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Phospholipids of some marine microalgae: Identification, antivirus, anticancer and antimicrobial bioactivities

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ABSTRACT

Phospholipids (PL) of five species of marine macroalgae (two species of Rhodophyta (Laurencia popillose, Galaxoura cylindriea); one specie of Chlorophyta (Ulva fasciata), and two species of Phaeophyta (Dilophys fasciola, Taonia atomaria) collected from the Red and Mediterranean seas, respectively were extracted, purified on silicic acid column chromatography, and identified by liquid chromatography LC/ MS/MS. Macroalgal phospholipids content varied from 3.18 to 8.80 % of the total lipid; the maximum phospholipids content was recorded in G. cylindriea (8.80%) followed by L. papillose (8.7%) and U. fasciata (8.18%). Phosphorus contents of algal phospholipids were varied from 0.11 to 0.86%. All algal phospholipids have a high concentration of plamitic acid ($C_{16:0}$) which is ranged from 27.28 to 53.95% while, a high level of $C_{20:3}$ (46.67%) and $C_{18:2}$ (27.11%) were observed in D. fasciola phospholipids. The main structure of algal phospholipids fraction was identified as phosphatidyl serine, phosphatidic acid, Lysophosphatidyl choline, phosphatidyl ethanol amine and phosphatidyl glycerol. The Phospholipids of U. fasciata and L. popillose was found to inhibit antiviral activity of simplex virus type 1. All algal phospholipids possessed a high anticancer activity in vitro against breast and liver human cancer cells with IC_{50} values ranging from 0.47 to 3.15 µg/ml. Phospholipids of U. fasciata exhibited a remarkable activity against E. coli and B. subtili with MIC values ranging from 40 µg/ml, while T. atomaria showed the most potential selective activity with MIC of 80 µg/ml against A. niger and C. albicans.

Key words: Phospholipids, Marine macroalgae, Anticancer, Antiviral, Antibacterial, HSV-1 , MCF7 Cell , HepG2 Cell

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INTRODUCTION

Algae represent valuable sources of a wide spectrum of complex lipids with different potential applications especially, in food, cosmetic, and pharmaceutical industries [1-2].

Phospholipids are a class of lipids, major components of all cell membranes, and they can form lipid bilayers in the cell. Phospholipids are synthesized by both prokaryotic and eukaryotic organisms. They are the major component of most eukaryotic cell membranes, which play a fundamental role in compartmentalizing the biochemistry of life. The quantity and composition of phospholipids are so regulated in a way that enables membranes for maintaining their structure and function, in spite of their developmental and environmental changes [3]. Most phospholipids are characterized by a common backbone of phosphatidic acid (PA), formed from L-glycerol 3-phosphate with two fatty acids esterified on positions 1 and 2. They play important structural and metabolic roles in living cells [4]. The phospholipids with sphingolipids, glycolipids, and lipoproteins are called complex lipids [5-6]. The algae contains three major phospholipids, phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and phosphatidylcholine (PC) [7-8]. Phospholipids possess various biological activities such as antibacterial, antiviral, and antitumoral activities [9]. For instance, *P. yezoensis* phospholipid fraction were found to possess a potent antitumor activity against Meth A fibrosarcoma [10] and phospholipids fraction of *Sargassum marginatum* has high effect in inhibiting the growth of HL-60 cells cancer [11].

This study aims to isolate and identify of phospholipids fraction, characterize their chemical constituents in some Egyptian marine algae, and assess their antiviral, anticancer and antimicrobial activities.

MATERIALS AND METHODS

1- Collection of algal samples

Five macroalgal species were used throughout this study. *Laurencia popillose* and *Galaxoura cylindriea* species were collected from west coast of Red Sea (Faied and Ein Al-Sokhna sector of Suez Galf). *Ulva fasciata* and *Taonia atomaria* (Abu-Qir sector Alexandria governorate) and *Dilophys fasciola* (Marsa Matrouh governorate) were

collected from the Mediterranean Sea. All algal samples were washed several times with water, air dried in shaded area. The dried samples were grinded into fine particle by Brown mill and stored in glass containers at room temperature for further experiments

2-Identification of marine macroalgae species
After preparation of herbarium specimens of the algae species, they were identified by Dr**.** Rauhaiya Abdul-Latif, Professor of Botany Department of Botany, Faculty of Science, Al-Azhar University**,** to whom the authors are very indebted.

3- Extraction and determination of total lipids
Total lipids of marine algae (10 g) were extracted with 100 ml methanol: chloroform solvent (2:1, v/v) mixture [12]. After filtration, the mixture was evaporated at 40^{o}C to a minimum volume (15 ml) and dried under N_2. Then, the total lipids content were determined by weighing

4- Separation and determination of macroalgal phospholipids
Zwitterionic and polar were separated from total lipid extracts using diethylaminoethyl-cellulose (DEAE-cellulose) column chromatography (0.6 × 6 cm, i.d), then eluted with 21.5 ml mixture of chloroform/ methanol (20 ml, 3:2, v/v) [12]. Then, phospholipids fraction were separated from zwitterionic and polar lipid fraction by using silicic acid column (100- 200 mesh, 15x 2.5cm i.d) and eluted with methanol (100%) as described by Maktoob et al., [13] The methanol extracts were evaporated under N_2 to dryness at 40^{o}C then total phospholipids content was calculated by weighing.

