} value with HepG2 (16.3 µg/ml), HCT-116 (13.6 µg/ml) cell lines and moderate IC_{50} value with MCF-7 cell line (21.29 µg/ml). For *S. platensis* methanol extract, moderate IC_{50} values (41.07, 26.96, 37.80 µg/ml) were noticed for liver, colon and breast cell lines, respectively. These data were in agreement with the findings of Konickova et al. (2014) who reported the role of *S. platensis* with promising potential for its use in the treatment of cancer. *Oscillatoria sp*. secondary metabolites extracted as fatty acids methyl esters (FAME) proved significant pharmaceutical potentials to control tumor activities (Sutharsana et al. 2016). In addition, phenolic compounds found in algae, have been reported to exert several biological effects including anti-apoptosis, anti-aging and anticarcinogenic properties and as chemo-protective agents (Namvar et al. 2014).

GC-MS analysis of cyanobacteria methanol extracts

As shown in Table VI, *S. platensis* methanolic extract contained some valuable biomolecules of antimicrobial and antitumor potent activity. According to the depicted peak area percentage, alcohols (1-Propanol, 1-Pentanol, 2-Methyl-1-propanol (isobutanol) and 1-Butanol, 2-methyl-(S)) constituted 18.179% of the total recorded peak areas in the chromatogram. The fatty

Table IV. The antioxidant activity of cyanobacterial species methanolic extracts.

Methanol extract	% inhibition of DPPH	% inhibition of ABTS^{+}
Ascorbic acid	6.14^{A} ± 0.62	49.30^{A} ±1.2
S. platensis	2.41^{B} ± 0.93	17.50^{B} ±0.62
O. acuminata	6.58^{A} ± 0.62	34.60^{C} ±0.95
O. amphigranulata	5.48^{A} ± 0.31	23.20^{B} ±0.80

Difference in superscript letters in each column indicate significance at $p \leq 0.05$ level.

