

# Antioxidant, cytotoxic, antitumor, and protective DNA damage metabolites from the red sea brown alga *Sargassum* sp

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## ABSTRACT

**Background:** Macroalgae can be viewed as a potential antioxidant and anti-inflammatory sources owing to their capability of producing compounds for its protection from environmental factors such as heat, pollution, stress, oxygen concentration, and UV radiations. **Objective:** To isolate major compounds which are mainly responsible for the pharmacological activity of brown alga under investigation, *Sargassum* sp. **Materials and Methods:** Algal material was air dried, extracted with a mixture of organic solvents, and fractionated with different adsorbents. The structures of obtained pure compounds were elucidated with different spectroscopic techniques, and two pure materials were tested for protection of DNA from damage, antioxidant, antitumor, and cytotoxicity. **Results:** Four pure compounds were obtained, of which fucosterol (1) and fucoxanthin (4) were tested; it was found that fucoxanthin has strong antioxidant and cytotoxicity against breast cancer (MCF-7) with  $IC_{50} = 11.5 \mu\text{g/ml}$ . **Conclusion:** The naturally highly conjugated safe compound fucoxanthin could be used as antioxidant and as an antitumor compound.

**Key words:** Antioxidant, cytotoxicity, fucoxanthin, *Sargassum* sp

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## INTRODUCTION

The genus *Sargassum* belongs to brown algae of class Phaeophyceae in the order Fucales. Numerous species are distributed throughout the tropical and subtropical oceans of the world, where they generally inhabit shallow water and coral reefs. However, the genus may be well known for its planktonic (free-floating) species.<sup>[1]</sup> It was clear from the literatures that more than 62 species of the genus *Sargassum* has been investigated,<sup>[2]</sup> and indicated its productivity with impressive diversity of natural compounds. For instances, the secondary metabolites contain different structural classes such as plastoquinones,<sup>[3-5]</sup> chromanols,<sup>[6]</sup> chromenes,<sup>[7,8]</sup> steroids,<sup>[9]</sup> and glycerides.<sup>[10]</sup> The publications showed that, *Sargassum* metabolites have wide range of biological activities, which include antibiotic, anti-HIV, anticoagulant, anticonvulsant, anti-inflammatory, antineoplastic, and antitumor.<sup>[11-14]</sup>

In continuation of our search program, which interested in the isolation of secondary metabolites from marine sources, especially macroalgae collected from Red Sea,<sup>[15-17]</sup> a marine macroalgae, identified as *Sargassum* sp., collected from El- Shuaiba lagoon 80 km south of Jeddah, was investigated. The total extract (Pet. ether: Chloroform: Methanol [1 : 1 : 1]) had been fractionated using different chromatographic techniques and afforded four metabolites; fucosterol (1), saringosterone (2), saringosterol (3), and fucoxanthin (4). **1** and **4** had been tested toward the Bleomycin-dependent DNA damage, cytotoxicity against HepG2 (human hepatocellular liver carcinoma cell line), WI 38 (Skin carcinoma cell line), Vero (cell line was initiated from the kidney of a normal adult African green monkey), MCF-7 (breast cancer cell lines), antitumor, and antioxidant using 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS).

## MATERIALS AND METHODS

### Apparatus and material

Chromatographic material: Aluminum oxide type 60-120 mesh was used for column chromatography. Thin-layer

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chromatography (TLC) silica gel GF 254 was used for TLC. Preparative thin-layer chromatography (PTLC) was performed on aluminum oxide plates (20 x 20 cm) of 250- $\mu$ m thickness. Electron impact mass spectra were determined at 70 eV on a Kratos MS-25 instrument. 1D and 2D NMR (Nuclear Magnetic Resonance) spectra were recorded on Bruker AVANCE III WM 600 MHz spectrometers and  $^{13}$ C NMR at 150 MHz. Chemical shifts are given in  $\delta$  (ppm) relative to TMS (Tetramethyl silane) as internal standard. The Infra Red (IR) spectra were recorded on a Perkin Elmer spectrometer model 100. The spray reagent used is 50%-sulfuric acid in methanol as spraying reagent. The chromatoplate was heated after spraying at 100 to 105°C until the spots attained maximum color intensity. The alga was described as *Sargassum* (Family *Sargassaceae*) and was collected by hand from El-Shuaiba lagoon 80 km south of Jeddah, Saudi Arabia, in the Red Sea, in June 2010.

