



Original Article

 Bioassay guided purification of cytotoxic natural products from a red alga *Dichotomaria obtusata*

 Amir Reza Jassbi^{a,*}, Younes Mirzaei^a, Omidreza Firuzi^a, Jima N. Chandran^b, Bernd Schneider^b
^a Medicinal and Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

^b Research Group Biosynthesis/NMR, Max Planck Institute for Chemical Ecology, Jena, Germany

ARTICLE INFO

Article history:

Received 21 January 2016

Accepted 7 June 2016

Available online 12 August 2016

Keywords:

Dichotomaria obtusata

Red alga

Trans-phytol palmitate

Tocopherol

Cytotoxic activity

ABSTRACT

Different solvent extracts of *Dichotomaria obtusata* (J. Ellis & Solander) Lamark, Galaxauraceae, a red algae collected from the coast of Bushehr in the Persian Gulf, was investigated for its cytotoxic properties and chemical constituents. The fresh alga, after extraction with methanol and dichloromethane were combined and partitioned between water, dichloromethane and ethyl acetate. The above fractions were then tested against MOLT-4 (human lymphoblastic leukemia) cancer cell line. The IC₅₀ values of the dichloromethane and ethyl acetate layers of the crude extract were 29.8 ± 3.1 and 30.6 ± 7.9 μg/ml against MOLT-4 cells, respectively, while the water layer showed a weak activity with IC₅₀ > 50 μg/ml. After fractionation of the active extracts using open column chromatography over silica gel and preparative thin layer chromatography purification, two terpenoid derived compounds, *trans*-phytol palmitate and γ-tocopherol were isolated from the dichloromethane and ethyl acetate extracts. The structures of the compounds were elucidated using different spectral data including ¹H NMR, ¹³C NMR, HSQC, HMBC and EI-MS. The IC₅₀ values of compounds *trans*-phytol palmitate, γ-tocopherol and an undetermined mixture of compounds (F-13-14) were determined as 43.4 ± 1.6, – and 20.3 ± 6.2 μg/ml against LS180 (human colon adenocarcinoma); 53.2 ± 9.3, >100 and 27.6 ± 6.9 μg/ml against MCF-7 (human breast adenocarcinoma) and 40.0 ± 4.1, 48.8 ± 1.8 and 15.9 ± 0.3 μg/ml against MOLT-4 cell lines, respectively, which were comparable to the IC₅₀ values of standard anticancer agent, cisplatin against the same cell lines. The red algae collected from the Persian Gulf contained substances that could inhibit the growth of human cancer cell lines and may represent a natural source for the discovery of novel anticancer agents.

© 2016 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

The northern coasts of the Persian Gulf and the Gulf of Oman in the Iranian territory are suited natural habitats for the growth of different types of marine organisms such as red, brown and green algae. *Dichotomaria obtusata* (J. Ellis & Solander) Lamark is a red alga from the family Galaxauraceae reported in different coastal area from Indian and Pacific oceans in Kenyan (Bolton et al., 2007) and Cuban coastal area (Vázquez et al., 2011; Delgado et al., 2013) and also from Gavater and Joud in the Gulf of Oman (Sohrabipour and Rabei, 2008). The red and brown algae are known as a source of biologically active natural products with cytotoxic, and antimicrobial activity against cancer cells, and pathogenic bacteria and fungi, respectively. For instance, different solvent extracts of *Hypnea flagelliformis*, *Cystoseira myrica* and *Sargassum*

boveanum showed antimicrobial activity against both gram positive and gram negative bacteria and also exhibited antioxidant potential in an 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay (Jassbi et al., 2013). The phytochemical analyses of the algal extracts resulted in identification and quantification of their fatty acids and steroids (Jassbi et al., 2013).