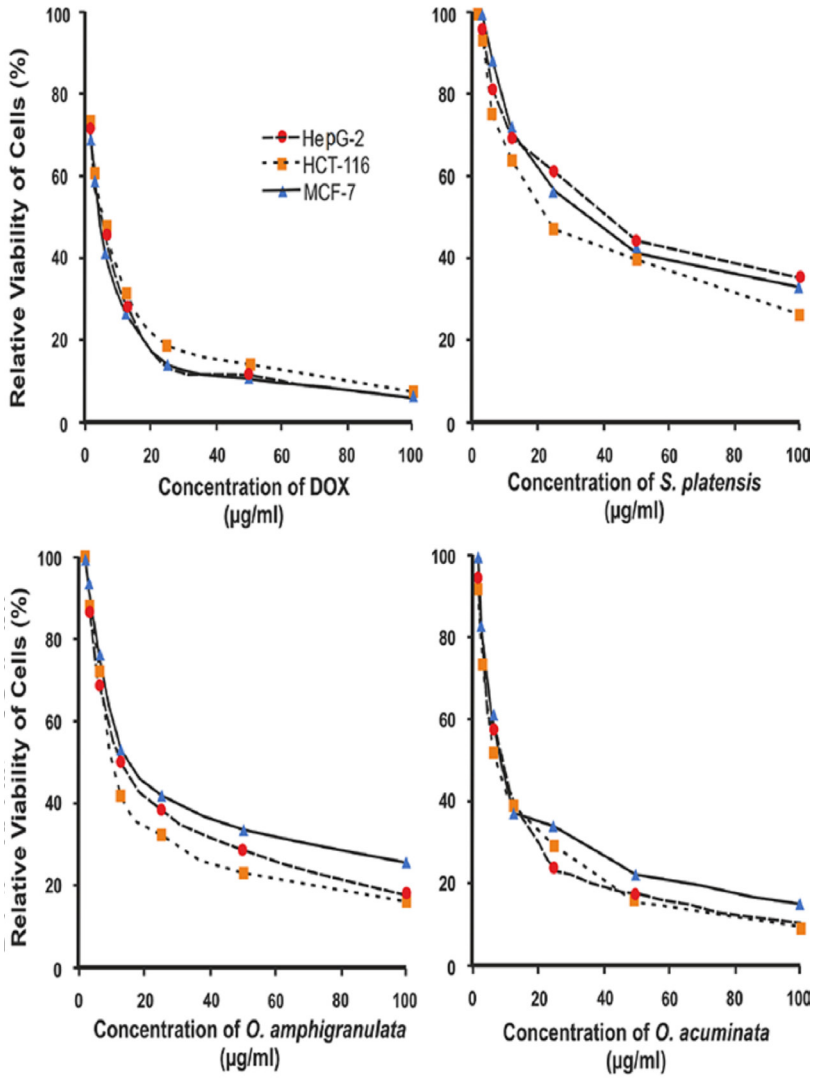

Figure 1. Cytotoxicity assay of cyanobacterial concentrations on different cell lines by MTT method.

Table V. Cytotoxicity (IC_{50}) of the tested cyanobacterial methanolic extracts on different cell lines*.

Methanolic extracts	*In vitro* Cytotoxicity IC50 (µg/ml)		
	HePG2	HCT-116	MCF-7
Doxorubicin	4.50^{aA} ± 0.2	5.23^{aA} ±0.2	4.17^{aA} ±0.2
S. platensis	41.07^{aC} ± 3.0	26.96^{bC} ±2.2	37.80^{cC} ±2.5
O. acuminata	8.91^{aA} ± 0.8	8.43^{aA} ±0.6	11.16^{bD}±1.2
O. amphigranulata	16.31^{aB} ± 1.6	13.58^{bB} ±1.4	21.29^{cB} ±1.9

***IC_{50} (µg/ml): 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic). Difference in superscript letters in each row or column indicate significance at $p \leq 0.05$ level.**

acids content was noticeable (20.003%) of which 9-Hexadecenoic acid, methyl ester, (Z), Hexadecanoic acid, methyl ester, ç-Linolenic acid, methyl ester, 9, 12-Octadecadienoic acid (Z,Z)-, methyl ester and 9-Octadecenoic acid, methyl ester, (E). The GC-MS profile also contained valuable active compounds of 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol alcohol, 3.444%) and Diisooctyl phthalate (2.166%). In addition to betaine, guanidine, L-Isoleucine, N-benzoyl, Benzaldehyde, Oxime-, methoxy-phenyl and m-Xylene in small ratios.

Results listed in Table VII showed the GC-MS profile of *O. acuminata* methanolic extract. Alcohols of 1-Propanol and 2-Methyl-1-propanol (isobutanol) were present forming 2.821% of the total recorded peak areas in the chromatogram. Fatty acids and their esters were of pronounced ratios recording 72.763% of which 11-Octadecenoic acid, methyl ester (38.344%), 9,12-Octadecadienoic acid (Z,Z), methyl ester (22.771%) and Hexadecanoic acid, methyl ester were forming the bulk percentage. Other valuable components were 3-Aminobutanoic acid, á-Hydroxyisovaleric acid, L-Isoleucine, N-benzoyl, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol alcohol), Diisooctyl phthalate, Octacosane and Heptacosane hydrocarbon. In addition to L-(+)-Ascorbic acid 2, 6-dihexadecanoate.