### Extraction of *Sargassum* sp.

The air-dried algal material (350 g) was extracted by equal volume of mixture of Pet. ether, chloroform, and methanol (2 x 6 l, 24 hours for each batch) at room temperature. The extract was concentrated under reduced pressure to obtain 10 g residue. This material was chromatographed on a column of Silica gel.

### Column chromatography

Silica gel column (500 g, 80 x 2.5 cm) was used for resolution. The residue (10 g) was homogenized with small amount of silica gel (50 g) and poured on to the top of the column which was packed in Pet. ether (40: 60). The eluent were used successively (Pet. ether- Ether, Pet. ether- Ethyl acetate). Fractions were collected (50 ml); Pet. ether- Ether mixture of increasing polarity by diethyl ether and then ethyl acetate and followed by TLC using silica-gel chromatoplates, appropriate solvent system and 50% sulfuric acid in methanol as spraying reagent. If the material was not pure, preparative TLC was applied using the appropriate solvent system and the adsorbent aluminum oxide for purification.

### Isolated compounds

The fraction A eluted by Pet. ether-Ether (6 : 4) was collected and rechromatographed over PTLC of silica gel using Pet. Ether-ethyl acetate (8 : 2) to give three compounds: **1**, **2**, and **3**. The fraction B eluted by Pet. ether-Ethyl acetate (6 : 4) was collected and rechromatographed on Sephadex LH-20 using a mixture of MeOH-CHCl<sub>3</sub> (9 : 1) and then finally purified by preparative TLC of silica gel using chloroform methanol (9.5 : 0.5) to afford a pure compound **4**.

Fucosterol (**1**), (24*E*)-Stigmasta-5, 24(28)-diene-3 $\beta$ -ol: white solid (30 mg, 0.008% dry wt) m.p. 133-135°C. IR

(cm<sup>-1</sup>): 3480 (OH), 1945 (C = C-H), 1640 (C = C), 1370 (gem. Di-Me); EI-MS *m/z*: 412 [M, C<sub>29</sub>H<sub>48</sub>O]<sup>+</sup>, 397 [M-CH<sub>3</sub>]<sup>+</sup>, 379 [M-CH<sub>3</sub>-H<sub>2</sub>O]<sup>+</sup>, 314 (100) [M-C<sub>6</sub>H<sub>10</sub>O]<sup>+</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  (ppm) 0.86-0.88 (d, *J* = 6.6 Hz, 6H, Me-26 and Me-27), 0.91 (d, *J* = 6.6 Hz, 3H, Me-21), 0.68 (s, 3H, Me-18), 1.00 (s, 3H, Me-19), 1.59 (d, *J* = 6.6 Hz, 3H, Me-29), 3.53 (m, H, H-3), 5.35 (m, H, H-6), 5.11 (q, H, H-28), 2.22 (sep, *J* = 6.6 Hz, H, H-25). <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  (ppm) (39.75, C-1), (35.20, C-2), (71.80, C-3), (42.33, C-4), (140.74, C-5), (121.70, C-6), (36.14, C-7), (35.78, C-8), (50.10, C-9), (39.72, C-10), (28.23, C-11), (42.28, C-12), (42.30, C-13), (56.74, C-14), (31.64, C-15), (34.78, C-16), (56.72, C-17), (11.85, C-18), (19.40, C-19), (39.50, C-20), (24.32, C-21), (37.23, C-22), (31.90, C-23), (146.00, C-24), (33.90, C-25), (22.50, C-26), (22.23, C-27), (115.94, C-28), (18.90, C-29).

