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Structural characterization and Biological Activity of Sulfolipids from selected Marine Algae

By **F.K. El Baz**[1], **G.S. El Baroty**[2], **H.H. Abd El Baky**[1, ✉], **O.I. Abd El-Salam**[3] and **E.A. Ibrahim**[1]

[1] Plant Biochemistry Department, National Research Centre, Dokki, Cairo, Egypt
[2] Biochemistry Department of, Faculty of Agriculture, Cairo University, Cairo, Egypt
[3] Applied Organic Chemistry Department, National Research Centre, Dokki, Cairo, Egypt
✉ Corresponding author: abdelbakyh@hotmail.com

RESUMEN

Caracterización estructural y actividad biológica de sulfolípidos de algas marinas seleccionadas

Se separaron diferentes clases sulfolípidos (SL) a partir de los lípidos totales de cinco especies de algas marinas: una especie de Chlorophyta (Ulva fasciata), dos especies de Phaeophyta (Dilophys fasciola, Taonia atomaria) y dos especies de Rhodophyta (Laurencia popillose, Galaxoura cylindriea) que se purificaron mediante cromatografía en columna de DEAE-celulosa. Los components de SLs fueron identificados por IR, cromatografía de gases MS/MS y cromatografía líquida MS/ MS. Los contenidos de SL en relación al total de lípidos varió de 1,25% (en L. papilosa) al 11,82% (en D. fasciola). Sin embargo, no hay diferencias significativas en el contenido de sulfato observado entre todas estas especies de algas (desde 0,13 hasta 0,21%). Todos los SL se caracterizaron por un alto contenido de ácido palmítico (C16:0), que osciló entre 30,91% en G. cylindriea a 63,11% en T. atomatia. Sulfoquinovosyl-di-acilglicerol y acilglicerol sulfoquinovosyl fueron identificados como los principales constituyentes de los sulfolípidos de estas algas.

Los sulfolípidos de las diferentes especies de algas estudiadas mostraron una notable actividad antiviral contra el virus del herpes simple tipo 1 (VHS-1) con una IC50 que osciló entre 18,75 y 70. 2 g mL^{-1}. Por otra parte, los sulfolípidos de estas algas inhibieron el crecimiento de células tumorales de mama y células de cáncer de hígado humano con valores de IC50 que van desde 0,40 hasta 0,67 g mL^{-1} para las células de adenocarcinoma de mama humano (MCF7).

PALABRAS CLAVE: Algas marinas contra el cáncer – Antibacterianas – Antivirales – Células – HepG2 – HSV-1 – MCF7 – Sulfolípidos.

SUMMARY

Structural characterization and Biological Activity of Sulfolipids from some Marine Algae

The sulfolipid classes (SLs) in the total lipids of five species of marine algae, two species of Rhodophyta (*Laurencia popillose*, *Galaxoura cylindriea),* one species of Chlorophyta (*Ulva fasciata*), and two species of Phaeophyta (*Dilophys fasciola*, *Taonia atomaria*) were separated and purified on DEAE-cellulose column chromatography. The SLs component was identified by IR, gas chromatography MS/MS and liquid chromatography MS/MS. The level of SLs contents varied from 1.25% (in *L. papillose*) to 11.82% (in *D. fasciola*) of the total lipid contents. However, no significant differences in sulfate content (0.13 – 0.21%) were observed among all these algae species. All SLs were characterized by high contents of palmitic acid ($C_{16:0}$), which ranged from 30.91% in *G. cylindriea* to 63.11% in *T. atomatia***.** The main constitutes of algal sulfolipids were identified as sulfoquinovosyl-di-acylglycerol and sulfoquinovosyl acylglycerol.

The sulfolipids of different algal species exhibited remarkable antiviral activity against herps simplex virus type 1 (HSV-1) with an IC_{50} ranging from 18.75 to 70. 2 μg mL^{-1}. Moreover, algal sulfolipid inhibited the growth of the tumor cells of breast and liver human cancer cells with IC_{50} values ranging from 0.40 to 0.67 μg mL^{-1} for human breast adenocarcinoma cells (MCF7).

KEY-WORDS: Antibacterial – Anticancer – Antiviral – Cells – HepG2 – HSV-1 – Marine algae – MCF7 – Sulfolipids.

1. INTRODUCTION

Glycolipids (GL), sulfolipids (SL) and phospholipids (PL) are usually present in all photosynthetic membranes in plants, algae and various bacteria. However, Sulfolipids (SLs) represent up to 29% of total lipid and have been regarded as predominant lipid components in both prokaryotic and eukaryotic organisms (Norman *et al.,* 1996; Benning and Somerville, 1992). SLs are located in the thylakoid membrane as an important component to preserve the membrane's structure and function. Recently, lipid constituents have been recognized as potentially important factors in the processes of signal transduction and endomembrane transport (Simons and Toomre 2000). However, algae represent valuable sources of a wide spectrum of complex lipids, and their components have promising potential applications especially in the food, cosmetic, and pharmaceutical industries (Hossain *et al.,* 2005). The various biological actions of SLs include: inhibitory effects on DNA polymerase and viral reverse transcriptase, antitumor, anti-inflammatory, and inhibition and promotion of cell growth (Mizushina *et al.,* 1998; Liptak *et al.,* 2004; Maeda *et al.,* 2008; Naumann, 2009). Several reports have indicated that SLs compounds isolated from

different algae species inhibited the growth of several viruses (such as HIV, HSV-1 and the AIDS virus) as well as cancer types (Gustafson *et al.,* 1989; Xue *et al.,* 2002; Chirasuwan *et al.,* 2009). For instance, the sulfolipids of *S. horneri* were found to possess a potent anticancer activity against colon cancer cells (Caco-22 ceils) (Hossain *et al.,* 2005). In addition, the SLs of *Porphyridium cruentum* showed inhibitory effects on human colon (DLD-1), breast (MCF-7), prostate adenocarcinoma (PC-3) and malignant melanoma (M4 Beu) cancer cells (Bergé *et al.,* 2002).

The aim of the present work is to characterize the chemical structure and evaluate the biological activity of the sulfolipids of some marine algae collected from the Red and Mediterranean seas in Egypt.

2. MATERIALS AND METHODS

2.1. Collection of marine algal samples

Samples of *Laurencia popillose* and *Galaxoura cylindriea* were collected from the Red Sea (Faied and Ein Al-Sokhna at Suez gulf canal). *Ulva fasciata* and *Taonia atomaria* were collected from the Mediterranean Sea at (Abu-Qir city) Alexandria Governmate and *Dilophys fasciola* from (Matroh city) Marsa Matrouh Government. All algal samples were washed several times with tap water and then left to air dry. Samples of 500 g were ground and stored in brown glass containers at room temperature for further analysis.