5- Identification of marine macroalgal phospholipids
5. 1. Determination of total phosphorus of algal Phospholipids
Total phosphorus was spectrophotometracilly determined using ammonium molybdate reagent at 800 nm as reported by Rouser et al., [14]**.**

5.2. Identification of marine macroalgal phospholipid fatty acids:
Macroalgal phospholipids were subjected to direct transmethylation in 1.5% sulfuric acid: methanol mixture at 95^{o}C for 2 h [15]. Fatty acid methyl ester were analyzed by gas chromatography (Perkin Elmer Autosystem XL) equipped with a flame ionization detector and fused silica capillary column (DB-5 (American) 60 m x 0.32 mm, i.d.) with a film thickness of 0.25/25µm. The column temperature was initially 150^{o}C and was then gradually increased at rate of 3^{o}C/ min up to 250 ^{o}C. The injector and detector temperature were 230^{o}C and 250^{o}C, respectively. The helium was used as a carrier gas (at 1ml /min). The split ratio was 1/100. Fatty acids were identified by comparison between retention times of samples with those of methyl fatty acid standards mixture (Sigma, > 99% purity by GLC).

5.3. LC / Ms/Ms analysis of macroalgal phospholipids
An aliquot of phospholipids fraction was analyzed by LC–MS-MS (LCQ Advantage Max, Thermo Finnegan, USA) using triple mass spectrometer operated in positive electro spray ionization (ESI). The heated capillary and voltage were maintained at 255^{o}C and 4.5 KeV, respectively. The full scans of mass spectra of the phospholipids were carried out from m/z 500 to 2000 using 500 ms for collection of ion in the trap. MS/MS was used to break down the most abundant $[M+H]^+$ ion from MS with depended Collection Induced Dissociation (CID) [16].

6. Biological evaluation of algal phospholipids
6.1. Antiviral Activity of algal phospholipids
6-1-1- Preparation of macroalgal extract for bioassay:
Stock solution of algal PL was freshly prepared by dissolved 100 mg of phospholipids fraction in 10 ml of dimethyl sulfoxide (DMSO) in water (9:1, v/v) and kept at 4^{o}C until use, appropriate dilutions of solution were used in each assay. All the tests were carried out in three independent assays, and the means were used.

6-1-2- Antiviral screening of macroalgal phospholipids
The antiviral activity of algal phospholipids was evaluated for antiviral activity against herps simplex virus type- 1 (HSV-1). The virus was obtained from Virology Laboratory, Water Pollution Research Dept., National Research Centre (NRC), Egypt. The virus was propagated in viro cell cultures. Inhibition % of virus was calculated as plaque reduction as a result of being subjected to a given extracts [17].

6-1-3- Mode of action of macroalgal phospholipids as antiviral agent
Virus inhibition mechanism was studied for the algal phospholipids extracts in three categories.

a- Effect of macroalgal phospholipids on virus a replication
The inhibitory effect of phospholipids of algae extracts on the replication of HSV-1 in Vero cells was studied by the plaque reduction assay, which was performed according to Amoros et al., [18] method**.** Mono-layers of Vero cells (African green monkey, Kidney cell lines) were grown on 6-well culture plates. Virus was diluted at 10^7 PFU (Plaque forming Unit) / ml, then 50 µl was applied to cells. After incubated for 1 hour at 37^{o}C, the phospholipids were added at different concentrations (25, 50, 75 and 100 µg/ml). Cell sheets were fixed in 10% formalin solution for 2 h, and stained with 0.1% crystal violet, then the number of plaques was counted. The percentage of inhibition of plaques formulation was calculated as follows: Initial virus count (PFU/ml) – virus count of treatment with phospholipids fraction 100= % of reduce.

b- Effect of macroalgal phospholipids on virus adsorption.
Vero cells grown in 6-well plates were infected with HSV-1, in the presence of different concentrations of macroalgal phospholipids (75 and 100 µg/ml). The plates were incubated at 4 ^{o}C for 2h. Then, cells were washed with phosphate buffer to remove any unabsorbed virus. The number of infectious bound visions was then measured by the plaque reduction assay [19].

c- Veridical effect of macroalgal phospholipids.
For studying the direct effect of phospholipids on HSV-1, assays were performed according to Schuhmacher et al., [20] method. Briefly, fifty µl of viral suspension (HSV-1) containing 10^{10} PFU/ml were added to the extracts at concentrations giving maximum viral inhibition. Then, the volume of the mixture was adjusted to 100 µl, and added to the cell monolayer. After 1 hour incubation, 6 ml medium with 2% agarose were added to cell monolayer. Virus inoculated to cells and treated identically without the addition of algal extracts which served as control. Viral plaques were counted and the percentage of virus reduction was calculated. Acyclovir was used as the reference compound for antiviral activity.

6.2. Antitumor activity of macroalgal phospholipids
Potential antitumor activity of algal phospholipids was tested using the method of Skehan et al., [21]. Human hepato cellular carcinoma cells (Hep G2) and breast adenocarcinoma cells (MCF-7) were plated in 96 multi-well plate for 24 h before treatment with the algal phospholipids to allow attachment of cells to the well of the plate. Phospholipids and antitumor reference drug (Novantron) were added at serial concentrations to cell monolayer. After incubation for 48 h at 37^{o}C in atmosphere of 5% CO_2, the cytotoxicity was determined spectrophotometrically by measure the developed color at 570 nm by ELISA reader (Tecan Sunrise absorbance reader (No. 3008746), software Magllan V.4 was used, Germany).

6.3. Antimicrobial activity of macroalgal phospholipids
The antimicrobial activities were determined by conventional agar diffusion assay [22] using one gram positive (*Bacillus subtilis* NRRL B-94) one gram negative (*Escherichia coli* NRRL B-3703) bacteria, fungi (*Aspergillus niger* NRRL 313), and yeast (*Candida albicans* NRRL 477). The microbial growth inhibition zone was measured after incubation at 30˚C by the appearance of clear microbial free inhibition zone, beginning within 24 h for yeast, 24-48 h for bacteria and 72-96 h for fungus. The most active phospholipids fractions were tested for its MIC according to Hammer et al., [23]; MIC was determined as the lowest concentration of phospholipids fractions inhibiting the visible growth of each organism on the agar plate.