Results in Table VIII listed the biologically active components of *O. amphigranulata* methanolic extract. Most of these compounds were of valuable medicinal activities. Alcohols of 1-Propanol, 2-Methyl-1-propanol (isobutanol) and 1-Butanol, 3-methyl- constituted 9.37% of the total recorded peak areas in the chromatogram in addition to 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Phytol alcohol, 2.978%). Fatty acids and their methyl esters were obvious recording 35.382% of which Hexadecanoic acid, methyl ester, 9-Hexadecenoic acid, methyl ester, (Z)-, 11-Octadecenoic acid, methyl ester, Myristic acid and cis-10-Nonadecenoic acid were the dominant. Phenol compounds were represented by Cyclotrisiloxane, hexamethyl (1.040%) and Phenol, 2,4-bis(1,1-dimethylethyl)-(1.424%). The extract also contained other biologically active biomolecules as (ñ)-3-Hydroxybutyric acid (12.85%), D-2-Aminobutyric acid (0.768%), Benzoyl bromide (3.98%), p-Xylene (0.695%), Esculetin (0.692%), Heptadecane (0.724%) and Diisooctyl phthalate (2.866%).

The GC-MS profiles revealed that methanolic extracts of *S. platensis*, *O. acuminata* and *O. amphigranulata* were represented consistent sources of bioactive compounds. The ingredient ratios were different between these species. However, there were basic compounds such as fatty acids, alcohols including phytol, phthalate and hydrocarbons

which were commonly abundant and could be responsible for the recorded biological activity of these species (Tables VI, VII and VIII). The saturated and unsaturated fatty acids were of concentrated ratios in *O. acuminata* extract while were more distributed with lower ratios in *O. amphigranulata* extract. The mechanism of fatty acids as antimicrobial agent may be through disruption of the microorganism cellular membrane, leakage and/or reduction of nutrient uptake and inhibiting cellular respiration. According to (Kumar et al. 2011), the fatty acids may diffuse into the peptidoglycan meshwork of the microbial cell wall causing disruption of the cellular membrane and its disintegration. As reported by (Desbois et al. 2009), fatty acids cause peroxidative stress to the microbial cells. Fatty acids longer than 10 carbon atoms in chain length, such as palmitoleic and oleic acids, may induce lysis of microbial cell protoplast. The polyunsaturated arachidonic acid exhibited a strong activity against methicillin resistant *S. aureus* (MRSA), *E. coli, P. aeruginosa* and *S. epidermidis* (Smith et al. 2010). Different volatile compounds from *S. platensis* extracts exhibited antibacterial activities (El-Sheekh et al. 2014). Furthermore, Chauhan et al. (1992) reported that ether extract of *Oscillatoria sp*. demonstrated antibiotic activity which may be due to the isolated and identified saturated fatty acids (C14:0, C16:0 and C18:0). In the present study, the G +ve bacteria were more susceptible than the G -ve bacteria. Similar results were obtained with other studies (Bharat et al. 2013, Abo-State et al. 2015)**.** These differences may be due to the hydrophobicity of the G -ve bacteria outer membrane, of lipopolysaccharide composition, which acts as effective barrier against permeability of long chain fatty acids as antimicrobial substances. However, lauric, palmitic, linolenic, linoleic, oleic, stearic and myristic acids were known to have potential antibacterial and antifungal and to inhibit G-ve bacteria like *E. coli* with neither bacterial resistance to free fatty acids nor resistance phenotype has been developed (Desbois et al. 2009). Furthermore, antimicrobial activity of lipids may be attributed to the constituents of fatty acids and their ratios in a certain extract which could disrupt the oxidative phosphorylation, the electron transport chain and the energy production of microbial cells. Similarly, fatty acids may cause inhibition of enzyme activity, prompting peroxidation and auto-oxidation and degradation of microbial cells products (Desbois & Smith 2010). In the same context, the activity of antifungal compounds may be due to altering vital components of the fungal cell causing membrane permeability impairment, inhibition of spore germination and/ or inhibition of B-(1,3)-D-glucan synthesis. Furthermore, antifungal compounds may prevent lipid synthesis in the targeted fungal species by decreasing the ratio of unsaturated to saturated fatty acids or inhibiting the biosynthesis of ergosterols (Gupta et al. 2013).

