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Polyphenol-rich seaweed (*Eucheuma cottonii*) extract suppresses breast tumour via hormone modulation and apoptosis induction

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ABSTRACT

The edible red seaweed *Eucheuma cottonii* is abundantly cultivated for carrageenan production. This study investigated the effects of dietary *E. cottonii* polyphenol-rich extract (ECME) on breast cancer. *In vitro* assays showed that ECME was antiproliferative against oestrogen-dependent MCF-7 and oestrogen-independent MB-MDA-231 human breast-cancer cells (IC_{50} values of 20 and 42 μg/ml, respectively) but was non-toxic to normal cell lines. The ECME (150 and 300 mg/kg BW) was fed to female rats and, after 4 weeks, rat mammary tumour was induced using LA7 cells (inoculated subcutaneously). The ECME inhibited tumour development and erythrocyte lipid peroxidation in the cancer-induced rats, dose-dependently. It showed anti-oestrogenic effects on the rat estrous cycle and serum hormone levels. Electron microscopy and histopathology observations confirmed apoptosis in the rat mammary tumours. The polyphenol-rich ECME was tumour-suppressive via apoptosis induction, downregulating the endogenous oestrogen biosynthesis, and improving antioxidative status in the rats.

1. Introduction

The cultivated edible red seaweed, *Eucheuma cottonii* (*Kappaphycus alvarezi*), grows very rapidly in pristine water and can be harvested every 45 days for human use. It is abundantly cultivated in south east Asia, with potential in Africa and the pacific islands. *E. cottonii* is a commercial source of carrageenan (a gelling agent and stabiliser in the food and pharmaceutical industries). It contains high amounts of dietary fibres, minerals, vitamins, antioxidants, polyphenols, phytochemicals, proteins, polyunsaturated fatty acids and may have medicinal uses (Matanjun, Mohamed, Mustapha, & Muhammad, 2009).

Breast cancer is the leading cause of cancer deaths (14% of all cancer deaths) in women worldwide. Populations with typical Asian diets have a lower incidence of hormone-dependent cancers than have populations consuming western diets. These low rates have been partly attributed to their high intake of green tea, soy-products and possibly seaweed (Yoon, Seok-Jin, Gu, & Mi, 2010). Cancer prevention is the most cost-effective effort for cancer control. Chemoprevention is a strategy to inhibit, delay or reverse human carcinogenesis, using especially naturally occurring mediators. The increasing cancer incidence indicates a desperate need for the exploration of more anti-breast cancer alternative and complementary preventive or therapeutic agents.

Dietary antioxidants help to reduce oxidative damage, and the related chronic diseases risk. Many of them interfere with signal transduction regulation at different levels: modulate hormones/growth factor activities, inhibit oncogenes and activate tumour suppressor genes, induce terminal differentiation, activate apoptosis (cancer cell death), restore immune response, inhibit angiogenesis and decrease inflammation (Russo, 2007).

The seaweed composition varies with species, maturity and environmental conditions (Matanjun, Mohamed, Noordin, Kharidah, & Cheng, 2008). The protective effects of dietary kelps and other red and green algae against mammary (Funahashi et al., 2001), skin (Higashi-Okai, Otani, & Okai, 1999) and intestinal cancer (Lee & Sung, 2003) are supported by epidemiological data and rodent model studies.

The present study aims to evaluate the *in vitro* antiproliferative, apoptotic and cell cycle effects of *E. cottonii* alcohol extracts on the growth of oestrogen-dependent and oestrogen-independent human breast cancer cell lines, and to determine its *in vivo* anticancer modes of action on breast tumour induced by LA7 cells.

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2. Materials and methods

2.1. Chemicals

3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) was from Sigma–Aldrich, Canada (Oakville, Ontario). Roswell Park Memorial Institute (RPMI) 1640 growth medium, L-glutamine, $NaHCO_3$, amino acids, sodium pyruvate, fetal bovine serum (FBS) and phosphate buffered saline (PBS) were from Invitrogen Corporation (Burlington, Ontario), and methanol from Merck (Damstadt, Germany). All reagents were of analytical grade.

2.2. Raw materials

E. cottonii samples from Kudat (north coast of Sabah, Borneo), were washed and stored at −20 °C. Ground, freeze-dried seaweed samples were methanol-extracted (Matanjun, Mohamed, Kharidah, & Noordin, 2010), filtered and rotary-evaporated at 40 °C to give a dark green viscous mass (stored at −20 °C), containing 22.5 mg of phloroglucinol (phlorotannins) equivalents/g extract.

