Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2019, 11(10):22-35

Research Article

ISSN: 0975-7384
CODEN(USA): JCPRC5

Bioactive Potentiality of Some Secondary Metabolites Extracted from Microalga *Spirulina platensis*

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ABSTRACT

Cyanobacteria inhabit a range of diverse and extreme habitats and have potential to produce an elaborate array of secondary metabolites with unusual structures and potent bioactivity. The concept of biological control for the health maintenance has received widespread attention during the last few years. Therefore, the main project of this investigation focuses for active phytochemical substances that could be used as antioxidants, antimicrobials agents, anticancer and cholesterol-lowering agents. To achieves this target, four proceeding treatments (soaking, autoclaving, NaCl 10% and soxhlet) and four different solvents (Methanol, acetone, chloroform and petroleum ether) used for preparation of Spirulina platensis extracts. The common major secondary compounds like, carbohydrate, lipid, phenol, flavonoids and tannins were extracted using four polar and nonpolar solvents. Of the four solvents tested, acetone was the best solvent for isolation of antimicrobial compounds, anticancer agents, antioxidants compounds and cholesterol-lowering agents from the tested algae followed by methanol. The soaking treatment was the best method for extraction the active compounds followed by autoclaving. The present study elaborates the bioactive content of Spirulina platensis during gas chromatography mass spectrometry (GC-MS) analysis.

Keywords: *Spirulina platensis*; Antimicrobial activity; Anticancer efficiency; Biomedical properties

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INTRODUCTION

Spirulina, a cyanobacterium, is commonly distributed in nature. Earlier interest in *Spirulina* was as being a resource of protein, vitamins, especially vitamin B_{12} and provitamin A (β-carotene) as well as fatty acids like γ-linolenic

acid. Latterly, more attention has been given to study its curative effects that include cholesterol reduction and nephrotoxicity by heavy metals, anticancer properties, protection against radiation, and enhancement of the immune system [1]. *Spirulina* also have other biological functions as antiviral, antibacterial, antifungal, and antiparasite activities [2].

It has been consumed by many countries as human food for many years because of their high protein content (35-65%) and nutritional value. At now, some countries are culturing it on a large scale [3].

Spirulina are present in different habitats, so it is the excellent material for investigation by the ecologists, physiologists, biochemists, pharmacists and molecular biologists. Accordingly, looking for *Spirulina* with antimicrobial activity has acquired significance in recent years. Many biologically active substances were recognized to be extracted by *Spirulina* [4-7]. In large-scale screening of marine microalgae and cyanobacteria have led to the isolation and chemical determination of over 15000 compounds, inclusive fatty acids, sterols, phenolic compounds, terpenes, enzymes, polysaccharides, alkaloids, and flavonoids. More recent investigation revealed that marine microalgae as considerable sources of antioxidant compounds with potential free radical scavenging activity [8,9].

Phenolic compounds can be doing as antioxidants by chelating metal ions, banning radical formation and enhance the antioxidant endogenous system. The expression “phenolic compound” characterizes several hundred molecules found in eaten plants which have on their structure a benzenic ring exchanged by, at least, one hydroxyl group [10]. Tannins are known as natural occurring polyphenolic compounds and are widespread through terrestrial and marine plants [11]. Also, flavonoids, the major group of phenolic compounds is famous to contain a broad spectrum of chemical and biological activities including antioxidant and free radical scavenging properties. [12].

At the present time, Antibiotic resistance bacteria are one of the appearing health problems in the world. Algae are worthy natural sources effective against infectious agents. Great efforts for the extraction and identification of bioactive compounds obtained from natural resources have been made throughout the world.

Marine algae and cyanobacteria have the best choice among natural resources within aquaculture. Checking bioactivity of algal crude extracts is mandatory in biomedical practice, where antibacterial [13] and antifungal [14].

Cancer is one of the most dangerous impendence to human health in the world and chemotherapy is still the standard treatment method till now. Currently, most of the anticancer drugs used in chemotherapy are cytotoxic to normal cells and cause immunotoxicity that influences not only tumor development, but also aggravates patient’s recovery. The detection and identification of new antitumor drugs with minimum side-effects on immune system have become by liver which is required for different functions, found in some foods. It is a substance that plays an essential role, either as parent compound of hormones, bile acids and vitamin D, or as a structural component of the cell membranes and it enhances their fluidity and permeability [15]. However, the overabundant of cholesterol is serious; it is embroiled as a danger factor for cardiovascular disease [16]. Excess of cholesterol is risk factor, cause of death worldwide, which responsible for the death of more than 17 million people per year or about 30% of all death worldwide and 25 million deaths are expected in 2020 [17]. *Spirulina* is used in the therapy of many diseases, including cholesterol regression [18,19] as well as to decrease body weight in humans [20]. The present

study was performed with the microalga *Spirulina platensis.* The study was performed with the following objectives: (1) To investigate the preliminary phytochemical constituents present in microalgal extracts by different treatments (2) To estimate the secondary metabolites of the selected alga. (3) To evaluate the biological activities of selected microalgal. (4) To reveal the chemical constituents in the microalgal extracts using GC–MS analysis.