An aqueous extract of a fresh collection of *D. obtusata* has shown antineoplastic and anti-inflammatory activities in a mouse model of 12-*O*-tetradecanoylphorbol acetate-induced ear edema and acetic acid writhing in a dose-dependent manner (Vázquez et al., 2011). The presence of different kinds of natural products in the algal water extract, like terpenoids, coumarins, lactones, phenolics and polysaccharides has been explored by chemical tests (Vázquez et al., 2011). Furthermore, the analgesic effect of a methanolic extract of *D. obtusata* has also been examined in the croton oil-induced ear edema model and its phospholipase A2 inhibition has been studied (Delgado et al., 2013). The phytochemical analyses of different solvent extracts showed the presence of triterpenoids, flavonoids, phenolics, steroids and fats (Delgado et al.,

* Corresponding author.

E-mail: jassbiar@sums.ac.ir (A.R. Jassbi).

2013). However, to the best of our knowledge, no further phytochemical investigation has been performed on this alga species.

We report here the chemical constituents and cytotoxic activity of a DCM-EtOAc extract of *D. obtusata* and its chemical constituents collected from the coasts of Bushehr in the Northern parts of the Persian Gulf of Iran.

Materials and methods

Instruments and adsorbents

NMR spectra of the purified compounds were recorded on a Bruker Avance 500 spectrometer (Bruker Biospin, Karlsruhe, Germany), operating at resonance frequencies of 500.13 MHz for ^1H and 125.75 MHz for ^{13}C , respectively. The NMR spectrometer was equipped with a 5 mm TCI cryoprobe. Standard Bruker pulse sequences were used for measuring ^1H NMR, ^{13}C APT, ^1H - ^1H COSY, ^1H - ^{13}C HSQC and ^1H - ^{13}C HMBC spectra. Tetramethylsilane (TMS) was used as an internal standard for referencing ^1H and ^{13}C NMR spectra. NMR tubes (5 mm i.d. and 2 mm i.d.) were used for measurements. Data acquisition and processing was accomplished using Bruker Topspin 2.1. EI-MS was recorded on an Agilent 5975C inert GC/MS instrument. The chromatography separations were performed using open CC using silica gel 60 (0.2–0.04 mm particle size), FCC using silica gel 60 (0.040–0.063 mm particle size) and TLC using silica gel 60 F₂₅₄ pre-coated plates (0.25 mm film thickness). The adsorbents were purchased from Merck, Darmstadt, Germany.

Algal material and extraction procedure

All parts of *Dichotomaria obtusata* (J. Ellis & Solander) Lamarck, Galaxauraceae, were collected in April 2011 from the coastal area of Bushehr City at the coordinate (28° 58' 42" N; 50° 49' 53" E) in the west part of the coast of Bushehr in the Persian Gulf, Iran, and was identified by Dr. Jelveh Sohrabipour (taxonomist). A voucher specimen (PC-94-6-7-1.1) has been deposited at the herbarium of MNCRC. The fresh alga (9.5 kg) was macerated for two days in methanol (MeOH; 2 × 10 l) followed by the same volume of DCM successively at room temperature. The DCM and MeOH extracts were mixed and after removal of their solvents in vacuum subjected to liquid extraction to afford DCM (3.1 g), EtOAc (18.6 g) and water soluble (180 g) extracts.

Isolation and purification of the plant extracts

The bioactive DCM and EtOAc extracts (21.4 g) were pooled and subjected to column (60 × 3.5 cm) chromatography over silica gel (100 g, 0.2–0.04 mm). The column was eluted using *n*-hexane with gradient of DCM up to 100% and then followed by increasing the polarity of the mobile phase with MeOH to afford 27 fractions. The fractions eluted from the column were checked by TLC for their purity and similar fractions were pooled. The fractions were subjected to a cytotoxicity bioassay on MOLT-4 cell lines and the following fractions were found to be active (F7-F9 = 2.164 g), (F10-F12 = 10 g), (F13-F15 = 0.942 g) and (F16-F21 = 0.918 g). F-7-F9 was subjected to silica gel FCC using hexane-EtOAc as the mobile phase to afford 25 fractions. The pure compound (**1**) was obtained as a gummy material from fractions F7-12, 104 mg. The F13-15 was analyzed by the same FCC method as for F97-F9 and the purity of the two substances F13-13 (**2**, 52.8 mg) and F-13-14 (80 mg) were checked by silica gel TLC. Although F-13-14 detected as a single spot on silica gel TLC (CHCl₃:MeOH; 9:1), after sparing and subsequent heating at 110 °C, by 1% vanilline in H₂SO₄/ethanol reagent, but both ^{13}C and ^1H NMR spectral data (data are not shown) and