2.3. Compounds identification of ECME

The ECME (500 mg) was hydrolysed at 90 °C in 6 M HCl and refluxed for 2 h on a steam bath. The sample was cooled and filtered on a 0.45 μm (Whatman) nylon membrane filter before being injected into a RP-HPLC, 300 × 3.9 mm Symmetry 5 μm C18 column, (Waters Milford, MA, USA), using acidified water (TFA at pH2.5) and 80% methanol as mobile phase, on a gradient run, with a Waters Alliance 2996 photodiode array detector. Pure standards (e.g. rutin, quercetin, kaempferol, naringenin, catechin and epicatechin) and epigallocatechin (EGC), were used as internal and external standards, respectively, to identify the compounds.

2.4. Cell lines

A normal African green monkey kidney Vero cell line (ATCC CCl-81,) was cultured in the growth medium (GM) containing Eagle's Minimum Essential Medium (MEM, GIBCO, USA), supplemented with 10% FBS and 0.1% gentamicin, 20 mM HEPES and 2 mM glutamine, and harvested and plated in flat bottom 96-well plates at 1.5×10^3 cell/ml in GM and incubated as above for 24 h. After removing the old medium, the cells were treated with varying concentrations of test samples in MEM containing 1% FBS (MM), then incubated for 3 days. Cells overlaid with only MM were used as control. The cytotoxicity of ECME on Vero cells, was evaluated daily under inverted microscope (Nikon CMM 214, Japan) for morphological changes (Dzhambazov, Daskalova, Monteva, & Popov, 2002), cell shape and level of adhesion. The maximal non-toxic concentration (MNCC), of ECME under microscopic monitoring, was recorded.

Oestrogen-dependent MCF-7 and oestrogen-independent MB-MDA-231 cancer cells, purchased from the American Type Culture Collection (ATCC, Rockville, Maryland, USA), were cultured in RPMI 1640 medium (GIBCO, USA), and supplemented with FBS and gentamicin as above. The anti proliferative activities of the ECME on MCF-7 and MB-MDA-231 (initial density 1×10^3 cells/well) in 96-well plates were investigated, using MTT assay (Mosmann, 1983). All cells were maintained at 37 °C in a humidified incubator with 5% CO_2.

2.5. Scanning electron microscopy (SEM)

In each T-plate, treated and untreated cells were washed with PBS, fixed and dehydrated, then mounted onto stubs using standard procedures before coating with gold in a sputter coater for 1.5 min and viewed immediately using SEM or stored in a silica gel desiccator.

The MCF-7 cells (plated density 5×10^5 cells in a 35 mm dish) were exposed to ECME (25 and 100 μg/ml) for 48 h, then harvested and treated with the propidium iodide (PI) DNA stain (following the kit protocol), or stained with Acridine Orange (AO) and PI for observations of chromatin condensation. The percentage viable, necrotic, late apoptosis or secondary necrotic and apoptotic population for each time point were determined from >500 cells. The populations of each cell cycle phase were counted by flow cytometry (FACScan flow cytometer with CellQuest software).

2.6. Animals study

Adult female Sprague Dawley rats (from Sapphire Enterprise, Malaysia), weighing 200–250 g, with regular estrous cycles (4–5 days) for three consecutive cycles, were individually housed in wire cages, and maintained under controlled standard animal housing conditions with access to food and water *ad libitum*. Procedures were used to minimise pain or discomfort, and experiments were carried out with the University Animal Ethics Committee approval. The rats were randomly divided into three groups ($n = 8$): a vehicle control (0 mg/kg), a low dose (150 mg/kg), and a high dose (300 mg/kg) ECME. Weighed extracts were applied in the morning daily dose to a 2 g fresh apple wedge as vehicle (Skibola et al., 2005). There apple vehicles eliminated stress associated with gavage, and enhanced rat's eagerness to eat the apple-containing extracts, making it easy to monitor and ensure complete extract deliverance. Vaginal smears from each rat were monitored daily between 09:00 and 10:00 h using staging criteria. The vaginal epithelium cells observed under the microscope were classified into three types: leucocyte cells (L), nucleated cells (O) and cornified cells (Co), based on the majority of the cell type.

2.7. Mammary gland tumour inhibitory effects of ECME in rats

2.7.1.General

The LA7 cells (also called CRL 2283: a rat mammary gland tumour cell line with stem cell characteristics) were kindly provided by Dr. Teo Guan Young, (IBS-UPM), and maintained in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% FBS, 100 μg/ml streptomycin and 100 IU/ml penicillin. The LA7 cells were grown to 80% confluence, harvested using 0.25% Trypsin–EDTA, counted for cell viability using a trypan blue exclusion test, and then resuspended in a serum-free medium. Cells were inoculated subcutaneously into the mammary fat pad (right flank) of female Sprague Dawley rats with 200 μl of cell mixture (total 6×10^7 cells) using a 26-gavage needle. Rats were palpated weekly to monitor tumour development. The tumour diameters were measured with callipers and the volume calculated as follows: largest diameter × (smallest diameter) 2 × 0.4.

