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In vitro antioxidant and antibacterial activities of two fresh water Cyanobacterial species, *Oscillatoria agardhii* and *Anabaena sphaerica*

Azza M. Abd El-Aty*[1], Amal A. Mohamed[2], Farag A. Samhan[1]

[1]Water Pollution Research Department, National Research Centre, Cairo, Egypt, [2]Plant Biochemistry Dept., National Research Centre, Cairo, Egypt.

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ABSTRACT

Cyanobacteria are potential sources of biologically active compounds with antiviral, antibacterial, antifungal, and anticancer activities. In the present investigation, the effect of different solvents, including methanol, acetone, and water on the total phenolic, flavonoid, antioxidant and antibacterial activities of *Oscillatoria agardhii* and *Anabaena sphaerica* extracts were evaluated. The results showed that solvents with different polarities have various effects on phenolic content and antioxidant activity. Among the tested solvents, methanolic extract of *Oscillatoria agardhii* showed the highest antioxidant activity as well as the highest phenolic content. Organic extracts (with methanol or acetone) of the tested species actively inhibited the growth of bacteria compared to aqueous extracts. The highest antibacterial activity was detected by acetone extract of *Oscillatoria agardhii* against *Salmonella senftenberg*. By GC-MS analysis some important heterocyclic compound were identified in both *Oscillatoria agardhii* and *Anabaena sphaerica* crude extracts. This appeared to be responsible for such excellent antioxidant and antibacterial activity together with other unidentified compounds. Further exploration of antibacterial potential of cyanobacteria can open new horizons.

INTRODUCTION

Algae have a significant attraction as natural source of bioactive molecules with a broad range of biological activities, including antimicrobial, antiviral, anticancer, antioxidant, and anti-inflammatory effects (Tuney *et al.*, 2006 and Patra *et al*., 2008). Algae contain minerals, polysaccharides, amino acid derivatives, carotenoids, and phenolic compounds. Some of these compounds can display antioxidant properties at very low concentrations (Yuan and Walsh, 2006). Microalgae have the potential to produce natural bioactive compounds (in culture), which are difficult to be produced by chemical synthesis (Goud *et al*., 2007). Cyano-bacteria are morphologically, physiologically, and metabolically very diverse group, which makes them as a promising group of organisms for research on drugs discovery. Additionally, the bioactive products are active against bacteria, fungi, and virus (Abed *et al*., 2011 and, Rama-Murthy *et al*., 2012). Even though cyanobacteria are beneficial, they are often regarded as an environmental nuisance, which are hazardous to both humans and aquatic organisms. Cyanobacterial pigments are not only used as nutritional ingredients and natural dyes for food and cosmetics, but also used as pharmaceuticals and fluorescent markers in biomedical research (Venugopal *et al*., 2005). Antioxidants play an important role in inhibiting and scavenging radicals, thus providing protection to humans against infections and degenerative diseases.

The two most commonly used synthetic antioxidants; butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are restricted because of their toxicity and DNA damage induction. Therefore, natural antioxidants from plant and algal extracts have attracted much attention because of safety. Recent researches have been interested in finding novel antioxidants to combat and / or prevent reactive oxygen species (ROS) mediated diseases. Algae generally have higher antioxidant activity due to higher contents of non-enzymatic antioxidant components, including ascorbic acid, reduced glutathione, phenols, and flavonoids (Wu *et al*., 2010). Natural antioxidant compounds exhibit their antioxidant activity through various mechanisms; including chain breaking (by donation of hydrogen atoms or electrons that convert free radicals into more stable species) and decomposing lipid peroxides into stable final products (Hussain *et al*., 2008).

* *Corresponding Author*
Email: azzamy@hotmail.com, Phone: +201222338835

The ability of algae to produce antimicrobial substances could be used not only as a defense agent (against pathogens) but also as pharmaceutical bioactive natural compounds. Though much is known about the chemistry and the antimicrobial action of several phytochemicals, very few reports are available on the possible mechanism of action. For phenols and phenolic compounds, an injury of membrane functions has been proposed as a mechanism of action (Sashidhar, 2002). The present work aims to evaluate the antioxidant and antibacterial activities of two algal species (*Oscillatoria agardhii and Anabaena sphaerica*) along with studying total phenolic and flavonoid content of the extracts using different solvents (methanol, acetone, and water) as well as identifying their chemical profiles using GC/MS.

