

Bioassay-guided isolation of glycolipids from the seaweed *Gracilaria corticata*

Vajihe Akbari¹, Mahshid Abedi², and Afsaneh Yegdaneh^{2,*}

¹Department of Pharmaceutical Biotechnology and Isfahan Pharmaceutical Sciences Research Centre, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

²Department of Pharmacognosy and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

Abstract

Background and purpose: In some countries, seaweeds are famous traditional food which contain different types of secondary metabolites. These marine organisms have several bioactive secondary metabolites. The aim of this study was to perform bioassay-guided isolation of glycolipids from a Persian Gulf seaweed *Gracilaria corticata* J. Agardh.

Experimental approach: *G. corticata* was collected from the Persian Gulf. The plant was extracted by maceration with methanol-ethyl acetate solvent. The extract was partitioned by the Kupchan method to yield *n*-hexane, dichloromethane, butanol, and water partitions. The most active partition found in the cytotoxicity assay was further fractionated using medium pressure liquid chromatography and high-performance liquid chromatography (HPLC) methods to yield two pure compounds. The structures of the isolated compounds were elucidated using various spectroscopic methods. The cytotoxic activities of all fractions were also tested.

Findings/Results: *n*-hexane and dichloromethane partitions exhibited higher and significant cytotoxicity against the HeLa cell line with IC₅₀s of 117.41 and 291.38 µg/mL, respectively. The cytotoxic effects of nine fractions of the *n*-hexane partition against HeLa and HUVEC cells were also ranging from 96.33 to 243.56 µg/mL and 85.38 to 290.5 µg/mL, respectively. Two sulfoquinovosyldiacylglycerides were isolated and their structures were elucidated.

Conclusion and implications: From the spectral characteristics, the isolated compound from the extract was confirmed to be α-D-glucopyranosyl-1,2-O-diacyl-glycerols with moderate cytotoxic activity.

Keywords: Cytotoxic; *Gracilaria*; Persian Gulf; Seaweed; sulfoquinovosyldiacylglycerides.

INTRODUCTION

Marine habitat is an important source of biologically active metabolites. A large number of marine compounds are different from terrestrial natural metabolites due to the unique physical and chemical conditions in the marine litter (1). Marine organisms such as seaweeds, sponges, corals, fungi and ascidians contain potent metabolites with characteristic structural and chemical features. In the field of marine plants, there are more than 2400 marine natural compounds isolated only from seaweeds of subtropical and tropical populations (2).

A number of researches exhibited that seaweeds are important resources of natural

drug metabolites. They have various kinds of metabolites such as terpenoids, amino acids, phlorotannins, steroids, phenolics, and halogenated compounds particularly ketones, glycolipids, and cyclic polysulphides (3). Novel structures and also novel mechanisms of action of these metabolisms have led to the isolation of bioactive substances which show great potential as anti-inflammatory, antimicrobial, antiviral, antioxidant, antitumor, and anti-Alzheimer activities (4-6).

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*Corresponding author: A. Yegdaneh
Tel: +98-9133137184, Fax: +98-3116680011
Email: yekdaneh@pharm.mui.ac.ir

In recent years, isolation of the lipid composition of marine seaweeds has increased considerably. Glycoglycerolipids are one of the main groups of lipids present in the marine algae which are well-known for their interesting biological activities, such as improving the intestinal condition, antitumor activity, anti-inflammatory activity, and protecting against cell death (7). Glycolipids are a less studied class of secondary metabolites in natural organisms that are mainly isolated from seaweeds (8). Seaweeds produce three major types of glycolipids: monogalactosyldiacylglycerides (MGDG), digalactosyldiacylglycerides (DGDG), and sulfoquinovosyldiacylglycerides (SQDG). A number of algae are able to convert simple polyunsaturated fatty acids into complex eicosanoids and related oxylipins (9) and these derivatives of arachidonic acids play an important role in maintaining homeostasis in mammalian systems. In addition, in various diseases such as psoriasis, asthma, arteriosclerosis, heart disease, ulcers, and cancer, this class of metabolisms are abnormally produced (10,11). Therefore, isolation, purification, identification, and bioactivity analysis of glycoglycerolipid molecular structure are an interesting area in the field of marine chemistry.