Saringosterone (**2**), 24-vinyl cholest -4-ene -3-one: a colorless oil (10 mg, 0.003% dry wt). IR (cm<sup>-1</sup>): 3420 (OH), 1675 (C = O), 1630 (C = C); EIMS *m/z* (rel. int.): 426 (12) [M, C<sub>29</sub>H<sub>46</sub>O<sub>2</sub>]<sup>+</sup>, 383 (21) [M<sup>+</sup>-C<sub>3</sub>H<sub>7</sub>], 313 (30), 271(35), 269 (100). HREIMS: *m/z* 426.3413 (calcd. 426.3349) C<sub>29</sub>H<sub>46</sub>O<sub>2</sub>; <sup>1</sup>H-NMR (CDCl<sub>3</sub>)  $\delta$  5.76 (dd, *J* = 17, 12 Hz, H, H-28), 5.73 (br s, H, H-4), 5.27 (d, *J* = 12 Hz, H, H-29), 5.15 (d, *J* = 17 Hz, H, H-29), 1.16 (s, 3H, Me-19), 0.95 (d, *J* = 6.5 Hz, 3H, Me-21), 0.86 (d, *J* = 7.0 Hz, 3H, Me-26), 0.84 (d, *J* = 7.0 Hz, 3H, Me-27), 0.72 (s, 3H, Me-18). <sup>13</sup>C-NMR  $\delta$  (36.54, C-1), (34.65, C-2), (200.35, C-3), (124.43, C-4), (172.35, C-5), (33.64, C-6), (32.65, C-7), (36.32, C-8), (54.41, C-9), (39.25, C-10), (21.65, C-11), (40.22, C-12), (43.08, C-13), (56.26, C-14), (24.85, C-15), (28.86, C-16), (56.55, C-17), (12.63, C-18), (17.34, C-19), (36.84, C-20), (18.35, C-21), (32.54, C-22), (28.95, C-23), (89.76, C-24), (29.05, C-25), (19.46, C-26), (18.07, C-27), (137.78, C-28), (117.12, C-29).

Saringosterol (**3**), 24-vinyl cholest-5-ene-3 $\beta$ , 24-diol, saringosterol: colorless oil (5 mg, 0.001% dry wt.). IR (cm<sup>-1</sup>): 3445 (OH), 1644 (C=C); EIMS *m/z* (rel. int.): 428 (12) [M, C<sub>29</sub>H<sub>48</sub>O<sub>2</sub>]<sup>+</sup>, 410 (6) [M<sup>+</sup>-H<sub>2</sub>O], 314 (40), 273(20), 271 (100), 255 (28), 228 (22), 213 (40), 145 (64). <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  5.73 (dd, *J*=17, 12 Hz, H, H-28), 5.34 (br s, H, H-6), 5.28 (d, *J* = 12 Hz, H, H-29), 5.17 (d, *J* = 17 Hz, H, H-29), 3.53 (m, H, H-3), 1.02 (s, 3H, Me-19), 0.97 (d, *J* = 6.6 Hz, 3H, Me-21), 0.88 (d, *J* = 6.6 Hz, 3H, Me-26), 0.86 (d, *J* = 6.6 Hz, 3H, Me-27), 0.67 (s, 3H, Me-18). <sup>13</sup>C-NMR  $\delta$  (37.17, C-1), (32.54, C-2), (72.43, C-3), (37.94, C-4), (141.43, C-5), (122.31, C-6), (32.35, C-7), (36.84, C-8), (50.74, C-9), (37.93, C-10), (21.75, C-11), (40.41, C-12), (42.97, C-13), (57.43, C-14), (24.95, C-15), (29.06, C-16), (56.55, C-17), (12.54, C-18), (17.32, C-19), (36.54, C-20), (18.35, C-21), (32.33, C-22), (28.95, C-23), (89.85, C-24), (29.19, C-25), (20.06, C-26), (19.53, C-27), (137.82, C-28), (117.04, C-29).

Fucoxanthin (**4**): red residue (60 mg, 0.017% dry wt.). IR (cm<sup>-1</sup>): 3439 (OH), 2361 and 2332 (sp-hybrid carbon