7-Statistical analysis:-
Data were statistically analyzed through analysis of variance (ANOVA) and Duncans test at P> 0.01 probability level was applied [24].

RESULTS AND DISCUSSION

Total lipids content of marine macroalgal
The total lipids content (TL) of marine algae was ranged from 0.09 to 2.35%. *U. fasciata* (2.35%) had the highest TL contents followed by *D. fasciola* (1.11%), whereas the lowest level was found in *T. atomaria* (0.66%) and *L. popillose* (0.81%) as shown in Table (1). However, the levels of TL among all algae species were within the ranges of several algae species (1- 3%) [25]. In previously report by Manivannan et al., [26], the total lipid content in twelve species of marine algae belong to 3 families from Chlorophyceaean, Phaeophyceae and Rhodophyceae were ranged from 1.33±0.20% to 4.6±0.17%. The total lipids content was varied among algae depending on algae species, genetic origin, climate and geography of development of the algae [26-27].

Phospholipids contents of marine macroalgae
Total Phospholipids content of total lipids was ranged from 3.18 to 8.8% of total lipids (Table 1). Maximum phospholipids content was detected in *G. cylindriea* (8.8%) followed *by L. papillose* (8.7%) and *U. fasciata* (8.8%). On the other hand the minimum phospholipids content was observed in *T. atomaria* (3.18%).

Phosphorus content of marine macroalgal phospholipids
Total Phosphorus content of marine algal phospholipids fraction was presented in Table (1). The results showed that the maximum content was observed in *D. fasciola* (0.86 %) followed by *T. atomaria* (0.71%) and *G. cylindriea* (0.61%). The minimum phosphorus content was observed in *U. fasciata* (0.11%) and *L. papillose* (0.23%). Kulikova and Khotimchenko [28] found that phospholipids content of *S. miyabei* reached maximum content (40.8%) of the total lipids in the lower thallus region, while it was 27.6% in the upper thallus. The Phospholipids fraction of three species macroalgae were 10.9% of total lipids in *A. spicifera*, 10.2 of total lipids % in *G. folifera* and 7.7 % of total lipid in *G. edulis* [11]. Goncharova et al., [29] reported that the phospholipids content of macroalgae i.e (*A. tobuchiensis, L. japonica, S. pallidum, U. fenestrate and Z. marina*) constituted the substantial value (9.1– 49.7% of total lipids) and was lower in summer than in spring. Shevchenko et al., [30] found that the phospholipids of *L. gurjanova* represented 1.1% of total lipids.

Fatty acids composition of marine macroalgal phospholipids
The fatty acids composition of macroalgal phospholipids is summarized in Table (2). All algal phospholipids have a high concentration of plamitic acid ($C_{16:0}$) which is ranged from 27.28 to 53.95% while, $C_{20:3}$ was found in relatively higher amount in three alga *D. fasciola* (46.67%), *T. atomaria* (17.42%) and *G. cylindriea* (13.34%). The content of margaric acid ($C_{17:0}$) was found in moderate contents which were ranged from 9.12 to 18.65%. Whereas the highest content of $C_{22:5}$ was found in *U. fasciata* (29.94%) followed by *T. atomaria* (14.58%) and *L. papillose* (12.22%). A relatively high amount of oleic ($C_{18:1}$) was detected in phospholipid fraction of *D. fasciola* (11.48%) and $C_{14:0}$, $C_{18:0}$, and $C_{22:4}$ were existed in relatively smaller amount in all algal species. Similar results were obtained by Sanina et al., [31], they found that six major FAs, 16:0, 18:0, 18:1n-9, 18:1n-7, 20:4n-6 and 20:5n-3 were observed in phospholipids of *A. tobuchiensis*. Dembitsky and Rozentsvet [32] found that The major fatty acids compositions of seven green algal phospholipids were $C_{16:0}$, $C_{16:4}$, $C_{18:1}$, $C_{18:3}$, and $C_{18:4}$, while two species of algae *U. penicilliformis* and *U. rigida* have a high content of eicosapentaenoic acid (11.5% and 18.3%), respectively. Bhaskar et al., [11] reported that the red algae *Acanthophora spicifera* phospholipids had significantly higher amounts of eicosapentaenoic acid (EPA) and arachidonic acid (AA) as compared to the same fatty acids in *Gracilaria edulis* and *Gracilaria folifera*. Goncharova et al. [29] found that the main fatty acids of two brown marine macrophyte phospholipids were characterized by the significant contents of 16:1 (8.7-14.7% in *Z. marina* and 12.1% in *L. japonica*).

Identification of macroalgal phospholipids compounds by LC-MS

The proposed chemical structure of the active constituents of the algal phospholipid were determined using LC-MS-MS. LC/MS analysis of *U. fasciata*, *T. atomaria*, *G. cylindriea* and *L. papillose* phospholipid gave total ion at retention time ranged from 0 to 12.46 min. without any ion fragmentation except ions of *D. fasciola* phospholipids induced fragmentation. The following are the identification of phospholipids compounds of the different macroalgal species by LC/ MS.

a*. Identified phosophlipids compounds of *U. fasciata

Mass spectrum of *U. fasciata*, phospholipids showed five ions at m/z 793.42, 618.33, 604.46, 590.38 and 555.23 with retention time ranged from 0 to 12.46 min. The two ions; at m/z 793.42 (Rt =12.46) and at m/z 618.33 (Rt = 8.07) have high intensity ratio and were only identified by comparing their mass spectra with those previously reported in the literature. Ion of m/z at 793.42 ($C_{18:1}$ and $C_{20:4}$) corresponding to the phosphatidylserine (Table 3), that is confirmed by Wang et al. [33] and at m/z 618.33 corresponds to phosphatidic acid ($C_{14:0}$ and $C_{16:0}$) (Table 3), that is consistent with Mileykovskya et al. [34].