MATERIALS AND METHODS

Chemicals

Folin-ciocalteu reagent, gallic acid and methanol were purchased from Merck Company (Darmstadt, Germany). DPPH (2,2-diphenyl-1-picrylhydrazyl) and quercetin (QU) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents such as aluminum chloride and sodium hydroxide were obtained from BDH, Dorset, UK. The HPLC-grade organic solvents were obtained from Merck. All other chemicals were of analytical-grade purity and available from the National Research Centre, Dokki, Egypt.

Collection and Growth Condition of Algal Strains

Two algal species were selected to evaluate their antioxidant and antibacterial activity against some species of bacteria. These algae belong to cyanobacteria, *Anabaena sphaerica* (N_2-fixing blue algae) and *Oscillatoria agardhii*. The tested algal species were isolated from phytoplankton community structure of River Nile at Ismailia canal, Egypt. BG11 medium was used for maintenance of *Oscillatoria agardhii* (Carmichael, 1986) and Nitrogen free formula for *Anabaena sphaerica.* The cultured media were incubated at 30 ± 2 ºC without aeration and under continuous illumination of fluorescent lamps with intensity 2500 lux. The cultures were shaken every day to prevent algal cell clumping and adherence of algal cells to the containers.

Preparation of Algal Extracts

After 10-day cultivation period, exponentially grown algal cells were harvested, washed with distilled water (3 times), weighted, and used for the following analysis. The algal pellets (37 g) were extracted using serial Exhaustive Extraction Method (Das *et al*., 2010) with methanol, acetone, and water. All extracts were dried and weighed to estimate the concentration in 1 ml. Dried extracts were either reconstituted in ethanol or deionized water.

Estimation of Total Phenolic Content

Total phenolic (TP) contents were determined by the spectrophotometric method (Slinkard and Singleton, 1977). In brief, a 0.5 ml of each extract was made up to 3 ml with distilled water, and then mixed with 0.5 ml of Folin-Ciocalteu's phenol reagent. After 5 min, 2 ml of a 2 % Na_2CO_3 solution were added to the mixture and thoroughly mixed. The mixture was kept at 30 °C for 60 min in dark place, and then the absorbance was recorded at 650 nm. The TP was determined from extrapolation of calibration curve that was constructed by standard concentrations of gallic acid solution. Estimation of phenolic compounds was carried out in triplicate. The TP was expressed as milligrams of gallic acid equivalents (GAE) per g of dried sample.

Determination of Total Flavonoid Content

Total flavonoid (TF) was determined by a colorimetric method as described by Zhishen *et al*., (1999). A 0.5 ml of each extract was made up to 1 ml with methanol. Afterwards 0.4 ml of distilled water was added followed by 0.3 ml of 5 % $NaNO_2$ solution and the mixture was left for 5 min. Thereafter, 0.3 ml of (10%) $AlCl_3$ solution was added and allowed to stand for 6 min. Two ml of (1 M) NaOH solution was added to the mixture and the final volume was adjusted to 10 ml with distilled water. The mixture was thoroughly shaken and allowed to stand for 15 min. Absorbance of the reaction mixture was read at 510 nm. The concentrations of total flavonoids were determined as quercetin equivalents (mg/g of dry weight).

DPPH Radical Scavenging Activity Assay

Quantitative measurement of radical scavenging activity of algal extracts was carried out according to the method described by Blois (1958). Briefly, one ml of 0.1 m M 2, 2-dipheny-l-picryl-hydrazyl (DPPH) solution in methanol was added to 3 ml of methanolic extract prepared at different concentrations (50 – 150 µg/ml). Butyl-4-hydroxyanisole (BHA) was used as a positive control. Discoloration was measured (in triplicate) at 517 nm after incubation for 30 min. The capacity to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = [ADPPH - AS / ADPPH] ×100

where, ADPPH is the absorbance of the DPPH solution and AS is the absorbance of the solution when the sample extract was added.