[allenic]), 1721 and 1253 (ester), and 1654 and 1605 (polyene); HRFAB: 658.4224 (calcd. 658.4233) [ $C_{42}H_{58}O_6$ ], 460(5), 391(5), 307(50), 154(100),  $^1H$ -NMR ( $CDCl_3$ )  $\delta$  7.15(d,  $J = 11.0$  Hz, H, H-10), 6.75 (m, H, H-15), 6.66 (d,  $J = 15.0$  Hz, H, H-12), 6.63 (m, H, H-15'), 6.60 (m, H, H-11'), 6.57 (m, H, H-11), 6.41 (d,  $J = 11.5$  Hz, H, H-14), 6.35 (d,  $J = 15.0$  Hz, H, H-12'), 6.27 (d,  $J = 11.5$  Hz, H, H-14'), 6.13 (d,  $J = 11.0$  Hz, H, H-10'), 6.05(br s, H, H-8'), 5.38 (m, H, H-3'), 3.81(m, H, H-3), 3.65 and 2.60 (qAB,  $J = 18.5$  Hz, 2H, H-7), 2.33 and 1.78 (m, 2H, H-4), 2.27 and 1.51 (m, 2H, H-4'), 2.04 (s, 3H, Ac), 2.01 and 1.41(m, 2H, H-2'), 1.99 (s, 6H, H-20, H-20'), 1.95 (s, 3H, H-19), 1.81 (s, 3H, H-19'), 1.47 and 1.37 (m, 2H, H-2), 1.38 (3H, s, H-18'), 1.35 (s, 3H, H-17'), 1.22(s, 3H, H-18), 1.07 (s, 3H, H-16'), 1.03 (s, 3H, H-17), 0.96 (s, 3H, H-16).  $^{13}C$ -NMR ( $CDCl_3$ )  $\delta$  (35.18, C-1), (47.11, C-2), (64.34, C-3), (41.68, C-4), (66.19, C-5), (67.11, C-6), (40.83, C-7), (197.87, C-8), (134.54, C-9), (139.14, C-10), (123.39, C-11), (145.05, C-12), (136.67, C-13), (135.44, C-14), (132.19, C-15), (28.16, C-16), (25.06, C-17), (21.46, C-18), (11.85, C-19), (12.80, C-20), (35.79, C-1'), (45.45, C-2'), (68.03, C-3'), (45.25, C-4'), (72.71, C-5'), (117.52, C-6'), 202.36, C-7'), (103.39, C-8'), (132.19, C-9'), (128.54, C-10'), (125.71, C-11'), (138.09, C-12'), (137.11, C-13'), (132.52, C-14'), (129.44, C-15'), (31.31, C-16'), (32.14, C-17'), (29.22, C-18'), (14.04, C-19'), (12.94, C-20'), (170.45, Ac-1), (21.18, Ac-2).

### Biological evaluation of the isolated compounds

DNA (Calf Thymus type1), bleomycin sulfate, butylated hydroxyanisole, thiobarbituric acid (TBA), ethylenediaminetetraacetic acid (EDTA), and ascorbic acid were obtained from sigma. 2,2'-azo-bis-(2-amidinopropane) dihydrochloride and ABTS were purchased from Wako Co., USA.

### Cell viability and Bleomycin-dependent DNA damage assay in negative control (Fucoxanthin on MCF-7)

The reaction mixture contained DNA (0.5 mg/ml), bleomycin sulfate (0.05 mg/ml),  $MgCl_2$  (5 mM),  $FeCl_3$  (50  $\mu$ M), and samples to be tested in a conc. of 0.1 mg/ml. L-ascorbic acid was used as positive control. The mixture was incubated at 37°C for 1 hour. The reaction was terminated by addition of 0.05 ml EDTA (0.1 M). The color was developed by adding 0.5 ml TBA (1% w/v) and 0.5 ml HCl (25% v/v), followed by heating at 80°C for 10 minutes. After centrifugation, the extent of DNA damage was measured by increase in absorbance at 532 nm.<sup>[18,19]</sup> The viability of the cells was determined by the microscopical examination<sup>[20]</sup> using a hemocytometer and using trypan blue stain (stains only the dead cells).

### Antioxidant activity screening assay 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid method

For each of the investigated compounds, 2 ml of ABTS

solution (60  $\mu$ M) was added to 3 ml  $MnO_2$  solution (25 mg/ml), all prepared in 5 ml aqueous phosphate buffer solution (pH, 7; 0.1 M). The mixture was shaken, centrifuged, filtered, and the absorbance of the resulting green-blue solution (ABTS radical solution) at  $\lambda$  734 nm was adjusted to approximately ca. 0.5. Then, 50  $\mu$ l of (2 mM) solution of the tested compound in spectroscopic grade MeOH/phosphate buffer (1: 1) was added. The absorbance was measured and the reduction in color intensity was expressed as inhibition percentage. L-ascorbic acid was used as standard antioxidant (positive control). Blank sample was run without ABTS and using MeOH/phosphate buffer (1: 1) instead of tested compounds. Negative control was run with ABTS and MeOH/phosphate buffer (1: 1) only.<sup>[21]</sup>