2.2. Identification of marine algae 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.

2.3. Extraction and determination of total lipid

The total lipids of marine algae (10 g) were extracted with 100 mL of the mixture: methanol: chloroform (2:1, v/v) (Roughan and Bratt, 1968). After filtration, the mixture was evaporated at 40 °C to a minimum volume (15 mL) and dried under N_2. Then, the total lipid contents were determined by weight.

2.4. Isolation and determination of algal sulfolipids

The sulfolipids were separated from the total lipid extracts using diethylaminoethyl-cellulose (DEAE-cellulose) column chromatography (0.6 × 6 cm, i.d), then eluted with a 21.5 mL mixture of chloroform/ methanol (20 mL; 6:4, v/v) and concentrated NH_3 (1.5 mL) (Roughan and Bartt 1968)**.** The mixture, which contained purified sulfolipids, was dried at 40 °C, under N_2 then the total sulfolipids were calculated by weight.

2.5. Identification of algal sulfolipids

2.5.1. Determination of total sulfate of algal sulfolipids

The sulfate content of algal sulfolipids was determined using a sodium rhodizonate reagent, which in the presence of barium forms a red compound. The reduction of color intensity indicates the quantity of sulfate present in the sample. A standard calibration curve was prepared using sodium sulfate (Na_2SO_4), scaled for 1.0 – 12.0 µg sulfate (Terho and Hartiala, 1971).

2.5.2. Identification of algal sulfolipid fatty acids

The algal sulfolipids were subjected to direct transmethylation in 1.5% sulfuric acid:methanol at 95 °C for 2 h (Luddy *et al.,* 1960**)**. Fatty acid methyl esters 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 °C and was then gradually increased at rate of 3 °C min^{-1} up to 250 °C. The injector and detector temperatures were 230 °C and 250 °C, respectively. Helium was used as the carrier gas (at 1mL min^{-1}). The split ratio was 1/100. The fatty acids were identified by comparison between the retention times of the samples and those of methyl fatty acid standard mixtures (Sigma, > 99% purity by GLC).

2.5.3. Identification of function group of marine algal sulfolipids using IR

The IR spectra of algal sulfolipid samples were recorded on a JASCO FT/IR 6100A spectrometer in the wave number range 4000-400 cm^{-1}, according to the KBr disk method.

2.5.4. LC-MS-MS analysis of algal sulfolipids

An aliquot of sulfolipid fraction was analyzed by LC-MS-MS (LCQ Advantage Max, Thermo Finnegan, USA) using a triple mass spectrometer operating in positive electro spray ionization (ESI). The heated capillary and voltage were maintained at 255 °C and 4.5 KeV, respectively. The full scans of mass spectra of the sulfolipids were carried out from m/z 500 to 2000 using 500 ms for the 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) (Pons *et al*., 2002).

2.5.5. Identification of algal sulfolipids using GC-MS

Sulfolipids were identified using GC-MS (Chrompack France, Les Ullis, France) equipped

with a 0.25 µm film phase and fused CP-Sil5 CB Low bleed-MS capillary column (25 m x 0.32 mm). The temperature of the injector was 280 °C. Sulfolipids were analyzed using the following temperature program: 90 °C for 3 min then gradually increased at the ratio of 5 °C min^{-1} until 260 °C. The analyses were performed in the EI mode (ionization energy 70 eV; source temperature 150 °C) (Pons *et al.,* 2002)

2.6. Biological evaluation of marine algal sulfolipids

2.6.1. Antiviral activity of algal sulfolipids

Preparation of the algal extract for bioassay. A stock solution of algal SLs was freshly prepared by dissolving 100 mg of sulfolipid fraction in 10 mL of dimethyl sulfoxide (DMSO) in water (9:1, v/v) and kept at 4 °C until use; appropriate dilutions of the solution were used in each assay. All the tests were carried out in three independent assays, and the means were applied.

Antiviral screening of algal sulfolipids. Algal SLs were evaluated for antiviral activity against herps simplex virus type- 1 (HSV-1). The virus was obtained from the Virology Laboratory, Water Pollution Research Dept., National Research Center (NRC), Egypt. The virus was propagated in viro cell cultures. The Inhibition % of the virus was calculated as plaque reduction as a result of being subjected to a given extract (Tebas *et al*., 1995).

2.6.2. Antitumor activity of algal sulfolipids

The potential antitumor activity of algal sulfolipids was tested using the method of Skehan *et al*. (1990). Human hepato cellular carcinoma cells (Hep G2) and breast adenocarcinoma cells (MCF-7) were plated on 96 multi-well plates for 24 h before treatment with the algal sulfolipid to allow for the attachment of cells to the well of the plate. Sulfolipids and antitumor reference drugs (Novantron) were added at serial concentrations to cell monolayers. After incubation for 48 h at 37 °C in an atmosphere of 5% CO_2, the cytotoxicity was determined spectrophotometrically by measuring the developed color at 570 nm by the ELISA reader (Tecan Sunrise absorbance reader (No. 3008746), software Magllan V.4 , Germany).

2.6.3. Antimicrobial activity of algal sulfolipids

The antimicrobial activities were determined by the conventional agar diffusion assay (Greenwood, 1983) 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 a 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 sulfolipid fractions were tested for their MIC according to Hammer *et al.,* (1999). MIC was determined as the lowest concentration of sulfolipid fractions inhibiting the visible growth of each organism on the agar plate.

2.7. Statistical analysis

Data were statistically analyzed through the analysis of variance (ANOVA) and Duncan's test and the P> 0.01 probability level was applied (Gomes and Gomes, 1984)

3. RESULTS AND DISCUSSION

3.1. Marine algal total lipid contents

The total lipid contents (TL) of marine algae ranged from 0.09 to 2.35% (Table 1). *U. fasciata* (2.55%) had the highest TL content followed by *D. fasciola* (1.11%), whereas the lowest level was found in *G. cylindriea* (0.09%) followed by *T. atomaria* (0.66%) and *L. popillose* (0.88%). However, the concentrations of TL in the tested algae species were within the ranges (1.0 – 5.0%) reported in the literature for several algae species (Matanjun *et al.,* 2009; Manivannan *et al.,* 2008). For instance, the TL contents in 12 species of marine algae belong to 3 families from

Table 1
Total lipid, total sulfolipid contents and sulfate contents of some marine algae

Algae species	Total lipid Content %	Total sulfolipid (% of total lipid)	Sulfate %
U. fasciata	2.35[d]	3.60	0.13[a]
T. atomaria	0.66[b]	4.50	0.16[a]
D. fasciola	1.11[a]	11.80	0.21[a]
L. papillose	0.81[c]	1.25	0.16[a]
G. cylindriea	0.09[a]	10.00	0.17[a]
LSD	0.09		0.07

The mean (n = 3) difference is significant at $P \leq 0.01$.

the *Chlorophyceaean* (4 species), *Phaeophyceae* (5 species) and *Rhodophyceae* (3 species) and ranged from 1.33 ± 0.20% to 4.60 ± 0.17% (Manivannan *et al.* 2008). In general, the total lipid contents varied among alga species depending on species, genetic origin, climate and the geography of the development of the algae (Araki *et al.* 1990).