The tumour masses were removed, examined for abnormalities and fixed immediately in 10% formalin overnight, embedded in paraffin, cut into 4 μm sections and stained with haematoxylin–eosin (H&E). Five random sections from each H&E slide were viewed at 20× magnification and the mean score calculated. Morphological alterations induced by ECME were examined using phase contrast, fluorescent and transmission electron microscope.

2.7.2. Biochemical measurement

Blood samples were obtained from unfasted rats via cardiac puncture under anaesthesia (50 mg ketamine/kg, 5 mg xylazine/kg). Blood in heparinised tubes was kept on ice and the plasma obtained by centrifugation at 4000*g* at 4 °C for 15 min. and stored at −80 °C. Blood serum 17 β-estradiol, was assayed by a

radioimmunoassay COAT.A.COUNT (Siemens Medical Solutions Diagnostics). The erythrocyte antioxidant enzyme activities, catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) were determined (Matanjun et al., 2010). The erythrocytes glutathione (GSH) activity was estimated by the 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) method.

2.8. Statistics

All data were expressed as means ± standard error of the mean (SEM). One-way analysis of variance (ANOVA), using Statistical Package for the Social Sciences (SPSS) software version 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA), was used to test for differences between different treatment concentrations. Differences at a $p < 0.05$ significance level were identified by the Student–Newman–Keuls multiple range test.

3. Results

3.1. Cytotoxic and anti-oestrogenic properties of ECME

Observations on cellular morphological changes showed that the ECME was not toxic to a normal Vero cell line, but dose- and time-dependently inhibited the proliferation of both oestrogen-dependent and independent human breast cancer cell lines. The ECME was more potent against the oestrogen-dependent MCF-7 (IC_{50} 20 μg/ml after 72 h) than was the oestrogen-independent MB-MDA-431 (IC_{50} 42 μg/ml after 72 h) (Table 1). The apoptotic morphological changes (cell shrinkage, DNA fragmentations, cell membrane blebbing, microvillus disappearance or reduction, condensation of chromosomes and apoptotic bodies with complete membrane and activation of the caspase cascade) were found in all ECME-treated cancer cells. When the growth inhibited cells were stained with AO/PI, apoptotic cell death was observed in all ECME treated cancer cell cultures.

3.2. Cell cycle analysis

Apoptosis was confirmed with the flow cytometric analysis, with the sub-G1 population, (indicating apoptotic cells). It increased in a dose-dependent manner from 4.25% at 0 μg/ml (control) to 7.53% at 25 μg/ml, 12.1% at 50 μg/ml, and 22.4% at 100 μg/ml, after 48 h exposure to ECME (Fig. 1 and 2). Although the G1 population decreased, along with an increase of sub-G1, the other portion of non-apoptotic cells did not show significant changes. These results suggested that ECME could induce apoptosis in MCF-7 cells without cell cycle arrest.

Table 1
IC_{50} (μg/ml) of *Euchema cottonii* methanolic extract (ECME) against human breast cancer cells and estradiol level (pg/ml) of experimental groups at different times. ECME showed no toxicity on normal Vero cells at 1 mg/ml.

Cell line	IC_{50} (μg/ml)		
	24 h	48 h	72 h
MCF-7	25 ± 0.1	22 ± 0.3	20 ± 0.2
MDA-MB- 231	50 ± 0.4	50 ± 0.6	42 ± 0.3
Group	MCF-7 Cell cycle data: % of each cell cycle phase after 48 h		
	Sub G_1	G_1	S	$G_{2/M}$
0 μg/ml control	4.24	60.54	28.70	9.20
25 μg/ml ECME	7.53	54.70	29.84	10.88
50 μg/ml ECME	12.01	50.23	30.63	9.18
100 μg/ml ECME	22.04	49.60	31.33	6.76

Fig 2 illustrates the morphologies seen with AO/PI staining in untreated and ECME-treated apoptotic cells. Untreated cells show a diffuse green fluorescence while, in ECME-treated apoptotic cells, condensed chromatin material resulted in clumps of intense green fluorescent spots within the cell. The characteristic condensation patterns observed were the crescent shape at the nuclear periphery and the more numerous round clumps. Purely necrotic cells stain red with PI with no evident green fluorescence. Cells that were initially apoptotic but then lost membrane integrity later, stained red with PI but still exhibited intense green spots. These were the 'secondary necrotic' cells.