MATERIALS AND METHODS

Test Cyanobacterium

The cyanobacterium *Spirulina platensis* (Nordstedt) Geitler (Oscillatoiales) was attained from the Culture Collection of the Algal laboratory, Faculty of Science, Alexandria University, Egypt. *Spirulina* was cultivated in Zarrouk's medium [21] under continuous illumination (35 µmol/m^2/s) with temperature adjusted to 30 ± 2.0°C in pH range from 9-10. Cells were harvested within stationary phase, i.e., after 24 days of incubation by filtration, washed thoroughly with distilled water and finally dried at 70°C until constant weight.

Extraction Methods

Four scenarios of *S. platensis* biomass extraction were applied (soaking, soxhlating, autoclaving and Na Cl pretreatment) using the following solvents petroleum ether, chloroform, acetone and methanol separately. *S. platinsis* dry biomass specimens (2 g) were soaked in the dark for 72 h at room temperature (cold percolation) with shaking. Then, the filtrates (crude extracts) were stored under 5°C until further uses. The second method of extraction, two grams of algal powdered material was extracted by soxhlet, using the same solvents as mentioned above for 10 h. Two grams of the dried algal material were mixed thoroughly with 10 mL dist. H_2O in 50 ml capacity conical flask and autoclaved at 121°C with for 15 min, after cooling the samples were dried and let to be extracted by the above mentioned solvents (soaking for 72 h.) separately. *S. platensis* biomass specimens (2 g) were primed in 10 % Na Cl solution for 24 hours, then centrifuged (3000 rpm for 5 min.), washed with dist. water and after drying samples were subjected to extraction by the above mentioned solvents.

Phytochemical Analysis

Detection of some phytochemical constituents was performed according to the standard protocol described [22] for screening presence of triterpenoids, steroids, saponins, cumarins, terpenoids, quinines, glycosides, Cardiac glycosides, Phlorotannins and Antharaquinones. Total flavonoids, tannins, phenolic compounds, reducing power, antioxidant capacity were determined [23-27]. Cholesterol reduction assay was investigated after the method of Richmond [27,28].

Antibacterial Activity Assay

The agar diffusion techniques were used to determine the antibacterial activity of various crude extract of *Spirulina platensis* against two, Gram positive bacteria *Staphylococcus aureus* and, *Bacillus subtilus* SH04, Gram negative bacteria *Escherchia coli* ATCC 8739 and *Salmonella sp.* Bacteria achieved from microbiology lab of GEBRI University of Sadat city Egypt» Briefly 50 µl of crude extracts were encumbered on sterile and air dried filter paper (Whitman No. 1, 3 mm in diameter). The papers were put on the surface of nutrient agar media and 10^5 CFU/ml of bacteria cells were strewn on the surface of the nutrients agar plate. The plates were incubated with 30°C, after 48

hrs, the clear zones were determined around the paper disc that stated, of antibacterial activity of *S. platensis* crude extract.

Cytotoxic Effect of *S. platensis* Crude Extract against Cancer Cell Line MCF-7,CaCo-2 and HepG-2

Cytotoxicity was determined by MTT [3-(4,5-dimetheylthiazol-2)-2,5-diphenyl tetrazolium bromide] assay according to Mosmann [29]. The cancer cell lines, Fetal Calf Serum (FCS) was supplied by Vacsera.

GC-MS Analysis of *S. platensis* Crude Extract

The chemical composition of the different *Spirulina* extracts were achieved using Agilent GC-MS-5975C with a Triple–Axis Detector organized with an auto sampler according to Musharraf et al. [30].

Statistical Analysis

Statistical analysis of results was expressed as standard error of means.

RESULTS

Phytochemical Screening of Alga

The quantitative phytochemical screening of different chemical compounds was investigated by four different treatments by using four different extracts of crude powder of *S. platensis* carried out in order to evaluate the presence of antimicrobial potentiality, anticancer, antioxidant activity and cholesterol reduction. Results were illustrated in Tables 1. showed the presence of saponins, quinines, cardiac glycosides, terpenoids, triterpenoids, coumarins and steroids in all extracts except for glycosides which was absent in all the extracts of all treatments. Also, antharaquinones absent in all extracts by autoclaving and soxhlet treatments as well as pholotannins and saponins are absent in all extracts by autoclaving treatment.

Table 1. Preliminary phytochemical screening of *Spirulina platensis* of various extracts by different treatments

Test	Autoclaving				NaCl10%				Soxhlet				Soaking			
	PE	Ch	Ac	Me	PE	Ch	Ac	Me	PE	Ch	Ac	Me	PE	Ch	Ac	Me
Saponins	-	-	-	++	-	+++	+++	+++	-	-	+++	+++	-	-	+++	+++
Quinines	+	+	+	+	+	+	++	+	+	+	++	++	+	+	++	++
Glycosides	-	-	-	-	-	-	++	++	-	-	-	-	-	-	-	-
Cardiac glycosides	+++	+++	+++	+	+++	+++	+++	++	+	++	+++	++	++	++	++	++
Terpenoids	-	++	+++	++	-	++	+++	++	+	++	+++	++	+	++	+++	++
Triterpenoids	++	++	++	++	++	+++	++	++	++	+++	+++	+++	++	+++	+++	++

Coumarins	++	+++	+++	+++	++	+++	+++	+++	+	+++	+++	+++	+	++	++	++
Steroids	+	++	+++	+++	-	++	+++	+++	++	++	+++	+++	++	++	+++	+++
Pholotannins	-	-	-	-	-	-	+	+	-	+	+	+	-	+	++	+
Antharaquinones	-	-	-	-	-	-	++	++	-	-	-	-	-	+	+	+

(+): low , (++): average , (+++):high and (-): absent.