The methanolic extracts of the tested cyanobacterial species proved potent chemo-preventive or chemotherapeutic agents. The cytotoxicity percentages were proportional to the increase in the extracts concentrations and dependent on the extract ingredients of each species, especially the fatty acids content. *O. acuminata* extract was rich in fatty acids methyl esters content (72.76%) of 11-Octadecenoic acid, 9,12-Octadecadienoic acid (Z,Z) and Hexadecanoic acid; in addition to the other biologically active compounds in the extract (Table VII), interacted together to manifest the highest antioxidant and anti- proliferative activity of the tested extracts. Although of more diversity in composition, the fatty acids profile of *O. amphigranulata* extract exhibited lower antioxidant and cytotoxic activity than *O. acuminata,* and so

applied for *S. platensis* extract. This may be due to the lower relative ratios of these fatty acids and their esters as 35.4% and 20% for *O. amphigranulata* and *S. platensis*, respectively. However, it should be noted that most of the principal bioactive components were common in the three chromatograms yet with different ratios. This implied the role of synergistic action between the ingredients in each extract to show its activity. According to (Kim et al. 2009), fatty acids as α-linolenic, linoleic and their derivatives exhibited pro-apoptotic and growth inhibitory activities on breast cancer-oestrogen positive cells. In their study using diethyl ether extracts of (10) cyanobacterial species, (Bharat et al. 2013) stated that saturated fatty acids of tetradecanoic, eicosanoic, docosanoic and heptadecanoic acids as well as PUFAs of α-Linolenic and Linoleic were abundant and responsible for the antimicrobial and cytotoxicity effects of these extracts. The mechanism(s) of action by which fatty acids trigger tumor cell death is still controversial. As described by (Dai et al. 2013), the tumor killing activity of PUFAs may be due to: (a) increased ROS generation (b) caspase enzymes activation (c) accumulation of lipid peroxidation toxic products leading to cell apoptosis (d) activation of peroxisome proliferator-activated receptors (PPARs) (e) modifying the expression of gene/ anti-oncogene, and (f) chromosomal damage stimulation of the cancer cells. Recently, Ahmed et al. (2017) recorded that *Plectonema* and *Cyanothece sp*. exhibited a pronounced cytotoxicity against hepatocellular carcinoma by inhibition of cell proliferation, stimulation of apoptosis and cell cycle arrest at diverse phases. Moreover, Ahmed et al. (2018) recorded that *Fischerella* BS1-EG crude extract has a pronounced influence on liver cancer (HepG-2), lung cancer (A549), colon cancer (HCT-116) and breast cancer (MCF-7).

CONCLUSION

In this study, different organic extracts of some soil cyanobacterial species showed a substantial antimicrobial activity. The methanolic extracts of *S. platensis, O. acuminata* and *O. amphigranulata* exhibited a significant antibacterial and antifungal action especially against *B. subtilis* and *A. flavus*. The phytochemical analysis revealed that these extracts contained many

Table VI. GC-MS analysis of *Spirulina platensis* methanolic extract**

Retention time	Compound Name/ Activity	Peak area%	Compound nature	Molecular formula
2.443	**1-Propyne, 3-chloro-**	3.524	Alkyne	C_3H_3Cl
2.483	**2-Propanone, 1-(1-methylethoxy)**	1.964	Ketone	$C_6H_{12}O_2$
2.503	**Betaine** organic osmolyte, treatment of hypochlorhydria, gastrointestinal disturbances	1.596	Alkaloid	$C_5H_{11}NO_2$
2.523	**Guanidine** Anti-inflammatory agents, antidiabetic, chemotherapy	1.589	Alkaloid	CH_5N_3
4.219	**1-Propanol** Antimicrobial	10.682	Alcohol	C_3H_8O
4.479	2-Methyl-1-propanol (isobutanol) Antimicrobial	4.063	Alcohol	$C_4H_{10}O$
5.195	**L-Isoleucine, N-benzoyl** Antitumor activity.	9.509	Amino acid derivative	$C_{13}H_{17}NO_3$

