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

SALY F. GHEDA & GEHAN A. ISMAIL

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 showed the highest antioxidant activity (6.58 and 34.60 % using DPPH (2, 2'- diphenyl-1- picrylhydrazyl) and ABTS’ (2, 2'-azino-bis (3-ethybenzthiazoline-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, 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 polyunsaturated 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 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 μEm⁻²s⁻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 aeruginosa AE-4091, Escherichia 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 10⁶ 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:

\[
\text{Free radical scavenging } \% = \frac{(Ac - As)}{Ac} \times 100
\]

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:

\[
\left( \frac{A_{570 \text{ of treated samples}}}{A_{570 \text{ of untreated sample}}} \right) \times 100.
\]

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 unrelated 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 (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 | 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±0.3          | 12±0.1                 | 10±0.3               | 12±0.5             | 13±0.3             | NA                  | NA                  |
|                       | Hexane    | 11±0.02         | NA                     | 9±0.05               | 11±0.1             | 12±0.2             | NA                  | NA                  |
|                       | Methanol  | 14±0.04         | 10±0.03                | 10±0.03              | NA                 | 19±0.2             | NA                  | NA                  |
| N. muscorum           | Ethyl acetate | 11±0.01         | NA                     | 9±0.1                | NA                 | NA                 | NA                  | NA                  |
|                       | Hexane    | 13±0.4          | 13±0.4                 | 10±0.4               | NA                 | 11±0.2             | 13±0.1             | NA                  |
|                       | Methanol  | 14±0.1          | 14±0.1                 | 9±0.1                | NA                 | 12±0.2             | 14±0.6             | 17±0.3             |
| N. linchiae           | Ethyl acetate | 13±0.04         | NA                     | 13±0.02              | 15±0.04            | 17±0.3             | 12±0.1             | 15±0.02            |
|                       | Hexane    | 13±0.06         | NA                     | 12±0.03              | 13±0.04            | 15±0.6             | 18±0.3             | 22±0.4             |
|                       | Methanol  | 14±0.3          | 14±0.3                 | 12±0.04              | 20±0.01            | 22±0.2             | 18±0.3             | 26±0.3             |
| O. acuminata          | Ethyl acetate | NA              | NA                     | 13±0.01              | 15±0.04            | 17±0.3             | NA                 | NA                  |
|                       | Hexane    | NA              | NA                     | 12±0.01              | 13±0.04            | 15±0.6             | 18±0.3             | 22±0.4             |
|                       | Methanol  | 14±0.6          | 17±0.02                | 12±0.04              | 20±0.01            | 22±0.2             | 18±0.3             | 26±0.3             |
| O. amphigranulata     | Ethyl acetate | 16±0.05         | NA                     | 16±0.02              | 15±0.05            | 19±0.4             | 25±0.02            | 31±0.02            |
|                       | Hexane    | 18±0.05         | NA                     | 21±0.02              | 18±0.01            | 27±0.06            | 30±0.03            | 31±0.02            |
|                       | Methanol  | 18±0.05         | NA                     | 20±0.02              | 19±0.04            | 27±0.06            | 30±0.03            | 31±0.02            |
| S. platensis          | Ethyl acetate | 13±0.1          | 13±0.2                 | 15±0.03              | 19±0.02            | 14±0.2             | NA                 | NA                  |
|                       | Hexane    | 12±0.01         | 10±0.01                | 16±0.01              | 23±0.04            | 24±0.01            | 21±0.03            | 27±0.01            |
|                       | Methanol  | 16±0.3          | 17±0.02                | 19±0.3               | 24±0.01            | 24±0.01            | 21±0.03            | 27±0.01            |
| Ampicillin            |           | 26±0.3          | 29±0.1                 | 28±0.2               | 29±0.4             | 30±0.3             | --                 | --                 |
| Fluconazole           |           | --              | --                     | --                   | --                 | 28±0.4             | 32±0.6             | --                 |