### Cytotoxicity and antitumor assay

Samples were prepared for assay by dissolving in 50  $\mu$ l of DMSO (Dimethyl Sulfoxide), and diluting aliquots into sterile culture medium at 0.4 mg/ml. These solutions were sub-diluted to 0.02 mg/ml in sterile medium and the two solutions were used as stocks to test samples at 100, 50, 20, 10, 5, 2, and 1 mg/ml in triplicate in the wells of microtiter plates. The compounds were assayed in triplicate on monolayers grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) calf serum (HyClone Laboratories, Ogden, UT), 60 mg/ml Penicillin G, and 100 mg/ml streptomycin sulfate maintained at 37°C in a humidified atmosphere containing about 15% (v/v)  $CO_2$  in air. All medium components were obtained from Sigma Chemical Co., St. Louis, MO, unless otherwise indicated. Cell stocks were maintained at 34°C in culture flasks filled with medium supplemented with 1% (v/v) calf serum. Subcultures of cells for screening were grown in the wells of microtiter trays (Falcon Microtest III 96-wells trays, Becton Dickinson Labware, Lincoln Park, NJ) by suspending cells in medium following trypsin-EDTA treatment, counting the suspension with a hemocytometer, diluting in medium containing 10% calf serum to  $2 \times 10^4$  cells per 200  $\mu$ l culture, aliquoting into each well of a tray, and culturing until confluent. Microtiter trays with confluent monolayer cultures of cells were inverted, the medium shaken out, and replaced with serial dilutions of sterile compounds in triplicate in 100 l medium followed by titered virus in 100  $\mu$ l medium containing 10% (v/v) calf serum in each well. In each tray, the last row of wells was reserved for controls that were not treated with compounds. Trays were cultured for 96 hours. Trays were inverted onto a paper towel pad, the remaining cells rinsed carefully with medium, and fixed with 3.7% (v/v) formaldehyde in saline for at least 20 minutes. The fixed cells were rinsed with water, and examined visually. The cytotoxic activity is identified as confluent, relatively unaltered monolayers of stained cells treated with the investigated compounds. Cytotoxicity was estimated as the concentration that caused approximately 50% loss of the monolayer. 5-fluorouracil was used as a positive control.<sup>[22]</sup>

## RESULTS AND DISCUSSION

Compound **1** showed in its EI MS a molecular ion at  $m/z$  412, (Liebermann-burchard reaction gave the typical slow reacting green color of a  $\Delta^5$  sterol) which<sup>[23]</sup> together with  $^{13}\text{C}$  NMR data suggested a molecular formula of  $\text{C}_{29}\text{H}_{48}\text{O}$ . The loss of part of the side chain ( $\text{C}_7\text{H}_{14}$ ) is characteristic of sterols<sup>[23]</sup> with a  $\Delta^{24(28)}$ .  $^1\text{H}$  NMR spectroscopic data were typical of sterols, in which a signal at  $\delta$  0.88 (6H, d,  $J = 6.6$  Hz) attributable to the two methyls of an isopropyl group, a signal at  $\delta$  0.91 (3H, d,  $J = 6.6$  Hz) and two signals at  $\delta$  1.00 (3H, s) and at  $\delta$  0.68 (3H, s) assigned to two methyl groups attached to two quaternary carbons are assigned to C-26, C-27, C-21, C-19 and C-18, respectively. In addition, a doublet at  $\delta$  1.59 ppm ( $J = 6.6$  Hz) for the proton at C-29 ( $\delta_c$  18.70), a quartet  $\delta$  5.18 ppm ( $J = 6.6$  Hz) at C-28 proton ( $\delta_c$  115.54), a multiplet at  $\delta$  5.35 for C-6 proton ( $\delta_c$  121.70), and a multiplet at 3.53 for C-3 proton ( $\delta_c$  71.80) were observed.  $^{13}\text{C}$  NMR of compound **1** indicated 29 carbons, of which two double bonds and a secondary alcohol (*cf exp.*) were present. The DEPT spectra showed that compound **1** contains six methyl, ten methylene, nine methane, and four quaternary carbons. The stereochemistry at the side chain double bond can be explained by observing the  $^1\text{H}$ NMR spectra at the C-25 proton which resonates at  $\delta$  2.32 ppm ( $\delta_c$  36.50) in case of (*E*) isomer (Frost, ward, 1968). The position of the ethylidene group was proved from HMQC, HMBC correlations, and COSY experiment. These data are in agreement with literature<sup>[24]</sup> which can allow us to assign compound **1** as fucosterol [Figure 1].