Gracilaria is a genus of red algae (Rhodophyta) notable for its economic importance as an agarophyte, as well as its use as a food for humans and various species of shellfish. Various species within the genus are cultivated among Asia, South America, Africa, and Oceania (12). Despite the high value of this marine algae, there have been only a few studies on the pharmacological properties of these seaweeds. The main objective of the present study was to evaluate the cytotoxic activity of brown alga *Gracilaria corticata* J.Agardh (*G. corticata*) collected from the Persian Gulf and also isolate the bioactive compounds.

MATERIALS AND METHODS

Instrumentations

Column chromatography (CC): silica gel 63-200 μm (Merck, Germany); thin-layer

chromatography (TLC): silica gel GF254 plates (20 \times 20 cm, 0.5 mm, Merck). Preparative high-performance liquid chromatography (HPLC): Agilent 1100 Series with a normal phase column (250 \times 20 mm i.d.) packed with 5 μm silica (YMC Co., Ltd., Kyoto, Japan). Nuclear magnetic resonance (NMR): Bruker AV-400 (1H) and AV-100 (13C), δ in ppm rel. to Me4Si and J in Hz. High-resolution electrospray ionization mass spectrometry (HR-ESI-MS): Waters Q-TOF Micro YA019 mass spectrometer in m/z and EI-MS spectra. The gas chromatography (GC)-MS was Agilent Technologies 6890N GC equipped with a mass-selective detector 5973 Network MSD, a split injector, and a silica-capillary GC column HP-5MS (30 m \times 0.25 mm; i.d. 0.25 μm film, Agilent Technologies, Inc.).

Authentication of plant material

The seaweeds were collected in 2012 from the Persian Gulf coasts of Iran close to Bushehr Province. They were identified by the Agricultural and Natural Resources Research Center of Bushehr and their voucher specimens coded as 2662 were deposited in the herbarium of the School of Pharmacy and Pharmaceutical Sciences of Isfahan University of Medical Sciences, Isfahan, I.R. Ira).

Extraction and bioassay-guided fractionation of lipids

The *G. corticata* powder was successively extracted at room temperature with EtOAc/methanol 1:1 (v/v). After filtration, the extracts were combined, dried, and partitioned to *n*-hexane, dichloromethane, butanol, and water through Kupchan partitioning method (11). *n*-hexane partition was further fractionated by medium pressure liquid chromatography. Silica gel was used as a stationary phase and the column was eluted with a gradient solvent system from 100% *n*-hexane to pure EtOAc. The eluates were monitored by TLC and ¹HNMR and divided into 14 fractions (Frs. 1-9). Fraction F9 was further purified on a silica gel column, which was sequentially eluted with chloroform/methanol with increasing concentrations of methanol (95:5, 90:10, 80:20, 50:50 v/v) and finally 100% methanol. The resulting fractions were

combined in twelve final fractions. Fractions F9i was further isolated by HPLC separation (C18 column, methanol: water 70: 30), yielded the pure compounds 1 and 2 (Fig. 1).

Alkaline hydrolysis

A solution of the compound in MeOH (0.5 mL) was treated with sodium methoxide (NaOMe) 0.5 M in MeOH (1 mL) and stirred at room temperature for 5 h. Afterward, the reaction mixture was neutralized with Dowex 50W \times 4, and the resin was removed by filtration (9). The filtrate was extracted with *n*-hexane and the *n*-hexane layer was concentrated and analyzed by GC-MS. The column oven temperature was kept at 80 °C for 1 min and then increased at a rate of 15 °C/min up to 310 °C, carrier He, flow rate 0.8 mL/min. Their retention times and fragmentation peaks were in good agreement with those of standard fatty methyl esters.