b. Identified phosophlipids compounds of *T. atomaria*

The ion of *T. atomaria* at m/z 570.33 (Rt = 7.78) was found to correspond to the lysophosphatidylcholine ($C_{22:5}$) (Fig. 3), that agreed with the results obtained by Chen and Li [35] and the ion at m/z 694.55 (Rt =10.90) corresponds to the phosphatidylglycerol ($C_{16:0}$ and $C_{16:0}$) (Table 3), that is confirmed by Mazzella *et al*. [36].

c. Identified phosophlipids compounds of *G. cylindriea*

Total ion chromatogram of *G. cylindriea* phospholipids at retention time 12.49 and at m/z 793.48 was attributed to the phosphatidylserine (Table 3), m/z 779.43 (Rt= 12.11) was attributed to the phosphatidylglycerol ($C_{18:0}$ and $C_{18:0}$), which is agreed with the results of Zink *et al*. [37] and 714.72 (Rt =10.47) was attributed to the phosphatidylethanol amine ($C_{18:1}$ and $C_{16:0}$) (Table 3), that is confirmed by [36] and [34] and 618.42 (Rt = 8.10) was attributed to the phosphatidic acid (Table 3). However other molecular of *G. cylindriea* phospholipids was observed at another retention times and had smaller molecular, which might resulted from fragmentation.

d. Identified phosophlipids compounds of *L. papillose*

The ESI- Ms of *L. papillose* phospholipids showed one ion at m/z 632.47(Rt = 8.37), which is the corresponding to the phosphatidylethanol amine ($C_{14:0}$ and $C_{14:0}$) (Table 3)., that is confirmed by Mileykovskya et al. [34] and Mazzella et al. [36]

e. Identified phosophlipids compounds of *D. fasciola*

Four molecular ions $[M + H]^+$of *D. fasciola* phospholipids at retention time 0 to 60 min. were identified by ESI-Ms. The molecular ions $[M + H]^+$of *D. fasciola* phospholipids were m/z 601.30(Rt = 24.50), m/z 1148 (Rt = 0.22), 1395.20 (Rt = 0.88) and 566.69 (Rt =1.62). ESI/ MS of the major component of *D. fasciola* phospholipids was consistent with the molecular ion $[M + H]^+$at *m/z*= 1148 corresponding to the molecular di-phosphatidylglycerol (formula of $C_{71}H_{137}O_8P$) (Table 3). The fragmentations of this compound (Fig.1) were the peak at m/z = 895.71 and this was due to the loss of $C_{18}H_{35}$. The peak at m/z = 812.63 was due to loss C_8 H_{11} and also the peak at m/z 730 was due to the loss C_8 H_{11}. The fragmentation of the compound may resulted in di-phosphatidylglycerol (Fig. 1). Meanwhile the molecular ion of peak at 1395.20 (Rt = 0.88) was identified as tri-acylphospatidylinositol di-mannosides as reported by Yague *et al*. [38] they come in harmony with the results of the present study, that the ion at 1395 correspond to tri-acylphosphatiylinositol di-mannosides. In this concern Khotimchenko and Tittlyanova [39] found that The phospholipids composition of marine brown algae were phosphatidyl choline (PC), phosphatidylethanolamine (PE), phosphatidyl glycerol (PG), phosphatidyl inositol (PI) and new amino phospholipid (PX), which are in harmony with the result of the present study. Hanus *et al.* [40] found that Phosphatidylcholine (PC) contained in red algal species varies from 61.6 to 77.8 %.

The mass spectrum of phospholipids from soybean indicated the presence of peaks at m/z = 761. Each precursor scan showed a clear protonated molecular peak along with a sodium adduct molecular ion peak, simultaneously. 1-oleoyl-2-palmitoyl-PC (18:1-16:0 PC)), [M +H]+ along with $[M + Na]^+$ peaks were shown exclusively at *m/z* 761.0 and 782.5, respectively. The MS–MS spectrum of the selected molecule (*m/z*: 761.0) contained fragment ions at *m/z* 496.5 and 522.5, corresponding to the neutral loss of the fatty acid group as a ketone at *sn*-1 ($[M+H$ R1-CH= C O$]^+$) and *sn*-2 ([M +H- R2-CH= C O]+), respectively [41].

Biological evaluation of marine algal phospholipids fraction.

1. Antiviral activity of marine algal phospholipids

Antiviral activity of algal phospholipids was evaluated by plaques reduction method and the results are illustrated in Table (4). Algal phospholipids showed high antiviral inhibitions against HSV-1, which were ranged from 21.87 to 75.25 %. The maximum inhibition % of HSV1 was found at the concentration of 20 µg/ml in *U. fasciata* (75.25%) followed by *L. papillose* (75%) and *G. cylindriea* (68.75%). A moderate inhibition % of HSV-1 was found at 10 µg/ml in *U. fasciata* (56.25%) and at 20 µg/ml in *T. atomaria* (53.12%). The minimum inhibition % against HSV1 was observed at 10 µg/ml in *G. cylindria* (21.87%) and *D. fasciola* (25.00%)**.**

1.1. Antivirus activity confirmation of *U. fasciata* and *L. papillose* phospholipids

U. fasciata and *L. papillose* phospholipids were selected for confirmation according to inhibition % which reached ≥ 75%. The antiviral activity was confirmed using HSV1 with different concentrations of phospholipids 25, 50, 75 and 100 µg/ml. The effectiveness of inhibition (100% of HVS1 virus) was found in *L. papillose* at all concentrations, while *U. fasciata* phospholipids inhibited virus maximum by 78% (Table 5). IC_{50} of antiviral were 10, 29 µg/ml for *U. fasciata* and *L. papillose*, respectively.