Phytochemical Analysis of *Spirulina platensis*

Chemical analysis screening demonstrated that flavonoids, tannins and phenols are depicted in Figures 1 and 2 where acetone extracts were recorded the highest amounts of flavonoids and tannins. Flavonoids increased by the following descending order: autoclaving>soaking>soxhlet>NaCl (0.86, 0.77, 0.72 and 0.65 mg/g) respectively. Total amount of tannins increased in the following descending order: soaking>autoclaving>NaCl>soxhlet (0.92, 0.87, 0.62 and 0.46 mg/ g) respectively. Phenol amounts of acetone and methanol extracts, which recorded as the following soaking>soxhlet>autoclaving>NaCl 10% with the amount; 0.56, 0.43, 0.42 and 0.35 mg/ g in methanol extracts, respectively.

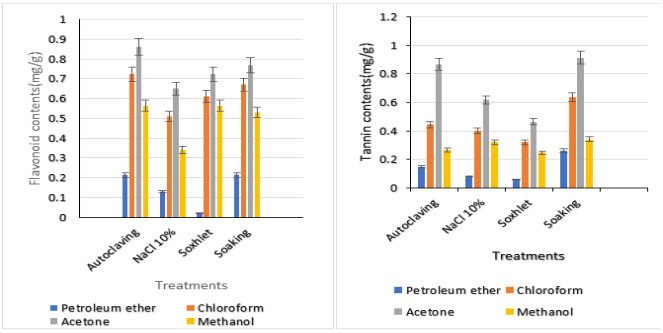

Figure 1. Flavonoid contents and tannin contents of *S. platensis* extracted by using different treatments and different solvents.(mean and standard deviation of three replicates are shown)

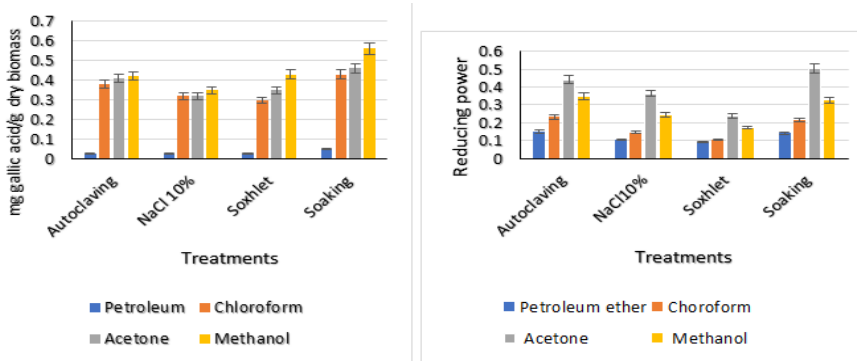

Figure 2. Phenol contents and Reducing power of *S. platensis* extracted by using different treatments and different solvents. (mean and standard deviation of three replicates are shown)

Antioxidant Activity

Reducing power assay: The reducing activity of different solvent extract of *S. platensis* illustrated in Figure 2. The highest amount of reducing power was recorded to acetone extract of the tested alga as follow: soaking>autoclaving>NaCl 10%>soxhlet, with value; 0.51, 0.44, 0.36 and 0.24 respectively. Followed by methanol extract, autoclaving>soaking>NaCl 10%>soxhlet, with value; 0.35, 0.33, 0.24 and 0.17 respectively. The minimum reducing power of petroleum ether extract, recorded in order: autoclaving>soaking>NaCl 10%>soxhlet, with the value 0.15, 0.14, 0.11 and 0.09 respectively.

Total antioxidant capacity (TAC): The total antioxidant activity of both methanol and chloroform extracts of the tested alga are comparable, while the maximum total antioxidant activity of acetone was recorded as following: soaking>autoclaving>NaCl 10%>soxhlet, with values; 0.643>0.613>0.551>0.550 mg ascorbic acid equivalent, respectively, at the same concentrations. The minimal total antioxidant activity was observed in petroleum ether extracts of four treatments at the same order as the following; soaking>autoclaving>NaCl 10%>soxhlet: 0.227>0.147>0.137>0.087mg ascorbic acid equivalent (Figure 3).

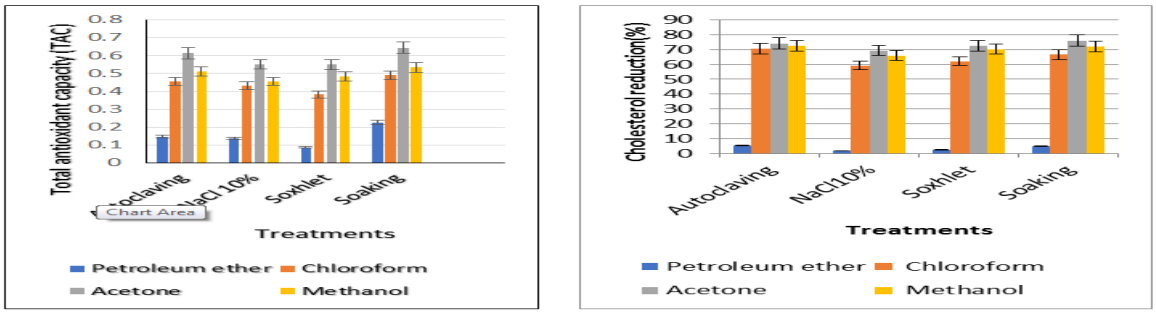

Figure 3. Total antioxidant capacity and Cholesterol reduction of S. platensis extracted by using different treatments and different solvents. (mean and standard deviation of three replicates are shown)