NA, no activity recorded. Difference in superscript letters in each row or column indicate significance (p ≤ 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 (20 mm). 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. amphigranulata different extracts (except for E. coli MG-1655 with the hexane extract). The highest inhibition zones were recorded by the methanolic extracts of O. amphigranulata as 31, 27 and 25 mm 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 angustissima 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 (Bílková 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±1.5        | 6.25±1.3              | 9.37±0.5              | 9.37±0.5          | 12.5±1.5            | 4.68±1.5            | 1.56±0.2           |
| O. acuminata        | 25.0±1.5        | 9.37±1.0              | 6.25±1.3              | 37.5±2.5          | 18.75±1.1           | 6.25±1.3            | 3.13±0.3           |
| O. amphigranulata   | 4.68±0.7        | 3.13±0.3              | 2.34±1.0              | 3.13±0.3          | 2.34±1.0            | 1.17±0.2            | 0.78±0.5           |
| Ampicillin          | 1.56±0.2        | 2.34±1.0              | 1.17±0.2              | 0.78±0.5          | 1.17±0.2            | 1.17±0.2            | 0.58±1.0           |
| Fluconazole         | ----            | ----                  | ----                  | ----              | ----                | ----                | ----               |

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)         | 18.38±0.3    | 32.63±1.3    | 15.69±0.5         |

*+ = Presence of constituents; – = Absence of constituents.*
glycosides (Table III), it may react 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<sub>50</sub> value (the concentration that caused 50% loss of the cell monolayer) of *O. acuminata* extract was of insignificant difference at p ≤ 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<sub>50</sub> value with HepG2 (16.3 µg/ml), HCT-116 (13.6 µg/ml) cell lines and moderate IC<sub>50</sub> value with MCF-7 cell line (21.29 µg/ml). For *S. platensis* methanol extract, moderate IC<sub>50</sub> 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 ± 0.62            | 49.30 ± 1.2           |
| *S. platensis*     | 2.41 ± 0.93            | 17.50 ± 0.62          |
| *O. acuminata*     | 6.58 ± 0.62            | 34.60 ± 0.95          |
| *O. amphigranulata*| 5.48 ± 0.31            | 23.20 ± 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.](image-url)
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 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%).

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 chemopreventive 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

| Retention time | Compound Name/ Activity | Peak area% | Compound nature | Molecular formula |
|---------------|-------------------------|------------|-----------------|------------------|
| 2.443         | 1-Propyne, 3-chloro-     |            | Alkyne          | C3H3Cl           |
| 2.483         | 2-Propanone, 1-(1-methylethoxy) |     | Ketone          | C3H7O2           |
| 2.503         | Betaine                 |            | Alkaloid        | C5H11NO2         |
|               | organic osmolyte, treatment of hypochlorhydria, gastrointestinal disturbances | | | |
| 2.523         | Guanidine               |            | Alkaloid        | CH3N3            |
|               | Anti-inflammatory agents, antidiabetic, chemotherapy | | | |
| 4.219         | 1-Propanol              |            | Alcohol         | C3H8O            |
|               | Antimicrobial            |            |                 |                  |
| 4.479         | 2-Methyl-1-propanol (isobutanol) |   | Alcohol         | C3H8O            |
|               | Antimicrobial            |            |                 |                  |
| 5.195         | L-Isoleucine, N-benzoyl  |            | Amino acid derivate | C6H11NO3        |
|               | Antitumor activity.     |            |                 |                  |