Compound **2** showed in its EI MS a molecular ion at  $m/z$  426, which together with  $^{13}\text{C}$ -NMR and HREI MS suggested a molecular formula of  $\text{C}_{29}\text{H}_{46}\text{O}_2$  ( $m/z$  426.3413; calcd. 426.3349). The double doublet at  $\delta$  5.75 ( $J = 18, 13$  Hz), doublet at 5.29 ( $J = 13$  Hz), and doublet at 5.17 ( $J =$

18 Hz) in the  $^1\text{H}$ -NMR and signals at  $\delta_c$  137.79 and 117.10 in the  $^{13}\text{C}$  NMR were attributable to terminal vinyl protons, three methyl doublets at 0.96, 0.87, 0.85 in the  $^1\text{H}$ -NMR spectrum. These data, together with the presence of a fragment at  $m/z$  271 in the mass spectrum due to the loss of the side-chain  $\text{C}_{10}\text{H}_{19}\text{O}$ , suggest a stigmastane skeleton with unsaturation at C-28. The IR spectrum showed strong bands at 3420 and 1675  $\text{cm}^{-1}$ . The above data, together with the presence of signals at  $\delta_c$  200.38, 172.38, 124.42, and 89.75 in the  $^{13}\text{C}$  NMR spectrum and a signal at  $\delta$  5.74 in the  $^1\text{H}$  NMR spectrum, suggested the presence of  $\alpha,\beta$ -unsaturated carbonyl group and a tertiary hydroxyl function in the molecule. As the compound contains two double bonds and two oxygen atoms, one as carbonyl and the second as tertiary hydroxyl, also has seven degrees of unsaturation revealed by mass spectrometry, it must be a tetracyclic product. The structure of known compound **2** [Figure 1] was established by comparison of its data (*cf exp.*) with literature.<sup>[25]</sup>

Compound **3**, its structure was established by comparing their physical and spectral data (*cf exp.*) with those in the literature<sup>[25,26]</sup> as 24-vinyl cholest-5-ene-3 $\beta$ , 24-diol.

Compound **4** was isolated as a bright orange solid and the IR showed the presence of the hydroxyl (3439  $\text{cm}^{-1}$ ), sp-hybrid carbon (allenic) (2361, 2332  $\text{cm}^{-1}$ ), ester (1721, 1253  $\text{cm}^{-1}$ ), and polyene (1654, 1605  $\text{cm}^{-1}$ ) groups. Its molecular formula was deduced as  $\text{C}_{42}\text{H}_{58}\text{O}_6$  based on HRFAB-MS analysis [ $\text{M}^+$ ],  $m/z$ : 658.4224 (Calcd. for  $\text{C}_{42}\text{H}_{58}\text{O}_6$ , 658. 4233), and NMR spectroscopic data (*cf exp.*). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of active compound revealed signals assignable to polyene having acetyl, conjugated ketone, two quaternary geminal dimethyls, two quaternary geminal methyls of oxygen, four olefinic methyls, and allene functionalities. The physicochemical features outlined above suggested that the active compound was a carotenoid in which one of