3.2. Algal total sulfolipid contents

The sulfolipid fractions of five macroalgal lipids are illustrated in Table (1). The sulfolipid fraction content ranged from 1.25 to 11.80% of the total lipids. The highest sulfolipid content was found in *D. fasciola* (11.80%) followed by *G. cylindriea* (10.00%). The lowest sulfolipid fractions were detected in *T. atomaria* (4.50%), *U. fasciata* (3.60%) and *L. papillose* (1.25%).

3.2.1. Sulfate content of marine algal sulfolipids (SLs)

The sulfate content among the algae sulfolipids ranged between 0.13 and 0.21%. The highest sulfate content was found in *D. fasciola* (0.21%) followed by *G. cylindriea* (0.17%), *T. atomaria* (0.16%), *L. papillose* (0.16%) and *U.fasciata* (0.13%). These results agree with those obtained by Sanina *et al.* (2004) in which the sulfolipid contents of the total lipids in *A. tobuchiensis* (Rodophyta), *L. japonica* and *S. pallidum* (Phaeophyta), *U. fenestrata (*Chlorophyta) and *Z. marina* (Embriophyta) were 10.0%, 15.0%, 7.1%, 9.7% and 6.2%, respectively. In addition, these results are in accordance with those of Gerasimenko *et al.* (2010), who stated that the sulfolipid contents of juvenile and adult brown algae *Costaria costata* ranged from 0.4 to 10.6% of the total lipids.

3.2.2. Fatty acid compositions of marine algal sulfolipids

The fatty acid compositions of the algal sulfolipid fraction are presented in Table 2. All algal SLs have high contents of plamitic acid ($C_{16:0}$), ranging from 30.9% (*G. cylindriea*) to 63.1% (*T. atomaria*); while $C_{22:5}$ was detected in *T. atomaria* (13.8%), *G. cylindriea* (13.8%) and *L. papillose* (10.80%). Margaric acid ($C_{17:0}$) was identified as the main fatty acid in the SLs of *U. fasciata* (21.6%) and *L. papillose* (12.9%). A remarkable content of arachidonic acid ($C_{20:4}$) was detected in *G. cylindriea* (37.4%). Oleic acid ($C_{18:1}$) and linoleic ($C_{18:2}$) were also found in considerable amounts at 11.4% in sulfolipid of *U. fasciata* and 27.1 and 10.7% in the sulfolipid fraction of *D. fasciola* and *L. papillose,* respectively. These results reveal that fatty acid composition differed significantly among all the algae species. The FA composition of the sulfolipid fraction is similar to those previously reported for green, brown and red algae (Sanina *et al.,* 2004). Similar results were obtained by Araki *et al.,* (1989), who found that the sulfoquinovosyl diacylglycerols (SQDG) of *G. verrucosa* were characterized by high levels of $C_{16:0}$ and $C_{20:4\acute{\omega}6}$ fatty acids. *P. incisa* sulfolipid (SQDG) were rich in $C_{16:0}$, $C_{18:1\acute{\omega}7}$, $C_{18:2}$ and $C_{18:3\acute{\omega}3}$ (Bigogno *et al.,* 2002). Xue *et al.* (2002) found that the main fatty acids of *Spirulina platensis* SQDG were $C_{16:0}$, and $C_{18:2n-6}$.. The major fatty acids of sulfoquinovosyl-di-acylglyceride in Phaeophyta, Chlorophyta and Rhodophyta were saturated and monounsaturated

Table 2
Fatty acid composition of marine algal sulfolipids

Fatty acids	Algae strains				
	Mediterranean sea			Red sea	
	U. fasciata	*T. atomaria*	*D. fasciola*	*L. papillose*	*G. cylindriea*
$C_{14:0}$	0.90	10.9	3.9	4.7	2.8
$C_{16:0}$	49.9	63.1	57.7	34.5	30.9
$C_{16:1}$	–	–	1.2	2.1	1.2
$C_{17:0}$	21.5	4.51	0.78	12.8	1.0
$C_{18:0}$	2.8	4.0	1.9	6.4	4.0
$C_{18:1}$	11.3	3.0	3.1	6.4	3.1
$C_{18:2}$	2.4	–	27.1	10.7	4.4
$C_{20:3}$	1.7	0.5	0.1	0.75	1.2
$C_{20:4}$	1.3	–	–	1.6	37.4
$C_{22:5}$	7.4	13.8	3.9	10.8	13.8
SFAs %	75.2	82.6	64.4	58.5	38.7
MUFAs %	11.3	3.0	4.3	8.5	4.4
PUFAs %	5.5	14.3	31.1	23.6	43.0

FA, fatty acid; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, Polyunsaturated fatty acids.

fatty acids except for SQDG from Chlorophyta (*Z. marina*), which contained the highest contents of $C_{18:\ 2n-6}$ and $C_{18\ :\ 3n-3}$ acids (Khotimchenko, 2003). Hossain *et al.* (2005) found that the major fatty acids of SQDG from *S. horneri* (brown algae) were $C_{16:0}$, $C_{18:1}$, $C_{18:4}$, $C_{20:1}$, $C_{20:4}$, and $C_{20:5}$

3.3. Proposed algal sulfolipid structures of bioactive constituents

3.3.1. IR analysis

As illustrated in Figure 1, the infrared spectra of all algal sulfolipid fractions showed two characteristic absorption bands for sulpher-containing compounds. The first one appeared at 927cm^{-1}, indicating the presence of a strong dehydration of SO_3 and the second was at 771cm^{-1} , indicating a symmetrical C-O-S associated with a C-O-SO_3 group (Ermanno *et al*., 1994 and Ranjaniv and Steven 1995). Regarding other absorption bands, there were large amounts of $^{-}$OH stretching at 3400 cm^{-1}, symmetric CH_3 bending at 1380 cm^{-1} and C-H stretching at 2930 cm^{-1}.

