

GC/MS spectroscopic approach, antimicrobial activity and cytotoxicity of some marine macroalgae from Qusier and Marsa Alam Seashore (Red Sea), Egypt

Doaa A. Abdelrheem¹, Aziz A. Rahman², Khaled N. M. Elsayed³,
Sayed A. Ahmed^{1,*}

- 1- Department of Chemistry, Faculty of Science, Bani-Suef University 62511, Egypt
- 2- Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh
- 3- Department of Botany and Microbiology, Faculty of Science, Bani-Suef University, Egypt

*Corresponding Author: Sayed.hassan@science.bsu.edu.eg

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ABSTRACT

In the present work, three macroalgae viz; *Halimeda tuna*, *Padina gymnospora* and *Phacelocarpus tristichus*, collected from Egyptian Red Sea shores were investigated for their chemical composition and pharmacological properties. Volatile compounds of 70% methanol extracts of three selected algae were analyzed by GC-MS. Antimicrobial and cytotoxic activities of algal extracts (70% methanol) were tested by agar well diffusion and *in vitro* cell viability assays, respectively. *P. gymnospora* showed the maximum antibacterial activity against *E. coli* (13.90±0.66 mm), followed by *P. tristichus* (12.97± 0.65mm), while *H. tuna* inhibited significantly the growth of *S. aureus* (13.17± 0.67mm). Furthermore, the highest antifungal activity was obtained by *P. gymnospora*, followed by *P. tristichus* and finally *H. tuna* against *C. neoformans* and *A. fumigatus*. Also, *P. gymnospora* showed more cytotoxicity against HepG-2 and MCF-7 cell lines than *P. tristichus* and *H. tuna*. Moreover, it is the first report of chemical composition, antimicrobial activity and cytotoxicity of *P. tristichus* and also this research showed new reports on cytotoxicity of *P. gymnospora* and *H. tuna* against new cell lines. In conclusion, the Egyptian marine macroalgae possess antimicrobial and cytotoxic activities, that could be investigated for future application in medicine and recognizing novel drugs from the marine resources after checking their bioavailability *in vivo*.

INTRODUCTION

Recently, attention is placed on possible discovery of drugs from natural source rather than synthetic chemicals (Blunt *et al.*, 2017). Marine algae or seaweeds are one of the natural resources essentially for producing various bioactive secondary metabolites with potential for use in the development of new pharmaceutical and industrial agents (Rico *et al.*, 2017). Macroalgae are mainly classified into three taxonomic groups depend

on pigmentation, these are green algae (Chlorophyta), red algae (Rhodophyta) and brown algae (Phaeophyta) (Pereira, 2010).

Several research studies have shown that macroalgae play a significant role in pharmaceutical industry due to their ability to produce secondary metabolites with a broad range of pharmacological activities, such as anti-inflammatory, antioxidant, antimicrobial, cytotoxicity, antiviral and anticoagulant activities (Al-Enazi *et al.*, 2018a). As an aid to protect themselves against other organisms in their environment, macroalgae produce various bioactive metabolites including, phlorotannins, polyketides, sterols, cyclic peptide, alkaloids, polysaccharide, diterpenoids, quinones, glycerols and lipids that have a wide range of biological activities (Al-Saif *et al.*, 2014).

Halimeda tuna and *Padina gymnospora* are members of green and brown macroalgae, respectively. These macroalgae have already been studied for antimicrobial activity, but a very few results were obtained regarding their chemical composition and cytotoxicity (Araujo *et al.*, 2013; Murugan & Iyer, 2014; Milović *et al.*, 2017; Madkour *et al.*, 2019). Moreover, there is no specific report about chemical and biological properties of red alga *Phacelocarpus tristichus*.

The algal richness of Egyptian Red Sea coasts is undeniable in terms of diversity and quantity. However, there have been only a few studies on the pharmacological effects and chemical composition of the marine algae in this region (Madkour *et al.*, 2019). Therefore, this study was designed to determine chemical composition, antimicrobial activity against nine microbial strains and cytotoxicity against two human cancer cell lines (HepG-2 and MCF-7) of the total MeOH (70%) extracts obtained from three macroalgae viz; *Halimeda tuna*, *Padina gymnospora* and *Phacelocarpus tristichus*, collected from Egyptian Red Sea shores. It is worth mentioning that, this study is the first report of evaluating the chemical and biological properties of the red alga *Phacelocarpus tristichus*. Besides, our investigation showed new reports on cytotoxicity of *P. gymnospora* and *H. tuna* against new types of cell lines.

MATERIALS AND METHODS

Algal samples

Algal samples of *Halimeda tuna*, *Padina gymnospora* and *Phacelocarpus tristichus* were collected from Quseir and Marsa Alam at the Red Sea shores in Egypt in (2016-2017) (Figure 1). The voucher specimen of the algae was deposited at the Department of Chemistry, Faculty of Science, Beni-Suef University, Egypt. The identification of the collected algae was done by Dr. Khaled NM Elsayed, Associate Professor of algal biotechnology at Botany and Microbiology Department, Faculty of Science, Beni-Suef University, Egypt.



Figure 1: Marine algae collected from the Red Sea (Qusier and Marsa-Allam, Egypt).

Preparation of the algal extracts

The air-dried powdered algal samples (50 g each) were macerated in 70% methanol (500 ml) for 3 days with regular shaking. After evaporation of solvent under reduced pressure, each extract of *H. tuna* (0.51 g), *P. gymnospora* (1.53 g) and *P. tristichus* (1.4 g) were stored at -20°C until further use.

GC-MS analysis of algal extracts

The analysis of chemical constituents present in the total extracts (70% methanol) of *H. tuna*, *P. gymnospora* and *P. tristichus* were achieved by gas chromatography–mass spectrometry at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Egypt, utilizing a DB5-MS column [30 m \times 0.25mm ID (J&W Scientific, USA)] with 1 mL/min flow of helium as carrier gas. The WILEY & NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) libraries were used for recognition of the main peaks (Araujo *et al.*, 2013).