Table VI. Continuation

Retention time	Compound Name/ Activity	Peak area%	Compound nature	Molecular formula
5.270	**1-Pentanol** Antimicrobial	2.316	Alcohol	$C_5H_{12}O$
5.300	**1-Butanol, 2-methyl**- (S)- Antimicrobial	1.118	Alcohol	$C_5H_{12}O$
6.405	**p-Xylene** Antifungal, antioxidant, antimicrobial.	0.833	Aromatic hydrocarbon	C_8H_{10}
6.725	**Butane, 1,1-diethoxy-**	3.870	Alkane	$C_8H_{18}O_2$
6.930	**Benzaldehyde** Antimicrobial, antioxidant, insecticidal	1.030	Aromatic aldehyde	C_7H_6O
7.305	**m –Xylene** Antifungal, antioxidant, antimicrobial	1.248	Aromatic hydrocarbon	C_8H_{10}
7.485	**Oxime-, methoxy-phenyl-** Antifungal, antibacterial, anticancer, antitumor	0.667	Ether	$C_8H_9NO_2$
21.331	**Eicosane** Antifungal, antibacterial, antitumor, cytotoxic.	0.659	Alkane	$C_{20}H_{42}$
24.057	**9-Hexadecenoic acid, methyl ester, (Z)-** Antioxidant, hypochloesterolemic, haemolytic, antiandrogenic, 5-alpha reductase inhibitor	0.866	ω7fatty acid	$C_{17}H_{32}O_2$
24.322	**Hexadecanoic acid, methyl ester** Antioxidant, anti-inflammatory, antifibrinolytic, cancer preventive, hemolytic, antialopecic, hepatoprotective, antihistaminic, antiacne,	7.195	Palmitic acid	$C_{17}H_{34}O_2$
26.168	**ç-Linolenic acid, methyl ester** Antibacterial, anticandidal, anticancer, hypocholesterolemic, hepatoprotective, antiinflammatory, antihistaminic, antiarthritic, antieczemic, antiacne, antiandrogenic.	1.597	ω3fatty acid	$C_{19}H_{32}O_2$
26.363	**9,12-Octadecadienoic acid (Z,Z), methyl ester** Anti-cancer, antiinflammatory, analgesic, hypocholesterolemic, ulcerogenic, antiarthritic, hepatoprotective, antihistaminic.	4.007	ω6 Linoleic acid	$C_{19}H_{34}O_2$
26.443	**9-Octadecenoic acid, methyl ester, (E)-** Antibacterial, antitumor, antiinflammatory, 5-α reductase inhibitor, allergenic, anemiagenic, antialopecic, choleretic.	6.368	ω9 Oleic acid	$C_{19}H_{36}O_2$
26.573	**3,7,11,15-Tetramethyl-2-hexadecen-1-ol** Vitamins E and K1 precursor, anticancer, antimicrobial, anti-inflammatory, chemopreventive, vaccine formulations.	3.444	Diterpene (Phytol)	$C_{20}H_{40}O$
31.680	**Diisooctyl phthalate** Antimicrobial, antifouling, plasticizer, melanogenesis inhibitor.	2.166	Ether	$C_{24}H_{38}O_4$

**** (Source: Dr. Duke's Phytochemical and Ethnobotanical Databases).**

Table VII. GC-MS analysis of *Oscilatoria acuminata* methanolic extract**.