3.3.2. Identification of marine algal sulfolipid compounds by GG-MS and LC-MS/MS

The proposed chemical structure of the active constituents of the algal sulfolipids was determined using GC-MS and LC-MS-MS. Several compounds of sulfolipids were separated from algae and two of them were identified by EI-MS and ESI/ MS fragmentations. The ESI/ MS and EI/ MS of the major component (compound 1) of sulfolipids consisted of molecular ion $[M + H]^+$ at *m*/*z*= 820 corresponding to the molecular formula of $C_{43}H_{78}O_{12}S$ (Fig. 2 and 4a). The main fragmentations of compound 1 were the peak at m/z = 564.3 (Fig. 2) and was due to the loss of fatty acyl (plamitic acid $C_{16:0}$, m/z 255.3). The peak at m/z = 329 was due to the loss of linoleic acid ($C_{18:2}$), which is characteristic for SQDG. The peak at m/z= 243 was due to the loss of glycerol (m/z= 87). The fragmentation of compound 1 may result in sulfoquinovosyl-di-acylglycerol (Figure 4a).

The LC/MS/MS data revealed that the minor second compound was consistent with the molecular ion $[M + H]^+$ at *m*/*z*= 556.7 (Figure 3 and 4b) corresponding to the molecular formula of $C_{25}H_{48}O_{11}S$. The main fragmentations of SQMG were the peak at m/z = 293.71 due to the loss of palmitic acid. The peak at m/z = 206.71 was due to the loss of glycerol. The fragmentation of compound 2 may result in sulfoquinovosyl acylglycerol (SQMG, fig. 4b). Furthermore, this structure was provided by the GC-MS, and LC- MS/ MS fragmentation pattern and was similar to that reported by Keusgen *et al.,* (1997).

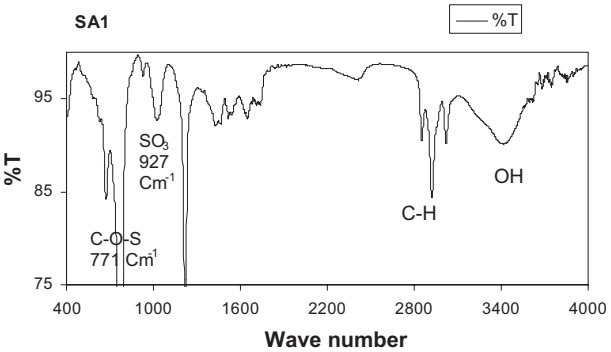

Figure 1
IR of marine algal sulfolipids.

The SQDG of the *Chondria armata* fraction was consistent with the sodiated molecular ion $[M + Na]^+$ at *m*/*z* 629, which have a mass difference corresponding to a likely loss of sulfonic acid (SO_3H) and sodium salt (SO_3Na), respectively. The product ion observed at *m*/*z* 345 appears to have originated by the loss of a fatty acyl side chain as corresponding acid (palmitic acid, $C_{16:0}$). Based on the fragmentation pattern, the sulfolipid with pseudo-molecular ion $[M–H + 2Na]^+$ at *m*/*z* 601 was characterized as 2-*O*-palmitoyl-3-*O*-(6′-sulfoquinovo-pyrano-syl)-glycerol (Al-Fadhli *et al.,* 2006). SQDG structures gave three molecular ions by ESI/MS at *m*/*z* 737.46, 765.77 and 793.77. These ions showed that the glycerol moiety was esterified by the following combinations of fatty acids: ($C_{14:0}$ + $C_{16:0}$) giving *m*/*z* 737.46; ($C_{14:0}$ +$C_{16:0}$) giving *m*/*z* 765.77 and ($C_{14:0}$ + $C_{18:0}$ or $C_{16:0}$ + $C_{16:0}$) giving *m*/*z* 793.77. Tandem-MS gave fragments corresponding to sulfoquinovosyl (*m*/*z* 243.07 and 225.44); and sulfoquinovose linked to glycerol (*m*/*z* 283.35). Minor ions at *m*/*z* 509.71 and 537.83 are probably due to the respective loss of palmitic ($C_{16:0}$) and myristic ($C_{14:0}$) acids (De-Souza *et al*., 2007).

3.4. Biological evaluation of sulfolipid fractions

3.4.1. Antiviral activity

The antiviral activity of algal sulfolipids was evaluated according to the plaque reduction method and the results are summarized in Table 3. Algal sulfolipids showed high antiviral inhibitions against HSV-1, which ranged from 18.75 to 70.12 %. All algal sulfolipids had low virus inhibition at the concentration of 10.0 µg, while the highest inhibition HSV1 was observed at 20 µg mL^{-1} for *D. fasciola* (70.12%). Thus, the sulfolipid fraction had dose-dependant antiviral activity. Among several antiviral available, acyclovir was used as a positive control in this study. The inhibitory effect of algae SLs (IC_{50} ranged from 15.0 to 25.0 µg mL^{-1}) was shown to be quite a bit more potent than that of acyclovir (IC_{50} 5.5 µg mL^{-1}). These results are in agreement with the results obtained by Chirasuwan *et al*., (2009) who found that sulphoquinovosyl-di-acylglycerol compounds extracted from *Spriulina platensis* have antiviral activity (HSV-1) in virus cells. Ohta *et al.,* (1998) found that the new sulfolipid, 6-sulfo-alpha-D-quinovopyranosyl-1',2'-diacylglycerol (SQDG) isolated from *G. tenella* (marine red alga) contains a potent inhibitor of eukaryotic DNA