RP-18 TLC (MeOH:H₂O; 6:4) analyses showed that the mixture is consisted of at least four–five compounds.

Transesterification of compound 1 and GC-MS analyses of the resulting fatty acid methyl ester

Compound **1** (1 mg) was transesterified using 250 μl of a 10% BF₃ in MeOH as described previously (Jassbi et al., 2013). The GC-MS analyses of the resulting ester was performed on a 7890A GC coupled to HP-6890 mass spectrometer operating in EI mode at 70 eV. The column was a DB-5 MS (J&W Scientific column, 30 m × 0.25 mm i.d., 0.25 μm film thickness). One microliter of the fatty acid methyl esters in a hexane solution was analyzed in the same condition described previously; the oven temperature was set at 150 °C and after 4 min, rose to 250 °C at 4 °C/min and kept for 10 min at 250 °C (Jassbi et al., 2013).

Spectroscopic data

Trans-phytyl palmitate (codioester, **1**): EI-MS *m/z* (rel. int.%): 534 [C₃₆H₇₀O₂]⁺ (trace), 296 [M-palmitate]⁺ (1), 292 (3), 278 (6), 256 (45), 227 (5), 213 (18), 185 (11), 157 (10), 123 (32), 111 (20), 97 (31), 71 (100), 55 (54). ^1H NMR (500 MHz, CDCl₃): δ 5.40 (1H, *t*, *J* = 7.1 Hz, H-2), 4.16 (2H, *d*, *J* = 7.0 Hz, H-1,1'), 2.33 (2H, *t*, *J* = 7.6 Hz, H-2'), 1.2–1.65 (20 H, *m*, CH₂), 1.66 (3H, *s*, H-3a), 0.88 (3H, *t*, *J* = 7.0 Hz, H-16'), 0.84 (12 H, *d*, *J* = 6.5 Hz, H-7a, 11a, 15a, 16). ^{13}C NMR (125 MHz, CDCl₃): δ 179.9 (C-1'), 140.4 (C-3), 122.9 (C-2), 59.4 (C-1), 39.88 (C-4), 39.4 (C-14), 37.45 (C-10), 37.37 (C-8), 37.3 (C-12), 36.75 (C-6), 34.12 (C-2'), 32.81 (C-11), 32.73 (C-7), 29.78–29.02 (C-3'-13'), 28.01 (C-15), 24.82 (C-13), 25.1 (C-5), 24.51 (C-9), 22.72 (C-15a), 22.68 (C-16), 19.71 (C-11a), 19.76 (C-7a), 16.2 (C-3a), 14.14 (C-16').