Cell culture

The epithelioid cervix carcinoma cell line, HeLa, and human umbilical vein endothelial cells, HUVEC, were obtained from the Pasteur

Institute of Iran, Tehran. Cells were incubated in a humidified incubator with 5% CO₂ at 37 °C. HeLa and HUVEC cells were fed with Roswell Park Memorial Institute medium (RPMI) and Dulbecco's modified eagle's medium (DMEM), respectively. The media were supplemented with 10% (v/v) fetal bovine serum (FBS) and penicillin-streptomycin (100 IU/mL and 100 µg/mL, respectively).

in vitro cytotoxicity

To identify the bioactive compounds from the algae, cytotoxicity of four different partitions including water, *n*-butanol, dichloromethane, and *n*-hexane, were initially evaluated, and then the most effective partition was subjected to further fractionation by HPLC. Twelve fractions were also screened for their cytotoxic effects. *in vitro* cytotoxicity of samples against HeLa as well as HUVEC cells was evaluated using 3-(4,5-dimethyl-2-thiazolyl) - 2,5 -diphenyl -2H- tetrazolium bromide (MTT) assay as described previously (6). Briefly, a cell suspension of 2×10^5 cells/mL was seeded in 96-well plates and incubated overnight to allow cell attachment.

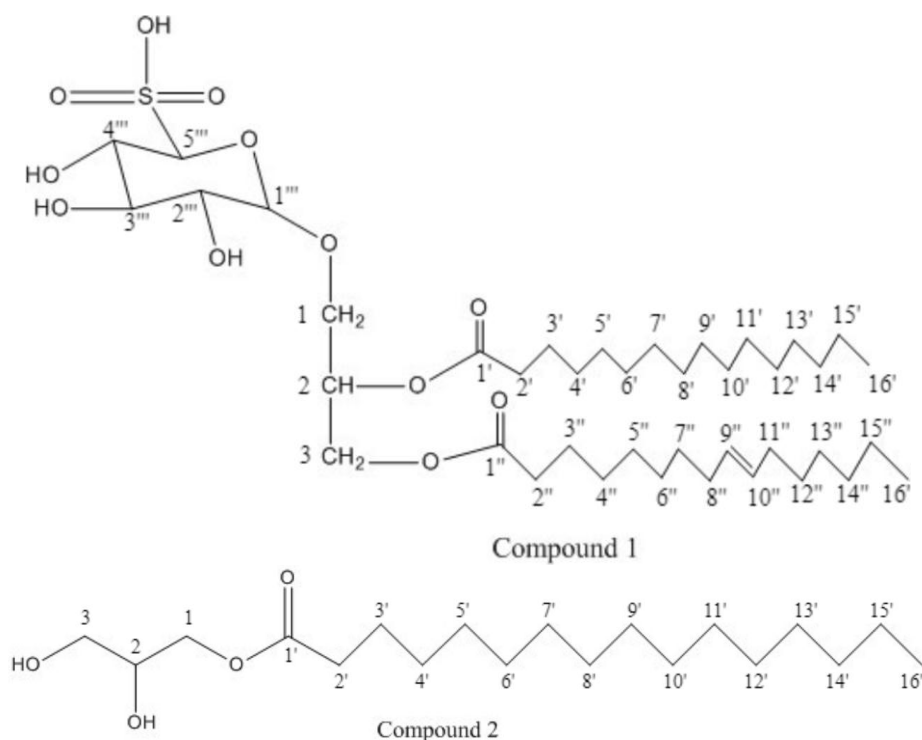


Fig. 1. Structure of compounds 1 and 2.