1.2. Mode of action of *U. fasciata* and *L. papillose* phospholipids as antivirus

a. *U. fasciata* and *L. papillose* phospholipids as antireplication of virus cells

Virus replication into cells is also one of the indicators of antiviral targets. As shown in (Figs. 2 & 3), at 100 µg/ml of *L. papillose* phospholipids completely inhibited virus replication (100%), while the phospholipids of *U. fasciata* inhibited the virus replication by about 95 %.

b. *U. fasciata* and *L. papillose* phospholipids as anti-adsorption of virus on host cells.

The effect of algal phospholipids on adsorption was determined by the inhibition of HSV-1 binding to host cells pretreated with various concentrations of macroalgal phospholipids. At concentrations of 75 and 100 µg/ml, phospholipids of *L. papillose* showed inhibition of virus adsorption to host cell membranes by approximately 83 and 90 % respectively, while the phospholipids extract of *U. fasciata* did not induce any effect on virus adsorption (Figs. 2 & 3) .

c. *U. fasciata* and *L. papillose* phospholipids as veridical

Figs. 2 and 3 showed the dose-dependent veridical effect of phospholipids of *L. papillose* and *U. fasciata* against HSV-1. At the concentration of *L. papillose* phospholipids (75 µg /ml) the residual infectivity of these viruses was approximately 90%, while high concentration of 100 µg /ml was inactivated HSV-1 by about 95%. *U. fasciata* phospholipids were not affected of virus.

D and L-isomers of phosphatidyl choline, phosphatidyl glycerol, phosphatidic acid, and phosphatidyl serine have approximately equal antiviral activity [42]. Tuchnaya et al. [43] investigated the antivirus activity of different synthetic compounds which contained phosphate and sulfate derivatives. They found that 2, 3, 4, 5-tetra-O-benzyl-*D, L*-iditoldi-phosphate XIII and 1,12-dodecanediolmonophosphate IX were active against herpes simples viruses HSV1 and HSV2. The structures were highly active inhibitors of viral penetration into cells.

2-Antitumor activity of marine algal phospholipids

2-1- Antitumor activity of algal phospholipids against human breast carcinoma (MCF-7)

The cytotoxic activities of five algal phospholipids fraction was tested against MCF-7 and the results are illustrated in Table (6). All algal phospholipids showed highly inhibition of MCF-7 cell line at all concentrations which is ranged from 42.73 to 93.39%. The most potential selective activity $IC_{50,}$ was ranged from 0.47 to 1.28 µg/ml against MCF-7cell line (Table 7). The minimum IC_{50} of algal phospholipids against MCF-7 cell was found in *D. fasciola, L. papillose and G. cylindriea* (0.47 µg/ml) followed by *U. fasciata* (1.28 µg/ml) and *T. atomaria* (1.21 µg/ml) (Table 6 & 7). It is interested to note that all algal species had IC_{50} lower than the IC_{50} of antitumor drug Novantron.

2-2- Antitumor activity of marine algal phospholipids against human hepato carcinoma (HepG2)

Phospholipids of all algal species showed inhibition against HepG2 which is ranged from 14.59 to 84.8% (table 6). The highest inhibition HepG2 was recorded in all algal species phospholipids at 5, 10 µg/ml. The IC_{50} of algal phospholipids varied from 1.81 to 3.15 µg/ml against HepG2 (Table 7). The minimum IC_{50} of algal phospholipids against HepG2 was recorded in *L. papillose* (1.81 µg/ml) followed by *D. fasciola* (2.40 µg/ml) (Table. 7). The results of the present study are in agreement with those obtained by [10]. They found that low doses of macroalgal phospholipids isolated from *Laminaria angustata, L. angustata, S. ringgoldianum* and *Porphyra yezoensis* showed significant antitumor activity against Meth-A fibrosarcoma. The anticancer activity of lipids classes extracted from brown alga *Sargassum marginatum* showed high effective inhibition to the growth of human pro-melocytic leukemia (HL-60) cells [11]. The mechanism of the antiproliferative of synthetic phospholipids conducts through indirect ways of action, such as activation of macrophages, inhibition of invasion of tumor cells in T-cell lymphoma, and also mechanisms which directly influence cellular signaling and lipid metabolism [44].

3- Antimicrobial activity of marine macroalgal phospholipids

All algal phospholipids did not show any antifungal and antiyeast effect except *T. atomaria*, which has an antifungal activity against *A. niger* and inhibition the yeast growth of *C. albicans*. *T. atomaria* showed the most potential selective antimicrobial activity with MIC of 80 µg/ml against *A. niger* and MIC against *Candida albicans* was 60 µg/ml (Table 8).

Table 1: Phospholipids and Phosphorous contents of some Egyptian marine macroalgae

Algae strains	Total lipid %	Total phospholipids (% of total lipid)	Phosphorous %
U. fasciata	2.35[d]	8.18	0.11[a]
T. atomaria	0.66[b]	3.18	0.71[c]
D. fasciola	1.11[a]	7.27	0.86[c]
L. papillose	0.8[1c]	8.70	0.23[ab]
G. cylindriea	0.09[a]	8.80	0.61[ab]
LSD	0.09	1.45	0.55

The mean difference is significant at P ≤0.01.

