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Full Length Research Paper

Antimicrobial Activity of Microalgal Extracts Isolated From Baharia Oasis, Egypt

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In the present study, the methanolic extract of five species (four cyanobacterial and one green alga) namely *Nostoc caeruleum*, *Spirulina platensis*, *Cylindrospermum majus*, *Oscillatoria formosa* and *Chlorella vulgaris* were tested with the agar well diffusion method for their antibacterial against three Gram positive bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*) and three Gram negative bacteria (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*) as well as for their antifungal activity against (*Aspergillus fumigatus*, *Candida albicans*, *Geotricum candidum*n and *Trichophyton mentagrophytes*). The results indicated that the extract of *Chlorella vulgaris* was more efficient against the tested bacteria and fungi strains followed by *Spirulina platensis*. Chemical analyses showed that *Chlorella vulgaris* recorded the highest percentages of the total phenolic and total flavonoid contents. So, *C. vulgaris* crude extract was tested for their effectiveness as antioxidant activity. Also, *Chlorella vulgaris* extract screened for their anticancer activities against selected cancer cell lines; breast cancer cell lines MCF-7, colon cancer HCT-116 and liver cancer cell lines HepG-2. The results indicates that the HepG-2 was most sensitive followed by the HCT-116 than MCF-7 at (IC50) with 40.5, 43 and75.3. µg/ml respectively. Finally the result of GC mass analysis proved the presence of quercitin compounds.

Keyword: Soil microalgae, antimicrobial activity, *Chlorella vulgaris*, Baharia Oasis, Egypt.

INTRODUCTION

Algae a diverse group of plant kingdom, contains different bioactive compounds. The bioactive substances produced by actively growing cells of algae includes proteins, fats, lipids, carbohydrates, phenol, flavonoid, vitamins, free amino acids, enzymes, growth regulators, pigments, toxins and antibiotics. Algae are admirable sources of antibiotics which in laboratory tests, inhibited bacteria and fungi that incite diseases of humans(Kulik, 1995). Microalgae and cyanobacteria offer numerous advantages for antimicrobial investigations because of their enormous biodiversity and fast growth rate (Pulz and Gross, 2004). The cell extracts and active constituents of various algae shown to have antibacterial activity in vitro against Gram positive and Gram negative bacteria (Goud *et al*., 2007). A wide range of in vitro anti-fungal activities have also been reported from extracts of green algae, diatoms and dinoflagellates (Ely *et al*., 2004). Screening efforts aimed to identify antimicrobial agents in microalgae have revealed several promising lead compounds. Some of the substances identified include Chlorellin (Metting and Pyne, 1986),

Nostocyclyne A (Ploutno and Carmeli, 2000), Nostofungicidine (Kajiyama *et al*., 1998). Najdenski *et al*. (2013) stated that ethanolic extract of *Scenedesmus obliqus, Chlorella* sp. and *Nostoc* sp. has antibacterial effect against *Staphylococcus aureus* and *Bacillus cereus*.

Flavonoids comprise a large groups of naturally occurring compounds widely distributed in the plant kingdom and some of these compounds have been reported to contain various and potent biological activities including antioxidative tissue protective and tumoristatic effects as well as the inhibition of hepatic cholesterol biosynthesis (Krant *et al.,* 2005; Kim *et al*., 2007; Volk, 2009). Some studies reported that cancer was prevented by algae extracts because of their antioxidant properties (Mirada *et al*., 1998). Substances known as antioxidants are bioactive compounds that are able to inactivate free radicals, which are instable molecules that may cause several deleterious effects to human health (Bolanho *et al*., 2014). Thus the usefulness of antioxidants in protecting cellular components against oxidative stress is well established (Bermejo-Bescos *et al*., 2008).

Therefore the present study was aimed to isolate as many algae as possible from Bawety soil at Baharia Oasis Egypt, and test them for their antimicrobial, antifungal, anticancer and antioxidant activities. Furthermore, GC/MS for *Chlorella vulgaris* extract was employed for detection of active constituents.

MATERIALS AND METHODS

Isolation and culture conditions

The soil samples were collected at different localities at Km 16 Bawety at Baharia Oasis, Egypt during March 2014. Samples were grown in Bold basal medium and BG-11 according to (Bold and Wynne, 1978; Browitzka and Browitzka, 1988) respectively. After colonization, the algal species were transferred to the same medium. Each isolated was cultured in a 250 ml flask containing 100 ml of BG11 and Bold media without shaking, for 30 days. The incubation temperature was 28°C ± 2 and illumination at 3000 lux with a white continuous light and a regime of 16 hrs light 8 hrs dark. The isolated algae were identified using morphological variation studies and taxonomical approaches according to (Prescott, 1962) then purified and free from bacteria according to the methods described by (Pringsheim, 1949; Hoshaw and Rosowski, 1973) respectively.