γ-Tocopherol (**2**): EI-MS *m/z* (rel. int.): 416 [M]⁺ (100), 191 [C₁₂H₁₅O₂]⁺ (20), and 151 [C₉H₁₁O₂]⁺ (85). ^1H NMR (500 MHz, CDCl₃): δ 6.94 (1H, *s*, H-5), 2.50 (2H, *m*, H-4), 2.09 (*s*, C7-CH₃), 2.00 (*s*, C8-CH₃), 1.58 (2H, *m*, H-3), 1.54 (*m*, H-12'), 1.50 (*m*, H-1'), 1.4 (*m*, 4H, H-9', 8', 7', 3'), 1.33 (*m*, H-2'), 1.27 (*m*, H-10'), 1.22 (*m*, H-6'), 1.15 (*m*, H-11'), 1.08 (2H, *m*, H-4', 5'), 1.07 (*s*, C-2-CH₃), 0.88 (*d*, *J* = 6.6 Hz, H-13', C12'-CH₃), 0.86 (*d*, *J* = 6.6 Hz, C-8'-CH₃), 0.85 (*d*, *J* = 6.6 Hz, C-4'-CH₃) (Baker and Myers, 1991). ^{13}C NMR (125 MHz, CDCl₃): δ 149.3 (C-9), 142.3 (C-6), 128.8 (C-7), 125.3 (C-8), 119.7 (C-5), 118.3 (C-10), 75.8 (C-2), 40.9 (C-1'), 39.4 (C-11'), 37.59 (C-3'), 37.6 (C-7'), 37.5 (C-5'), 37.3 (C-9'), 32.8 (C-4'), 32.8 (C-8'), 31.1 (C-3), 28.0 (C-12'), 24.8 (C-10'), 24.5 (C-6'), 23.5 (C2-CH₃), 22.7 (C12'-CH₃), 22.6 (C-13'), 21.9 (C-4), 21.0 (C-2'), 19.7 (C8'-CH₃), 19.6 (C4'-CH₃), 13.0 (C7-CH₃), 11.9 (C8-CH₃) (Baker and Myers, 1991).

Statistical analyses

The IC₅₀ values in Table 1 are the average of 3–5 replicates. One-way analyses of variance (ANOVA) and post hoc multiple comparison tests were used for determination of signification $p \leq 0.05$ between different measurements using SPSS (version 11.5 for windows) software.

Cytotoxicity assay

Cell lines

LS180 (human colon adenocarcinoma), MCF-7 (human breast adenocarcinoma) and MOLT-4 cells (human acute lymphoblastic leukemia) cells were obtained from the National Cell Bank of Iran, Pasteur Institute, Tehran, Iran. LS180 and MCF-7 cells were grown in monolayer cultures, while MOLT-4 cells were grown in suspension. Cell lines were maintained at 37 °C in humidified air containing 5% CO₂ and were grown in RPMI 1640 supplemented with 10% FBS,

Table 1

Cytotoxic activity of the algal extracts, fractions from column chromatography and the isolated pure compounds against selected human cancer cell lines.

Algal extracts/fractions/pure compounds	IC ₅₀ LS180 ^A	IC ₅₀ MCF-7	IC ₅₀ MOLT-4
Aqueous fraction	– ^B	–	>50 ^A
Ethyl acetate fraction	–	–	30.6 ± 7.9bc ^C
Dichloromethane fraction	–	–	29.8 ± 3.1bc
F7-9	–	–	21.8 ± 2.2cd
F10-12	–	–	25.1 ± 3.4c
F13-15	–	–	27.1 ± 4.8c
F16-21	–	–	28.4 ± 4.1c
F7-12 (1)	43.4 ± 1.6a	53.2 ± 9.3a	40.0 ± 4.1b
F13-13 (2)	–	>100	48.8 ± 1.8a
F13-14	20.3 ± 6.2b	27.6 ± 6.9b	15.9 ± 0.3d
Doxorubicin	0.017 ± 0.006c	0.036 ± 0.004c	0.013 ± 0.002e
Cisplatin	1.7 ± 0.7c	2.4 ± 0.8c	0.7 ± 0.4e

^A IC₅₀ values are expressed as µg/ml and are the mean ± S.D. of 3–4 experiments.^B Not determined.^C Values that do not share any letter have a statistically significant difference at $p < 0.05$.

100 units/ml penicillin-G and 100 µg/ml streptomycin. The passage number after thawing cells was kept below twelve.