Antibacterial Activity

The antimicrobial activity of *Spirulina platensis* against *Aspergillus flavus*, *Aspergillus sp.*, *Staphylococcus aureus*, *Bacillus subtilus*, *Escherchia coli* and *Salmonella sp.* presented in Table 2. The agar diffusion method was used to assess the antimicrobial activity by determine the zone of inhibition against the tested fungi and bacteria. Acetone extracts of *Spirulina platensis* has showed the prominent antibacterial activity, whereas petroleum ether extracts recorded the minimum activities of all tested microorganisms.

Table 2. Antimicrobial activity of *Spirulina platensis* against human pathogens

	Autoclaving				**NaCl**				**Soxhlet**				**Soaking**			
	PE	**Ch**	**Ac**	**Me**	**PE**	**Ch**	**Ac**	**Me**	**PE**	**Ch**	**Ac**	**Me**	**PE**	**Ch**	**Ac**	**Me**
1	0.53 ± 0.01	0.63 ± 0.01	0.72 ± 0.01	0.67 ± 0.02	0.58 ± 0.01	0.64 ± 0.02	0.63 ± 0.01	0.58 ± 0.01	0.52 ± 0.01	0.63 ± 0.01	0.77 ± 0.01	0.59 ± 0.01	0.53 ± 0.01	0.63 ± 0.01	0.80 ± 0.01	0.72 ± 0.01

2	0.55 ± 0.02	0.86 ± 0.03	0.92 ± 0.01	0.76 ± 0.01	0.55 ± 0.02	0.73 ± 0.02	0.72 ± 0.01	0.65 ± 0.02	0.53 ± 0.03	0.77 ± 0.01	0.66 ± 0.01	0.63 ± 0.01	0.55 ± 0.01	0.89 ± 0.02	0.80 ± 0.01	0.72 ± 0.01
3	0.55 ± 0.02	0.86 ± 0.03	0.92 ± 0.01	0.76 ± 0.01	0.55 ± 0.02	0.73 ± 0.02	0.72 ± 0.01	0.65 ± 0.02	0.57 ± 0.03	0.78 ± 0.01	0.66 ± 0.01	0.72 ± 0.01	0.54 ± 0.01	0.89 ± 0.02	0.81 ± 0.01	0.63 ± 0.01
4	0.57 ± 0.03	0.75 ± 0.03	0.85 ± 0.03	0.72 ± 0.01	0.55 ± 0.03	0.58 ± 0.01	0.65 ± 0.03	0.55 ± 0.03	0.049 ± 0.01	0.75 ± 0.03	0.83 ± 0.03	0.65 ± 0.03	0.65 ± 0.03	0.82 ± 0.01	1.18 ± 0.04	0.75 ± 0.02

1: *Staphylococcus aureus,*2: *Bacillus subtilus*, 5: *Escherchia coli*, 6: *Salmonella sp.*

PE: Petroleum ether, Ch: Chloroform, Ac: Acetone, Me: Methanol (mean and standard deviation of three replicates are shown).

Cholesterol Reduction

The percentage reduction of Cholesterol reduction percentage by different extraction protocols by *Spirulina platensis* was obtained in Figure 3. Results indicate that different treatments of *Spirulina platensis* have a highly effective on the cholesterol reduction by using various solvents except petroleum ether was recorded minimum effects in the reduction of cholesterol under all treatments. Maximums percentage reduction of cholesterol were obtained by acetone followed by methanol under all treatments as the following soaking>autoclaving>soxhlet>NaCl 10%: 76.12>74.16>72.53>69.40% respectively.

Effects of Various Spirulina Extracts on McF-7, CaCo, HePG-2 Cell Viability

Results in table 3 investigated that IC50 µg/ml for alga extracts using various solvents and methods against CaCo-2 (Colon Cancer), MCF-7 (Breast Cancer) and HepG-2 (Hepatocellular Carcinoma) cell lines. Chloroform by NaCl extracts showed the strongest cytotoxic effect against MCF-7, while acetone by autoclave, acetone cold and chloroform by NaCl, respectively showed the strongest cytotoxic effect against CaCo-2. Furthermore, acetone by autoclave, acetone cold, and acetone NaCl, showed the strongest cytotoxic effect against HepG-2.Among the tested extraction methods with acetone NaCl showed promise cytotoxic activity against Hepatocellular Carcinoma cell lines and those suggest this extract for further investigation as anticancer agent.