Antibacterial Assay

Seven bacterial strains including Gram-negative strains, *Escherichia coli ATCC 13706*, *Salmonella typhimurium* ATCC6538, *Salmonella senftenberg*775w, ATCC43845, *Pseudomonas aeruginosa* ATCC10145, and Gram+ve strains; *Enterococcus faecalis* ATCC 43845, *Enterococcus facium* DSMZ[i] 25389, and *Staphylococcus aureus* ATCC43300 were tested using agar disc diffusion (ADD) bioassays. Bacterial strains were grown overnight in nutrient broth (Merck) at 37 ºC. The agar media were seeded by bacterial suspensions adjusted to 10^6CFU/ml. Sterile filter paper discs (6 mm diameter) were saturated with 750 µg/ml of the crude extracts. The Petri dishes were placed for 5 h at 4 °C till the metabolites release into the medium. The plates were incubated at 37 °C for 24 h. The antibacterial activity was determined by measuring the diameters of the clean inhibitory zone (IZ) around each paper disc **(**Jassbi *et al*., 2002).

Gas Chromatography/Mass Spectrometry (GC/MS)

The volatile constituents from methanol and acetone extracts of *Oscillatoria agardhii* and *Anabaena sphaerica* were analyzed by GC/MS using a Thermo Scientific Capillary Gas Chromatography (model Trace GC ULTRA) directly coupled to ISQ Single Quadruple MS. The dried extracts were re-dissolved in petroleum ether (40–60 °C). Soluble constituents from the organic fraction were collected using separating funnel. The fraction was re-dissolved in diethyl ether and subjected to GC/MS analysis. The GC/MS analysis was performed on a TG-5MS non-polar 5 % phenyl methyl polysiloxane capillary column (30m × 0.25 mm ID × 0.25 µm) under the following conditions: oven temperature program from 40 °C (3 min) to 280 °C at 5 °C/min, then isothermal at 280 ºC for 5 min, flow rate of carrier gas (Helium) was 1 ml/min, the injected sample volume was 1 µl, splitless injection technique, and ionization energy of 70 eV in the electron ionization (EI) mode. Identification was carried out by comparing the retention indices and fragmentation pattern in mass spectra with those of published mass spectra data (Fujise *et al*., 2010). In very few cases, identification of some components was conducted by means of commercial libraries (Wiley9 and NIST08).

Statistical Analysis

The experimental results were expressed as mean ± standard error of mean (SE) of three replicates using the website http://easycalculation.com/statistics/standard-error-calculator.php.

RESULTS AND DISCUSSION

Total Phenolic (TP) and Total Flavonoid (TF) Contents

Phenolic compounds, including flavonoids, phenolic acids, and tannins are considered to be the major contributors to the antioxidant property of higher plants. These compounds also possess diverse biological activities, such as anti-inflammatory, anti-atherosclerotic, and anti-carcinogenic activities (Fresco *et al*., 2006). The total phenolics and flavonoids of three different solvent extracts (methanol, acetone and water) of *Oscillatoria agardhii* and *Anabaena sphaerica* are presented in Table (1). The highest phenolic content was found in both methanol and acetone extracts (20.91 ± 0.21 and 16.23 ± 0.03 mg/g gallic acid equivalent) of *Oscillatoria agardhii* in comparison to aqueous extract. However *Anabaena sphaerica* species gave 14.81±0.02 and 5.27± 0.11 mg/g gallic acid equivalent, respectively. For flavonoid, the highest value of 12.11± 0.02 mg/g quercetin equivalent was observed in methanol extract of *Oscillatoria agardhii*, followed by the acetone and water extract of (11.09 ± 0.02 and 5.06 ± 0.07 mg/g quercetin equivalent) in *Oscillatoria agardhii*. The lowest flavonoid content was noticed from the water extract of *Anabaena sphaerica* (3.21 ± 0.03 mg/g quercetin equivalent). Variations in TP, and TF contents across species were observed using three solvents with different polarities, as shown in Table (1). The difference perhaps could be attributed to genetic factors between both algal species.