Cell viability following exposure to plant extracts was measured by the MTT reduction assay (Firuzi et al., 2010; Mosmann, 1983). Briefly, 5000 cells were seeded in 96-well microplates and after overnight incubation, 3–4 different concentrations of the extracts or pure compounds (5, 10, 25 and 50 µg/ml) were added to the wells in duplicate. Extracts or isolated compounds were first dissolved in dimethyl sulfoxide (DMSO) and then diluted in the growth medium at the desired concentration. DMSO concentration in the wells was kept under 0.25%. Cells were further incubated for 72 h and their growth medium was replaced with fresh medium containing 0.5 mg/ml MTT and incubated for 4 h at 37 °C. The media was removed and the formazan crystals were dissolved by adding 200 µl DMSO to each well. The absorbance was measured at 570 nm with background correction at 655 nm using a Bio-Rad microplate reader (Model 680). For MOLT-4 cells that grow in suspension, the plates were centrifuged at 500 × g for 10 min before each step that total or partial removal of the media was necessary. The percent inhibition of viability for each concentration of the extract or pure compound was calculated with reference to the untreated cells and IC₅₀ values were calculated with the software Curve Expert version 1.34 for Windows (Fig. 1). Each experiment was repeated 3–4 times. Known cytotoxic agents, doxorubicin and cisplatin were tested as positive controls.

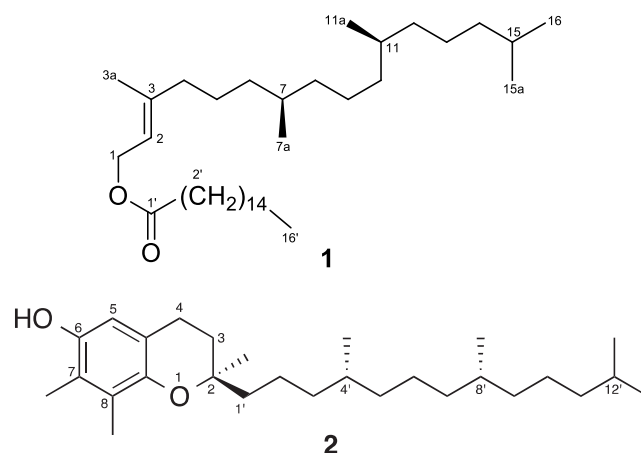
Result and discussion

The cytotoxic activity of EtOAc, water and DCM soluble parts of the DCM-MeOH extract of *D. obtusata* were assayed against MOLT-4 cell line in MTT colorimetric assay. The EtOAc and DCM extracts could effectively lower the viability of cancer cells with IC₅₀s of 30.6 ± 7.9 and 29.8 ± 3.1 (mean ± S.D.), respectively and were more effective than the aqueous phase (IC₅₀ > 50 µg/ml) (Table 1). Therefore, we combined both DCM and EtOAc extracts and subjected them to different chromatography techniques to separate the bioactive constituents of the extracts in bioassay guided purification (Table 1).

Trans-phytyl-1-palmitate (Codioester) (1) and γ -tocopherol (2) and fraction F-13-14 exhibited cytotoxic activities against three cancer cell lines LS180 (human colon adenocarcinoma), MCF-7 (human breast adenocarcinoma) and MOLT-4 cells (human acute lymphoblastic leukemia) with IC₅₀s ranging from 15.9 to more than 100 µg/ml (Table 1). All of 2 compounds and F-13-14 showed activities that were significantly lower than standard cytotoxic agents, doxorubicin and cisplatin.

Compounds 1 and 2 were purified using silica gel CC, flash column chromatography (FCC) and preparative TLC. The chemical

structures of the compounds were elucidated using different spectral data including 1D and 2D NMR and EI-MS and comparing their spectral data with those described in the literature (Ali et al., 2001; Baker and Myers, 1991).