Retention time	Compound Name	Peak area %	Compound nature	Molecular formula
2.453	**3-Aminobutanoic acid** Induces plant disease resistance, affect virus, fungal and nematodes plant pathogens.	0.382	Amino acid	$C_4H_9NO_2$
2.513	**3-Hydroxyisovaleric acid** Dietary supplement in medical foods, increases lean body mass and muscle strength, inhibits breakdown of proteins in muscle tissue, promotes wound healing,	2.516	Organic acid	$C_5H_{10}O_3$
4.139	**1-Propanol***	1.818	Alcohol	C_3H_8O
4.404	**2-Methyl-1-propanol (isobutanol)***	0.667	Alcohol	$C_4H_{10}O$
5.129	**L-Isoleucine, N-benzoyl***	0.856	Amino acid	$C_{13}H_{17}NO_3$
5.205	**1-Pentanol***	0.336	Alcohol	$C_5H_{12}O$
23.912	**7,10-Hexadecadienoic acid, methyl ester** Antioxidant, antimicrobial, anti-inflammatory	0.685	Fatty acid	$C_{17}H_{30}O_2$
24.067	**9-Hexadecenoic acid, methyl ester, (Z)***	0.283	ω7 fatty acid	$C_{17}H_{32}O_2$
24.362	**Hexadecanoic acid, methyl ester***	7.104	Palmitic acid	$C_{17}H_{34}O_2$
26.453	**9,12-Octadecadienoic acid (Z,Z)-, methyl***	22.771	ω6 Linoleic acid	$C_{19}H_{34}O_2$
26.578	**11-Octadecenoic acid, methyl ester** Anti carcinogenic, anemiagenic, hypocholesterolemic, dermatitigenic.	38.344	Vaccenic acid	$C_{19}H_{36}O_2$
26.633	**3,7,11,15-Tetramethyl-2-hexadecen-1-ol***	1.299	Diterpene (phytol)	$C_{20}H_{40}O$
26.783	**Octadecenoic acid** Cosmetic, lubricant, hypocholesterolemic, surfactant, softening, perfumery, flavor.	2.444	Stearic acid	$C_{18}H_{34}O_2$
27.519	**Hexadecanoic acid, ethyl ester***	0.301	Palmitic acid	$C_{18}H_{36}O_2$
28.014	**Heptacosane** Antibacterial, antifungal, antioxidant, cytotoxic	0.616	Alkane	$C_{27}H_{56}$
28.494	**6-Octadecenoic acid, methyl ester, (Z)-** Cancer preventive, insectifuge**.**	0.501	Fatty acid	$C_{19}H_{36}O_2$
28.759	**Heptacosane***	0.871	Alkane	$C_{27}H_{56}$
28.989	**Eicosanoic acid, methyl ester** Alpha-glucosidase inhibitor activity, detergents, lubricants.	0.330	Arachidic acid	$C_{21}H_{42}O_2$
29.069	**Octacosane** Antibacterial, antifungal, antioxidant, cytotoxic	0.544	Alkane	$C_{28}H_{58}$
29.709	**Hexanedioic acid, bis (2-ethylhexyl) ester** A plasticizer	0.826	Ester	$C_{22}H_{42}O_4$
31.200	**L-(+)-Ascorbic acid 2,6-dihexadecanoate** Antioxidant, anti-inflammatory, antiscorbutic, cardio-protective, anticancer, flavor, wound healing property, antimutagenic, anti-infertility	0.412	Ester	$C_{38}H_{68}O_8$
31.730	**Diisooctyl phthalate***	2.638	Ester	$C_{24}H_{38}O_4$

**(Source: Dr. Duke's Phytochemical and Ethnobotanical Databases).* Activity was mentioned in table VI.

Table VIII. GC-MS analysis of *Oscilatoria amphigranulata* in methanolic extract**.