These variations could be attributed to the polarity of the solvents, the type of phenolic, and flavonoids mixtures present in each species (Hemalatha *et al*., 2013). Methanol was considered to be the best solvent for extraction of TP and TF. These findings agreed with the earlier investigation by Uma *et al.,* (2011) and they clearly explain that the methanolic extract found to be having higher phenolic content in *D. olivaceous* and flavonoid content was high in acetone extract of *C. humicola*. Flavonoids of marine algae are widely distributed with different biological activities which may lead to the useful adjunct for the treatment of multiple disease categories from marine resources (Markham, 1988). According to Manivannan *et al*., (2012) methanol extract of *Chlorella marina* exhibited higher activity which was followed by diethyl ether and hexane extracts. This may be due to the differences in the polarity of the solvents used. Results also showed that, among all the solvent; methanol and acetone were better solvents for effective extraction of phenolic compounds as compared to other solvent like water. This might be due to the higher solubility of phenolics in highly polar solvents. The way of determination of the level of total phenolic is not based on absolute measurements of the amounts of phenolic compounds, but is in fact based on their chemical reducing capacity relative to gallic acid. It is very important to point out that; there was a positive relationship between antioxidant activity and amount of phenolic compounds of the crude extracts (El-Baz *et al*., 2010).

Table 1: Total phenolic and flavonoid contents of *Oscillatoria agardhii and Anabaena sphaerica* in different solvent extracts.

Algal species	Solvents extract		
	Methanol	Acetone	Water
	Total phenolic (mg/g d.wt)		
Oscillatoria agardhii	20.91 ±0.21	16.23±0.03	8.04±0.02
Anabaena sphaerica	14.81±0.02	5.27±0.11	4.34±0.07
	Total flavonoid (mg/g d.wt)		
Oscillatoria agardhii	12.11±0.04	11.09±0.02	5.06±0.07
Anabaena sphaerica	3.54±0.01	4.52±0.09	3.21±0.03

(n= 3, value= mean ± SE)

DPPH Radical Scavenging Activity

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Pinelo *et al*., 2004). In fact, free radical scavenging method (DPPH) show the reduction of alcoholic DPPH solutions in the presence of an hydrogen donating antioxidant (Koleva *et al*., 2002) and phenolic compound have been reported to be potent hydrogen donators to DPPH because of their excellence structural chemistry (Von Gadow *et al*., 1997).The reduction capability of DPPH radical is determined by the decrease in its absorbance at 5l7 nm, induced by antioxidants. The decrease in absorbance of DPPH radical is caused by antioxidants, because of the reaction between antioxidant molecules and radicals, progresses, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to yellow. Results obtained from antioxidant activity evaluation of two microalgae strains extracts by different solvents using DPPH free-radical reduction method at three different concentrations (50

µg/ml, 100 µg/ml, and 150 µg/ml) have been shown in Fig. (1). As depicted in this figure, the extract of *Oscillatoria agardhii* obtained by methanol solvent possessed the highest antioxidant activity, with (49.2 %) of inhibition of DPPH radical at 50µg/ml followed by the acetone and water extracts with activity (45.1% and 23.9 % of inhibition of DPPH radical, respectively) at the same concentration (50 µg/ml). We used BHA as standard synthetic antioxidant compound. The scavenging effects of methanolic extracts from *Oscillatoria agardhii* and BHA standard on the DPPH radical decreased in the order of BHA > methanol extract > acetone extract > water extract which were 92.4, 89.1, 82.5 and 52.9% at the concentration of 150 µg/ml, respectively. The free radical scavenging activity of methanolic extract was superior to that of other extracts. Similar to *Oscillatoria agardhii*, the *Anabaena sphaerica* was useful in DPPH radical scavenging activity and its antioxidant activity increased by increasing sample concentration. But generally, the antioxidant activity of *Anabaena sphaerica* was less in comparison to *Oscillatoria agardhii*. The scavenging effect of different solvent extracts of *Anabaena sphaerica* and BHA standard on the DPPH radical decreased in following order: BHA >methanol extract > acetone extract >water extract having inhibition value, 92.4, 70.7, 64.7, 48.1 % respectively at 150 µg/ml as shown in (Fig 2). As illustrated in Fig (1, 2), it was found that the methanolic extracts of *Oscillatoria agardhii* and *Anabaena sphaerica* had a noticeable effect on scavenging free radicals. However, the scavenging effect of BHA still higher than methanolic extracts of *Oscillatoria agardhii* and *Anabaena sphaerica* respectively**.**