The dried fractions or partitions were dissolved in dimethyl sulfoxide (DMSO; the final concentration of DMSO in the palate was less than 1%) and 20 μ L of different concentrations of samples were added and incubated for 72 h at 37 °C in a humidified atmosphere. Doxorubicin was used as the positive control and 1 % (v/v) DMSO was applied as the negative control. Then cells were incubated with 20 μ L of MTT solution (5 mg/mL) at 37 °C for 3 h. The medium was removed and to dissolve MTT-formazan crystals 150 μ L of DMSO was added and the absorbance was measured at 570 nm by a plate reader (Stat Fax-2100; Awareness Technology Inc., Palm City, FL, USA). The cell survival was calculated according to the following equation:

$$\text{Cell survival} = \frac{\text{Absorbance of treated wells} - \text{Absorbance of blank}}{\text{Absorbance of negative control} - \text{Absorbance of blank}} \times 100$$

Statistical analyses

All data are expressed as mean \pm standard deviation (SD). Significant differences were calculated by analysis of variance (ANOVA) using SPSS version 20 and Post Hoc test was

used to evaluate the difference between groups. $P < 0.05$ was considered statistically significant.

RESULTS

Evaluation of cell toxicity

The effect of *n*-hexane, dichloromethane, *n*-butanol, and water-soluble parts of the EtOAc/methanol extract of *G. corticata* on the viability of HeLa and HUVEC cells were evaluated by MTT assay. As shown in Table 1, the *n*-hexane and dichloromethane partitions exhibited the higher and significant cytotoxicity against the HeLa cell line with IC₅₀s of 117.41 and 291.38 μ g/mL, respectively and more effectively inhibited the viability of cancer cells than polar phases (*i.e.*, *n*-butanol and water). The cytotoxic effects of nine fractions of the *n*-hexane partition against HeLa and HUVEC cells were also evaluated and as shown in Table1, the IC₅₀s ranged from 96.33 to 243.56 μ g/mL and 85.38 to 290.5 μ g/mL, respectively. Fraction B showed the highest cytotoxicity against cancer cells with an IC₅₀ of 96.33 μ g/mL. Additionally, this fraction exhibited the highest selectivity index among other fractions.

Table 1. IC₅₀ values for different algal extracts, fractions from column chromatography, and the isolated pure compounds against HeLa and HUVEC cells. Data represent mean \pm SD, n = 3

Algal partitions / fractions / pure compounds	IC ₅₀ (μ g/mL)		Selectivity index*
	HeLa cells	HUVEC cells	
<i>n</i> -hexane partition	117.41 \pm 8.83	152.76 \pm 13.26	1.3 \pm 0.21
Dichloromethane partition	291.38 \pm 15.43	321.60 \pm 11.98	1.1 \pm 0.10
<i>n</i> -butanol partition	1148.3 \pm 65.33	1384.07 \pm 70.68	1.2 \pm 0.08
Water partition	2686.1 \pm 86.87	3111.05 \pm 113.97	1.16 \pm 0.08
Fraction A	121.82 \pm 9.05	127.75 \pm 10.31	1.04 \pm 0.01
Fraction B	96.33 \pm 10.22	134.91 \pm 11.80	1.40 \pm 0.28
Fraction C	145.90 \pm 9.35	145.35 \pm 9.40	0.99 \pm 0.12
Fraction D	132.65 \pm 9.46	98.01 \pm 10.49	0.74 \pm 0.13
Fraction E	116.46 \pm 9.05	119.29 \pm 9.21	1.02 \pm 0.16
Fraction F	123.37 \pm 7.36	85.38 \pm 6.65	0.69 \pm 0.10
Fraction G	109.84 \pm 9.57	120.45 \pm 5.96	1.10 \pm 0.15
Fraction H	202.53 \pm 8.16	204.29 \pm 11.05	1.00 \pm 0.01
Fraction I	173.56 \pm 9.00	141.4 \pm 11.2	0.81 \pm 0.10
Compound 1	106.88 \pm 5.8	290.50 \pm 11.63	2.72 \pm 0.04
Compound 2	243.56 \pm 9.44	244.71 \pm 12.16	1.00 \pm 0.09
Doxorubicin	1.50 \pm 0.05	3.75 \pm 0.13	2.50 \pm 0.14

*Selectivity index was calculated by dividing IC₅₀ of the HUVEC cells to that of the HeLa cells.