Antimicrobial activity

The methanol algal extract was tested for antimicrobial activity by using agar well diffusion method (Scott, 1989). All algal extracts were tested in vitro for their antibacterial activity against Gram positive bacteria (*Staphylococcus aureus* (RCMB 010027), *Staphylococcus epidermidis* (RCMB 010024), *Streptococcus pyogenes* (RCMB 010015) and Gram negative bacteria (*Klebsiella pneumonia* (RCMB 0010093), *Pseudomonas aeruginosa* (RCMB 010043) and *Escherichia coli* (RCMB 010056)). Antifungal activity was carried out against *Aspergillus fumigatus* (RCMB 02564), *Candida albicans* (RCMB 05035), *Geotricum candidum* (RCMB 05096) and *Trichophyton mentagrophytes* (RCMB 0925) using Sabouraud Dextrose Agar medium. Amphotericin B Ampicillin and Gentamycin were used as standard drugs for Gram position, Gram negative and antifungal activity respectively. DMSO was used as solvent control. The extracts were tested at different concentration of 10 mg/ml against both bacterial and fungal strains. In this study the bacterial and fungal strains were obtained from Culture Collections of the Regional Center for Mycology and Biotechnology Antimicrobial unit test organisms at Al- Azhar University. Cairo, Egypt.

Also, the minimum inhibitory concentration (MIC) was determined against tested microorganisms using agar dilution method adopted by Saini *et al*. (2005).

Chemical analysis

The total phenolic compounds were determined according to the method described by Malik and Singh (1980). Where, the aluminum chloride method was used for the determination of the total flavonoids according to the method described by El Far and Taie (2009).

Isolation of flavonoid compounds

The crude extract was subjected to Thin Layer chromatography, TLC (System1: butanol: acetic acid: water (4:1:5), System 2: acetic acid: water (15%) and System 3: ethyl acetate –methanol –water (30:5:4). Band of flavonoid was exposure to ammonia vapor. Then observed under UV light at 254-365nm (CAUTION Ultra-Violet Radiation CN-6T) and /or spraying with $FeCl_3$, about 30 chromatogram were scratched then dissolved in methanol and submitted to a column of sephadex LH-20. Three compounds were isolated and identified by GC/Mass.

Antioxidant Assay:

The antioxidant activity of extract was determined by DPPH free radical scavenging assay in triplicate and average values were considered by Yen and Duh (1994).

Antitumor activity assay:

Antitumor activity assay of extract was determined by Human hepatocellular carcinoma (HepG2), breast cancer cell lines MCF-7and colon cancer HCT-116. Cell line was obtained from the American Type Culture Collection

(ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The cells were maintained at 37°C in a humidified with 5% CO_2 and were subcultures two to three times a week according to (Mosmann, 1983; Gomha *et al.,* 2015).

GC/MS. Analysis of *Chlorella vulgaris* crude extract:

Pure compound was subjected to GC/Mass investigation using (SHIMADZU GC/Mass QP 5050 A) instrument employing the following conditions: column: DB5, (0.53mm IDX-1.5µm.film) carrier gas: He (1ml/min); injector temp. (280^{O}C) detector temp. (280^{O}C) column temp. 60^{O}C, (0.5 min.) -150^{O}C (1min.) at 10^{O}C/min.-250^{O}C (2min) at 10^{O}C/min. Mass spectra: Electron empact70ev. All previous analysis was determined at the Regional Center for Mycology and Biotechnology (RCMB) at Al- Azhar University.

RESULTS AND DISCUSSION

Nine species were isolated from the soils, eight species of cyanobacteria and one species of green alga were identified *Spirulina platensis, Oscillatoria Formosa*, *Cylindrospermum majus*, *Anabaena* sp., *Anabaena spiroides*, *Nostoc caeruleum*, *Anabaena circinalis*, *Chroococcus* sp. and *Chlorella vulgaris*. Antimicrobial preliminary test were done for all algal species. The result proved that five species named *Nostoc caeruleum*, *Spirulina platensis*, *Cylindrospermum majus*, *Oscillatoria Formosa* and *Chlorella vulgaris* have the best growth and the concentration of 10 mg/ml were best result as antimicrobial.

The methanolic algal extracts effects on antibacterial and antifungal activities were evaluated in Table (1). The result showed that *Chlorella* extract has the highest antifungal activities against *Candida albicans* and *Geotricum candidum* with the inhibition zones of (20.6 ± 1.5 and 22.3 ± 0.72 mm) respectively, followed by *Nostoc caeruleum* (16.3 ± 0.5 and 22.4 ± 0.6) respectively, in agreement with Ghasemi *et al*. (2004) reported that *C. vulgaris* have antifungal activity against (*A. niger* and *C. albicans*), in contrast with (Salem *et al*., 2014) showed that the *Chlorella* methanol extract have no antifungal activity against the five fungal strains tested this contradictory may be due different type of algal species.

While *C. majus* showed 18.4 ± 0.58 and19.1 ± 1.2 mm respectively against *Candida albicans* and *Geotricum candidum*, but not record antifungal activity against *A. fumigatus*, in contrast with Malathi *et al*. (2015) showed that the aqueous extract showed maximum inhibition zone (15.33 mm) against *A. fumigatus* so we can say that the solvent type and algal species affect the extract activity against the different pathogens species, on the other hand *S. platensis*, *O. formosa* were recorded the inhibition zones at *Geotricum candidum* and *Candida albicans* 16.2±0.63 mm while at *Candida albicans* recorde the highest inhibition 20.3±0.5 and 17.3±0.5 mm respectively. However, the methanolic extract of all algal species did not record antifungal activity against *A. fumigatus* and *T. mentagrophytes*.