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

| Retention time | Compound Name/ Activity | Peak area% | Compound nature | Molecular formula |
|----------------|-------------------------|------------|-----------------|-------------------|
| 5.270          | 1-Pentanol              | 2.316      | Alcohol         | C₅H₁₂O            |
| 5.300          | 1-Butanol, 2-methyl-(S)- | 1.118      | Alcohol         | C₅H₁₂O            |
| 6.405          | α-Xylene                | 0.833      | Aromatic hydrocarbon | C₈H₁₀     |
| 6.725          | Butane, 1,1-diethoxy-   | 3.870      | Alkane          | C₅H₁₀O₂           |
| 6.930          | Benzaldehyde            | 1.030      | Aromatic aldehyde | C₆H₊O            |
| 7.305          | m-Xylene                | 1.248      | Aromatic hydrocarbon | C₈H₁₀     |
| 7.485          | Oxime-, methoxy-phenyl- | 0.667      | Ether           | C₈H₉NO₂           |
| 21.331         | Eicosane                | 0.659      | Alkane          | C₂₀H₄₂            |
| 24.057         | 9-Hexadecenoic acid, methyl ester, (Z)- | 0.866 | ω7fatty acid | C₁₉H₃₂O₂ |
| 24.322         | Hexadecanoic acid, methyl ester | 7.195 | Palmitic acid | C₁₉H₃₄O₂ |
| 26.168         | γ-Linolenic acid, methyl ester | 1.597 | ω3fatty acid | C₁₉H₃₄O₂ |
| 26.363         | 9,12-Octadecadienoic acid (Z,Z), methyl ester | 4.007 | ω6 Linoleic acid | C₁₉H₃₄O₂ |
| 26.443         | 9-Octadecenoic acid, methyl ester, (E) | 6.368 | ω9 Oleic acid | C₁₉H₃₂O₂ |
| 26.537         | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 3.444 | Diterpene (Phytol) | C₃₀H₄₀O |
| 31.680         | Diisooctyl phthalate     | 2.166      | Ether           | C₂₈H₄₈O₄          |

* (Source: Dr. Duke's Phytochemical and Ethnobotanical Databases).
| Retention time | Compound Name | Peak area % | Compound nature | Molecular formula |
|----------------|---------------|-------------|-----------------|-------------------|
| 2.453          | 3-Aminobutanoic acid | 0.382 | Amino acid | C₄H₆NO₂ |
| 2.513          | 3-Hydroxyisovaleric acid | 2.516 | Organic acid | C₂H₈O₃ |
| 4.139          | 1-Propanol* | 1.818 | Alcohol | C₃H₈O |
| 4.404          | 2-Methyl-1-propanol (isobutanol)* | 0.667 | Alcohol | C₄H₁₀O |
| 5.129          | L-Isoleucine, N-benzoyl* | 0.856 | Amino acid | C₅H₇NO₃ |
| 5.205          | 1-Pentanol* | 0.336 | Alcohol | C₅H₁₀O |
| 7.10-Hexadecadienoic acid, methyl ester | 0.685 | Fatty acid | C₁₇H₃₄O₂ |
| 24.067         | 9-Hexadecenoic acid, methyl ester, (Z)* | 0.283 | ω7 fatty acid | C₁₈H₃₄O₂ |
| 24.362         | Hexadecanoic acid, methyl ester* | 7.104 | Palmitic acid | C₁₈H₃₆O₂ |
| 26.453         | 9,12-Octadecadienoic acid (Z, Z)-, methyl* | 22.771 | ω6 Linoleic acid | C₁₉H₃₈O₂ |
| 26.578         | 11-Octadecenoic acid, methyl ester | 38.344 | Vaccenic acid | C₁₁₀H₂₄O₂ |
| 26.633         | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol* | 1.299 | Diterpene (phytol) | C₂₅H₄₀O |
| 26.783         | Octadecenoic acid | 2.444 | Stearic acid | C₁₈H₃₆O₂ |
| 27.519         | Hexadecanoic acid, ethyl ester* | 0.301 | Palmitic acid | C₁₈H₃₄O₂ |
| 28.014         | Heptacosane | 0.616 | Alkane | C₁₉H₃₈ |
| 28.494         | 6-Octadecenoic acid, methyl ester, (Z)- | 0.501 | Fatty acid | C₁₉H₃₈O₂ |
| 28.759         | Heptacosane* | 0.871 | Alkane | C₂₁H₄₄ |
| 28.989         | Eicosanoic acid, methyl ester | 0.330 | Arachidic acid | C₂₂H₄₄O₂ |
| 29.069         | Octacosane | 0.544 | Alkane | C₂₃H₄₈ |
| 29.709         | Hexanedioic acid, bis (2-ethylhexyl) ester | 0.826 | Ester | C₂₇H₅₀O₄ |
| 31.200         | L-(+)-Ascorbic acid 2,6-dihexadecanoate | 0.412 | Ester | C₃₀H₄₀O₈ |
| 31.730         | Diisooctyl phthalate* | 2.638 | Ester | C₄₀H₇₀O₄ |