The involvement of free radicals, especially their increased production, appears to be a feature of most, if not all human diseases, including cardiovascular disease and cancer (Deighton *et al*., 2000). However, Sivakumar and Rajagopal, (2011) reported that the highest antioxidant activity was observed in methanol extract from eight green algal species. However Uma *et al*., (2011) observed that the methanolic extracts displayed greater potential in all antioxidant assays when compared to ethanolic and acetone extracts of green microalgae *Desmococcus olivaceous* and *Chlorococcum humicola*. Similarly, Lee *et al*., (2010) reported that 80 % methanol extract and organic solvent fractions (n-hexane, chloroform, and ethyl acetate of *Halochlorococcum porphyrae* and *Oltamannsiellopsis unicellularis* showed notable activities indicating the higher efficacy for scavenging of free radicals.

These implications are important as radical scavengers may protect cell tissues from free radicals, thereby preventing diseases such as cancer. Free radicals such as superoxide radical (O_2), hydroxyl radical (OH^-) and other reactive oxygen species are associated with multistage carcinogenesis and mutagenesis. In previous study, several polysaccharides have been described to be potent antioxidants and there was a direct relationship between the uronic acid contents and the radical-scavenging effects of *Spriulina platensis* polysaccharide conjugates (Mendiola *et al*., 2007).

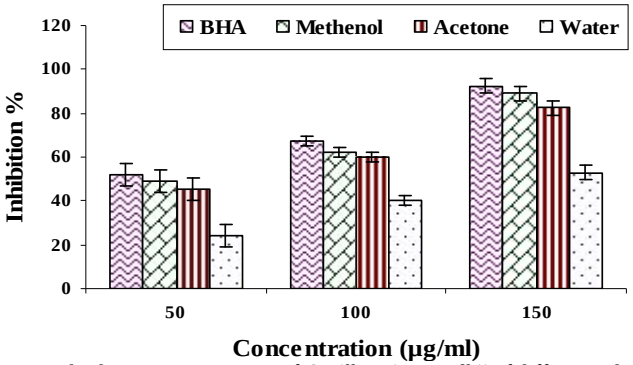

Fig. 1: Radical scavenging activity of *Oscillatoria agardhii* of different solvent extracts at different concentration by DPPH method. (n= 3, value= mean ± SE).

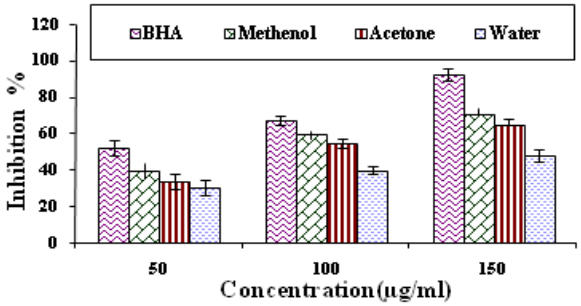

Fig. 2: Radical scavenging activity of *Anabaena sphaerica* of different solvent extracts at different concentration by DPPH method. (n= 3, value= mean ± SE).