Antimicrobial activity

Microbial strains

The extracts (70% methanol) of selected algae were subjected for their antimicrobial activity against nine micro-organisms. Three Gram positive bacteria *Streptococcus mutans* [RCMB 017 (1), ATCC 25175], *Bacillus subtilis* [RCMB 015 (1), NRRL B-543] and *Staphylococcus aureus* [RCMB010010], three Gram negative bacteria *Enterobacter cloaca* [RCMB 001 (1), ATCC 23355], *Salmonella typhimurium* [RCMB 006 (1), ATCC 14028] and *Escherichia coli* [RCMB 010052, ATCC 25955] and three fungi *Candida albicans* [RCMB 005003 (1), ATCC 10231], *Cryptococcus neoformans* [RCMB 0049001] and *Aspergillus fumigates* [RCMB 002008] were selected for the experiment. Microbial pathogens were got from the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Egypt.

Antimicrobial assay

Antimicrobial activity of algal extracts was determined by agar well diffusion technique following National Committee for Clinical Laboratory Standards (NCCLS) (National Committee for Clinical Laboratory Standards, 2006). Algal extracts were

dissolved in 5 mg/mL of dimethyl sulfoxide (DMSO, Merck), following control agents that were used, positive control agents: gentamycin (10 µg/ mL) (for bacteria) and ketoconazol (20 µg/ mL) (for fungi) as well as negative control agent: 5% DMSO. Mueller-Hinton agar was utilized as a culture media for bacteria and sabouraud dextrose agar was utilized for fungi. The tested algal extracts and controls (100 µl) were dispensed into the wells (diameter 6 mm). Plates were incubated for 24 h at 37 °C for bacteria and for 3 days at room temperature for fungi; all cultures were kept under aerobic conditions. The diameters of the growth inhibition zones were measured in mm. The experiments were realized in triplicates.

Cytotoxic activity

Cells and propagation

HepG-2 cells (human hepatocellular carcinoma) and MCF-7 cells (human breast carcinoma) were brought from VACSERA tissue culture unit, Cairo University, Egypt. Cells were kept in a 5% CO₂ atmosphere at 37 °C. The cancer cells were grown in RPMI-1640 medium involving 10% fetal bovine serum, HEPES buffer, 50µg/ml gentamycin and 1% L-glutamine (all purchased from Lonza).

Cell viability assay

The cells were plated in 96-well microplates (Falcon, NJ, USA) at concentration of (1×10⁴ cells/well) in 100µl of culture medium, and were allowed to stick for 24 h before treatment. Then, different concentrations of tested algal extracts in DMSO were added. 0.1% (v/v) was the maximum concentration of DMSO in wells where DMSO was used as a solvent for all the tested extracts which showed no effect on the test. Doxorubicin HCl (Sigma-Aldrich) was used as reference drug positive control tested at the same concentrations used for the tested extracts. The cells were incubated for 24 h in the presence and absence of tested samples. Cytotoxicity was evaluated by a colorimetric method where (1%) crystal violet solution was added to culture media for at least 30 minutes and then glacial acetic acid (30%) was added. Then, absorbance was measured on micro-plate reader (Sun Rise, TECAN, Inc, USA), at wavelength 490 nm. (**Gomha *et al.*, 2015**)

Statistical analysis

For antimicrobial activity: All the data were illustrated as means ± standard deviation (SD). The procedures were carried out in triplicates.

For cytotoxicity: The 50% inhibitory concentration (IC₅₀) was obtained by nonlinear regression curve utilizing Graph pad Prism software (San Diego, CA, USA).

RESULTS AND DISCUSSION

Chemical composition of algal extracts

The chemical components of 70% methanol extracts of *H. tuna*, *P. gymnospora* and *P. tristichus* were characterized by GC–MS analysis (**Figure 2, Table 1(a-c)**).

Obviously, there were various classes of chemical compounds in three algal extracts such as fatty acids, esters, sterols, terpenes, alkaloids, hydrocarbons, aldehydes, etc. Major chemical constituents present in *H. tuna* extract were lup-20(29)-en-3 α -ol, acetate (36.45%), dibutyl phthalate (29.52%), betulinaldehyde (4.26%), cyclooctasiloxane, hexadecamethyl (3.36%) and lupeol (3.02%). While hexadecanoic acid, methyl ester (14.09%), hexadecanoic acid (13.09%), cis-13-octadecenoic acid, methyl ester (10.99%), propanoic acid, 2-hydroxy-, ethyl ester (10.42%), 1-pentanol (6.99%), 2-pentanone, 4-hydroxy-4-methyl (6.67%), tetradecanoic acid (4.69%) and di-(9-octadecenoyl)-glycerol (3.26%) were the major components in *P. gymnospora* extract.

Moreover, *P. tristichus* extract contains the highest percentage of hexadecanoic acid, methyl ester (20.57%), followed by 5, 8, 11, 14-eicosatetraenoic acid, methyl ester, (all-Z) (11.73%), stigmasta-5,24(28)-dien-3 α -ol, (Z) (10.01%), methyl-9-octadecenoate (9.31%), methyl stearidonate (8.81%), trans-9-octadecenoic acid (4.70%), hexadecanoic acid (4.41%) and cis-5,8,11,14,17-eicosapentaenoic acid (3.82%) as major components.

Previous studies on the chemical constituents of *H. tuna* are limited (**Shahnaz & Shameel, 2006; Milović et al., 2017; Milović et al., 2019**). The methanol extracts of *Caulerpa scalpelliformis*, *Halimeda tuna* and *Udotea indica* from Karachi coast in Pakistan were distinguished by high amount of saturated fatty acids (61-77%) than unsaturated fatty acids (23-39%) with the most predominant palmitic acid and oleic acid as well as cholesterol is the common sterol in three algal extracts (**Shahnaz & Shameel, 2006**), whereas in our study, the 70% methanol extract of *H. tuna* contains the highest level of triterpenoids. In another study, the dichlorometane: methanol (1:1) dry extract of *H. tuna* from the Adriatic coast in Montenegro was characterized by high level of palmitic acid (32%) and linoleic acid (13.63%) with total 20 fatty acids (**Milović et al., 2017**). However, the cyclohexane extract of *H. tuna* from the same previous collected region was identified with its high percentage of β -sitosterol (73.90 \pm 0.08 μ g/g) and low amount of campesterol (3.78 \pm 0.12 μ g/g) as well as fatty acids with oleic acid, palmitic acid and α -linoleic acid as dominant fatty acids (**Milović et al., 2019**). However, in our investigation, lup-20(29)-en-3 α -ol, acetate (36.45%) is the most dominant compound.