The cytotoxic activity of two pure compounds obtained from fractions B and H were also evaluated against HeLa and HUVEC cells. As shown in Fig. 2A, compound 1 exhibited a higher inhibitory effect against HeLa cells while had a lower inhibitory effect against HUVEC cells (selectivity index of 2.7). Interestingly, the selectivity index for compound 1 was even higher than the positive control (selectivity index of 2.5). However, compound 2 showed lower cytotoxicity and selectivity (Fig. 2B) against HeLa cells.

Spectroscopic data

Compounds 1 and 2 were analyzed using different spectral data. Careful analysis of the

^1H - and ^{13}C -NMR data including 1H-1H COSY, DEPT, HSQC, and HMBC spectra (Fig. 1), allowed the assignment of all ^1H - and ^{13}C -NMR signals (Table 2).

Compound 2: Crystallin, MW (g/mol): 302; ^1H NMR (400 MHz, CDCl_3): 3.53(1H, dd, $J = 2.8, 11.2$ Hz, H-1), 3.61 (1H, dd, $J = 2.8, 11.2$ Hz, H-1), 5.21 (1H, m, H-2), 4.10 (1H, ddd, $J = 5.2, 11.6, 12.4$ Hz, H-3), 2.2 (2H, t, $J = 7.6$ Hz, H-2'), 1.47 (2H, m, H-3'), 1.2-1.3 (m, H-4'-13'), 0.81 (6H, t, $J = 6.8$ Hz, H-14'), 1.56 (2H, m, H-1').

^{13}C NMR (100 MHz, CDCl_3): 174.3 (C-1'), 70.2 (C-2), 65.1 (C-3), 63.3 (C-1), 34.1 (C-12'), 31.9 (C-2'), 29.6-29.7 (C-4'-11'), 24.9 (C-3'), 22.7 (C-13'), 14.1 (C-14').

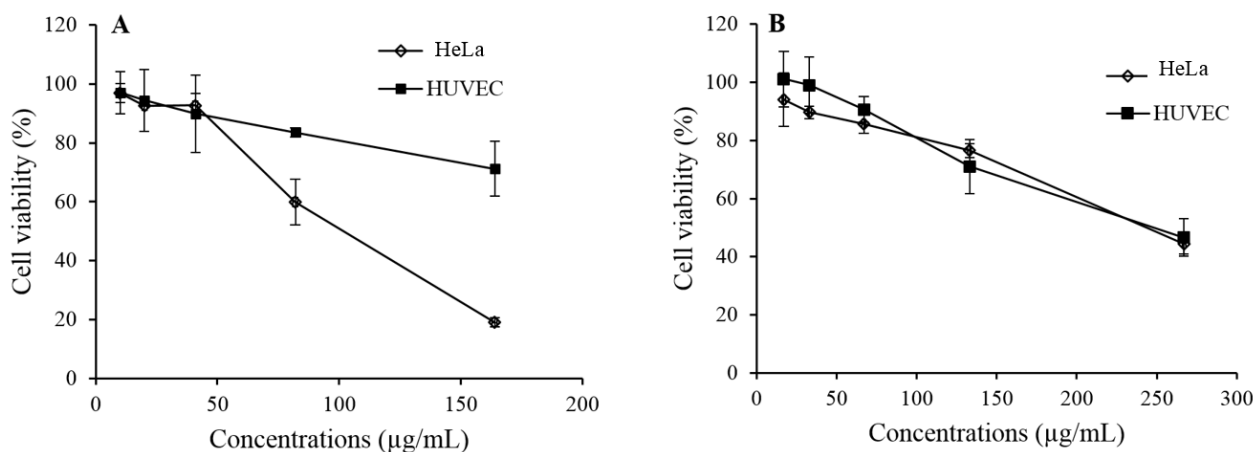


Fig. 2. Evaluation of cytotoxicity of (A) compounds 1 and (B) compound 2 against HeLa and HUVEC cell lines using MTT assay. Data are shown as mean \pm SD, $n = 12$.

Table 2. Nuclear magnetic resonance data of compound 2. Deuterated dimethyl sulfoxide was used as the solvent.