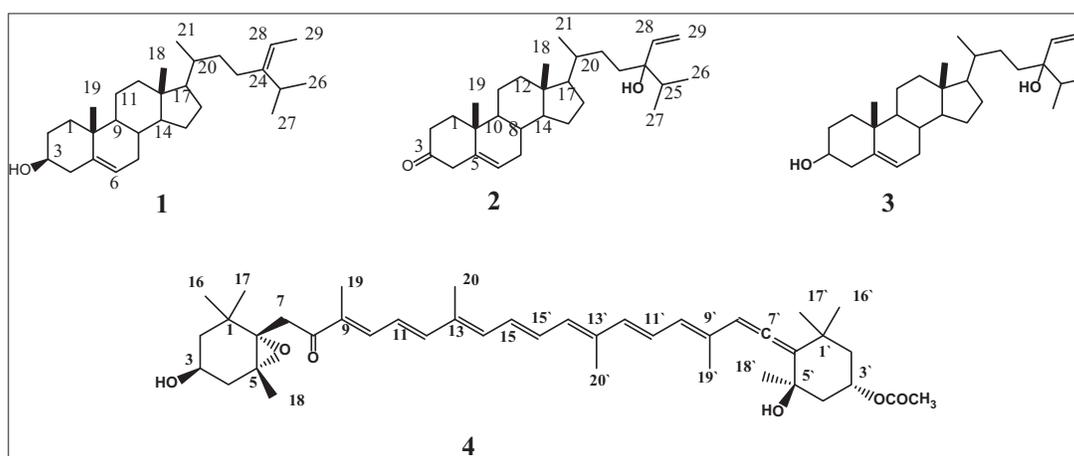


Figure 1: Structure of compounds 1-4

**Table 1: Cell viability and bleomycin-dependent DNA damage assay of fucoxanthin (4)**

	% cells <sup>a</sup>	% Viable cells after adding Bleomycin		
		1	24	48 hours
MCF-7	99.33 ± 1.16 <sup>c</sup>	59.33 <sup>b</sup> ± 3.24	22.00 <sup>b</sup> ± 3.00	12.67 <sup>b</sup> ± 4.04
Fucoxanthin <sup>d</sup>	99.33 ± 1.16	59.33 ± 3.24	61.00 <sup>b</sup> ± 3.00	32.67 ± 4.04
DNA damage	0.008 ± 0.000	0.211 ± 0.16	0.311 ± 0.002	0.334 ± 0.00

<sup>a</sup>Percentage of viable cells before adding bleomycin (negative control), <sup>b</sup>Means that values given in mean ± S.D. are significant, <sup>c</sup>Standard deviation, <sup>d</sup>Concentration of fucoxanthin 20 µg/ml

**Table 2: Antioxidant activity of tested compounds by ABTS method**

Compound	ABTS (% inhibition)
Fucoxanthin	72.06
Fucosterol	26.99
Vit. C	80.03

the hydroxyl groups was acetylated. This suggestion was further supported by the UV spectrum [448 (ε560,000), 248 (24,000)]. From detailed comparison of the data for the active compound with those of fucoxanthin [Figure 1], the active compound was in agreement with an authentic fucoxanthin in all aspects.<sup>[27-29]</sup>

The data obtained from Table 1 indicate that fucoxanthin (4) have some protective activity to DNA by certain mechanism. The isolated compounds were screened for their antitumor activity against MCF-7. The viability of the cells used in control experiments exceeded 95%; compound 4 proved to have cytotoxic activity sustained for 48 hours after adding the compound to MCF-7, as shown in Table 1. Compounds 1 and 4 were tested for antioxidant activity, where fucoxanthin (4) exhibited the highest antioxidant activity by 72% inhibition [Table 2]. On the other hand, compound 1 exhibited a weak to moderate activity.

Compounds 1 and 4 displayed significant antioxidant and anticancer activities [Table 3] against HepG2 (human hepatocellular liver carcinoma cell line), WI 38 (Skin carcinoma cell line), Vero (cell line was initiated from the kidney of a normal adult African green monkey), and MCF-7 (breast cancer cell lines). Fucoxanthin reduced the viability of MCF-7 cells with IC<sub>50</sub> (µg/ml) = 11.5.

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**Table 3: Cytotoxicity of tested compounds on different cancer cell lines**

Compounds	IC <sub>50</sub> (µg/ml) <sup>a</sup>			
	HepG2	WI-38	VERO	MCF-7
Fucoxanthin	20.2	24.6	22.4	11.5
Fucosterol	91.2	82.4	80.3	79.2
5-Fu	8.2	3.17	6.3	2.2

<sup>a</sup>(IC<sub>50</sub>, µg/ml): 1-10 (very strong), 11-25 (strong), 26-50 (moderate), 51-100 (weak), 100-200 (very weak), Above 200 (non-cytotoxic)

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