Briefly, compound 1 (104 mg) was purified as a white precipitate and then subjected to EIMS exhibiting ions at m/z 296 ($M^+ - C_{16}H_{32}O_2$), 278, 71 (100%) and m/z 256 suggesting the terpene fragments ($C_{20}H_{38}$), (C_5H_{11}) and the acyl moiety ($C_{16}H_{32}O_2$) of the molecule. However the molecular ion $[C_{36}H_{70}O_2]^+$ at m/z 534 in the spectrum was detected as trace peak due to easy loss of the ester fragment from the molecule. In the ¹H NMR spectrum of the compound 1, a group of multiplets at δ 0.84–0.88 represented 15 hydrogen atoms of four isoprenoid methyl groups and one terminal methyl group of the long chain acyl group. A singlet at δ 1.66 for the olefinic methyl group, a triplet of H-2 at δ 5.41 ($J = 7.1$ Hz) and a doublet at δ 4.16 ($J = 7.0$ Hz) of the phytol hydroxymethylene part of molecule completed the assignments. The configuration at the phytol double bond was determined using the attached proton test (APT) ¹³C NMR spectrum by observing signals at δ 59.4 (C-1), 122.9 (C-2) and 140.4 (C-3) recorded previously for *trans*-phytyl palmitate (Codioester) isolated from a green algae *Codium iyengarii* (Ali et al., 2001). Compound 1 was transesterified to its methyl ester using 10% BF₃ in methanol as described previously (Jassbi et al., 2013). The GC-MS analysis of the resulting fatty acid methyl ester of the compound resulted in detection of methyl palmitate.

Compound (2) has shown molecular ion at EIMS m/z 416 [M^+], 191 [$C_{12}H_{15}O_2]^+$], and 151 [$C_9H_{11}O_2]^+$]. Analysis of the ¹H NMR spectrum exhibited a singlet at δ 6.94 (1H), 2.1 (3H) and 2.0 (3H) for an aromatic proton and two methyls connected to a phenyl ring, respectively. The signals at δ 0.88 (d, 6H) and 0.86 (d, 6H) and a