No.	δC	δH
1	64	4.12 (1H, dd, $J = 2.8, 12$), 4.12 (1H, dd, $J = 2.8, 12$)
2	70.2	5.17 (1H, m)
3	68.7	3.86 (1H, dd, $J = 5.6, 10.8$), 3.63 (1H, dd, $J = 5.2, 10.8$)
1'''	105.3	4.12 (1H, d, $J = 7.6$)
2'''	71.8	3.62 (1H, dd, $J = 7.2, 9.6$)
3'''	72.4	3.36 (1H, t, $J = 9.2$)
4'''	74.8	2.92 (1H, t, $J = 9.2$)
5'''	76.8	3.75 (1H, ddd, $J = 4.8, 5.6, 10.4$)
6'''	62.4	2.58 (1H, dd, $J = 6, 14$), 2.57 (1H, dd, $J = 6.2, 13.9$)
2', 2''	33.1	2.21 (4H, m)
3', 3''	26.0	1.50 (4H, m)
4' - 7', 12' - 15', 4'' - 13''	30- 30.1	1.2 - 1.3 (m)
9', 10'	130.9, 130.8	5.23 (2H, t, $J = 4.8$)
18', 16''	14.5	0.84 (6H, t, $J = 6.8$)
17', 15''	23.7	1.2 - 1.3 (m)
16', 14''	35, 35.1	1.2 - 1.3 (m)
8', 11'	30.8	1.97 (4H, m)
1', 1''	174.7, 175	1.2 - 1.3 (m)

DISCUSSION

Our results were in agreement with our previous findings in which the polarity of solvent could influence the cytotoxic activity of partitions from algal extract, and chloroform and *n*-hexane partitions from *S. boveanum* had the highest anticancer activity (12). Additionally, in the present study, the *n*-hexane partition had a lower cytotoxic effect against the normal cell line, HUVEC (Table 2). Therefore, we subjected the *n*-hexane partition to more fractionation by HPLC, and fraction B showed the highest cytotoxic activity. For structure elucidation of isolated compounds, different spectroscopy data were combined. From NMR spectra of both compounds, one spin system was recognized, assigned to a glycerol moiety [δ H 4.32 and 4.12 (δ C 62.7); δ H 5.17 (δ C70.2); δ H 3.89 and 3.63 (δ C 68.7)].

The cross-peaks in the HMBC spectrum [δ H / δ C: 5.17 (Hsn-2)/174.5, 175(COO); 4.32 and 4.12 (Hsn-1)/172.4, 172.6 (COO); 2.28 (α -CH₂)/ 172.4, 172.6 (COO)] indicated the presence of acyl groups on the sn-1 and sn-2 positions of the glycerol moiety. Therefore, the second spin system signals were attributed to two fatty acyl groups whose terminal methyl signals appeared overlapped at δ H 0.84 (6H, t, J = 6.8 Hz, δ C 14.5).

The relative small coupling constant value of the anomeric proton (H-1'''), J = 3.5 Hz, indicated the α orientation of the glycosidic union, while the large vicinal coupling constants observed between H-2'''/H-3''', H-3'''/H-4''' and H-4'''/H-5''' (J = 9.6 Hz), indicated the glucopyranosyl nature of the sugar moiety.

On the other hand, the ¹H and ¹³CNMR characteristic chemical shifts of the methylene protons, H-6''' (δ H 2.90 and 2.54) and carbon C-6''' (δ C 54.5) indicated the presence of a sulphonyl group attach at C-6''' carbon, of the sugar, instead of the hydroxyl group of glucose (13). The presence of a small triplet at δ H 5.31 (J = 4.7 Hz) due to olefinic protons in the ¹HNMR, spectrum suggested the presence of small amounts of another compound with an unsaturated fatty acid (Table 1).

All proton and carbon NMR data are in good agreement with the presence of 6-deoxy-6-

sulpho- α -D-glucopyranosyl-1,2-O-diacylglycerols. To identify the acyl substituents at sn-1 and sn-2 in the mixture, alkaline hydrolysis was performed. On the other hand, to infer the exact nature of the fatty acids in sn-2 position, the mixture was subjected to alkaline hydrolysis with NaOMe in MeOH. After partitioning, the organic extract was analyzed by GC-MS, the composition of the fatty acid methyl esters was shown to be methyl myristate, methyl oleate, and methyl palmitate, being the last in greater proportion.