**(Source: Dr. Duke’s Phytochemical and Ethnobotanical Databases).** Activity was mentioned in table VI.
Table VIII. GC-MS analysis of *Oscillatoria amphigranulata* in methanolic extract**.

| Retention time (RT) | Compound Name/ Activity | Peak area % | Compound Nature | Molecular formula |
|---------------------|--------------------------|-------------|-----------------|-------------------|
| 2.488               | (ii)-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 | C₄H₈O₃ |
| 2.724               | D-2-Aminobutyric acid A substrate for D-amino acid oxidase, biosynthesis of non-ribosomal peptides. | 0.768 | Amino acid | C₄H₉NO₂ |
| 3.944               | 1-Propanol* | 6.319 | Alcohol | C₃H₇O |
| 4.239               | 2-Methyl-1-propanol (isobutanol)* | 2.190 | Alcohol | C₃H₇O |
| 5.004               | Benzoyl bromide Antibacterial, antifungal. | 3.980 | Aromatic ketone | C₆H₅BrO |
| 5.074               | 1-Butanol, 3-methyl Antimicrobial | 0.861 | Alcohol | C₄H₈O |
| 5.450               | Cyclotrisiloxane, hexamethyl- Antimicrobial, antioxidants. | 1.040 | Phenol | C₃H₆O₂Si₃ |
| 6.650               | Butane, 1,1-diethoxy- | 1120 | Alkane | C₄H₈O₂ |
| 7.250               | p-Xylene* | 0.695 | Aromatic alkyl | C₈H₁₀ |
| 7.856               | Esculetin Antitumor, anti-inflammatory, antimicrobial, antioxidant | 0.692 | Lactone | C₁₉H₂₂O |
| 18.55               | Phenol, 2,4-bis(1,1-dimethylethyl)- Antimicrobial. | 1.424 | Phenol | C₁₃H₂₂O |
| 21.34               | Heptadecane antibiotics, antifungal and anti-inflammatory | 0.724 | Alkene | C₁₇H₃₅ |
| 21.506              | Methyl myristoleate A cytotoxic component, induces apoptosis and necrosis in human prostate cancer LNCaP cells. | 2.456 | Ester | C₁₄H₂₆O₂ |
| 21.686              | Myristic acid Antioxidant, cancer preventive, nematicide, hypercholesterolemic, lubricant, cosmetic. | 3.039 | Fatty acid | C₁₄H₂₈O₂ |
| 22.547              | Pentadecanoic acid, methyl ester Antioxidant, antimicrobial | 1.586 | Fatty acid | C₁₅H₃₀O₂ |
| 23.907              | 7,10-Hexadecadienoic acid, methyl ester* | 0.687 | Fatty acid | C₁₅H₃₀O₂ |
| 24.072              | 9-Hexadecenoic acid, methyl ester, (Z)* | 6.509 | ω7 Fatty acid | C₁₅H₃₀O₂ |
| 24.192              | 9-Hexadecenoic acid, methyl ester, (Z)* | 3.837 | ω7 Fatty acid | C₁₅H₃₀O₂ |
| 24.342              | Hexadecanoic acid, methyl ester* | 9.582 | Palmitic acid | C₁₆H₃₂O |
| 26.373              | 9,12-Octadecadienoic acid (Z,Z), methyl* | 1.357 | Linoleic acid | C₁₈H₃₂O |
| 26.448              | 9-Octadecenoic acid, methyl ester, (E)* | 1.184 | Oleic acid | C₁₈H₃₄O |

**Notes:**
- * indicates that the compound is known to have significant biological activity.
- ** indicates that the table includes all compounds detected with their molecular formulas and nature.

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Table VIII. Continuation

| Compound                                    | Retention Time | 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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SALY F. GHEDA
https://orcid.org/0000-0002-4458-7945

GEHAN A. ISMAIL
https://orcid.org/0000-0002-3996-0047

Correspondence to: Saly Farouk Gheda
E-mail: Sally.gheda@science.tanta.edu.eg

Author contributions
Both authors contributed equally in the conception and design of the work. The practical part, analysis and interpretation of results were handled in equal share between the two authors, as well as the writing and revising of the manuscript.