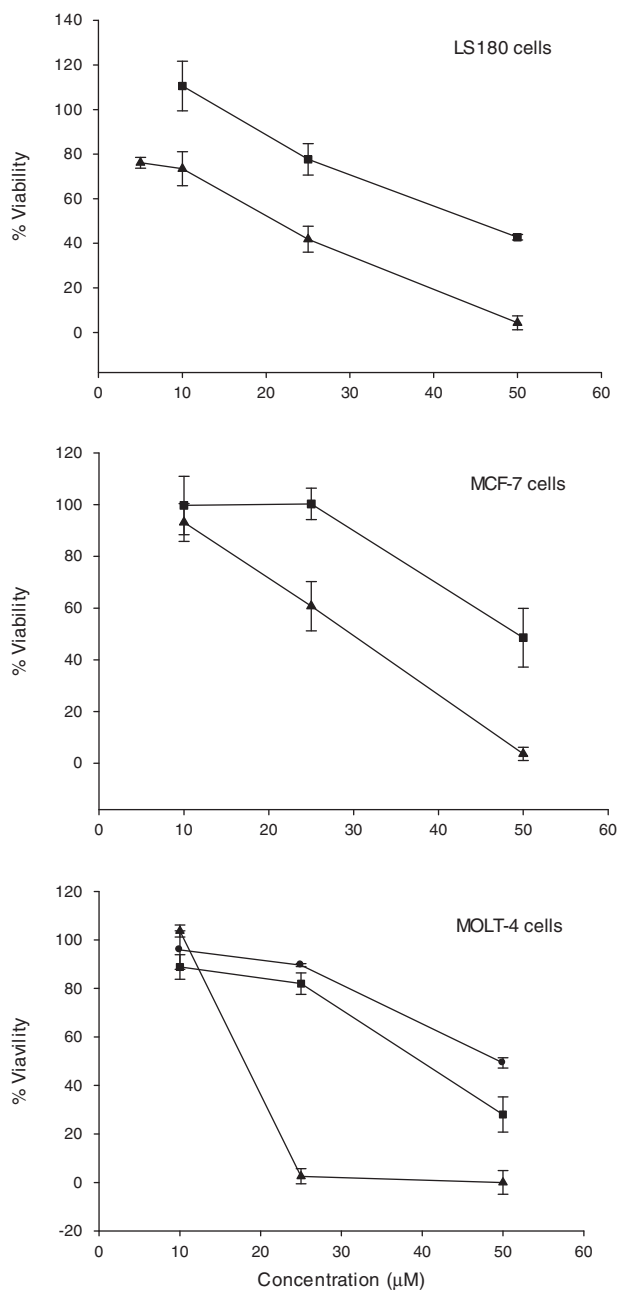


Fig. 1. Assessment of cytotoxicity of compounds **1**, **3** and F-13-14 against human cancer cell lines. Cytotoxic activity of the isolated compounds including compound **1** (■), compound **2** (●) and F-13-14 (▲) were tested at different concentrations against LS180, MCF-7 and MOLT-4 cancer cell lines by MTT reduction assay.

singlet at 1.13 (3H) suggested a saturated acyclic diterpene moiety in the molecule. The APT ^{13}C NMR spectrum was more informative with representing 29 signals consisting of five quaternary carbons, four methynes, fourteen methylene and six methyl signals. The quaternary carbons at δ 149.3, 142.3, 128.6, 125.3, 118.3 and a methyne at δ 119.7 suggested a five carbon substituted phenyl group in the molecule. A downfield signal at δ 75.8 together with its HMBC correlation with the methyl signal at δ 1.13 and two methylene signals at δ 21.9 and 31.18 were a part of a chromane system. An HMBC correlation between the H-5 and C-4 confirmed the structure of the compound **2** as gamma isomer of tocopherol (Fig. 2). The above mentioned spectral data were compatible with the γ -tocopherol (γT) structure biosynthesized by different algae (Baker and Myers, 1991). However there were differences between

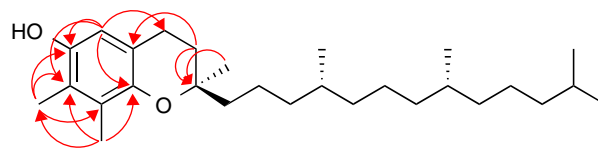


Fig. 2. The HMBC correlations (500 MHz, CDCl_3) for assignment of the substitution and chemical shifts of ^1H and ^{13}C NMR signals of γ -tocopherol (**2**).

chemical shifts of the aromatic ring of compound **2** with those recorded for d - γT (Baker and Myers, 1991).

Several biological activities including antioxidant, anticancer and anti-inflammatory activities have been reported for vitamin E including αT , βT , γT , δT and also their metabolic products such as tocopheryl quinines (Hensley et al., 2004). Our results that show the abundant presence of γT in *D. obtusata* may explain the anti-inflammatory and antinociceptive activity of the methanol and aqueous extracts of this alga previously reported for a collection from Cuban coasts (García Delgado et al., 2013; Vázquez et al., 2011).

In a recent study, phytol alcohol has shown anticancer activity in hepatocellular carcinoma cells (Kim et al., 2015). Phytol's cytotoxicity has also been reported against several other cancer cell lines (Pejin et al., 2014). This may suggest that the cytotoxic activity of compound **1** is mainly due to the presence of its diterpenoid part rather than its fatty acid moiety. However, to the best of our knowledge, this is the first report on the cytotoxic activity of a phytol ester against cancer cells.

Conclusion

The red algae collected from the Persian Gulf contained compounds **1** and **2** and other unidentified substances (F-13-14) that could inhibit the growth of human cancer cell lines and may represent a natural source for the discovery of novel anticancer agents. Our primary cytotoxic bioassays showed that we still need to characterize the structures of the phytochemicals of other active fractions (F-13-14) in the future.