Our findings showed that compound 1 as a SQDG has more cytotoxic effect than compound 2. SQDG is a group of sulfolipids which have been reported to have several biological activities including anticancer, antiviral, antimicrobial, anti-Alzheimer, antioxidant, and anti-inflammatory activities [14-16]. Different biological activities of SQDGs could be explained based on slight differences in the degree of sulfation and acetylation of these sulfolipids. The main mechanism that has been proposed for the anticancer activity of SQDGs is inhibition of eukaryotic DNA polymerase activity (17). Ohta *et al.* reported that KM043, a kind of SQDGs isolated from a marine red alga, exhibited a strong and dose-dependent inhibitory effect on DNA polymerase alpha (involved in DNA replication) and DNA polymerase beta (involved in DNA repair) and HIV-reverse transcriptase type 1 (17). The biological activity of SQDGs including the antiproliferative effect is mainly dependent on the chain size of fatty acids. However, the sulfonic acid head group in quinovose has also been considered to contribute to the suppression of cancer cell proliferation by the enzymes (18). Furthermore, Ohta *et al.* proposed the interaction of sulfolipids and oligosaccharides on the tumor cell surface plays an important role in their anticancer activity (19). Therefore, the anticancer activity of AQDGs might be also dependent on the type of cancer cells. In the future, it will be of interest to evaluate the antiproliferative effect of these two isolated SQDGs on different types of cancer cells. Further studies are still needed to understand the exact mechanism of SQDGs as an anticancer agent.

CONCLUSION

SQDG is an important type of glycosides which present in the seaweed collected from the Persian Gulf, and compounds of this type have shown the biological effects which make them suitable to be used in pharmaceutical and medical applications. Our bioassay-guided fractionation of *Gracilaria corticata* has led to the isolation of two known SQDG compounds which exhibited moderate cytotoxic activity. The cytotoxic activity of all fractions from the hexane partition of the extract was evaluated and showed that non-polar partitions are more potent than polar ones. Further studies are necessary to isolate the active compounds in hexane partition.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest in this study.