Retention time (RT)	Compound Name/ Activity	Peak area %	Compound Nature	Molecular formula
2.488	**(ñ)-3-Hydroxybutyric acid** An energy source for the brain when blood glucose is low, treatment of depression, anxiety, and cognitive impairment, polyesters, biodegradable plastics.	12.849	Organic acid	$C_4H_8O_3$
2.724	**D-2-Aminobutyric acid** A substrate for D-amino acid oxidase, biosynthesis of non-ribosomal peptides.	0.768	Amino acid	$C_4H_9NO_2$
3.944	**1-Propanol***	6.319	Alcohol	C_3H_8O
4.239	**2-Methyl-1-propanol (isobutanol)***	2.190	Alcohol	$C_4H_{10}O$
5.004	**Benzoyl bromide** Antibacterial, antifungal.	3.980	aromatic ketone	C_7H_5BrO
5.074	**1-Butanol, 3-methyl** Antimicrobial	0.861	Alcohol	$C_5H_{12}O$
5.450	**Cyclotrisiloxane, hexamethyl-** Antimicrobial, antioxidants.	1.040	Phenol	$C_6H_{18}O_3Si_3$
6.650	**Butane, 1,1-diethoxy-**	1.120	Alkane	$C_8H_{18}O_2$
7.250	**p-Xylene***	0.695	aromatic alkyl	C_8H_{10}
7.856	**Esculetin** Antitumor, anti-inflammatory, antimicrobial, antioxidant	0.692	Lactone	$C_9H_6O_4$
18.55	**Phenol, 2,4-bis(1,1-dimethylethyl)-** Antioxidant, antimicrobial.	1.424	Phenol	$C_{14}H_{22}O$
21.34	**Heptadecane** antibacterial, antifungal and anti-inflammatory	0.724	Alkene	$C_{17}H_{36}$
21.506	**Methyl myristoleate** A cytotoxic component, induces apoptosis and necrosis in human prostate cancer LNCaP cells.	2.456	Ester	$C_{15}H_{28}O_2$
21.686	**Myristic acid** Antioxidant, cancer preventive, nematicide, hypercholesterolemic, lubricant, cosmetic.	3.039	Fatty acid	$C_{14}H_{28}O_2$
22.547	**Pentadecanoic acid, methyl ester** Antioxidant, antimicrobial	1.586	Fatty acid	$C_{16}H_{32}O_2$
23.907	**7,10-Hexadecadienoic acid, methyl ester***	0.687	Fatty acid	$C_{17}H_{30}O_2$
24.072	**9-Hexadecenoic acid, methyl ester, (Z)***	6.509	ω7 fatty acid	$C_{17}H_{32}O_2$
24.192	**9-Hexadecenoic acid, methyl ester, (Z)***	3.837	ω7 fatty acid	$C_{17}H_{32}O_2$
24.342	**Hexadecanoic acid, methyl ester***	9.582	Palmitic acid	$C_{17}H_{34}O_2$
26.373	**9,12-Octadecadienoic acid (Z,Z)-, methyl***	1.357	ω6 Linoleic acid	$C_{19}H_{34}O_2$
26.448	**9-Octadecenoic acid, methyl ester, (E)***	1.184	ω9 Oleic acid	$C_{19}H_{36}O_2$

Table VIII. Continuation

26.518	**11-Octadecenoic acid, methyl ester***	5.145	Vaccenic acid	$C_{19}H_{36}O_2$
26.583	**3,7,11,15-Tetramethyl-2-hexadecen-1-ol ***	2.978	Diterpene (Phytol)	$C_{20}H_{40}O$
26.798	***cis*-10-Nonadecenoic acid** Potential antitumor activity, prevent LPS-induced tumor necrosis factor, inhibit p53 activity.	0.851	Fatty acid	$C_{19}H_{36}O_2$
31.695	**Diisooctyl phthalate ***	2.866	Esters	$C_{24}H_{38}O_4$

****(Source: Dr. Duke's Phytochemical and Ethnobotanical Databases). * Activity was mentioned in tables VI or VII.**

compounds of antimicrobial and antioxidant activities. The results of *O. acuminata* extract recommended the potent anticancer activity of 90.7, 91.2 and 85.2% against HepG2, HCT-116 and MCF-7 cell lines, respectively. According to the results of GC-MS analysis, fatty acids and their esters were responsible for the antimicrobial and the antitumor activities of these extracts. In addition, considerable ratios of biologically active compounds like organic alcohols including phytol, benzene derivatives, hydrocarbons, phenolics and phthalates were also detected which contributed synergistically to manifest the recorded activities. However, further studies should be conducted to visualize the crude extracts from cyanobacteria as a safe, natural and low-cost source for medicinal uses and drug industry after compass clinical trials.

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