On the other hand, *C. vulgaris* crude extract showed antibacterial activity against *S. aureus* and *St. pyogenes*, *K. pneumoniae* and *E. coli* with inhibition zones (19.3 ± 2.1, 21.1 ± 0.63 , 20.1 ± 0.58 and 22.4 ± 0.63 mm respectively). These results were compatible with the study of Salem *et al*. (2014) on the antibacterial of *C. vulgaris* extract activity against *B. subtilis*, *S. aureus*, and *K. pneumonia* with inhibition zones 17.5, 17 and 14.5 mm respectively. Meanwhile, *Nostoc caeruleum* and *S. platensis* exhibited antimicrobial activity against *E. coli*, *S. marcescens* and *B. cereus* in addition to *K. pneumonia*, *S. aureus* and *St. Pyogenes* respectively (Table 1) in agreement with Shaieb *et al*. (2014) found that *Nostoc commune* and *S. platensis* showed antibacterial activity against all species tested (*Escherichia, Bacillus, Micrococcus, Staphylococcus, Klebsiella and Pseudomonas*).

Gupta and Shrivastava (1965) found that extracts from *Oscillatoria princeps* were active against *B.subtilis*, *S. aureus*, *E. coli* and *Brucella bronchiseptica* while in the present study *Oscillatoria formosa* had the minimum activity against the test organisms *S. aureus*, *St. pyogene* and *E. coli* respectively. *Cylindrospermum majus* inhibited *K. pneumoniae* only with inhibition zone of (18.5 ± 1.2mm). This result is in harmony with Malathi *et al*. (2015) that showed the maximum antibacterial sensitivity of inhibition zone (17.33 mm) against *K. pneumoniae* by *C. majus* was noticed in the Chloroform extract.

Chlorella vulgaris had the greatest frequency among the species that showed antibacterial and antifungal activity and exhibited the most prominent effect. The effect of antimicrobial activity of *Chlorella* species has been reported in other studies such as (Kellam and Walker, 1989; Ordog *et al*., 2004), reported that antibacterial and antifungal activity was seen predominantly from the *Chlorella* species.

Minimum inhibitory concentration (MIC)

The MIC was estimated for all the algal extracts with the pathogenic bacteria and fungi given in Table (2). The results showed similar trend as the antifungal and antibacterial activity, the MIC values of the tested extracts against pathogenic fungi were ranged between (0.98 and 62.5µg/ml). The excellent antifungal activity and low MIC value was observed with crude extract of *C. vulgaris* and *Nostoc caeruleum* (0.98 µg/ml) against *Geotricum candidum*. Also, the MIC values of the tested algae extracts against pathogenic bacteria were ranged between (0.98-31.25 µg/ml); *C. vulgaris* was active against *E. coli*, *K. pneumonia*, *St. pyogenes* and *S. aureus* with MIC

Table 1: Antibacterial and antifungal activity of crude algal extracts against pathogenic bacteria and fungal (inhibition zone expressed as mm diameter), data are expressed in the form of mean ± SD

Tested microorganism		*N. caeruleum*	*S. platensis*	*C. majus*	*O. Formosa*	*C. vulgaris*	Standar treatment	
Fungi	*Aspergillus fumigatus*	NA	NA	NA	NA	NA	23.7 ± 0.10	Amphotericin B
	Candida albicans	16.3 ± 058	15.1 ± 1.2	18.4 ± 0.58	15.2 ± 0.63	20.6 ± 1.5	21.9 ± 0.12	
	Geotricum candidum	22.4 ± 0.63	20.3 ± 0.58	19.1 ± 1.2	17.3 ± 0.58	22.3 ± 0.72	26.4 ± 0.20	
	Trichophyton mentagrophytes	NA	NA	NA	NA	NA	25.4 ± 0.16	
Gram (+) Bacteria	*Staphylococcus aureus*	18.6 ± 0.58	17.8 ± 0.63	NA	15.8 ± 0.63	19.3 ± 2.1	28.9 ± 0.14	*Ampicillin*
	Staphylococcus epidermidis	NA	NA	NA	NA	NA	17.3 ± 0.12	
	Streptococcus pyogenes	17.1 ± 0.63	16.3 ± 0.72	NA	17.3 ± 0.18	21.1 ± 0.63	26.4 ± 0.34	
Gram (-) Bacteria	*Klebsiella pneumoniae*	NA	18.5 ± 1.2	18.5 ± 1.2	NA	20.1 ± 0.58	26.3 ± 0.15	*Gentamycin*
	Pseudomonas aeruginosa	NA	NA	NA	NA	NA	17.3 ± 0.12	
	Escherichia coli	19.7 ± 0.63	NA	NA	16.3 ± 1.5	22.4 ± 0.63	25.3 ± 0.18	

***NA : No activity**

Table 2: Antimicrobial Activity as minimum inhibitory concentration (MICS µg/ ml) of samples against tested microorganisms.

Tested microorganism		*N. caeruleum*	*S. platensis*	*C. majus*	*O. Formosa*	*C vulgaris*	Standar treatment	
Fungi		Minimum inhibitory concentration (µg/ml)						Amphotericin B
	C. albicans	31.25	62.5	7.81	62.5	1.95	0.98	
	G. candidum	0.98	3.9	3.9	15.63	0.98	0.49	

Table 2: Continue

Gram Positive Bacteria	*S. aureus*	7.8	7.81	NA	31.25	3.9	0.49	*Ampicillin*
	St. pyogenes	15.63	31.25	NA	15.63	1.95	0.49	
Gram negative Bacteria	*K. pneumoniae*	NA	1.95	7.81	NA	3.9	0.49	*Gentamycin*
	E. coli	3.9	7.81	NA	31.25	0.98	0.49	

values varying from (0.98 to 3.9 µg/ml). The only activity for *C. majus* was toward *K. pneumonia* with MIC value (7.81 µg/ml).