Table 3. IC50 µg/ml for algae extract using different solvents

		Autoclaving	**NaCl 10%**	**Soxhlet**	**Soaking**
Methanol	MCF-7	842	NA	NA	342
	CaCo-2	560	714	407	188
	HepG-2	NA	285	359	187

Chloroform	MCF-7	476	121	937	259
	CaCo-2	108	96	201	189
	HepG-2	232	351	730	161
Acetone	MCF-7	278	567	NA	253
	CaCo-2	99	133	625	88
	HepG-2	98	31	NA	38
Petrolium ether	MCF-7	NA	NA	NA	NA
	CaCo-2	NA	NA	NA	935
	HepG-2	NA	NA	NA	NA

GC/MS Analysis of the Most Effective Extract and Treatment

The GC/MS chromatograms showed a various compound present in different extracts of *Spirulina platensis* by soaking treatment. The crude extracts of tested alga showed a mixture of volatile compounds. The total number of the main peaks 22, 11, 11 and 12 were observed with retention times for acetone extract, petroleum ether extract, methanol extract and chloroform extract respectively. The components were identified using spectral database NIST 11 software installed in the GC-MS. Results indicated that twenty-two, eleven, eleven and twelve phytocompounds were characterized and identified in *Spirulina platensis* acetone extract, petroleum ether extract, methanol extract and chloroform extract respectively as shown in Table 4. The main chemical constituents found in high percentages are Heptadecane (retention time RT=16.812 min), Hexadecanoic acid (RT=22.014 min), Hexadecanoic acid methyl ester (RT=20.76 min), 9,12-Octadecadienoic acid (RT=25.187 min), Phytol (RT=24.320 min), Decanedioic acid, dibutyl ester (RT=25.402 min), Tributyl acetylcitrate (RT=27.019min) and Bis(2-ethylhexyl) phthalate (RT=31.385).

Table 4.The GC-MS analysis of chemical constituents in (acetone, petroleum ether, methanol and chloroform) extracts of *Spirulina platensis* by soaking

No	Compounds name	Retention time	Peak Area %	Molecular formula	Molecular weight
	Acetone extract				
1	Heptadecane	16.812	23	C17H36	240
2	Hexadecanoic acid, methyl ester	20.76	53.5	C17H34O2	270
3	n-Hexadecanoic acid	22.014	78.4	C16H32O2	256
4	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	23.914	8.98	C19H34O2	294
5	Phytol	24.32	63.8	$C_{20}H_{40}O$	296
6	9,12-Octadecadienoic acid (Z,Z)-	25.187	29.1	$C_{18}H_{32}O_2$	280

7	Decanedioic acid, dibutyl ester	25.402	46.4	$C_{18}H_{34}O_4$	314
8	Tributyl acetylcitrate	27.019	86.6	$C_{20}H_{34}O_8$	402
9	9-Octadecenamide, (Z)-	28.771	20.1	$C_{18}H_{35}NO$	281
10	Bis(2-ethylhexyl) phthalate	31.385	34.3	$C_{24}H_{38}O_4$	390
11	1,2-Cyclohexanedicarboxylic acid, cyclohexylmethyl nonyl ester	32.836	9.77	C24H42O4	394
12	1,2-Cyclohexanedicarboxylic acid, 2-methylcyclohexyl undecyl ester	33.162	4.36	C26H46O4	422
13	1,2-Cyclohexanedicarboxylic acid, cyclohexylmethyl tetradecyl ester	33.494	5.27	C29H52O4	464
14	1,2-Cyclohexanedicarboxylic acid, heptadecyl 2-methylcyclohexyl ester	33.653	6.73	C32H58O4	506
15	Didecyl 1,2-cyclohexanedicarboxylate	34.164	3.35	C28H52O4	452
16	1,2-Cyclohexanedicarboxylic acid, cyclohexyl isohexyl ester	34.287	3.8	C20H34O4	338
17	1,2-Cyclohexanedicarboxylic acid, dinonyl ester	34.502	5.64	C26H48O4	424
18	1,2-Cyclohexanedicarboxylic acid, didecyl ester	34.877	352	C28H52O4	452
19	1,2-Cyclohexanedicarboxylic acid, cyclobutyl undecyl ester	35.043	3.44	C23H40O4	380
20	1,2-Cyclohexanedicarboxylic acid, 3-pentyl undecyl ester	35.24	3.38	C24H44O4	396
21	1,2-Cyclohexanedicarboxylic acid, isobutyl nonyl ester	35.615	4.47	C21H38O4	354
22	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	54.479	20	C20H40O2	312
Petroleum ether extract					
1	Heptadecane	16.96	21.2	C17H36	240
2	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	19.278	28.3	C20H40O	296
3	1.2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	20.059	42.5	C20H40O	296
4	n-Hexadecanoic acid	22.5	73.6	C16H32O2	256
5	Phytol	24.455	53.1	C20H40O	296
6	9,12-Octadecadienoic acid (Z,Z)-	25.605	28.4	C1 8H32O2	280
7	Hexanedioic acid, mono(2-ethylhexyl)ester	29.226	40.3	C14H26O4	258
8	Bis(2-ethylhexyl) phthalate	31.427	40.5	C24H38O4	390
9	Rhodopin	41.075	16.2	C40H58O	554
10	psi.,.psi.-Carotene, 1,2-dihydro-1-hydroxy-	42.181	30.7	C40H58O	554
11	11,13-Dimethyl-12-tetradecen-1-ol acetate	54.798	14.4	C18H34O2	282
Methanol extract					
1	Heptadecane	16.757	21.8	C17H36	240
2	Hexadecanoic acid, methyl ester	20.772	51.9	C17H34O2	270
3	Palmitoleic acid	21.252	32.5	C16H30O2	254
4	n-Hexadecanoic acid	21.774	76.7	C1 6H32O2	256
5	γ-Linolenic acid, methyl ester	23.625	52.8	C19H32O2	292
6	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	23.914	19.6	C19H34O2	294
7	Phytol	24.277	59.6	C20H40O	296
8	Gamolenic Acid	24.664	53.2	C18H30O2	278