Table 2: Fatty acids composition of some macroalgal phospholipids

Algae strains / Fatty acids	Mediterranean sea			Red sea	
	U. fasciata	*T. atomaria*	*D. fasciola*	*L. papillose*	*G. cylindriea*
$C_{14:0}$	1.02	2.33	2.53	6.83	6.87
$C_{16:0}$	53.95	42.54	27.28	64.5	43.21
$C_{16:1}$	0.52	-	-	-	1.26
$C_{17:0}$	9.12	16.15	3.72	9.97	18.65
$C_{18:0}$	4.33	3.12	3.26	4.51	3.84
$C_{18:1}$	2.26	2.15	11. 48	1.43	2.20
$C_{20:3}$	2.78	17.42	46.67	8.83	13.34
$C_{20:4}$	-	1.70	-	1.72	1.59
$C_{22:5}$	25.94	14.58	5.07	12.22	9.04
Saturated FAs %	68.42	64.14	36.79	77.27	68.73
MUFAs %	2.78	2.15	11.48	1.43	3.46
PUFAs %	28.72	33.7	51.74	22.7	23.97

FA, fatty acid; MUFAs, mono unsaturated fatty acids; PUFAs, Polyunsaturated fatty acids

Table 3: Identified phosopholipids compounds in five marine macroalgae strains

Algae strains	Phosphatidyl serine	Phosphatidic acid	Lysophosphatidyl choline	Phosphatidyl glycerol	Phosphatidyl ethanol amine	Tri-acyl phosphatiyl inositol -di mannosides	Di-Phosphatidyl glycerol
	Exact lMass:793.56 MW:794.09	Exact Mass:619.43 MW:619.83	Exact Mass:570.36 MW:570.72	Exact Mass:694.55 R1 &R2 C16:0 Exact Mass:714.72 R1 &R2 C18:0	Exact Mass:632.47 R1 &R2 C14:0 Exact Mass:714 R1C16 &R2 C18:1	Exact Mass:632.47 R1 &R2 C14:0 Exact Mass:714 R1C16 &R2 C18:1	Exact Mass:1149.82
U. Fasciata	+	+					
T. atomaria	+		+	+	+		
G. cylindriea	+	+		+	+		
L. papillose				+	+		
D. fasciola	+					+	+

Table 4: Antiviral activity of phospholipids extracted from some marine algae against HSV-1

Algae strains	Viral inhibition %	
	10 µg / ml	20 µg / ml
U. fasciata	56.25	75.25*
T. atomaria	50.00	53.12
L. papillose	50.00	75.00*
G. cylindriea	21.87	68.75
D. fasciola	25.00	34.37

Table 5: Antiviral activity of *U. fasciata* and *L. papillose* phospholipids and antivirus drug acyclovir against HSV-1

Algal phospholipids concentrations	Acyclovir	Inhibition %	
		Mediterranean Sea	Red sea
		U. fasciata	*L. papillose*
25 µg /ml	5	51	100
50 µg /ml	60	54	100
75 µg /ml	70	59	100
100 µg /ml	100	78	100
IC_{50} µg/ml	55	29	10

Table 6: Antitumor activity of some algal phospholipids against MCF7 and HepG2 cells after 48h incubation

Algae species	Concentrations	Growth Inhibition %	
		MCF7 Cell	HepG2 Cell
U. fasciata	1 µg /ml	70.78[a]	14.59[a]
	2.5 µg /ml	86.03[a]	48.39[b]
	5 µg /ml	88.45[a]	*76.53*[c]
	10 µg /ml	91.09[a]	*84.8* [d]
T. atomaria	1 µg /ml	44.54[a]	16.76[a]
	2.5 µg /ml	77.38[a]	43.45[b]
	5 µg /ml	80.96[a]	68.16[c]
	10 µg /ml	82.55[a]	79.51[d]
D.fasciola	1 µg /ml	79.33[a]	35.78[a]
	2.5 µg /ml	89.70[a]	42.51[a]
	5 µg /ml	92.59[a]	74.2[b]
	10 µg /ml	92.46[b]	78.49[b]
L. papillose	1 µg /ml	87.91[a]	45.92[a]
	2.5 µg /ml	87.7[a]	48.6[de]
	5 µg /ml	88.83[a]	78.62[b]
	10 µg /ml	91.56[a]	83.36[b]
G. cylindriea	1 µg /ml	83.93[a]	45.92[a]
	2.5 µg /ml	88.93[a]	48.6[a]
	5 µg /ml	93.26[a]	78.62[b]
	10 µg /ml	93.39[a]	83.36[b]
Novantron (reference drug)	1 µg /ml	42.73	26.1
	2.5 µg /ml	52.81	42.27
	5 µg /ml	52.81	47.66
	10 µg /ml	52.81	59.5
LSD		7.3746	14.856

The mean (n=3) difference is significant at P ≤ 0.01.

Table 7: Antitumor activity of some algal phospholipids against MCF7 and HepG2

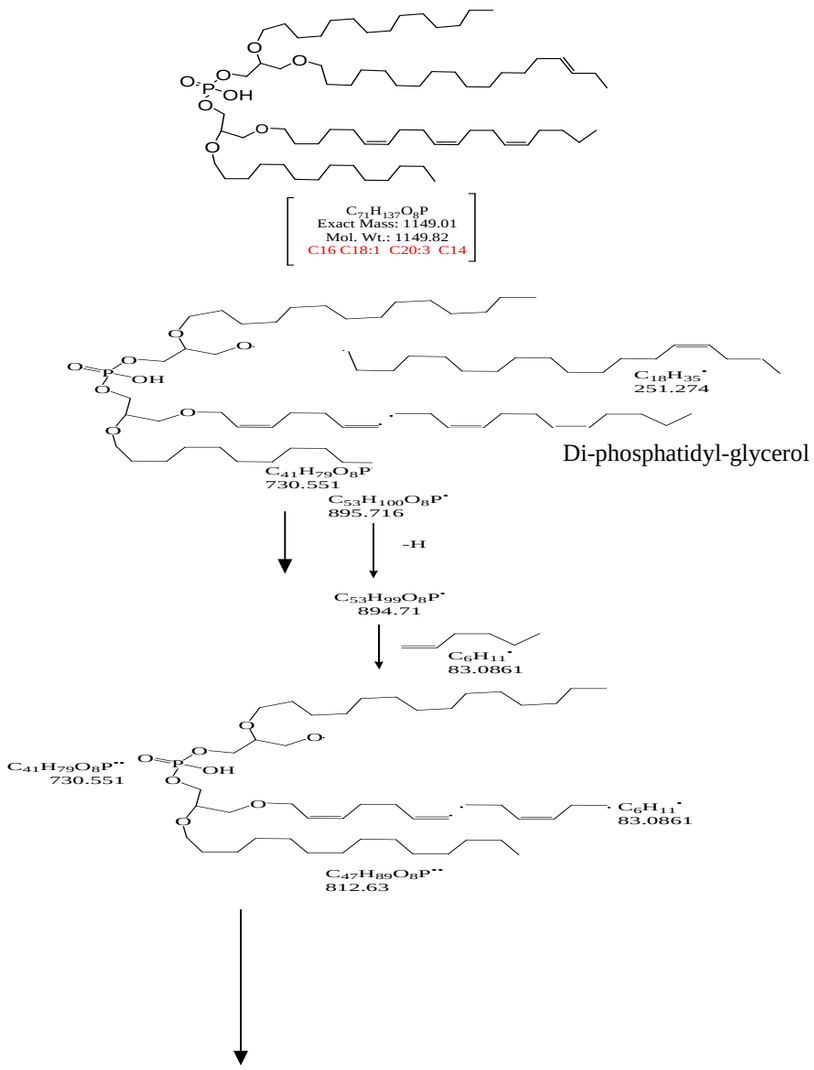
Algae species	IC_{50} µg/ml	
	MCF7 Cell	HepG2 Cell
U. fasciata	1.28	2.82
T. atomaria	1.21	2.82
D.fasciola	0.47	2.40
L. papillose	0.47	1.81
G. cylindriea	0.47	3.15
Novantron (reference drug)	1.4	4