Generally, the test microorganisms differ inhibition activity in relation to their susceptibility to algal extracts antimicrobial substances, *G. candidum* was the more sensitive than *C. albican* while Gram positive bacteria were low sensitive than the Gram negative bacteria. The reason for different sensitivity between the fungi and bacteria can be found in different transparency of the cell wall. The cell wall of the Gram positive bacteria consists of peptidoglycan (murein) and teichoic acids, while the cell wall of the Gram-negative bacteria consists of lipo polysaccharides and lipopoliproteins (van Heijenoort, 2001).

Whereas, the cell wall of fungi consists of polysaccharides such as hitchin and glucan (Farkaš, 2003) and or due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (Abu-Shanab *et al .,* 2005). Darah *et al*. (2013) reported the mechanism of actions involved in bacterial killing process. Among them are the interactions of antimicrobial compound with the cell membrane. Sahgal *et al*. (2011) attributed the differences of the MIC value could be due to the morphological structure of the bacterial cells and their composition in the cells.

The antimicrobial activity of the extract could be due to the presence of different chemicals that may include flavonoids and triterpenoids besides phenolic that may affect growth and metabolism of bacteria. Also, they could have an activating or inhibitory effect on microbial growth according to their constitution and concentration, compounds and free hydroxyl group (Yu *et al*., 2009) amides and alkaloids (Ghasemi *et al*., 2004).

The total phenolics and flavonoids compounds of five algal extract are presented in Table (3), the highest value of total phenolic was determined in *C. vulgaris* followed by *O. Formosa* than *C. majus* and *Nostoc caeruleum*, the lower value was recorded in *S. platensis*. Also, the highest value of total flavonoid was noted in *C. vulgaris*, additionally, moderate values of total flavonoid were detected in *Nostoc caeruleum*, *O. formosa* and *C. majus* (18.96, 18.52 and 17.67mg/g) then *S. platensis* was the lowest value of flavonoid (10.61 mg/g).

These results are in agreement with Ali *et al*. (2014) were recorded *Chlorella* sp. and *Scenedesmus obliquus* showed higher phenolic and carotenoid contents. Their studied cyanobacterial species plays a great role as a potential source of natural antioxidants, due to the phenolic compounds as well as carotenoids contents. Microalgae contain a variety of phenolic classes but they were very different from many other plant species like vegetables, fruits and medicinal plants. The microalgae could contain different antioxidant compounds from other plants (Manivannan *et al.* 2012).

Antioxidant activity of the methanol extract of *C. vulgaris* was determined in terms of IC50 value based on the percentage of free radical scavenging activity Table (4). High scavenging activity was observed at the concentration of 1000 µg/ml. IC50 value for the *C. vulgaris* determined by DPPH assay was (133.1 µg/ml). Annamalai and Nallamuthu (2014) showed that antioxidant activity of the methanol extracts of *C. vulgaris* and *C. reinhardtii* were determined in terms of IC50 value based on the percentage of free radical scavenging activity. In the extracts of both algal species, the higher scavenging activity was observed at the concentration of 1000 µg/mL. IC50 value for the *C. vulgaris* determined by H_2O_2 and Thiocyanate assay were 26.31 µg/mL and 28.18 µg/mL whereas for *C. reinhardtii* was 27.48 µg/mL and 58.05 µg/mL respectively.

The presence of flavonoids and phenols in the methanol extract might been responsible for free radical scavenging activity individually or by synergistic action. Klejdus *et al*. (2010) showed that several classes of flavonoids, such as isoflavones, flavanones, flavonols and dihydrochalcones are found in microalgae and cyanobacteria. This indicates

Table 3: Total phenolic and flavonoid contents of crude algal extract (mg/g).

Algal species	Total phenolic (mg/g)	Total flavonoid (mg/g)
N. caeruleum	**45.52**	**18.96**
S. platensis	**29.74**	**10.61**
C. majus	**64.43**	**17.67**
O. formosa	**70.12**	**18.52**
C. vulgaris	**108.66**	**32.73**

Table 4: Antioxidant activity of *Chlorella vulgaris* extract of (µg/ml) against DPPH radicals.

Chlorella vulgaris extract (µg/ml)	DPPH scavenging (%)
1000	**133.1**
800	**82.20**
400	**79.40**
200	**66.50**
100	**41.80**
50	**25.40**
25	**5.50**
0	**0**

that microalgae are more primitive than terrestrial plants and they are capable of producing relatively complex polyphenols.

The cytotoxicity of *Chlorella vulgaris* showed 50% inhibition (IC50) of Hepatocellular carcinoma cells (HepG-2) at 40.5 µg/ml higher compare to colon carcinoma cells (HCT-116) which inhibition (IC50) of cells at 43 µg/ml and breast cancer recorded low inhibition (IC50) at75.3 µg/ml (Figure 1). Mohd Syahril *et al*. (2012) found that the *Chlorella vulgaris* ethanol extract give the small effect on both MCF7 and no effect at all for HepG2 cell lines. Also, they showed that *Chlorella vulgaris* chloroform extract generated larger inhibition on cytotoxicity to the MCF7 with low doses such as 89 µg/ml compared to the HepG2 cell lines.