Antibacterial Activity

The results obtained from the present study concerning the antibacterial effects of algal extracts against seven species of bacteria were recorded in Table (2). It was concluded that the diameter of inhibition zone depends mainly on the type of the algal species, type of solvent used and the tested bacterial species. The experimental analysis of antibacterial effects indicated that all tested bacterial strains showed higher sensitivity to the acetone extract of *Oscillatoria agardhii* with the highest antibacterial activity against *Salmonella senftenberg* (24 mm inhibition zone) as shown in Table (2). Whereas the methanol extract showed moderate activity against all bacterial species with highest value in case of *Enterococcus facium* (9 mm inhibition zone) and *Salmonella typhimurium* (7 mm inhibition zone). At the same time the results of acetone and methanol extracts of *Anabaena sphaerica* revealed their antibacterial effect against all tested bacterial except *Salmonella typhimurium*. These results go in harmony with those obtained by Volk and Furkert, (2006) , they found that some microalgae had highest biological activity against *Bacillus subtilis, Bacillus thungiensis, Bacillus megaterium, Escherichia coli, Pseudomonas aeruginosa, Candida tropicalis* and *Sauatromyces cerevisiae*. The present results revealed the ineffectiveness of aqueous extracts against tested bacterial species except for *Escherichia coli* in case of aqueous extract of *Oscillatoria agardhii*. In contrast, Sethubati and Prabu, (2010), reported that aqueous algal extracts of *Oscillatoria* sp, *Phormidium* sp and *Lyngbya majuscule* exhibited antimicrobial activity on both Gram positive and Gram negative organisms.

The alcoholic extracts had more antibacterial effects than aqueous extract that may be due to the presence of phenolic and flavonoids which had poor water solubility leading to their lowered concentration in aqueous extract rather than in the alcoholic one. It was also reported that the phenolic content are active as antibacterial against different types of microorganisms like *Salmonella typhi* (Ouattara *et al*., 2011) and the flavonoids are reported as active against several strains like *Streptococcus* (Shu *et al*., 2011); *Escherichia coli* and *Staphylococcus aureus* (Gao and Zhang, 2010). Additionally, Ishida *et al*., (1997) mentioned that the acetone extract of cyanobacteria revealed antibacterial activity on *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. The results of Abdo *et al*., (2012), also proved that methanol and water extracts of several species of fresh water algae including *Anabaena sphaerica*, *Chroococcus turgidus*, *Oscillatoria limnetica* and *Spirulina platensis* (Cyanobacteria) and *Cosmarium* leave (green algae) exerted antibacterial activity against *Escherichia coli*, *Salmonella typhimurium and Streptococcus faecalis*. In contrast, our results of water extract showed no antibacterial effect against all bacterial species except *Escherichia coli*. Thus, our results proved that acetone and methanol were the best solvents for extracting the antibacterial and antioxidant agents from *Oscillatoria agardhii* and *Anabaena sphaerica*. In our study, fresh algal sample was used for extraction. It has been reported that extracts obtained from fresh algae exhibit more antibacterial activity than extracts from air-dried algae because some substances may be lost during drying process. For example, it has been shown that extracts of fresh *Gracilaria gracilis* and *Ectocarpus siliculosus* exhibit antibacterial activity against *Staphylococcus aureus*, *Streptococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*, however, extracts from dried samples of these algae do not show antibacterial activity on mentioned tested bacteria (Tüney *et al*., 2006).

Table. 2: Antibacterial activity (inhibition zone) of *Oscillatoria agardhii* and *Anabaena sphaerica* extracts at 750 µg/ml concentration.

Bacterial strains	Diameter of inhibition zone (mm)					
	Oscillatoria agardhii			*Anabaena sphaerica*		
	Acetone	methanol	Water	Acetone	methanol	Water
Escherichia coli	6	5	3	5	2	N.D
Salmonella typhimurium	6	7	N.D	2	N.D	N.D
Pseudomonas aeruginosa	10	3	N.D	3	4	N.D
Salmonella senftenberg	24	3	N.D	6	2	N.D
Enterococcus facium	12	9	N.D	2	6	N.D
Enterococcus faecalis	11	5	N.D	2	2	N.D
Staphylococcus aureus	15	3	N.D	5	4	N.D