Likewise, there were a few studies about the chemical constituents of *P. gymnospora* (**Al Easa et al., 1995; Murugan & Iyer, 2014; Shanmuganathan & Pandima, 2016; Vasanthi, 2016; Baliano et al., 2016; Ibraheem et al., 2017**). Fucosterol is the predominant sterol in *P. gymnospora* from the coast of Qatar among total 4 identified sterols (**Al Easa et al., 1995**). Also, fucosterol (12.45%) and L-(+)-ascorbic acid-2,6-dihexadecanoate (8.13%) were the most abundant compounds between total 16 compounds in *P. gymnospora* ethyl acetate extract from the coast of Tamil Nadu (**Murugan & Iyer, 2014**). *P. gymnospora* from the intertidal region of the Gulf of Mannar was characterized by high percentage of stearic acid of total 7 fatty acids (**Shanmuganathan & Pandima, 2016**). Oleic Acid (17.55%), stigmasterol (15.89%), trans-13-octadecenoic acid (8.69%), n-hexadecanoic acid (6.23%) and cis-vaccenic acid

(4.72%) were the main components in the hexane extract of *P. gymnospora* from Gulf of Mannar region (Vasanthi, 2016). From the bethonic area in Espirito Santo, *p. gymnospora* methanol extract contained eleven fatty acids among them linolenic, oleic, arachidonic and linoleic acids (Baliano *et al.*, 2016). Palmitic acid (36.35%) is the most predominant compound of total 9 fatty acids in the methanolic extract of *P. gymnospora* from Hurghada coastline, Red Sea, Egypt (Ibraheem *et al.*, 2017). But, in our study, the 70% methanol of *P. gymnospora* showed the highest percentage of fatty acids and their esters with the most abundant hexadecanoic acid, methyl ester and hexadecanoic acid.

Obviously, our study showed that the identified major components of 70% methanol extracts of *H. tuna* and *P. gymnospora* are not similar with the previous reports from other extraction solvent and other countries. Variations in the chemical components of *H. tuna* and *P. gymnospora* can be due to different factors such as seasonal periods, geographical location, soil condition, extraction methods, analysis procedure, storage conditions, etc. (Pérez *et al.*, 2016).

Moreover, this study is the first report about the chemical constituents of red alga *P. tristichus*, where its 70% methanol extract contained high level of esters of fatty acids, fatty acids and steroids with hexadecanoic acid, methyl ester and β -sitosterol as the most major compounds. In agreement to our study, red algae showed a variety of secondary metabolites as fatty acids, phenolics, phlorotannins, terpenes, polysaccharides, halogenated compounds, etc. (Alassali *et al.*, 2016).

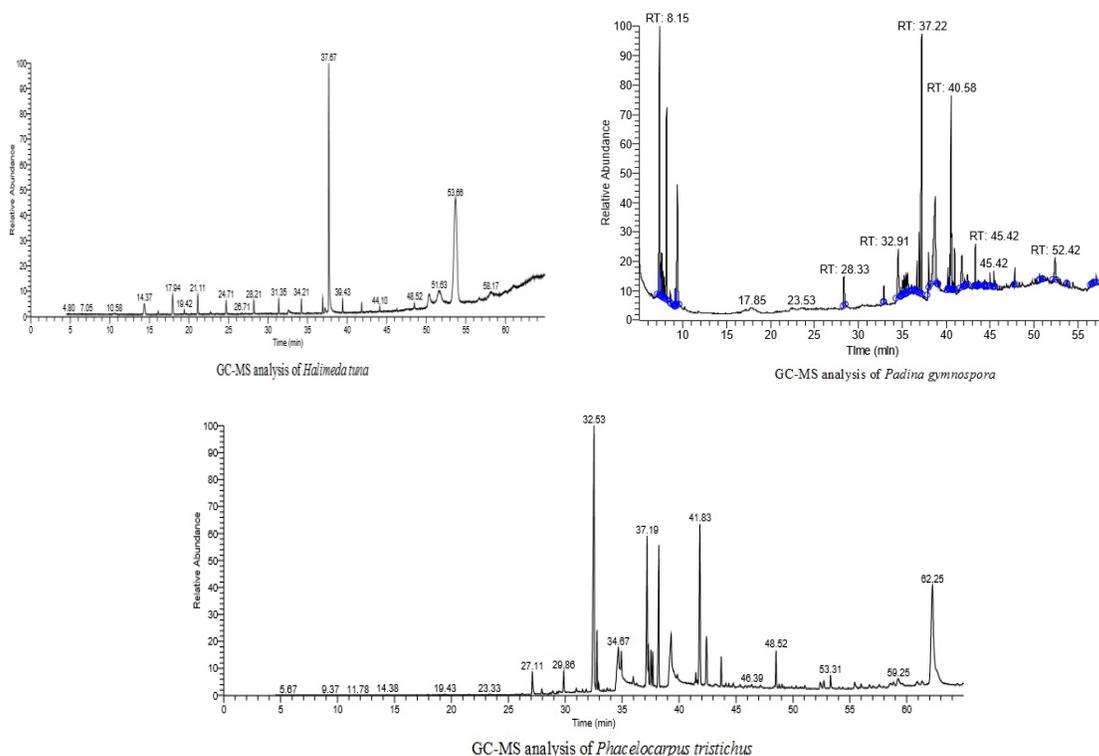


Figure 2: GC-MS chromatograms of 70% methanol extracts of three selected algae.