Flavonoids are polyphenolic compounds and the best-described property of almost every group of flavonoids is their capacity to act as antioxidants. In other words, flavonoids stabilize the reactive oxygen species by reacting with the reactive compound of the radical (Nijveldt *et al*., 2001).

Several reports have shown a close relationship between the total phenolic content and high antioxidant activity. Moreover, the results of Abdel-Raouf *et al*. (2011) suggested that flavonoids can be used clinically as antihyperlipidemic agent to treat alcohol-induced hepatic tissue damage. The present results were in accordance with an earlier report by (Jaganathan and Mandal, 2009) which stated that Quercetin and Kaempherol have evolved as promising pharmacological agents in the treatment of cancer.

GC/MS Analysis of *C. vulgaris* methanol crude extract:

The chemical composition of *C. vulgaris* methanol extract determined by GC/MS was illustrated in Figure (2) and Table (5) as well as the chemical structure in Figure (3). Present results, showed that the Quercitin present in the crude extract which was type of flvenoids analysis with Rf 0.7 and yellow color with UV. Al-Saif *et al*. (2014) showed that the highest values of rutin, quercetin and kaempherol were present in algal extract of *Gracilaria dendroides*, *Ulva*

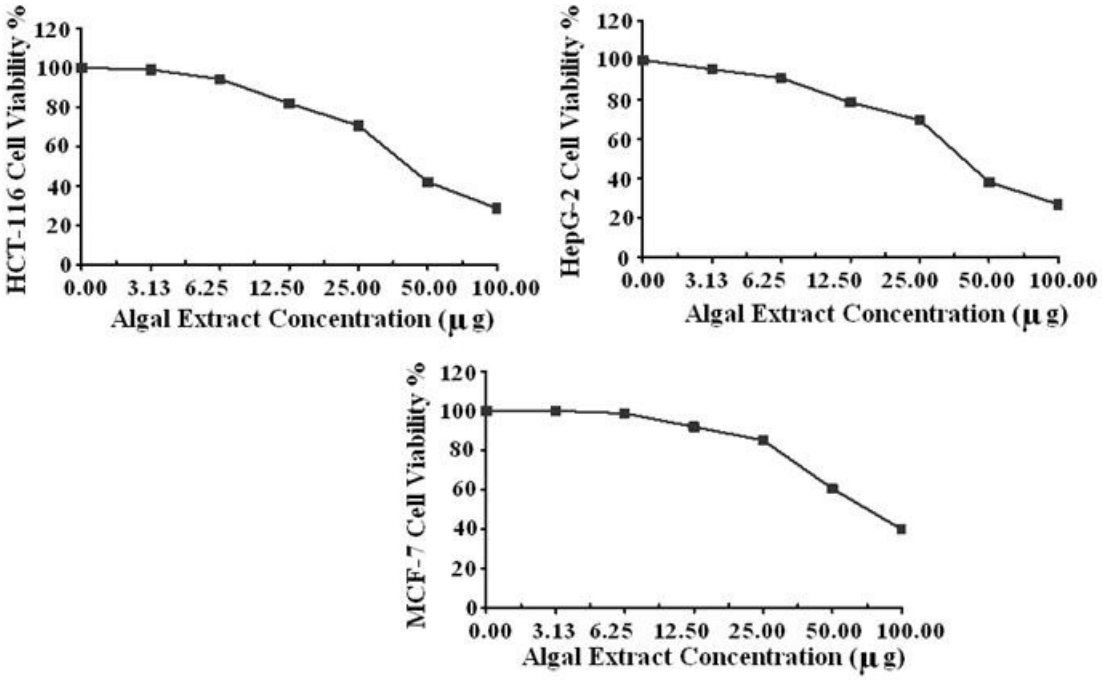

Figure 1: Antitumor activity of *Chlorella vulgaris* crude extract (µg/ml) against HCT-116, HepG-2 and MCF-7 cell line

Table 5: GC mass analysis of *C. vulgaris* methanol extract.

Name of compound	Group	Molecular formula	R_F value	Detection method			
Quercitin	Flavonoids	$C_{15}H_{10}O_7$	0.7	Visible	UV	UV/NH_4OH	$AlCl_3$
				Yellow	Yellow	Bright-Yellow	Orange
EI-MS	303(M^+) (100%), 165(20%), 228(30%), 257 (40%),and 274 (10 %).						