9	9,12-Octadecadienoic acid (Z,Z)-	24.953	31.5	C18H32O2	280
10	Tricyclo[20.8.0.0(7,16)]triacontane,1(22),7(16)-diepoxy	31.182	13.3	C30H52O2	444
11	1-Monolinoleoylglycerol trimethylsilyl ether	54.258	31.7	C27H54O4Si2	498
Chloroform extract					
1	Heptadecane	16.726	16.4	C17H36	240
2	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	19.204	17.5	C20H40O	296
3	n-Hexadecanoic acid	20.514	56.4	C16H32O2	256
4	Hexadecanoic acid, methyl ester	20.741	39.1	C17H34O2	270
5	Palmitic acid	21.319	56.6	C1 6H32O2	256
6	Pentadecanecarboxylic acid	21.743	75.8	C16H32O2	256
7	9,12-Octadecadienoic acid (Z,Z)-	23.871	7.8	C18H32O2	280
8	cis-9,cis-12-Octadecadienoic acid	24.264	23.5	C1 8H32O2	280
9	Gamolenic Acid	24.658	52	C18H30O2	278
10	cis,cis-Linoleic acid	24.916	30.4	C1 8H32O2	280
11	Isopropyl linoleate	31.575	9.09	C21H38O2	322
12	1-Monolinoleoylglycerol trimethylsilyl ether	54.27	44.9	C27H54O4Si2	498

DISCUSSION

Spirulina platensis are good source of bioactive constituents which cope with various applications in pharmaceutical industries. The chemical compounds synthesized by algae are usually sorted into primary and secondary metabolites based on chemical class, biosynthetic origin and functional groups. Chamorro et al. [31] reported that *Spirulina platens* has high medicinal potential extensively used in the drug and pharmaceutical industry due to its richness in secondary metabolites such as alkaloids, glycosides, flavonoids, tannins, steroids. Studies that revealed the presence of flavonoids, alkaloids saponins, quinines, cardiac glycosides, terpenoids, triterpenoids, coumarins and steroids in most studied algae is of great significance according to of their potentiality to probable usage as natural additive to replace synthetic antioxidants as well as antimicrobial preparations as suggested by Shan et al. [32]. Present results were in agreement with previous findings, which showed presence of saponins, quinines, cardiac glycosides, terpenoids, triterpenoids, coumarins and steroids.

Tannin compounds have been found to possess strong antioxidant activity, which may be attributed to their unique molecular structure [33]. Moreover, Tannins was reported to have antiviral, antibacterial, anti-inflammatory and antioxidant property for possible therapeutic applications [34]. The current study represented various methods of extraction with different organic solvents revealing variations in the investigated biological activities. According to Kuda [35] and Kahkonen et al. [12] phenolic constituents as flavonoids are responsible for most of the biological activities (antioxidants potentiality) attributed to algae. Duan et al. [36] demonstrated that phenols, tannins and flavonoids have been found in *Spirulina platensis* and considered as potential therapeutic agents, that counteract some diseases depending on their free radical scavenging activity Hirahashi et al. [37].

Generally, the reducing properties are attributed to presence of reductions which documented as terminators of free radical chain reactions via providing a hydrogen atom. In the present study, *Spirulina platensis* possesses various

constituents having reducing power. Results revealed that acetone extract exhibited the highest antioxidant activity in all extraction protocols.

The antioxidant activity of *Spirulina* can be explained on basis of presence of the two phycobiliproteins, phycocyanin and allophycocyanin. Piero Estrada et al. [38] attributed this activity to the phycocyanin content and depending on concentration. Alberto et al. [39] documented that Phenolic compounds represent the common chemical constituents of algal cells that may have either positive or negative biological effects on microbial growth depending on their chemical composition and concentration. In addition, the magnitude of this potentiality could also depend on extraction method and solvents used as well as on algal type, since some bioactive metabolites present in the algae varied in their solubility level in the organic solvents used [40]. Recently, *Spirulina platensis* has also exhibited antifungal activity [41].Cox et al. [42] reported that the isolation of bioactive substances from different algal species was solvent dependent. Pervious findings supported the present results; where higher antibacterial activity were obtained with acetone extract of *Spirulina platensis* as well as the potent inhibitory effect was precisely observed on both gram positive and gram negative bacteria. *Spirulina* possessed various positive nutritional and therapeutic properties. Prakash and Kumari [43] studied the preparation of low- fat and high-protein frozen yogurt enriched with papaya pulp and *Spirulina* with in order to find out the most favorable level of *Spirulina* that could be incorporated to obtain a best quality of frozen yogurt. Moreover, Kamal et al. [44] reported that the percentage reduction of cholesterol by *Spirulina platensis* is more effective than *Lactobacillus plantarum* and *Lactobacillus casei.* Present results indicated that maximum percentage reduction of cholesterol was obtained by acetone extract followed by methanol extract under all treatments. It was also found that, *S. platensis* have contributed antitumor and anticancer functions. Privalov et al. [45] discovered that significant to full tumor retreating was acquired with intravenous injection of a new chlorine photosensitizer (Radachlorin) that was obtained from *S. platensis*. Complex polysaccharides isolated from *Spirulina* resulted in inhibition of glioma cell growth through down regulating angiogenesis via partial regulation of interleukin-17 production [46]. High production of tumor necrosis factor-α (TNF-α), in macrophages, was observed due to the presence of acidic polysaccharides in *S. platensis* Parages et al. [47]) and Li et al. [48] have shown that with increased phycocyanin concentration, expression of CD59 proteins in HeLa cells was promoted while Fas protein that induces apoptosis was increased with an attendant decline in the multiplication of HeLa cells. These findings are an evidence for the multidimensional applications of phycocyanin content of *S. platensis.* All these pervious findings supported our results for anticancer effect of acetone extract of *S. platensis* was observed in Fetal Calf Serum Cell Line.