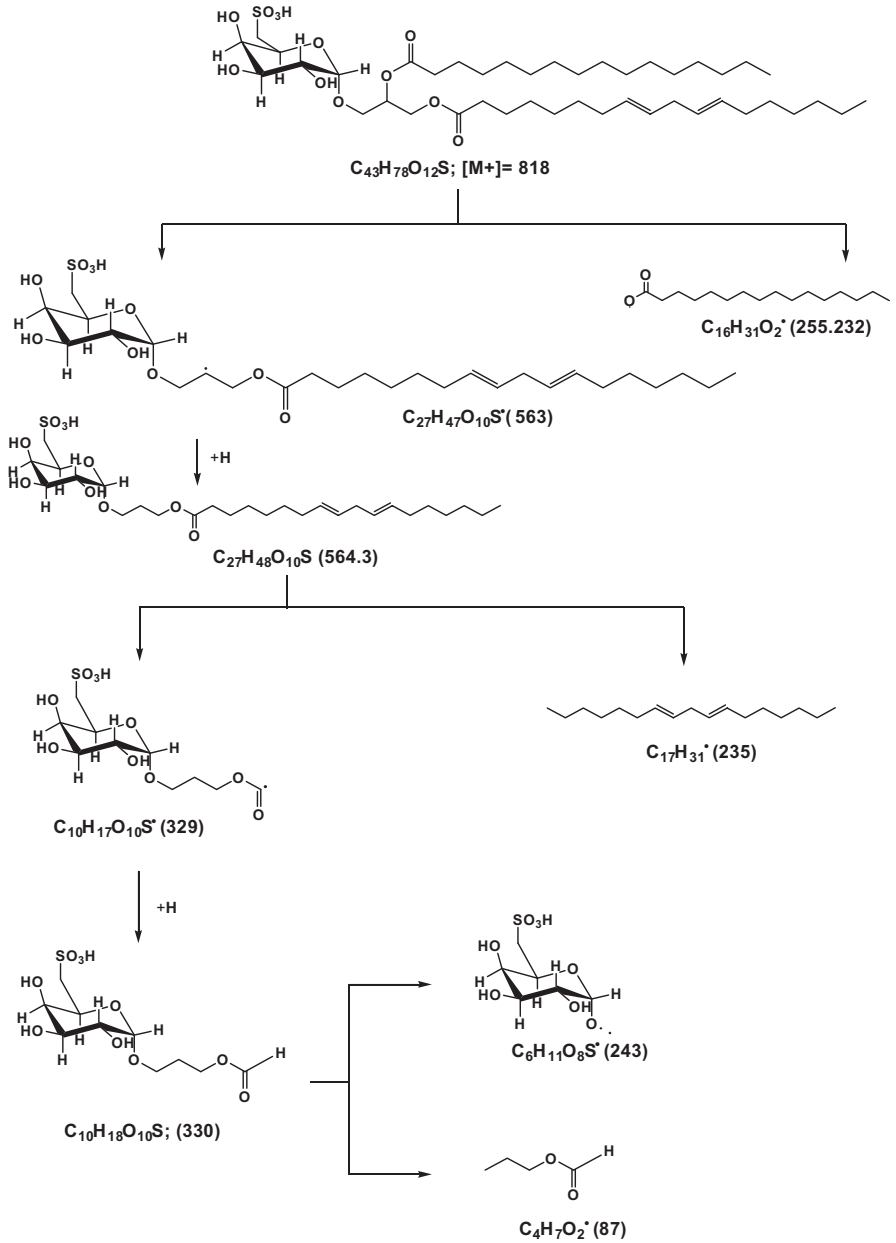

Figure 2
Fragmentation pattern of compound (1) sulfoquinovosyl-di-acylglycerol (SQDG) extracted from marine algal sulfolipids.

Table 3
Antiviral activity of some marine algae sulfolipids against HSV-1

Marine algae strains	Viral inhibition %	
	10 µg mL^{-1}	20 µg mL^{-1}
U. fasciata	18.75	46.87
T. atomaria	43.75	56.25
L. papillose	40.62	59.37
G. cylindriea	45.87	59.37
D. fasciola	46.87	70.12
Acyclovir IC50 (µg mL^{-1}) (reference drug)	5.5	

polymerases and HIV-reverse transcriptase type 1 (Gustafson *et al.* 1998). The SQDG fraction of *S. hofmanii* contains (C18:2/C16:0) and showed a higher antiviral activity against HCM-virus, with an IC_{50} of 19.0 µg mL^{-1} (Naumann, 2009).

3.4.2. Antitumor activity

Antitumor activity against human breast carcinoma (MCF-7). The cytotoxic activities of five algal sulfolipid fractions were tested against MCF-7 and the results are illustrated in Table 4. All algal sulfolipid fractions showed high inhibition percentages (%) toward the MCF7 cell, which ranged from 66.24 to 94.19%. *L. popillose* presented the highest antitumoral activity against the MCF-7 at all concentrations followed by *T. atomaria, G. cylindriea* and *U. fasciata.* In addition,

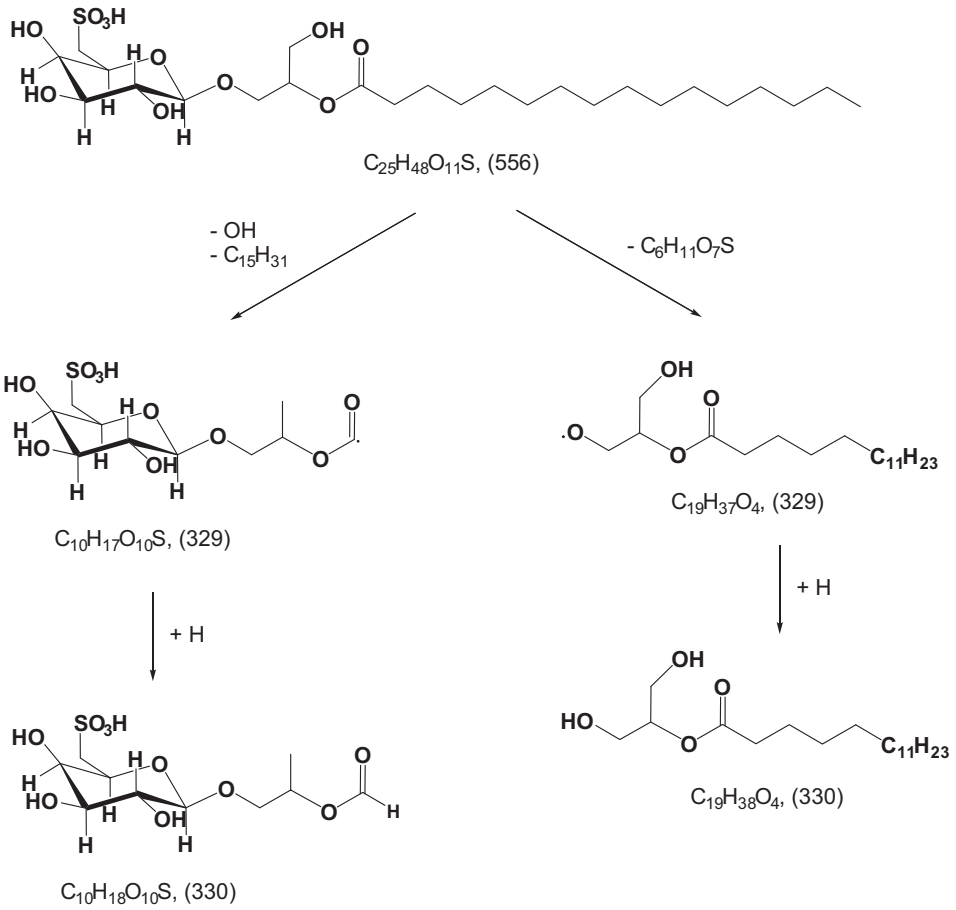

Figure 3
Fragmentation pattern of compound (2) Sulfoquinovosyl -acylglycerol extracted from marine algal sulfolipids.