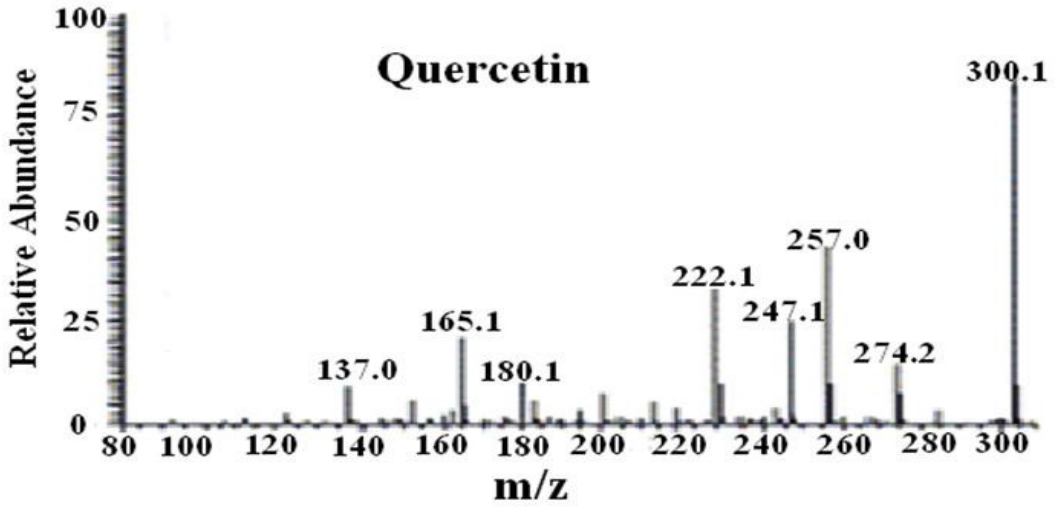

Figure 2: Gas chromatographic profile of the major constituents of *C. vulgaris*. methanol extract

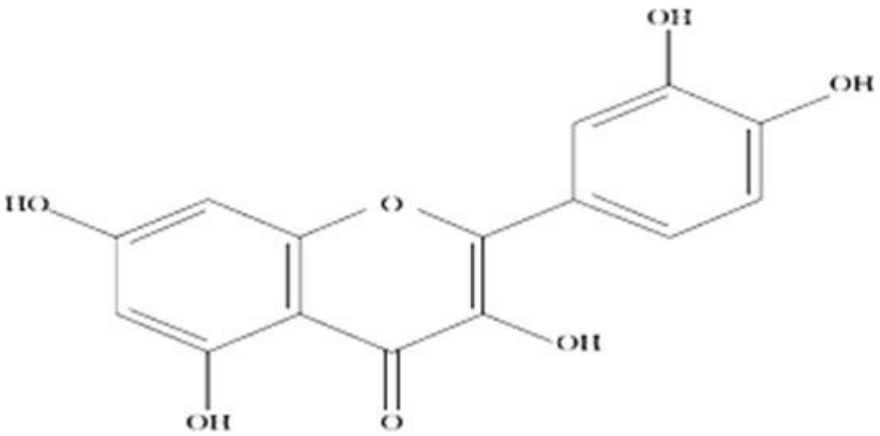

Figure 3: Chemical structure of Quercetin

reticulate and *Dictyota ciliolata*. The flavones are characterized by a planar structure because of a double bond in the central aromatic ring. One of the best described flavonoids, quercetin, is a member of flavones group. Quercetin (3, 5, 7, 3, 4-pentahydroxy flavon), is one of the most prominent dietary antioxidants (Paolillo *et al*., 2011). Quercetin has been reported to increase the genomic stability in rats and enhance the antioxidative defense system by up regulating antioxidant enzymes (Tieppo *et al*., 2007; Ishisaka *et al*., 2011). Pfeuffer *et al*. (2011) showed that the quercetin intake shows decreased incidence of cardiovascular and neoplastic diseases. Also, Quercetin has exhibited anticancer potential against a wide range of cancers such as prostate, cervical, lung, breast and colon by inhibiting cell proliferation by causing apoptosis and/or cell cycle arrest (Du G *et al*., 2010).

The results give an indication to the presence of promising antimicrobial compounds in methanol extract of soil algal species under study. Further phytochemical studies are needed to elucidate the components responsible for antimicrobial activity of these extracts against bacteria and fungi. The results clearly indicated scope for utilizing *Chlorella vulgaris* as source of antitumor effect, cancer chemoprevention properties, anti-inflammatory, antioxidant, and antimicrobial substances.

REFERENCES

Abdel-Raouf N, Ibraheem IBM, Abdel-Tawab S, Naser Yamna AG (2011). Antimicrobial and anti-hyperlipidemic activities of isolated quercetin from *Anabaena aequalis*. *Journal of Phycology*, 47:955-962.

Abu-Shanab B, Adwan G, Abu Safiya D, Adwan K, Abu-Shanab M (2005). Antibacterial activity of *Rhus coriaria* extracts growing in Palestine. *Journal of Islamic University Gaza, Palestine* (*Natural Sciences series*), 13(2):147-153.

Ali HEA, Shanab Sanaa MM, Abo-State Mervat AM, Shalaby EAA, El Demerdash UMN, Abdullah MA (2014). Screening of Microalgae for Antioxidant activities, Carotenoids and Phenolic Contents. *Applied Mechanics and Materials*, 625:156-159.

Al-Saif Sarah SA, Abdel-Raouf N, El-Wazanani Hend A, Aref IA (2014). Antibacterial substances from marine algae isolated from Jeddah coast of Red sea, Saudi Arabia. *Saudi Journal of Biological Sciences*, 21(1): 57-64.

Annamalai J, Nallamuthu T (2014). Antioxidant potential phytochemicals from methanol extract of *Chlorella vulgaris* and *Chlamydomonas reinhardtii*. *Journal of Algal Biomass Utilization*, 5(4):60-67.

Bermejo-Bescos P, Pinero-Estrada E, Villar del Fresno AM (2008). Neuroprotection by *Spirulina platensis* protean extract and phycocyanin against iron-induced toxicity in SH-SY5Y neuroblastoma cells. *Toxicology in Vitro*, 22(6):1496-1502.