The GC-Ms chromatograms analysis showed the presence of various chemical compounds in acetone extracts of *Spirulina platensis.* A large number of algal species have useful ingredients such as myristic acid, palmitic acid, stearic acid [49], phenols, indoles, acetogenins, terpenes, labdane diterpenes, brominated hydroquinones, tropodithietic acid and phlorotannins [50] that may produce antibiosis against fungi and bacteria which cause the antimicrobial activity of algae. Bergasson et al. [51] reported that lipids stop microbes by distracting the cellular membrane of bacteria, fungi and yeasts. Balamurugan et al. [49] isolated some compounds as n-hexadecanoic acid, tetradecanoic acid, oleic acid, 9-octadecenoic acid, 6-octadecenoic acid, hexadecanoic acid, ethyl ester, ethyl tridecanoate and octadecanoic acid from the ethanolic extract of *Hypnea musciformis.* Mohy El.Din and Elahwany

[52] reported that chemical constituents of algae can be characterized by GC-MS chromatograms. Moreover, Fathy and Essa [53] demonstrated the capability of *Spirulina platensis* to release detrimental bioactive metabolites into their surroundings. Usha and Maria Victorial Rani [54] identified several compounds in the methanolic extract of *Padania pavonica* including myristic acid, palmitic acid, linoleic acid, myristic acid ester, palmitic acid ester and oleic acid ester. Our results in this investigation indicated that twenty-two phytocompounds were characterized and identified in *Spirulina platensis* acetone extract by soaking. The main chemical constituents found in high percentages are Heptadecane, Hexadecanoic acid, Hexadecanoic acid methyl ester, 9,12-Octadecadienoic acid, Phytol, Decanedioic acid, dibutyl ester, Tributyl acetylcitrate, and Bis(2-ethylhexyl) phthalate.

CONCLUSIONS

Spirulina platensis may be considered as a potential candidate for conjugative therapy due to the possible synergetic effect of many phytochemicals in whole cell. It is concluded from this study that the analysis of bioactive natural compounds of *Spirulina platensis* and their antimicrobial efficiency, antioxidant capacity, anticancer efficiency and its cholesterol-lowering effects depends on solvent types used for algal extraction in addition to proceeding methods of extraction. Acetone was the most effective solvent for extraction of bioactive compounds from *Spirulina platensis* and the soaking treatment was the best methods for extraction to the effective compounds. The present study investigation presents adequate data on the phytochemical constituents of *Spirulina platensis* by using GC-MS techniques. It can be concluded that, this organism serves as very viable potential sources of bioactive products with commercial imports.

Conflict of Interest

The authors declare that there is no conflict of interest

REFERENCES

1. A Belay; Y Ota; K Miyakawa; H Shimamatsu. In: Phang *et al*., (eds.) Algal Biotechnology in the Asia-Pacific Region. **2009**, University of Malasya, 92-102.
2. M Khan; A Ather; K Thompson; R Gambari; *Antiviral Res*.**2005**, 67, 107-119.
3. C Steven; J Russell; *CRC Press* **1993**.
4. M A Borowitzka; *J Appl Phycol.* **1995**, 7, 3-15.
5. S Kreitlow; S Mundt; U Linequist; *J Biotechnol*. **1999**, 70, 61-63.
6. S Mundt; S Kreitlow; A Nowotny; U Effmert; *Intern J Hyg Environ Health.* **2001**, 203, 327-334.
7. RB Volk; FH Furkert; *Microbiol Res.* **2006**, 161, 180-186.
8. S Nallamuthu; S Viswanathan; CR Ebciba; S Santhiya; *Int J Innov Res Sci Eng Technol*. **2014**, 3, 2319-8753.
9. B Chauhan; W Chaitra Kapfo; *Sch Acad J Biosci.* **2015**, 3:762.
10. C Manach; A Scalbert; C Morand; C Rémésy; I Jiménez; *Am J Clini Nuti*. **2009**, 79, 762-765.
11. PG Waterman; S Mole; Analysis of Phenolic Plant Metabolites. In: Methods in Ecology, Blackwell Scientific Publications, 1994, Oxford, UK.
12. M Kahkonen; P Hopia; HJ Vuorela; H Rauha; JP Kujala; *J Agric Food Chem.***1999**, 47, 727-747.
13. I Tuney; BH Cadirci; D Unal; A Sukatar; *Turk J Biol*. **2006**, 30: 171-175.