Authors' contribution

ARJ: supervising the research, analyzing data and determining the structures of natural products, writing the paper, YM: chemical analyzing of the algal material, OF: performing biological activity, preparation of the manuscript; JNC: performing NMR spectroscopy and helping in structural elucidation of natural products, BS: helping in structural elucidation of natural products and editing the manuscript.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

We would like to express our acknowledgment to Iran National Science Foundation (Research Grant No. 88000545) and Vice Chancellor for Research, Shiraz University of Medical Sciences for the financial support of this project. The authors also wish to thank Professor Iraj Nabipour and Dr. Jelveh Sohrabipour for collection and identification of the algal material respectively.

References

- Ali, M.S., Saleem, M., Ahmad, V.U., Shameel, S., 2001. Phytol and glycerol derivatives from the marine green alga *Codium iyengarii* of the Karachi Coast (Arabian Sea). *Z. Naturforsch. B* 56, 837–841.
- Baker, J., Myers, C., 1991. One-dimensional and two-dimensional ¹H- and ¹³C-nuclear magnetic resonance (NMR) analysis of vitamin E raw materials or analytical reference standards. *Pharmacol. Res.* 8, 763–770.
- Bolton, J.J., Oyieke, H.A., Gwada, P., 2007. The seaweeds of Kenya: checklist, history of seaweed study, coastal environment, and analysis of seaweed diversity and biogeography. *S. Afr. J. Bot.* 73, 76–88.
- Delgado, N.G., Frías Vázquez, A.I., Sánchez, H.C., del Valle, R.M.S., Gómez, Y.S., Suárez Alfonso, A.M., 2013. Anti-inflammatory and antinociceptive activities of methanolic extract from red seaweed *Dichotomaria obtusata*. *Braz. J. Pharm. Sci.* 49, 65–74.
- Firuzi, O., Asadollahi, M., Gholami, M., Javidnia, K., 2010. Composition and biological activities of essential oils from four *Heracleum* species. *Food Chem.* 122, 117–122.
- García Delgado, N., Frías Vázquez, A.I., Cabrera Sánchez, H., del Valle, S., Menéndez, R., Sierra Gómez, Y., Alfonso, S., María, A., 2013. Anti-inflammatory and antinociceptive activities of methanolic extract from red seaweed *Dichotomaria obtusata*. *Braz. J. Pharm. Sci.* 49, 65–74.
- Hensley, K., Benaksas, E.J., Bolli, R., Comp, P., Grammas, P., Hamdheydari, L., Mou, S., Pye, Q.N., Stoddard, M.F., Wallis, G., 2004. New perspectives on vitamin E: γ -tocopherol and carboxyethylhydroxychroman metabolites in biology and medicine. *Free Radic. Biol. Med.* 36, 1–15.
- Jassbi, A.R., Mohabati, M., Eslami, S., Sohrabipour, J., Miri, R., 2013. Biological activity and chemical constituents of red and brown algae from the Persian Gulf. *Iran J. Pharm. Res.* 12, 339–348.
- Kim, C.W., Lee, H.J., Jung, J.H., Kim, Y.H., Jung, D.B., Sohn, E.J., Lee, J.H., Woo, H.J., Baek, N.I., Kim, Y.C., Kim, S.H., 2015. Activation of caspase-9/3 and inhibition of epithelial mesenchymal transition are critically involved in antitumor effect of phytol in hepatocellular carcinoma cells. *Phytother. Res.* 29, 1026–1031.
- Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65, 55–63.
- Pejin, B., Kojic, V., Bogdanovic, G., 2014. An insight into the cytotoxic activity of phytol at in vitro conditions. *Nat. Prod. Res.* 28, 2053–2056.
- Sohrabipour, J., Rabei, R., 2008. Rhodophyta of Oman Gulf (South East of Iran). *Iran J. Bot.* 14, 70–74.
- Vázquez, A.I.F., Sánchez, C.M.D., Delgado, N.G., Alfonso, A.M.S., Ortega, Y.S., Sánchez, H.C., 2011. Anti-inflammatory and analgesic activities of red seaweed *Dichotomaria obtusata*. *Braz. J. Pharm. Sci.* 47, 111–118.