N. D: Not detected

GC/MS Analysis

The GC/MS analysis of *Oscillatoria agardhii* methanolic and acetone extracts resulted in the identification of 40 compounds; however a few of them were predominant (Table 3). The major compound was 5,11,17,23-Tetra-t-butyl-25,26,27,28-tetrahydroxycalix-4-arene which presented in both methanolic and acetone extracts at (2.55 and 2.94 %), respectively. In Table (4) the GC/MS analysis of the methanol and acetone extracts of *Anabaena sphaerica* resulted in many compounds which have diverse use. Compounds having anti-inflammatory, antibacterial, antifungal, properties have been identified. For methanolic extract, the highest concentrations were cyclopentaneundecanoic acid, methyl ester (11.06 %), followed by methyl decadienoate (6.10%)**.** Whereas the major compounds estimated in acetone extract were, N-Methylyunaconitine-3-ol, (4.11%) followed by the 1-Pentanol (3.11%).This result is in agreement with a previous result of Castilho *et al*., (2012) who identified 1-hexacosanol, as one component of oregano essential oil using GC/MS. They also reported that non-esterified 1-hexacosanol could act as an antimicrobial and antioxidant compound. Moreover, the biological activity of some triterpenoid such as phytoene (PE) and phytofluene (PF) were reported by Engelmann *et al*., (2011).

Table. 3: Chemical composition of methanol and acetone extract of the *Oscillatoria agardhii* extracts using GC/MS analysis.

No.	Compounds	Composition (%)	
		Methanol	Acetone
1	Ç-Carotene,3',4'-didehydro- 1',2'dihydro-1',2'dihydroxy-	2.05	-
2	26,28-Dihydroxy-25,27-dioxaocta-4-ene-2,6-diynyl-p-tertbutylcalix[4]arene	1.93	-
3	5-(4-Hydroxyphenyl)-10,20-bis(3-methoxyphenyl)-15-propylporphyrin	1.72	-
4	8-O-Methyl-Falconerine	2.51	-
5	5"-(1,1-Dimethylethyl)-2,2',2",2"',2""-pentam ethoxy[1,1':3',1":3",1"':3"'.1""-quinquephenyl]-3,3""-dicarboxyaldehyde	2.26	2.11
6	5,10-bis-(3-aminophenyl)-15,20-diphenylporphyrin	2.32	2.41
7	Penitrem A	1.94	1.78
8	1-Hexacosanol	4	-
9	5,11,17,23-Tetra-t-butyl-25,26,27,28-tetrahydroxycalix-4-arene	2.55	2.94
10	Eicosanoic acid, methyl ester (CAS)	9.57	-
11	Cis-3,7-Dimethyl-1,3,6-octatriene	3.46	-
12	N-Methyl-yunaconitine-3-ol	2.21	2.04
13	Phytofluene	-	2
14	Lipo-3-episapelin A	-	4.62
15	Carotene,3,3',4,4'-tetradehydro-1,1',2,2'-tetrahydro1,1'-Dimethoxy-2,2'-dioxo-	-	2.78
16	2-Propenoic acid,2-methyl, dodecyl ester	-	1.76
17	Hexadecyl-phenol isomer	-	1.74
18	p-Nonylphenol	-	1.91
19	9-Octadecenal	-	4.01
20	1-Tetradecene	-	1.8
21	Tetra-tert-butyl-2,6-di-(3-propenyl)-3,7-dimethoxybicyclo[3.3.0]-octa-3,7-diene-2,4,6,8-dicarboxylate	2.32	1.85
22	N,N'-Dicyclohexyl-1-cyano-7-pyrrolidinylperylene-3,4:9,10-tetracar boxylic acid Bisimide	-	3.5

As it is well known that triterpenoid have antioxidant activit**y,** Inada *et al*., (1994) isolated Lipo-3-episapelin A (limonoid group) as a new triterpenoid derivative from *Trichilia connaroides* .Additionally, ginkgetinis known as a natural biflavone and was previously isolated from *Selaginella moellendorffii Hieron*,(Su *et al*., 2000), and the anticancer effect of ginkgetin was evaluated using MTT assay in three different human cell lines: ovarian adenocarcinoma (OVCAR-3), cervical carcinoma (HeLa) and foreskin fibroblast (FS-5). The authors reported that ginkgetin have cytotoxic effect in a human ovarian adenocarcinoma cell line. The methanol extract of the present work contains ginkgetin at

1.71%. Spectral data by GC/MS showed that a mixture of fatty acids characterized some bioactive compounds primarily consisting of 9-Octadecenal which may be involved in control of human pathogens, pests, termites and maggots (Manilal *et al*., 2011).