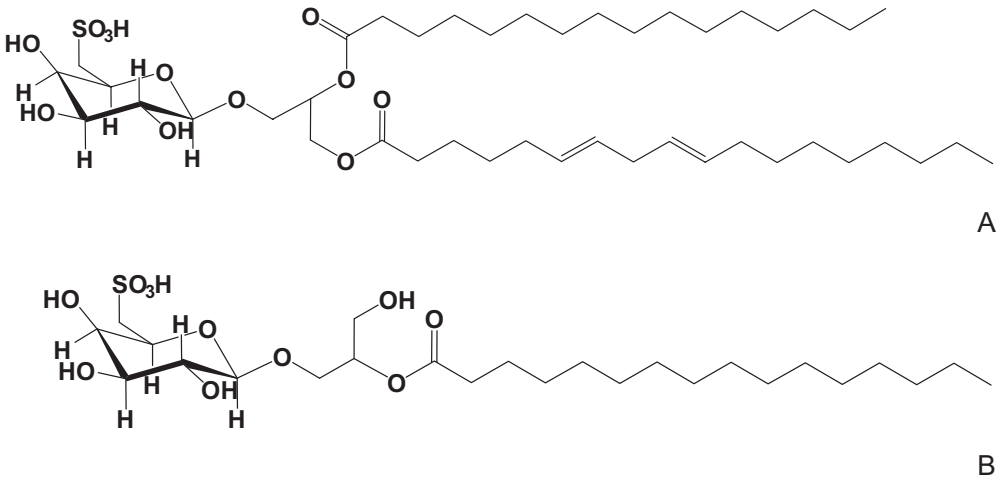

Figure 4
Suggested chemical structure of the different active compounds separated from the marine algal sulfolipids (SQDG). Compound A: sulfoquinovosyl-di-acylglycer. Compound B: sulfoquinovosyl-acylglycerol (SQMG).

all algal sulfolipid fractions showed high potential activities with an IC_{50} of 0.40-0.67 μg mL^{-1} against the MCF-7 cell (Table 5). In addition all algal sulfolipids have a significant antitumor activity compared to the reference antitumor drug novantron (IC_{50} = 1.40 μg mL^{-1}).

Antitumor activity against human hepato carcinoma (Hep G2). Table 4 illustrates that the algal sulfolipids of *T. atomaria* showed the highest inhibition activity (85.37%) against HepG2 cells followed by *D. fasciola* (80.29%), *L. papillose* (79.89%) and *U. fasciata* (72.97%). Therefore, all the algae sulfolipids showed potential antitumor activity against HEPG2 with IC_{50} values ranging from 0.60 to 2.75 μg mL^{-1} (Table 5). These results are in agreement with those obtained by

Table 4.
Antitumor activity of algal sulfolipids against MCF7 and HepG2 cells after 48h incubation

Algae species	Sulfolipids Concentrations (μg mL^{-1})	Growth Inhibition %	
		MCF7 Cell	HepG2 Cell
U. fasciata	1.0	65.40^{a}	48.15^{a}
	2.5	69.16^{bc}	54.39^{a}
	5.0	76.27^{ab}	71.93^{a}
	10.0	79.48^{c}	72.97^{a}
T. atomaria	1.0	79.46^{a}	71.19^{a}
	2.5	82.25^{a}	76.15^{a}
	5.0	85.35^{a}	76.70^{a}
	10.0	85.69^{a}	85.67^{a}
D. fasciola	1.0	71.12^{a}	57.36^{a}
	2.5	73.33^{a}	65.50^{a}
	5.0	74.43^{a}	72.95^{a}
	10.0	79.34^{a}	80.29^{a}
L. papillose	1.0	76.93^{a}	37.35^{a}
	2.5	90.66^{a}	44.85^{a}
	5.0	92.48^{a}	71.56^{b}
	10.0	94.19^{a}	79.89^{b}
G. cylindriea	1.0	66.37^{a}	28.31^{a}
	2.5	76.01^{b}	42.21^{b}
	5.0	77.30^{b}	69.06^{c}
	10.0	82.46^{c}	72.27^{c}
Novantron (reference drug)	1.0	42.73	26.1
	2.5	52.81	42.27
	5.0	52.81	47.66
	10.0	52.81	59.50
LSD		6.52	20.15

The mean (n = 3) difference is significant at $P \leq 0.01$.

Bergé *et al.,* (2002), who found that the sulfolipid compounds extracted from red algal *Porphyridium* possessed an inhibition effect against MCF-7. In another study carried out by Shao *et al.,* (2002), they found that the sulfolipid compounds ((2R)-1-O-myristoyl-2-O-palmitoyl-3-O-(6-sulpho-a-D-quinovo-pyranosyl)-glycerol) from the brown algae *Chondria crassicaulis* possessed an inhibition effect against HL60 and MCF-7 cell lines. Bhaskar *et al.,* (2004), reported that the total lipid and lipid classes of brown algae S. *marginatum* have potent inhibition of human pro-melocytic leukemia HL60. Also Hossain *et al.,* (2005) reported that the SQDG of brown algae *S. horneri* showed significant apoptosis activity towards Caco-2 cells. The SQDG fraction obtained from dried spinach was an inhibitor of mammalian DNA polymerases and a growth inhibitor of NUGC3 human gastric cancer cells (Maeda *et al.,* 2005). Sahara *et al.* (2002) and (Mizushina *et al.*, 2003) found that the synthetic sulfoquinovosyl-mono-acylglycerols had a highly significant effect in suppressing the growth of solid tumors (human lung adenocarcinoma A-549 cells), and showed a potent inhibition of DNA polymerase and potent antineoplastic agents against the gastric cancer cell line (NUGC3).

Antimicrobial activity of algal sulfolipids. Marine algal sulfolipids (SLs) presented a high growth inhibition of the bacterial strains (*B. subtilis* and *E. coli*) at the concentration of 100 μg/well**,** while all algal SLs did not show any inhibition effect against fungal or yeast cells (Table 6). The highest bacterial growth inhibition was obtained by *T. atomaria* sulifolipids (15.0 mm) against *E. coli* followed by *U. fasciata* sulifolipid*s* (13 mm) and *G. cylindriea* sulifolipids (11 mm). *L. papillose*

Table 5
Comparison of marine algal sulfolipid fractions against MCF7 and HepG2

Marine algae species	IC_{50} µg mL^{-1}	
	MCF7 Cell	HepG2 Cell
U. fasciata	0.54	1.41
T. atomaria	0.40	0.60
D.fasciola	0.60	0.60
L. papillose	0.67	2.21
G. cylindriea	0.40	2.75
Novantron (reference drug)	1.4	4.0