Table 1(a): Major chemical constituents of MeOH (70%) extract of *Halimeda tuna*

Serial n.	Compounds name	RT	Area (%)	Molecular formula	Molecular weight
1	Cyclooctasiloxane, hexadecamethyl	17.94	3.36	C ₁₆ H ₄₈ O ₈ Si ₈	592
2	Dibutyl phthalate	32.56	29.52	C ₁₆ H ₂₂ O ₄	278
3	Lupeol	37.67	3.02	C ₃₀ H ₅₀ O	426
4	Betulinaldehyde	48.52	4.26	C ₃₀ H ₄₈ O ₂	440
5	Lup-20(29)-en-3á-ol, acetate	50.30	36.45	C ₃₂ H ₅₂ O ₂	468

Table 1 (b): Major chemical constituents of MeOH (70%) extracts of *Padina gymnospora*

Serial n.	Compound name	RT	Area (%)	Molecular formula	Molecular weight
1	Propanoic acid, 2-hydroxy-, ethyl ester	7.34	10.42	C ₅ H ₁₀ O ₃	118
2	1-Pentanol	8.15	6.99	C ₅ H ₁₂ O	88
3	2-Pentanone, 4-hydroxy-4-methyl	9.38	6.67	C ₆ H ₁₂ O ₂	116
4	Tetradecanoic acid	34.57	4.69	C ₁₄ H ₂₈ O ₂	228
5	hexadecanoic acid, methyl ester	37.22	14.09	C ₁₇ H ₃₄ O ₂	270
6	Hexadecanoic acid	38.79	13.09	C ₁₆ H ₃₂ O ₂	256
7	cis-13-Octadecenoic acid, methyl ester	40.58	10.99	C ₁₉ H ₃₆ O ₂	296
8	Di-(9-octadecenoyl)-glycerol	41.80	3.26	C ₃₉ H ₇₂ O ₅	620

Table 1(c): Major chemical constituents of MeOH (70%) extract of *Phacelocarpus tristichus*

Serial n.	Compound name	RT	Area (%)	Molecular formula	Molecular weight
1	Hexadecanoic acid, methyl ester	32.52	20.57	C ₁₇ H ₃₄ O ₂	270
2	n-Hexadecanoic acid	34.66	4.41	C ₁₆ H ₃₂ O ₂	256
3	Methyl- 9-octadecenoate	37.19	9.31	C ₁₉ H ₃₆ O ₂	296
4	Methyl stearidonate	38.22	8.81	C ₁₉ H ₃₀ O ₂	290
5	trans-9-Octadecenoic acid	39.30	4.70	C ₁₈ H ₃₄ O ₂	282
6	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)	41.83	11.73	C ₂₁ H ₃₄ O ₂	318
7	cis-5,8,11,14,17-Eicosapentaenoic acid	42.41	3.82	C ₂₀ H ₃₀ O ₂	302
8	Stigmasta-5,24(28)-dien-3á-ol, (Z)	62.25	10.01	C ₂₉ H ₄₈ O	412

Antimicrobial activity

Recently, microbial resistance to pharmaceutical drugs is on rise, which demands an alternate source of antibiotics against fatal diseases. Marine macroalgae are potential source of bioactive compounds making them one of the major subjects for the

development of various pharmaceutical drugs. The present study revealed the antimicrobial activities of three algal extracts prepared by 70% methanol that were tested against nine micro-organisms (**Table 2**). The results showed that all three algal extracts exhibited relatively strong antimicrobial activity. These findings demonstrated that 70% methanol extracts contained significant amount of antimicrobial compounds. The finding is in agreement to our result by **Rangaiah *et al.* (2010)** that demonstrated that the methanolic extracts of seaweeds provided stronger antimicrobial activity than ethyl acetate and n-hexane extracts (**Rangaiah *et al.*, 2010**). In contrast, **Sastry and Rao (1994)** showed that the chloroform extract exhibited the highest antimicrobial activity rather than the methanol and benzene extracts (**Sastry & Rao, 1994**). This difference in the result probably because of many factors influenced the antimicrobial potency of marine macroalgae like the season and habitat of algal collection, different stages of plant growth, efficiency of extraction, resistance to the bacteria tested, etc. (**Seenivasan *et al.*, 2010**).

Consequently, the present study investigated that *P. gymnospora* exhibited the strongest antibacterial activity against *E. coli* (13.90±0.66 mm) followed by *P. tritichus* (12.97±0.65 mm). However, *H. tuna* showed potential antibacterial activity against *S. aureus* (13.17±0.67 mm). Additionally, the maximum antifungal activity (16.20±0.17 and 15.00±0.26 mm) was established by *P. gymnospora* against *C. neoformans* and *A. fumigatus*, respectively. *H. tuna* reported the lowest antifungal activity against *A. fumigatus* (7.98±0.18 mm). It could be noticed from the present investigation that the methanol extract of *P. gymnospora* (Phaeophyceae) exhibited both the highest value and the broadest spectrum of antimicrobial activity among selected seaweeds against tested fungal and bacterial pathogens. In agreement to our results, **Viachosi and Critchley (2001)** investigated that the Phaeophyta extracts showed the strongest antimicrobial activity followed by the Rhodophyta and then the Chlorophyta (**Viachosi & Critchley, 2001**).