14. JN Kumar; M Barot; NR Kumar; *Life Sci Leaf*. **2014**, 52, 57-70.
15. A Dilmi-Bouras; DE Suivi; *Alger*. **2002**, 99.
16. J Fabregas; L Ferron; J Abalde; B Cabezas; A Otero; *Technol*. **1995**, 53, 185-188.
17. R Saile; H Taki; *Les Technologies de Laboratoires*. **2007**, 2,1- 4.
18. N Nakaya; Y Honma; Y Goto; *Nutr Rep Int.* **1988**, 37, 1329-1337.
19. A Ramamoorthy; S Premakumari; *J Food Sci Technol*. **1996**, 33, 124-128.
20. EW Becker; B Jakober; D Luft; RM Schumuling; *Nutr Rep Int*. **1986,** 33, 565.
21. C Zarrouk; (Setch et Gardner) Geitler, **1966** University of Paris.
22. S Sadasivam; A Manickam; *New Delhi, India*, **1996**, 1-97.
23. C Chang; M Yang; H Wen; J Chern; *J Food Drug Anal.* **2002**, 10, 178-182.
24. R Julkunen-Titto; *J Agric Food Chem.* **1985**, 33, 213.
25. M Taga; E E.Miller; DE Pratt; *J Am Oil Chem Soc*. **1984**, 61, 928-993.
26. T Yamaguchi; H Takamura; T Matoba; *Biosci Biotech*. **1998**, 62, 1201-1204.
27. H Mitsuda; K Yuasumoto; SLU; *Eiyo to Shokuryo*. **1996**, 19, 210-214.
28. W Richmond; *Clin Chem.* **1973**, 19, 1350-1356.
29. T Mosmann; *J Immunol Methods*. **1983**, 65-55.
30. S Musharraf; MA Ahmed; N Zehra; M Choudhary; AU Rahman; *Chem Cent J*. **2012**, 6, 1-10.
31. G Chamorro; M Salazar; L Favila; H Bourges; *Revista de Investigacion Clinica*, **1996**, 48, 389-399.
32. B Shan; Y Cai; J Brooks; H Corke; *Inter J Food Micro.* **2007**, 117, 112-119.
33. RAS Hemat; *Dublin, Ireland*, **2007**, 83-85.
34. L Lu; S Wliu; SB Jiang; SG Wu; *Acta pharmacologica Sinica*. **2004**, 213-218.
35. T Kuda; T Kunii; H Goto; T Suzuk; T Yano; *Japan J Food Chem.* **2007**,103, 900-905.
36. XJ Duan; WW Zhang; W Xm; BG Li Wang; *Food Chem*. **2006**, 95, 1022 -1037.
37. T Hirahashi; M Matsumoto; K Hazeki; Y Saeki; M Ui; T Seya, *International Immunopharmacology*, **2002**, 2(4); 423-434.
38. JE Piero Estrada; P Bermejo Bescos; A M Villar Del Fresno; *IL Farmaco*, **2001**, 56, 497-500.
39. MR Alberto; M E Faryas; MC Manca De Nadra; *J Agric Food Chem*. **2001**, 49, 4359-4363.
40. WM Salem; H Galal Nasr; F El-Deen; *Afr J Microbiol Res.* **2011**, 5, 2160-2167.
41. C DUDA; *ACTA Scientiarum Polonorum Technologia Alimentaria.* 2013, 12, 33-39.
42. S Cox; N Abhu-Ghannam; S Gupta; *Int Food Res J*. **2010**, 17: 205-220.
43. DR Prakash; P Kumari; *Trends Biosci*. **2011**, 4,182-184.
44. S Kamal; RA Hamouda; H Mahrous; ML Salem; HA Hamza; E Abd Elhafez. *Inter J Pharma*. **2015**, 11, 638-643.
45. VA Privalov; AV Lappa; OV Seliverstov. *Proceedings of SPIE*, **2002**, 178-189.
46. Y Kawanishi; A Tominaga; H Okuyama. *Microbiology and Immunology*, **2013**, 57 (1). 63-73.
47. M L Parages; RM Rico; RT Abdala-D´iaz; M. Chabrill´on; TG Sotiroudis; C JIM´ENEZ; *Journal of Applied Phycology*, **2012**, 24, 1537-1546.

48. B Li; X Zhang, M Gao; X Chu; *Biomedicine and Pharmacotherapy*. **2005**, 59 (10), 551–560.
49. M Balamurugan; GG Selvam; T Thinakaran; K Sivakumar; *Am Eur J Sci Res.* **2013**, 8, 117-123.
50. KW Samarakoon; YJ Jeon. *Food Res Int*. **2012**, 48,948-660.
51. G Bergasson; H Hilmarsson; H Thormar; John Wiley et Sons Ltd, Chichester, UK, **2011**, 47-63.
52. SM Mohy El-Din; A.M.D. El-Ahwany; *J Taibah Univ Sci.* **2016**, 10:471.
53. SM Fathy; AF Essa; *Asian Pacific J Trop Biomed.* **2015,** 5, 451-457.
54. R. Usha; S Maria; R Victorial; *Biosci Discov*. **2015**, 6 (1), 1-5.