The antibacterial effect of all algal phospholipids on two bacterial strain *B. subtilis* and *E. coli* at concentration 100µg are shown in Table (8). The maximum inhibition zone was observed in *U. fasciata* (21mm) and (18mm) against *B. subtilis* and *E. coli ,respectively. T. atomaria* and *G. cylindriea* have relatively low antibacterial effect**.** *U. fasciata, T. atomaria* and *G. cylindriea* showed the most potential selective activity with MIC ranged for 40 to 80µg/ml against *B. subtilis* and *E. coli* . The result of the present study are in agreement with the results of Ramadan *et al.* [45].

Table 8: Antimicrobial activities of some macroalgal phospholipids (inhibition zone in diameter (mm) around the discs) at the concentration 100 µg/ well and MIC values

Microorganism / Algae strains	inhibition zone (mm)				MIC µg/ml			
	Bacteria		Fungi	Yeast	Bacteria		Fungi	yeast
	E. coli	*B. subtilis*	*A. niger*	*C. albicans*	*E. coli*	*B. subtilis*	*A. niger*	*C. albicans*
U. fasciata	18.0	21.0	-	-	40.0	40.0	--	--
T. atomaria	6.0	9.0	10.0	12.0	80.0	60.0	80.0	60.0
G. cylindriea	7.0	10.0	10.0	10.0	60.0	40.0	80.0	60.0

They found that phospholipids of *Spirulina platensis* inhibited the growth of all tested microorganisms (*B. subtilis, St. aureus, A. niger, A. flavus, S. cervisiae and C. albicans)* at concentration of 100µg except the *P. aeruginosa* and *E. coli* which did not show any activity. Ells *et al.* [46] reported that the Phospholipids and ergosterol of yeasts, containing arachidonic acid have susceptibility towards the antifungal effect. The antimicrobial activity of cationic parts is proposed to initiate electrostatic interaction with the negatively charged components of the membrane of microbes and disturbs its barrier function such as inhibit cell-wall, nucleic-acid, or protein synthesis or inhibit enzymatic activity [47 and 48]. Garg *et a*l. [49] indicated that the mechanism through which antimicrobial liysophosphatidic acid may regulate membrane trafficking events can involve (i) direct mechanisms, by altering membrane shape and/or binding proteins, (ii) indirect mechanisms, by stimulating or inhibiting signal transduction pathways through phosphatidic acid release, and (iii) a combination of both.