In the current study, the fungal strains were more susceptible to *P. gymnospora* extract than bacterial strains where the highest inhibition zone (16.20±0.17 mm) was exhibited by *P. gymnospora* against *C. neoformans*. Also, strong antibacterial activity was observed for *P. gymnospora* extract with high potent against *E. coli* (13.90±0.66 mm) followed by *B. subtilis* (12.10±0.75 mm), *S. aureus* (9.17±0.47 mm) and *S. typhimurium* (9.10±0.36 mm). Similar findings were reported by **Chander *et al.* (2014)** who investigated that methanolic extract of *P. gymnospora* from India exhibited strong inhibition for the growth of most tested bacterial and fungal strains (**Chander *et al.*, 2014**). In contrast, **Rosaline *et al.* (2012)** showed that the methanolic extract of *P. gymnospora* and *Sargassum wightii* from the coastal Tamil Nadu in South India showed no inhibition zone against *B. subtilis*, *S. aureus* and *E. coli* as well as the acetone extract of these algae showed the most efficiency antimicrobial activity (**Rosaline *et al.*, 2012**). Also, **Saliva *et al.* (2013)** investigated that the ethanol extracts of *Hypnea musciformes*,

P. gymnospora and *Ulva fasciata* from Pacheco showed stronger antimicrobial activity rather than methanol, hexane and acetone extracts in dissimilar to our results (Silva *et al.*, 2013). These variations could be because of the different solubility behavior of secondary metabolites that could be influenced by seasonal and geographical distribution of the species (Rajasulochana *et al.*, 2009).

In general, the antimicrobial activity of macroalgae is affected not only by natural factors as the environmental conditions, seasonality and the geographical location, but also antimicrobial phytoconstituents. Macroalgae contain a great variety of natural bioactive compounds with antimicrobial activity, like polyunsaturated fatty acids, phenolic compounds, carotenoids and polysaccharides. (Pérez *et al.*, 2016)

The findings revealed that 70% methanol extract of *P. gymnospora* could be used as a prominent source of antimicrobial agent in pharmaceutical industry. This may be due to the presence of synergetic bioactive compounds intracellularly in *P. gymnospora* extract. From the data of GC-MS, hexadecanoic acid, methyl ester (14.09%), hexadecanoic acid (13.09%) and cis-13-octadecenoic acid, methyl ester (10.99%) were the major compounds in crude extract of *P. gymnospora*. Previous studies showed that these secondary metabolites revealed several biological activities as antioxidant, antimicrobial, anti-inflammatory and cytotoxic activities (Pinto *et al.*, 2017).

In addition, the results investigated that *H. tuna* exhibited the least antimicrobial activity against both bacterial and fungal strains especially *E. coli* (9.03 ± 0.55 mm), *C. neoformans* (8.33 ± 0.29 mm) and *A. fumigatus* (7.98 ± 0.18 mm). In agreement with our results, Karthikaidev *et al.* (2009) showed that the ethanolic and chloroform extracts of *H. tuna* and *Ulva reticulata* from the coast of Vedalai, Gulf of Mannar, Tamilnadu exhibited a broad spectrum of antimicrobial activity compared to the methanolic extract (Karthikaidevi *et al.*, 2009). In disagreement to our results, Indira *et al.* (2013) investigated that the methanol extract of *H. tuna* from India showed higher inhibition zone against the growth of tested micro-organisms than the chloroform and ethanol extracts (Indira *et al.*, 2013). However, in the present study, *H. tuna* extract contained highly ratio of bioactive constituents as lup-20(29)-en-3-ol, acetate (36.45%) and dibutyl phthalate (29.52%) according to GC-MS (Yusuf-Babatunde *et al.*, 2019). This is weak antimicrobial activity of *H. tuna* probably because of the antagonistic effect of these compounds when they are found with each other in the *H. tuna* extract.

It is worth mentioned that, antimicrobial activity of red alga *Phacelocarpus tristichus* is reported for the first time in this study. The alga showed moderated antimicrobial activity among the selected algae. This is probably due to the synergetic bioactive compounds in *P. tristichus* extract. Hexadecanoic acid, methyl ester (20.57%), stigmasta-5, 24(28)-dien-3-ol (10.01%), 14-octadecenoic acid, methyl ester (9.31%), methyl stearidonate (8.81%), 5, 8, 11, 14-eicosatetraenoic acid, methyl ester (11.73%) and hexadecanoic acid (4.41%) were the major lipophilic components of *P. tristichus* according to GC-MS. The compounds were reported to possess antimicrobial, anti-

inflammatory, antioxidant and cytotoxic activities (Lavanya & Thangamathi, 2017). In agreement with our results, several studies reported that the methanol extract of red algae showed significant antimicrobial activity due to presence of lipophilic metabolites that have several pharmaceutical properties (Pérez *et al.*, 2016; Ibraheem *et al.*, 2017).

Our results indicated as well, the methanol extracts of *P. gymnospora* and *P. tristichus* were more effective as antifungal agents rather than bacterial agents when compared to *H. tuna*. This is evidence from the observation that the methanol extracts of the brown and red algae effectively inhibited most of the pathogens with the maximum inhibition zone of (16.20±0.17 mm) and (14.43±0.45 mm), *respectively* produced against *C. neoformans*. This is probably due to the difference in the composition and permeability of their cell wall. This observation is in accordance with many other studies, focused on antimicrobial activity that has investigated that structure and permeability of the cell wall are reasons for different sensitivities in Gram positive bacteria, Gram negative bacteria and fungi (Albouchi *et al.*, 2013; Kolanjinathan *et al.*, 2014). In contrast, Ballesteros *et al.* (1992) showed that *H. tuna* from Western Mediterranean exhibited strong antifungal properties (Ballesteros *et al.*, 1992). This difference may be due to seasonal or location variations (Lima-Filho *et al.*, 2002).

Also, the present study showed that a potent inhibitory activity of *P. gymnospora* extract was exactly observed on gram positive than on gram negative bacteria, except with *E. coli* that was highly affected with highest inhibition zone (13.90±0.66 mm). The strongest antibiotic activity of the brown algae against pathogens was supported by the recent findings of Manivannan *et al.* (2011) (Manivannan *et al.*, 2011). Furthermore, the findings of the present investigation has brought to light that the methanol extract of *H. tuna* is more effective against Gram positive bacteria (*S. aureus* and *B. subtilis*) than Gram negative bacteria (*E. cloaca*). A similar observation was recorded by Wan *et al.* (2018) who found that Gram positive bacteria (*B. subtilis*, *S. aureus* and *B. cereus*) were the most sensitive bacteria to methanol extract of *Halimeda* sp. from the coastal area of Peninsular Malaysia, while Gram negative bacteria (*E. coli* and *P. aeruginosa*) and fungi (*A. niger* and *C. albicans*) were the most resistance to *Halimeda* sp. (Wan *et al.*, 2018). In contrast, in this study, red algae extract of *P. tristichus* showed more effective against Gram negative bacteria (*E. coli* and *S. typhimurium*) compared to Gram positive bacteria (*B. subtilis*, *S. aureus*, and *S. mutans*).