Table 6
Antimicrobial activities of marine algal Sulfolipids (inhibition zone in diameter (mm) around the discs) and MIC

Marine algae species	Inhibition zone (mm) Microorganisms		MIC µg mL^{-1} Microorganisms	
	Bacteria		Bacteria	
	E. coli	*B. subtilis*	*E. coli*	*B. subtilis*
U. fasciata	13.0	16.0	60.0	40.0
T. atomaria	15.0	13.0	60.0	40.0
D. fasciola	8.0	10.0	–	–
L. papillose	8.0	11.0	–	–
G. cylindriea	11.0	11.0	80.0	80.0
Chloramphenicol (reference drug)	15.0	18.0	25.0	20.0

(8 mm) and *D. fasciola* sulifolipids (8 mm) showed the lowest growth inhibition against *E. coli*. The maximum inhibition zone against *Bacillus subtilis* was observed in *U. fasciata* sulifolipids (16 mm) followed by *T. atomaria* sulfolipids (13 mm). A moderate inhibition against *Bacillus subtilis* was observed in *G. cylindriea* and *L. papillose* sulfolipids (11 mm), followed by *D. fasciola* sulfolipids (10 mm). *U. fasciata*, *T. atomaria, L. papillose* and *G. cylindriea* algae sulfolipids showed the most potent activity with MIC ranging from 40.0 to 80.0 µg mL^{-1} against *E. coli* and *B. subtilis*. Al-Fadhli *et al.,* (2006) found that the sulfonoglycolipids of the red alga *Chondria. armata* inhibited the growth of bacteria strains (*P. aeruginosa* and *K. pneumoniae*) at 20 µg/disc, while they have poor activity against the fungi (*A. fumgatus*, *C. neoformans*, *A. niger* and *R.* spp). Sulfoglycerolipids, 1-0-palmitoyl-3-0(6′-sulpho-α-quinovopyranosyl)-glycerol isolated from the *S. wightii* had antibacterial activity against *Xanthomonas oryzae* which causes bacterial blight in rice **(**Arunkumar *et al.,* 2005).

In conclusion, the GC/MS and LC/Ms identified many compounds from extracted algal sulfolipids and two major compounds were identified by ESI/MS fragmentation. The ESI/MS of the major component of algal sulfolipids (SQDM) was a consistent molecular ion $[M + H]^+$ at *m/z*= 820 corresponding to the molecular formula of $C_{43}H_{78}O_{12}S$ and the second compound (SQMG) was the consistent molecular ion $[M + H]^+$ at *m/z*= 556.71 corresponding to the molecular formula of $C_{25}H_{48}O_{11}S$. The algal sulfolipid fraction has antitumor activity against (HepG2 and MCF-7 cell lines) and antibacterial activity against *Bacillus subtilis* and *Escherichia coli*. The antitumor and antibacterial activities of algal sulfolipids are probably owing to the presence of the sulfolipids as sulfoquinovosyl-di-acylglycerol, sulfoquinovosyl acylglycerol, unsaturated fatty acids and sulfate contents.

REFERENCES

Al-Fadhli A, Wahidulla S, D'Souza L. 2006. Glycolipids from the red alga *Chondria armata (*Kütz.) Okamura. *Glycobiology* **16**, 902-915.

Araki S, Sakurai T, Oohusa T, Kayama M, Sato N. 1989. Characterization of sulfoquinovosyl diacylglycerol from marine red algae. *Plant Cell Physiol.* **30**, 775-781.

Araki S, Sakurai T, Oohusa T, Kayama M, Nisizawa K. 1990. Content of arachidonic and eicosapentaenoic acids in polar lipids from Gracilaria (Gracilariales, Rhodophyta). *Hydrobiologia* **204/205**, 513-519.

Arunkumar K, Selvapalam N, Rengasamy R. 2005. The antibacterial compound sulphoglycerolipid 1-0 palmitoyl-3-0 (6′-sulpho-α-quinovopyranosyl)-glycerol

from *Sargassum wightii* Greville (Phaeophyceae). *Bot. Mar*. **48**, 441-445.

Bergé PJ, Debiton E, Dumay J, Duand P, Barthomeuf C. 2002. In vitro anti-inflammatory and anti-proliferative activity of sulfolipids from the red alga *Porphyridium cruentum*. *J. Agric. Food Chem*. **50**, 6227-6232.

Benning C, Somerville RC. 1992. Isolation and genetic complementation of a sulfolipid-deficient mutant of Rhodobacter sphaeroides. *J. Bacteriol*. **174**, 2352-2360.

Benning C, Garavito RM. 2009. Sulfolipid Biosynthesis and Function in Plants, Rüdiger Hell et al. (Eds.), Sulfur Metabolism in Phototrophic Organisms, 185-200.

Bhaskar N, Hosakawa M, Miyashita K. 2004. Growth inhibition of human pro- myelocytic Leukemia (HL-60) cells by lipid extracts of marine algae *Sargassum marginatum* (Fucales, phaeophyta) harvested off Goa (west coast of India) with special reference to fatty acid composition. *Indian J. Mar. Sci*. **33**, 335-360.

Bigogno C. Khozin-Goldberga I, Boussibaa S, Vonshaka A, Cohena Z. 2002. Lipid and fatty acid composition of the green oleaginous alga *Parietochloris incisa*, the richest plant source of arachidonic acid. *Phytochem.* **60**, 497-503.

Chirasuwan N, Chaiklahan R, Kittakoop P, Chanasattru W, Ruengjitchatchawalya M, Tanticharoen M, Bunnag B. 2009. Anti HSV-1 activity of sulphoquinovosyl diacylglycerol isolated from *Spirulina platensis. Sci. Asia* **35**, 137-141.

De-Souza ML, Iacomini M, Gorin AJP, Sari SR, Haddad AM, Sassaki LG. 2007. Glyco- and sphingophosphonolipids from the medusa *Phyllorhiza punctata*: NMR and ESI-MS/MS fingerprints. *Chem. Phys. Lipids* **145**, 85-96.

Ermanno A, Guido B, Gianguido R, Ronald JW. 1994. FT-IR study of the interaction of magnesium ferrite with SO_2. *Catal. Lett*. **23**, 353-360.

Gerasimenko IN, Busarova GN, Moiseenko PO. 2010. Age dependent changes in the content of lipids, fatty acids and pigments in brown alga *Costaria costata*. *Russ. J. Plant Physiol*. **57**, 62-68.

Gomes KA, Gomes AA. 1984. Statistical procedures for agricultural research. 2nd ed. Jon Willey and Sons Inc, New York, U.S.A.

Greenwood D. 1983. Antimicrobial chemotherapy, Part II-Laboratory Aspects of Antimicrobial Therapy Bailliere, Tindall, London, p. 71.