AUTHORS' CONTRIBUTION

All authors contributed equally to this work

REFERENCES

1. Yegdaneh A, Putschakan S, Yueyongsawad S, Ghannadi A, Plubrukarn A. 3-Oxoabolene and 1-oxocurcuphenol, aromatic bisabolanes from the sponge *Myrmekioderma* sp. *Nat Prod Commun.* 2013;8(10):1355-1357.
2. Blunt JW, Copp BR, Hu WP, Munro MH, Northcote PT, Prinsep MR. Marine natural products. *Nat Prod Rep.* 2014;31(2):160-258. DOI: 10.1039/c3np70117d.
3. Mtolera MS, Semesi AK. Antibacterial activity of extracts from six green algae from Tanzania. In: *Current trends in marine botanical research in the east african region.* Uppsala, Sweden: Gotab AB; 2000. pp. 211-217.
4. Ghannadi A, Plubrukarn A, Zandi K, Sartavi K, Yegdaneh A. Screening for antimalarial and acetylcholinesterase inhibitory activities of some Iranian seaweeds. *Res Pharm Sci.* 2013;8(2):113-118.
5. Mehdinezhad N, Ghannadi A, Yegdaneh A. Phytochemical and biological evaluation of some *Sargassum* species from Persian Gulf. *Res Pharm Sci.* 2016;11(3):243-249.
6. Ghannadi A, Shabani L, Yegdaneh A. Cytotoxic, antioxidant and phytochemical analysis of *Gracilaria* species from the Persian Gulf. *Adv Biomed Res.* 2016;5:139-147. DOI: 10.4103/2277-9175.187373.
7. Gerwick WH, Bernart MW. In: *Marine Biotechnology.* Attaway D, Zaborsky O. Plenum Press: New York. 1993;1:101-152.
8. Ireland C, Copp B, Foster M, McDonald L, Radisky D, Swersey J. In: *Marine Biology.* Attaway D, Zeborsky O. Plenum Press: New York. 1993;1:1-43.
9. Kind T, Meissen JK, Yang D, Nocito F, Vaniya A, Cheng YS, *et al.* Qualitative analysis of algal secretions with multiple mass spectrometric platforms. *J Chromatogr A.* 2012;1244:139-147. DOI: 10.1016/j.chroma.2012.04.074.
10. Yunoki K, Sato M, Seki K, Ohkubo T, Tanaka Y, Ohnishi M. Simultaneous quantification of plant glyceroglycolipids including sulfoquinovosyldiacylglycerol by HPLC-ELSD with binary gradient elution. *Lipids.* 2009;44(1):77-83. DOI: 10.1007/s11745-008-3248-4.
11. VanWagenen BC, Larsen R, Cardellina JH, Randazzo D, Lidert ZC, Swithenbank C. Ulosantoin, a potent insecticide from the sponge *Ulosa ruetzleri*. *J Org Chem.* 1993;58(2):335-337. DOI: 10.1021/jo00054a013.
12. Akbari V, Zafari S, Yegdaneh A. Anti-tuberculosis and cytotoxic evaluation of the seaweed *Sargassum boveanum*. *Res Pharm Sci.* 2018;13(1):30-37. DOI: 10.4103/1735-5362.220965.
13. Akasaka H, Mizushina Y, Yoshida K, Ejima Y, Mukumoto N, Wang T, *et al.* MGDG extracted from spinach enhances the cytotoxicity of radiation in pancreatic cancer cells. *Radiat Oncol.* 2016;11:153-163. DOI: 10.1186/s13014-016-0729-0.
14. El Baz F, El Baroty G, El Baky HA, El-Salam OA, Ibrahim E. Structural characterization and biological activity of sulfolipids from selected marine algae. *Grasas y Aceites.* 2013;64(5):561-571. DOI: 10.3989/gya.050213.
15. Hielscher-Michael S, Griehl C, Buchholz M, Demuth HU, Arnold N, Wessjohann LA. Natural products from microalgae with potential against Alzheimer's disease: sulfolipids are potent glutaminyl cyclase inhibitors. *Mar Drugs.* 2016;14(11):203-209. DOI: 10.3390/md14110203.
16. Yoon EY, Yang AR, Park J, Moon SJ, Jeong EJ, Rho JR. Characterization of a new trioxilin and a sulfoquinovosyl diacylglycerol with anti-inflammatory properties from the dinoflagellate *Oxyrrhis marina*. *Mar Drugs.* 2017;15(3):57-65. DOI: 10.3390/md15030057.
17. Ohta K, Mizushina Y, Hirata N, Takemura M, Sugawara F, Matsukage A, *et al.*

- Sulfoquinovosyldiacylglycerol, KM043, a new potent inhibitor of eukaryotic DNA polymerases and HIV-reverse transcriptase type 1 from a marine red alga, *Gigartina tenella*. Chem Pharm Bull (Tokyo). 1998;46(4):684-686.
DOI: 10.1248/cpb.46.684.
18. Mizushima Y, Kasai N, Iijima H, Sugawara F, Yoshida H, Sakaguchi K. Sulfo-quinovosyl-acylglycerol (SQAG), a eukaryotic DNA polymerase inhibitor and anti-cancer agent. Curr Med Chem Anticancer Agents. 2005;5(6):613-625.
DOI: 10.2174/156801105774574685.
19. Ohta K, Mizushima Y, Yamazaki T, Hanashima S, Sugawara F, Sakaguchi K. Specific interaction between an oligosaccharide on the tumor cell surface and the novel antitumor agents, sulfoquinovosylacylglycerols. Biochem Biophys Res Commun. 2001;288(4):893-900.
DOI: 10.1006/bbrc.2001.5852.