The more susceptibility of a particular group of bacteria was because of the difference in their cell wall structure and their composition (Taskin *et al.*, 2007). So, Gram positive bacteria were observed to be slightly more sensitive to both *P. gymnospora* and *H. tuna* extracts than Gram negative bacteria, possibly due to the hydrophilic cell wall structure of Gram negative bacteria (Silhavy *et al.*, 2010). The cell wall of Gram negative bacteria is constituted mainly by a lipo-polysaccharide, which blocks the penetration of hydrophobic components of fatty acids, esters, steroids and terpenoids that are the major components of these tested seaweeds (Beveridge, 1999).

However, the outer membrane of Gram negative bacteria act as a barrier to many environmental substances including antibiotics (Mendes *et al.*, 2013), the higher susceptibility noticed with the *P. tristichus* extract gives a promising indication of developing a potent drug from this marine natural source to be utilized in combating the infections because of these pathogens. Previous studies reported that the high sensitivity for Gram negative strains than Gram positive strains is probably due to the presence of phenolic compounds in the tested extracts that solubilized the lipopolysaccharide layer of Gram negative cell wall, inducing the entry of the inhibitory molecules (Butkhup *et al.*, 2010). However, other studies showed that phenolic extracts were more efficient against Gram positive bacteria (Klančnik *et al.*, 2009). However, the methanol extract of red alga *P. tristichus* in our study contained highly percentage of lipophilic compounds and trace amounts of slightly hydrophilic compounds according to GC-MS; it is more effective against Gram negative bacteria than Gram positive bacteria. This higher inhibitory effect is probably because of synergetic effects of these compounds with Gram negative strains. In agreement with our results, Tchinda *et al.* (2019) investigated synergistic effects of combination of mixture of stigmaterol and β -sitosterol (lipophilic) with β -sitosterol-3-*O*- β -D-glucoopyranoside (slightly hydrophilic) was observed in the inhibition of resistant Gram negative bacteria (Tchinda *et al.*, 2019).

Table 2: Antimicrobial screening test of (70% methanol) extracts of *Halimeda tuna*, *Padina gymnospra* and *Phacelocarpus tritichus*

Name of algae	Inhibition zones (mm)								
	Gram (+ve) bacteria			Gram (-ve) bacteria			Fungi		
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>S. mutans</i>	<i>E. coli</i>	<i>S. typhimurium</i>	<i>E. cloaca</i>	<i>A. fumigatus</i>	<i>C. neoformans</i>	<i>C. albicans</i>
<i>Halimeda tuna</i>	13.17±0.67	10.10±0.46	0.0±0.0	9.03±0.55	0.0±0.0	0.0±0.0	7.98±0.18	8.33±0.29	0.0±0.0
<i>P. gymnospora</i>	9.17±0.47	12.10±0.75	0.0±0.0	13.90±0.66	9.10±0.36	0.0±0.0	15.00±0.26	16.20±0.17	0.0±0.0
<i>P. tritichus</i>	0.0±0.0	9.10±0.36	0.0±0.0	12.97±0.65	11.10±0.36	0.0±0.0	13.93±0.15	14.43±0.45	0.0±0.0
Control	24.13±1.21	25.97±0.95	20.03±0.25	30.03±1.05	16.97±0.95	27.13±0.6	16.13±0.18	24.97±0.15	20.23±1.37

Data are means of three replicates (n = 3) ± standard error.

Gentamycin: control for gram (+ve) and gram (-) bacteria; **Ketoconazol:** control for fungi.

Cytotoxicity

Marine algae have showed several biological activities like cytotoxic and antitumor activities (Alves *et al.*, 2018). In this study, the anti-proliferative activity of the 70% methanol extracts of *H. tuna*, *P. gymnospora* and *P. tristichus* was tested against HepG-2 and MCF-7 cell lines. Cell viability assay revealed a dose-dependent decline in percent viability of the cells. Here, all the cell lines were applied with different concentrations of algal extracts for 24 h. The algal extract decreased the proliferation of liver and breast cancer cells after 24 h of incubation, presented in **Table 3** and **Figure 3**. The tested algal extracts especially *P. gymnospora* extract showed the maximum cytotoxicity (9.81 and 11 μ g/mL),

followed by *P. tritichus* (21 and 29.3 $\mu\text{g/mL}$) and finally *H. tuna* (22.4 and 37.3 $\mu\text{g/mL}$) against HepG-2 and MCF-7 cells, *respectively*.

Our results are the first published reports on the cytotoxic activity of red alga *P. tritichus* against HepG-2 and MCF-7 cell lines. However, there were a few reports on cytotoxicity of *P. gymnospora* and *H. tuna*, but our results are probably similar or contrary to these studies. These differences in the literature can be explained by the fact that the toxic effects of algae vary with the time of algae sampling and geological variations, solvent extraction and sampling protocol (Yamahara *et al.*, 2015).