Gustafson KM, Cardellina JH, Fuller RW, Weislow OS, Kiser RF, Snader KM, Patterson GML. 1989. AIDs-antiviral sulfolipid from cynobacteria (blue-green algae). *J. Nat. Cancer Inst*. **81**, 1254-1258.

Hammer KA, Carson CF, Riley TV. 1999. Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol*. **86**, 985-990.

Hossain Z, Kurihara H, Masashi H, Takahashp K. 2005. Growth inhibition and induction of differentiation and apoptosis mediated by sodium butyrate in CACO-2 cells with algal glycolipids. In Vitro Cell. Dev. Biol. Animal **41,** 154-159.

Keusgen M, Curtis MJ, Thibault P. 1997. Sulfoquinovosyl diacylglycerols from the alga Heterosigma carterae. Lipids **32**, 1101-1112.

Khotimchenko VS. 2002. Distribution of glycoglycerolipids in marine algae and grasses. *Chem. Nat. Compd*. **38**, 223-229.

Khotimchenko VS. 2003. The fatty acid composition of glycolipids of marine macrophytes. *Russ. J. Mar. Biol*. **29**, 126-128.

Liptak A, Balla E, Lorant J, Sajtosa F, Lászl S. 2004. The first synthesis of secondary sugar sulfonic acids by nucleophilic displacement reactions. *Tetrahedron Lett.* **45**, 839-842.

Luddy FE, Beerford RA, Riemen RW. 1960. Direct conversion of lipid component to their fatty acid methyl ester. *J. Am. Oil Chem. Soc*. **37**, 447-451.

Maeda N, Hada T, Murakami-Nakaia C, Kuriyamaa I, Hideki I, Fukumorid Y, Hiratsukaf J, Yoshidaa H, Sakaguchig K, Mizushinaa Y. 2005. Effects of DNA polymerase inhibitory and antitumor activities of lipase-hydrolyzed glycolipid fractions from spinach. *J. Nutr. Biochem*. **16**, 121-128.

Maeda N, Kokai Y, Ohtani S, Sahara H, Kumamoto-Yonezawa Y, Kuriyama I, Hada T, Sato N, Yoshida H, Mizushina Y. 2008. Anti-tumor effect of orally administered spinach glycolipid fraction on implanted cancer cells, colon-26, in mice. *Lipids* **43**, 741-748.

Matanjun P, Mohamed S, Mustapha MN, Muhammad K. 2009. Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*. *J. Appl. Phycol*. **21**, 75-80.

Manivannan K, Thirumaran G, Karthikai, Devi G, Hemalatha A, Anantharaman P. 2008. Biochemical composition of seaweeds from Mandapam coastal regions along Southeast Coast of India. *American-Eurasian J. Bot*. **1**, 32-37.

Mizushina Y, Watanabe I, Ohta K, Takemura M, Sahara H, Takahashi N, Gasa S, Sugawara F, Matsukage A, Yoshida S, Sakaguchi K. 1998. Studies on inhibitors of mammalian DNA polymerase alpha and beta: sulfolipids from a *Pteridophyte* and *Athyrium niponicum*. *Biochem. Pharmacol*. **55,** 537-541

Mizushina Y, Maeda N, Kawasaki M, Ichikawa H, Murakami C, Takemura M, Xu X, Sugawara F, Ukumori Y, Yoshida H, Sakaguchi K.,2003. Inhibitory action of emulsified sulfoquinovosyl acylglycerol on mammalian DNA polymerases. *Lipids* **38**, 1065-1074.

Naumann I. 2009. Sulfoquinovosyldiacylglyceride antiviral active Substanzen. ph.D. Thesis, Fakultät der Universitt Erlangen-Nürnberg.

Norman AH, Mischke FC, Allen B, Vincentt SJ. 1996. Semi-preparative isolation of plant sulfoquinovosyldiacylglycerols by solid phase extraction and HPLC procedures. *J. Lipid Res*. **37**, 1372-1376.

Ohta K, Mizushina Y, Hirata N, Takemure M, Sugawar F, Matsukage A, Yoshida S, Sakaguchi K. 1998. Sulfoquinovosyldiacylglycerol, KM043, a new potent inhibitor of eukaryotic DNA polymerases and HIV-reverse transcriptase type1 from a marine red alga. *Chem. Pharm. B*. **46,** 281-291.

Pons A, Timmerman P, Leroy Y, Zanetta JP. 2002. Gas-chromatography/mass-spectrometry analysis of human skin constituents as heptafluorobutyrate derivatives with special reference to long-chain bases. *J. Lipid Res*. **43**, 794-804.

Ranjaniv S, Steven W. 1995. FTIR characterization of the interaction of oxygen with zinc sulfide. *Indian Eng. Chem. Res*. **34**, 699-702.

Roughan PG, Bratt DR. 1968. Quantitative analysis of sulfolipid (sulfoquinovosyl diglyceride) and galactolipids (monogalactosyl and digalactosyl diglycerides) in plant tissue. *Anal. Biochem*. **22**, 74-88.

Sanina MN, Goncharova NS, Kostetsky YE. 2004. Fatty acid composition of individual polar lipid classes from marine macrophytes. *Phytochemistry* **65**, 721-730.

Sahara H, Hanashima S, Yamazak, Takahashi TS, Sugawara F, Ohtani S, Ishikawa M, Mizushina Y, Ohta K, Shimozawa K, Gasa S, Jimbow K, Sakaguchi K, Sato N, Takahashi N. 2002. Anti-tumor effect of

chemically synthesized sulfolipids based on sea Urchin's natural sulfonoquinovosylmonoacylglycerols. *J. Cancer Res*. **93**, 85-92.

Shao ZY, Cai JN, Ye QZ, Guo YI. 2002. Crassicaulisine, sulphonoglycolipid from red algae *Chondria crassicaulis* Harv. *J. Asian Nat. Prod. Res*. **4**, 205-209.

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. National Cancer Inst.* **82**, 1107-1112.

Simons K, Toomre D. 2000. Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Bio*. **1**, 31-39.

Tebas P, Stabell EC, Olivo PD. 1995. Antiviral susceptibility testing with a cell line which expresses beta-galactosidase after infection with herpes simplex virus. *Antimicrob. Agents Ch*. **39**, 1287-91.

Terho TT, Hartiala K. 1971. Method for determination of the sulfate content of glycosaminoglycans. *Anal. Biochem*. **41**, 471-476.

Xue C, Hu Y, Saito H, Zhang Z, Li Z, Cai Y, Ou C, Lin H, Imbs AB. 2002. Molecular species composition of glycolipids from *Sprirulina platensis*. *Food Chem*. **77**, 13.

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