Bolanho BC, Egea MB, Jácome ALM, Campos I, de Carvalho JCM, Danesi EDG (2014). Antioxidant and nutritional potential of cookies enriched with *Spirulina platensis* and sources of fibre. *Journal of Food Nutrition Research*, 53(2):171-179.

Bold HC, Wynne MJ (1978). Introduction to the Algae: Structure and Reproduction. Englewood Cliffs, New-Jersey, Prentice Hall, Inc. 07632. 720pp.

Browitzka MA, Browitzka LJ (1988). Micro-algal Biotechnology. Cambridge University Press, Cambridge, pp: 456-458.

Darah I, Lim SH, Ninthianantham K (2013). Effects of methanolic extract of *Wedelia chinensis* Osbeck (Asteraceae) leaves against pathogenic bacteria with emphasize on *Bacillus cereus*. *Indian Journal of Pharmaceutical Science*, 75:533-539.

Du G, Lin H, Yang Y, Zhang S, Wu X, Wang M, Ji L, Lu L, Yu L, Han G (2010). Dietary quercetin combining intratumoral doxorubicin injection synergistically induces rejection of established breast cancer in mice. *International Immunopharmacology*, 10(7):819-826.

El Far Mervat MM, Taie Hanan AA (2009). Antioxidant activities, total anthrocynins, phenolics and flavonoids contents of some sweet potato genotypes under stress of different concentrations of sucrose and sorbitol. *Australian Journal of Basic Applied Science*, 3: 3609-3616.

Ely R, Supriya T, Naik CG (2004). Antimicrobial activity of marine organisms collected off the coast of South East India. *Journal of Experimental Marine Biology and Ecology*, 309 (1): 121-127.

Farkaš V (2003). Structure and biosynthesis of fungal cell walls: Methodological approaches. Folia Microbiologica - *International Journal for General, Environmental and Applied Microbiology, and Immunology*, 48:469-478.

Ghasemi Y, Yazdi TM, Shafiee A, Amini M, Shokravi S, Zarrini G (2004). Parsiguine a novel antimicrobial substance from *Fischerella ambigua*. *Pharmaceutical Biology*, 42(4-5):318-322.

Gomha SM, Riyadh SM, Mahmmoud EA, Elaasser MM (2015). Synthesis and anticancer activities of Thiazoles, 1,3-Thiazines, and Thiazolidine using Chitosan-Grafted-Poly(vinylpyridine) as basic catalyst. *Heterocycles*, 91(6):1227-1243.

Goud MJP, Seshikala D, Charya MAS (2007). Antibacterial activity and biomolecular composition of certain fresh water microalgae from River Godavari (India). *Science World Journal*, 2(3): 19-23.

Gupta AB, Shrivastava GC (1965). On antibiotic properties of some fresh water algae. *Hydrobiologia*, 25:285-288.

Hoshaw RW, Rosowski JR (1973). Methods of Microscopic Algae. In. Stein, J.R., ed., Hand Book of Phycological Methods, Cambridge University Press. London, New York, Sidney.

Ishisaka A, Ichikawa S, Sakakibara H, Piskula MK, Nakamura T, Kato Y, Ito M, Miyamoto K, Tsuj A, Kawai Y, Terao J (2011). Accumulation of orally administered quercetin in brain tissue and its antioxidative effects in rats. *Free Radical Biology and Medicine*, 51(7):1329-1336.

Jaganathan SK, Manda M (2009). Antiproliferative effects of honey and of its polyphenols: A review. *Journal of Biomedicine and Biotechnology*, 2009:830616 (13pages).

Kajiyama SI, Kanzaki H, Kazuyoshi K, Kobayaashi A (1998). Nostofungicidine, an antifungal lipopeptide from the field-grown terrestrial blue-green alga *Nostoc commune*. *Tetrahedron Letters*, 39: 3737-3740.

Kellam SJ, Walker JM (1989). Antibacterial activity from marine microalgae in laboratory culture. *British Phycological Journal*, 24:191-194.

Kim IH, Lee DG, Lee SH, Ha JM, Ha BJ, Kim BJ, Lee JH (2007). Antibacterial activity of *Ulva lactuca* against Methicillin-resistant *Staphylococcus aureus* (MRSH). Biotechnology and Bioprocess Engineering, 12:579-582.

Klejdus B, Lojková L, Plaza M, Snóblová M, Stěrbová D (2010). Hyphenated technique for the extraction and determination of isoflavones in algae: Ultrasound-assisted supercritical fluid extraction followed by fast chromatography with tandem mass spectrometry. *Journal of Chromatography*, *A*, 1217: 7956- 7965.

Krant K, Schlesier K, Bitsch R, Hermann H, Rohe M, Bohm V (2005). Comparing antioxidative food additives and secondary plant products. Use of different assays. *Food Chemistry*, 93:171-175.

Kulik MM (1995). The potential for using cyanobacteria (blue green algae) and algae in the biological control of plant pathogenic bacteria and fungi. *European Journal of Plant Pathology*, 101: 585-599.

Malathi T, Ramesh Babu M, Lalitha Kumari K, Digamber Rao B (2015). Antimicrobial Activity of Soil Cyanobacteria *Cylindrospermum majus*. *International Journal of Recent Scientific Research*, 6(5):3859-3863.

Malik CP, Singh MB (1980). Plant Enzymology and Histo-Enzymology: A Text Manual. Kalyani Publishers, New Delhi, pp: 286.