Milovic *et al.* (2017) found that significant cytotoxicity of the dichloromethane: methanol (1:1) dry extract of *H. tuna* from the Adriatic coast in Montenegro against human colon carcinoma cell line (LS174), human adenocarcinoma cell line (HeLa) and human chronic myelogenous leukaemia cell line (K562) (Milović *et al.*, 2017). Kurt *et al.* (2014) showed that the methanol extract of *H. tuna* showed higher cytotoxicity against MCF-7 cell line than the chloroform extract because of increase in oxidative stress (Kurt *et al.*, 2014). Moo-Puc *et al.* (2009) explored cytotoxicity of dichloromethane: methanol (7:3) extract of *H. tuna* from Yucatán in Mexico against MDCK (normal canine kidney), Hep-2 (human laryngeal carcinoma), KB (human nasopharyngeal carcinoma) and HeLa cells (Moo-Puc *et al.*, 2009). Kurt *et al.* (2018) showed the cytotoxicity of methanol and chloroform extract of *H. tuna* against mouse neuroblastoma cell line (NA2B) due to the increase in oxidative stress (Kurt *et al.*, 2018). Compared with their studies, the findings of this research show that this study is the first report of cytotoxicity of *H. tuna* against HepG-2 cell line and that the *H. tuna* methanol extract showed weak cytotoxicity. Similarly, as mentioned in antimicrobial activity, the antagonistic effects of bioactive compounds were observed in weak cytotoxicity of *H. tuna* against MCF-7 and HepG-2 cell lines.

Our results are the first published reports on cytotoxic activity of extract of brown alga *P. gymnospora* on MCF-7 and HepG-2 cell lines. Exceptionally, it has been shown that the aqueous extract of *P. gymnospora* synthesizes gold Nanoparticles of 53-67 nm which exhibited significant cytotoxicity against HepG2 and lung (A549) cells (Singh *et al.*, 2015). Obviously, there is difference between cytotoxic activity of nanoparticles composite with macroalgae and algae only where there are changes in cell viability, cell morphology and metabolic activity (Singh *et al.*, 2015). Furthermore, others studies and findings reported the cytotoxicity of *P. gymnospora* from various location on other different cell lines (Koishi *et al.*, 2012; Guedes *et al.*, 2013; Murugan & Iyer, 2013; Baliano *et al.*, 2016; Sali, 2016; Gasparini *et al.*, 2017). In the literature there is no data on cytotoxicity activity of *P. gymnospora* extract on HepG-2 and MCF-2 cells, but cytotoxic activity of some other algal extracts were studied on these cell lines by other researches. Mashjoor *et al.* (2016) found cytotoxicity for ethyl acetate extracts of *Ulva flexuosa*, *Padina antillarum* and *Padina boergesen* on MCF7, HeLa and Vero cell lines (Mashjoor *et al.*, 2016). Al-Enazi *et al.* (2018b) reported a significant cytotoxicity of

ethanol extract of *Padina pavonica* against A-549, Caco-2, HCT-116, Hela, HEp-2, HepG-2, and MCF-7 cell lines (Al-Enazi *et al.*, 2018b). Compared with their results, the findings of this research suggest that the methanol extract of *P. gymnospora* showed potential cytotoxic effects.

In agreement with our results, several studies showed that brown seaweeds are probably good candidates for cancer therapies (Isnansetyo *et al.*, 2017). Moreover, the higher significant cytotoxicity of *P. gymnospora* rather than other tested algal species probably because of the synergetic effect of bioactive metabolites of this algal extract that is illustrated previously. Thus, brown alga *P. gymnospora* is the promising source of therapeutic agent against micro-organisms and cancer disease.

Additionally, cytotoxicity of *P. tristichus* was determined against HepG-2 and MCF-7 cells for the first time. The findings reported that *P. tristichus* (IC₅₀ range 20-30 µg/mL) exhibited moderate cytotoxicity against both two cell lines as compared to the rest two algae. This significant cytotoxicity may be because of synergetic effects of bioactive metabolites with predominant fatty acids and sterols that are indicated by GC-MS. In agreement with our results, Pacheco *et al.* (2018) showed that fatty acid extract of red alga *Adenocystis utricularis* has inhibitory effects against cancerous cells (Pacheco *et al.*, 2018).

Table 3: *In vitro* cytotoxicity of 70% methanol extracts of three selected algae in two cell lines

Algae	IC ₅₀ (µg/mL)	
	HepG-2	MCF-7
Doxorubicin (standard anti-cancer drug)	0.36	0.35
<i>Halimeda tuna</i>	22.4	37.3
<i>Padina gymnospora</i>	9.80	11
<i>Phacelocarpus tristichus</i>	21	29.3