Moreover, based on Biacs and Daood, (2000) lycoxanthin had antioxidant activity. The acetone extract of *Anabaena sphaerica* contains lycoxanthin at 2.15 %.This study explores the goodness of both Cyanobacteria species which has a commendable sense of purpose and can be advised as a phytopharmaceutical importance.

Table. 4: Chemical composition of methanol and acetone extract of the *Anabaena sphaerica* extracts using GC/MS analysis.

No.	Compounds	Composition (%)	
		Methanol	Acetone
1	2,7,12,17-Tetramethyl-3,5:8,10:13,15:18,20-tetrakis-(2,2-dimethylpropano) porphyrin	2.45	-
2	Tetra-tert-butyl-2,6-di-(3-propenyl)-3,7-dimethoxybicyclo-[3.3.0]-octa-3,7-diene-2,4,6,8-dicarboxylate	2.16	1.90
3	3-Hydroxy-1-(4-{13-[4-(3-hydroxy-3-phenylacryloyl)phenyl]-tridecyl}-phenyl)-3-phenylprop-2-en-1-one	2.86	-
4	26,28-Dihydroxy-25,27-Dioxaocta-4-ene-2,6-diynyl-p-tert-butylcalix [4] arene	1.66	2.21
5	5,11,17,23-Tetra-t-butyl-25,26,27,28-tetrahydroxycalix-4-arene	2.07	-
6	Penitrem A	1.66	2.22
7	5à-Cholestan-7à-yl-4-(à-Hydroxyphenylmethyl) phenylacetate-25,à-ether	1.52	-
8	1,1-Di(4-methylcyclohexyl) dodecane	2.04	-
9	5,10-bis-(3-aminophenyl)-15,20-diphenylporphyrin	1.70	2.18
10	Cyclopentaneundecanoic acid, methyl ester	11.06	-
11	17-Octadecynoic acid	3.88	-
12	Methyl decadienoate or 11,14,17-Eicosatrienoic acid, methyl ester	6.10	-
13	Ginkgetin (CAS)	1.71	-
14	psi.,.psi.-Carotene,3,3',4,4'-tetradehydro-1,1',2,2'-tetrahydro1,1'-Dimethoxy-2,2'-dioxo-	1.40	-
15	N-Methylyunaconitine-3-ol	-	4.11
16	3-Acetoy-8-deacetoxy-yunaconitine	-	2.31
17	5,15-Bis-(3-methoxyphenyl)-10-phenyl-20-propylporphyrin	-	2.37
18	Lycoxanthin	-	2.15
19	1-Pentanol (CAS)	-	3.11
20	10,13,14,17-Tetraethyl-11,16-dimethylphenanthrolinoporphyrin	-	2.38

CONCLUSION

Extraction with different solvents could affect the yield of total phenols, antioxidant, and antibacterial activities of *Oscillatoria agardhii* and *Anabaena sphaerica*. The most efficient solvent for phenolic extraction and radical scavenging activity are methanol and acetone. The existing data is not sufficient to explain the mechanism of action; however, it may enrich the strength of comprehensive data of antioxidant activity of *Oscillatoria agardhii* and *Anabaena sphaerica*. The antibacterial activity may be due to the presence of terpenes or other constituents in both crude extract. Methanol and acetone extracts were bacteriostatic against the tested micro-organisms. It is suggested that further work is needed to isolate and identify bioactive compounds from both Cyanobacteria species, which might be useful for therapeutic purposes.

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