Manivannan K, Anantharaman P, Balasubramanian T (2012). Evaluation of antioxidant properties of marine microalga *Chlorella marina* (Butcher, 1952). *Asian Pacific Journal of Tropical Biomedicine*, S342-S346.

Metting B, Pyne JW (1986). Biologically active compounds from microalgae. *Enzyme and Microbial Technology*, 8: 386-394.

Mirada MS, Cintra RG, Barros SB, Mancini-Filho J (1998). Antioxidant activity of the microalga *Spirulina maxima*. *Brazazilian Journal of Medical and Biological Research*, 31(8):1075-1079.

Mohd Syahril MZ, Roshani O, Nur hasyimah R, Mohamad Hafiz MS, Sharida MD, Ahmed HY (2012). Screening of anticancer activities of crude extracts of unicellular green algae (*Chlorella vulgaris*) and filamentous blue green algae (*Spirulina platensis*) on selected cancer cell lines. *Journal of Academia Univeriti Teknologi MARA Negeri Sembilan* (*My Jurnal*), 2:38-42.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1-2): 55-63.

Najdenski HM, Gigova LG, Iliev II, Pilarski PS, Lukavsky J, Tsvetkova IV, Ninova MS, Kussovski VK (2013). Antibacterial and antifungal activities of selected microalgae and Cyanobacteria. *International Journal of Food Science and Technology*, 48(7): 1533-1540.

Nijveldt RJ, van Nood E, van Hoorn DE, Boelens PG, van Norren K, van Leeuwen PA (2001). Flavonoids: a review of probable mechanisms of action and potential applications. *The American Journal of Clinical Nutrition*, 74(4): 418-425.

Ordog V, Stirk WA, Lenobel R, Bancirova M, Strand M, Van Standen J (2004). Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. *Journal of Applied Phycology*, 16: 309-314.

Paolillo R, Carratelli CR, Rizzo A (2011). Effect of resveratrol and quercetin in experimental infection by *Salmonella enterica* serovar Typhimurium. *International Immunopharmacology*, 11(2):149-156.

Pfeuffer M, Auinger A, Bley U, Kraus-Stojanowic I, Lau C, Winkler P, Rüfer CE, Frank J, Bösch-Saadatmandi C, Rimbach G, Schrezenmeir J (2011). Effect of quercetin on traits of the metabolic syndrome, endothelial function and inflammatory parameters in men with different APOE isoforms. *Nutrition, Metabolism and Cardiovascular Diseases*, 23(5):403-409.

Ploutno A, Carmeli S (2000). Nostocyclyne A, a novel antimicrobial cyclophane from the cyanobacterium *Nostoc* sp. *Journal of Natural Products*, 63: 1524-1526.

Prescott GW (1962). Algae of the Western Great Lake Areas. Publisher, Dubuque. Iowa, W. C. Brown Company, pp: 1004.

Pringsheim EG (1949). Pure Culture of Algae, Their Preparation and Maintenance. Cambridge University Press, London and New York. 119pp.

Pulz O, Gross W (2004). Valuable products from biotechnology of microalgae. *Applied Microbiology Biotechnology*, 65(6): 635-648.

Sahgal G, Ramanathan S, Sasidharan S, Mordi MN, Ismail S, Mansor SM (2011). In vitro and in vivo anticandidal activity of *Swietenia mahogany* methanolic seed extract. *Tropical Biomedicine*, 28(1):132-137.

Saini RK, Choudhary AS, Joshi YC, Joshi P (2005). Solvent Free Synthesis of Chalcones and their Antimicrobial Activities. *E-Journal of Chemistry*, 2(4):224-227.

Salem Olfat MA, Hoballah EM, Ghazi Safia M, Hanna Suzy N (2014). Antimicrobial activity of microalgal extracts with special emphasize on *Nostoc* sp. *Life Science Journal*, 11(12): 752-758.

Scott AC (1989). Laboratory control of antimicrobial therapy. In: Mackie & McCartney Practical medical microbiology (Edited by, Collee JG, Duguid JP, Fraser AG and Marmion B P) 13th Edn. Vol 2, United Kingdom-Edinburgh: Churchill Livingstone, p.161-181.

Shaieb FA, Issa AA, Meragaa A (2014). Antimicrobial activity of crude extracts of cyanobacteria *Nostoc commune* and *Spirulina platensis*. *Archives of Biomedical Sciences*, 2(2):34-41.

Tieppo J, Vercelino R, Dias AS, Silva Vaz MF, Silveira TR, Marroni CA, Marroni NP, Henriques JA, Picada JN (2007). Evaluation of the protective effects of quercetin in the hepatopulmonary syndrome. *Food and Chemical Toxicology*, 45(7):1140–1146.

van Heijenoort J (2001). Formation of the glycan chains in the synthesis of bacterial peptidoglycan. *Glycobiology*, 11: 25-36.

Volk RB (2009). Antialgal activity of several cyanobacterial exometabolites. *Journal of Applied Phycology*, 18:145-151.

Yen GC, Duh PD (1994). Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. *Journal of Agricultural and Food Chemistry*, 42(3):629-632.

Yu H, Jia S, Dai Y (2009). Growth characteristics of the cyanobacterium *Nostoc flagelliforme* in photoautotrophic, mixotrophic and heterotrophic cultivation. *Journal of Applied Phycology*, 21:127-133.