Two cell lines were treated with Doxorubicin as a positive control

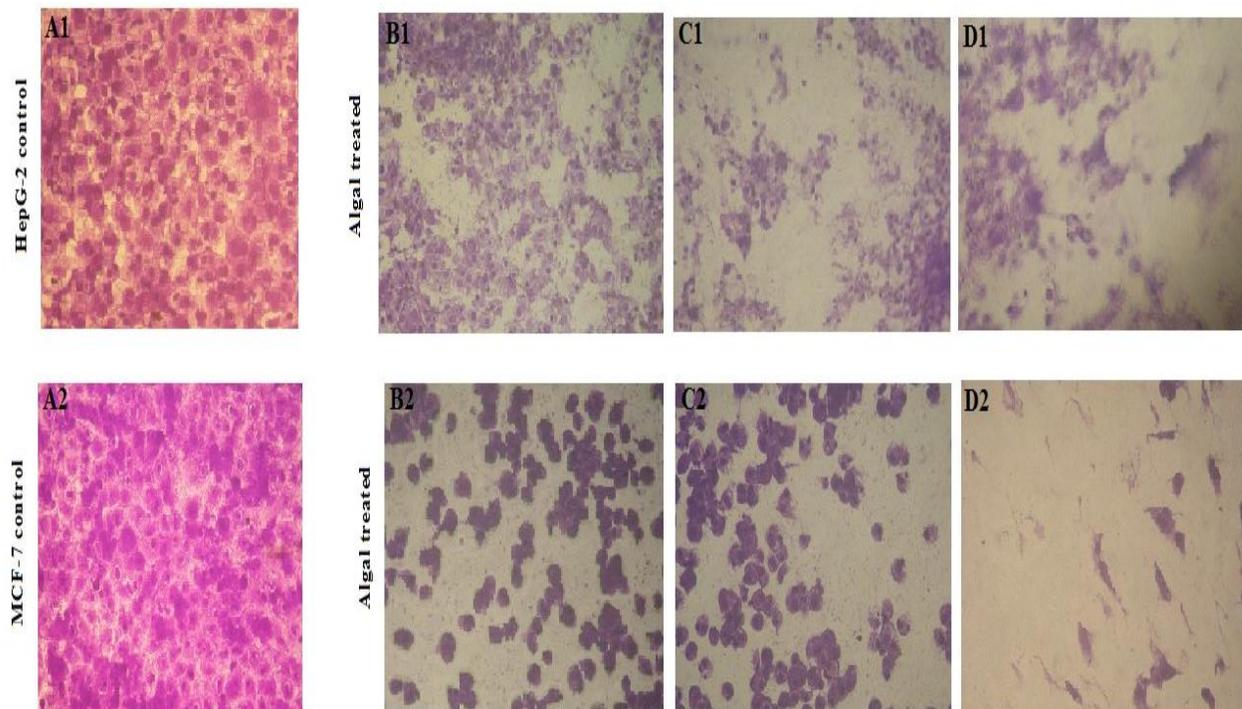


Figure 3: Photomicrograph of cell lines after 24 h of treatment with 70% methanol extracts of three selected algae at 125 $\mu\text{g/mL}$: (A1) HepG-2 control, (B1) HepG-2 treated with *H. tuna* extract, (C1) HepG-2 treated with *P. gymnospora* extract, (D1) HepG-2 treated with *P. tristichus* extract, (A2) MCF-7 control, (B2) MCF-7 treated with *H. tuna* extract, (C2) MCF-7 treated with *P. gymnospora* extract, (D2) MCF-7 treated with *P. tristichus* extract, all three selected algae-treated cell lines demonstrate shrinking and rounding of cells in contrast to control cells by different ratio.

CONCLUSION

The current study showed antimicrobial activity, cytotoxicity and GC-MS analysis for *Halimeda tuna*, *Padina gymnospora* and *Phacelocarpus tristichus* from Egyptian Red Sea shores. Where, the methanol (70%) extract of *P. gymnospora* exhibited the highest antimicrobial and cytotoxicity followed by *P. tristichus* and finally *H. tuna*. Thus, these results identified the potential use of *P. gymnospora* extract as pharmaceutical agent of future drug that requires *in vivo* study for more confirmation. Moreover, our study is the first antimicrobial and cytotoxicity report of *P. tristichus* due to probably its bioactive metabolites screened by GC-MS analysis that is already reported for the first time. Thus, this research creates attention for further evaluation of biological studies and chemical constituents of Egyptian red alga *phacelocarpus tritichus*.

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ARABIC SUMMARY

التقنية الطيفية كروماتوجرافيا الغاز/ الكتلة والنشاط المضاد للميكروبات و التأثير الخلوى السام ضد الخلايا السرطانية لبعض الطحالب البحرية الكبيره الموجودة على شواطى البحر الاحمر المصرية فى منطقتى مرسى علم والقصير

دعاء عبد المنعم عبدالرحيم^١، عزيز عبدالرحمن^٢، خالد ناجح السيد^٣ و سيد عبدالقادر أحمد^{١*}

١- قسم الكيمياء- كلية العلوم- جامعة بنى سويف ٦٢٥١١- مصر

٢- قسم الصيدليات- جامعة راجشاهى- راجشاهى ٦٢٠٥- بنجلاديش

٣- قسم النبات والميكروبيولوجى- كلية العلوم- جامعة بنى سويف ٦٢٥١١- مصر

تهدف الدراسات الحالية لاستكشاف التكوين الكيميائى والخصائص الفارماكولوجية لثلاثة طحالب كبيره وهم: *Halimeda tuna* و *Padina gymnospora* و *Phacelocarpus tristichus* التى تم تجميعها من شواطى البحر الأحمر المصرية. وقد استخدم الفصل الكروماتوجرافى الغازى/المقياس الطيفى الكتلى للتعرف على مكونات المستخلصات الميتانولية (تركيز ٧٠%) للطحالب المختارة كما تم اختبار تأثير هذه المستخلصات ضد الميكروبات بطريقة الانتشار بالأغار، كذلك تم اختبار تأثيراتهم الخلوية السامة المضادة للخلايا السرطانية بطريقة جدوى الخلية. أوضحت النتائج أن طحلب *P. gymnospora* كان الأعلى تأثيرا ضد بكتيريا إيشيريشيا كولاي بتركيز 13.90 ± 0.66 يتبعه طحلب *P. tristichus* بتركيز 12.97 ± 0.65 . بينما أظهر طحلب *H. tuna* تأثيرا فعالا ضد بكتيريا ستافيلو كوككاس أوريوس بتركيز 13.17 ± 0.67 . بالإضافة الى ذلك، التأثير الأعلى للطحالب *P. gymnospora*، *P. tristichus* و *H. tuna* بالتتابع ضد الفطريات كريبتوكوككاس نيوفورمانس و اسبيرجالس فيوميجنس. كما أن *P. gymnospora* كان الأكثر سمية ضد كل من خلايا الكبد والندى السرطانية ويليه فى التأثير طحلب *P. tristichus* ثم طحلب *H. tuna*. علاوة على ذلك تعتبر هذه الدراسة أول تقرير عن التكوين الكيميائى و التأثير المضاد للميكروبات و التأثير الخلوى السام ضد خلايا السرطان لطحلب *P. tristichus* وأيضا تقدم هذه الدراسة تقارير جديدة عن التأثير الخلوى السام للطحالب *P. gymnospora* و *H. tuna* ضد أنواع جديدة من خلايا السرطان. وطبقا لهذه النتائج، يعتبر الطحالب المصرية البحرية الكبيرة لها تأثيرا فعالا ضد الميكروبات وتأثيرا خلويا ساما ضد خلايا السرطان، لذلك من الممكن استخدام هذه الطحالب فى مجال الطب كاستخلاص علاجا جديدا فى المستقبل بعد دراسات مستقبلية على الفئران.