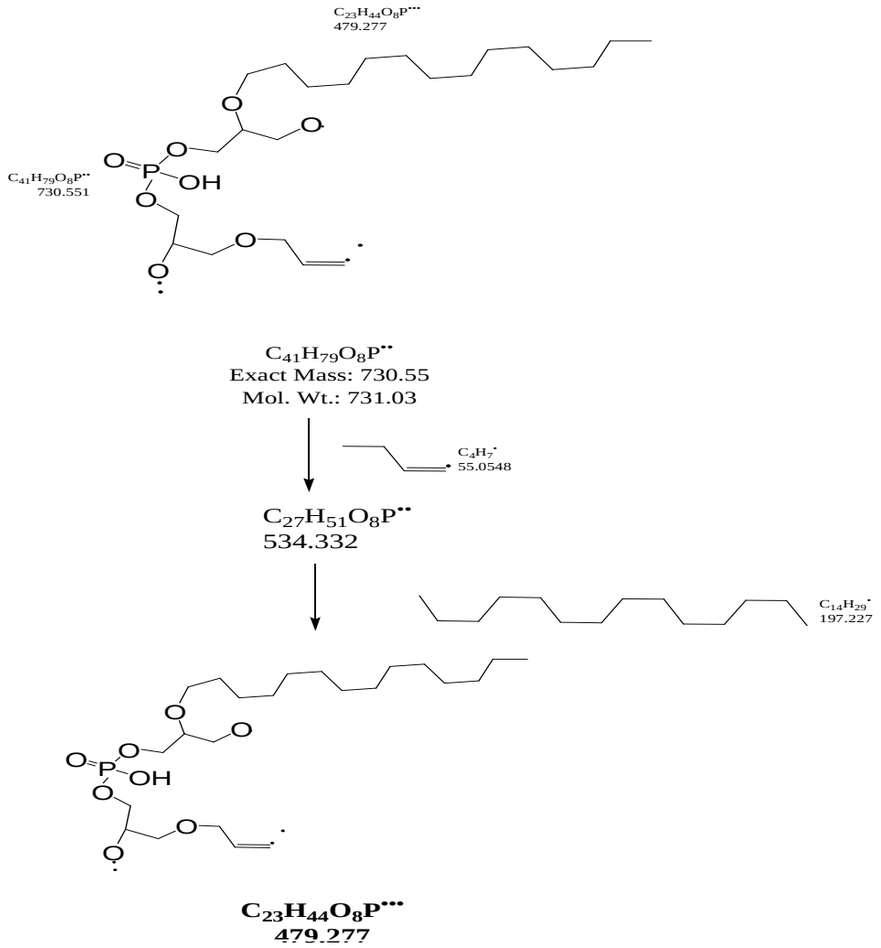


Fig.1. Fragmentation pattern of compound di-phosphatidyl glycerol extracted from *D. fasciola* phospholipids

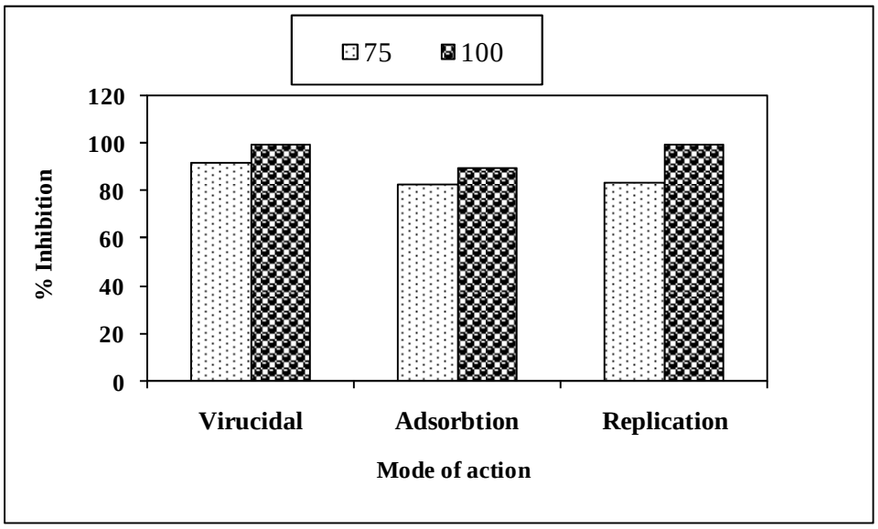


Fig.2: Mode of action of *L. papillose* phospholipids against HSV-1

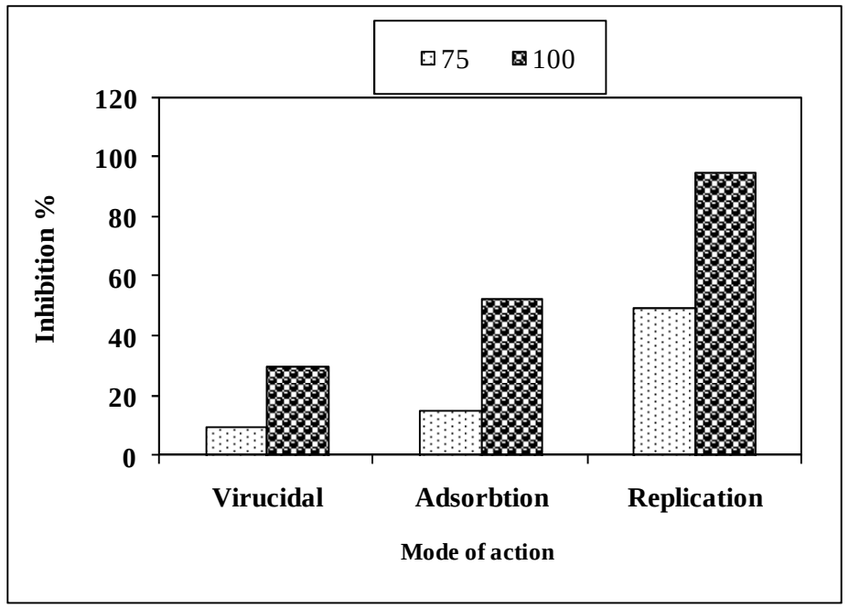


Fig. 3: Mode of action of *U. fasciate* phospholipids against HSV-1

In conclusion, the mass spectrum of *U. fasciata*, *T. atomaria* and *G. cylindriea* phospholipids indicated that the presence of peaks at m/z = 793.14, which may be attributed to phosphatidyl serine. The peaks observed in *U. fasciata* and *G. cylindriea* m/z = 619 is expected to be phosphatidic acid. The ion of *T. atomaria* was the distinct at m/z = 570, which may attributed to lysophosphatidyl choline. Whereas the ion of *T. atomaria* (m/z = 694.55) and ion of *G. cylindriea* (m/z 779.74) may be corresponds to phosphatidyl glycerol ($C_{16:0}$ and $C_{16:0}$)($C_{18:0}$ and $C_{18:0}$). *L. papillose* and *G. cylindriea* phospholipids showed spectrum peaks at m/z 632.47 and 714.72, which may be attributed to the phosphatidyl ethanol amine ($C_{14:0}$ and C $_{14:0}$)($C_{18:1}$ and $C_{16:0}$). *D. fasciola* phospholipids was characterized by the content of two high molecular at m/z =1148, this may be attributed di phosphatidyl-glycerol and m/z =1395, which is expected to be tri- acyl phospatidyl inositol di mannosides.

Algal phospholipids of *D. fasciola*, *L. papillose* and *G. cylindriea* presented highly inhibition of MCF-7 cell line growth . *U. fasciata* and *L. papillose* phospholipids inhibit virus replication and adsorption of virus on host cells. Antitumor and antivirus activities of phospholipids may be related to unsaturated fatty acid and phosphorus contents.

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