3.3. In vivo anti-oestrogenic effects

Based on the distribution and density of cell types, each daily vaginal smear was assigned one of four estrous cycle stages: proestrus, oestrus, metoestrus, and dioestrus. Untreated SD female rats displayed oestrus once every 4–5 days. Seaweed administration lengthened the rat estrous cycle from 4.2 ± 0.82 to 5.8 ± 1.1 days at 150 mg/kg BW and to 5.6 ± 1.8 days at 300 mg/kg BW. The ECME also dose- and time-dependently reduced serum estradiol levels significantly (Table 2).

Mammary tumours were observed 6–8 days after innoculation of LA7 cells. The tumour incidence rate and volume was also dose-dependently reduced by the ECME treatments (Table 2). The tumours of rats, pretreated with ECME, showed early apoptotic characteristics, which included, cell membrane blebbing, microvillus disappearance or reduction, and condensation of chromosomes under TEM (Fig 3).

The MDA levels were maximal in the untreated cancer control, and treatments with ECME reduced them dose-dependently. There was no significant difference in GSH, SOD or Cat levels, between the groups, although the GSH and CAT levels were low in the untreated cancer control group (Table 2).

3.4. Compounds identification of ECME

HPLC results, based on absorption profile, the retention time and spiking tests on the ECME, showed that catechin, rutin and quercetin were among the phenolic compounds in ECME. Quercetin was one of the four major peaks that were identified. Other peaks that were not identified may include seaweed phlorotannins.

4. Discussion

The ECME at the concentrations used in this study did not show a toxic effect on the normal Vero cells, but was cytotoxic to the MCF-7 cancer cell line. This suggests that the active substances affect special cancer-associated receptors, cancer cell signalling molecules or gene expression that triggers mechanisms causing cancer cell death. Cancer cell genotype is a sign of six vital alterations in cell physiology that collectively declare malignant development, i.e. independence of growth signals, insensitivity to growth-inhibitory (antigrowth) signals, avoidance of programmed cell death (apoptosis), unrestrained replicative potential, persistent angiogenesis and tissue invasion and metastasis. All of these new physiological capabilities are obtained during tumour growth and characterise the thriving infringement of an anticancer protection mechanism built into cells and tissues. Apoptosis induction is one mechanism for tumour suppression by ECME which is important in maintaining cellular homeostasis. The ECME caused irreversible cancer cell damage and induced apoptosis without cell cycle arrest.

Cancer increased the tissue oxidative stress and ECME administration significantly improved the oxidative status. This improved oxidative status contributed to the *in vivo* tumour suppression

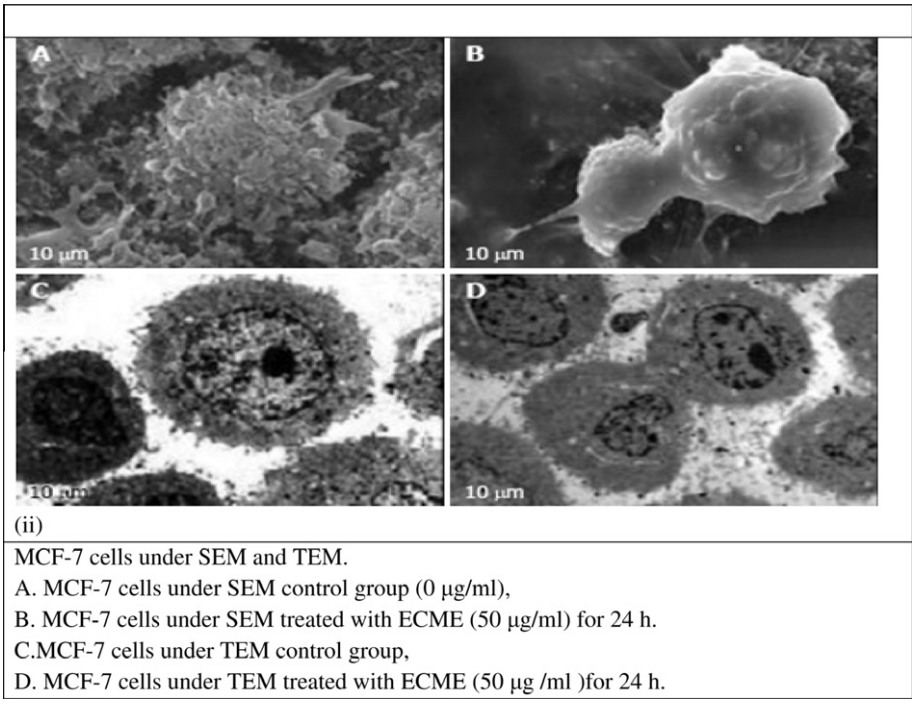


Fig. 1. Electron micrographs of MCF-7 treated with ECME.

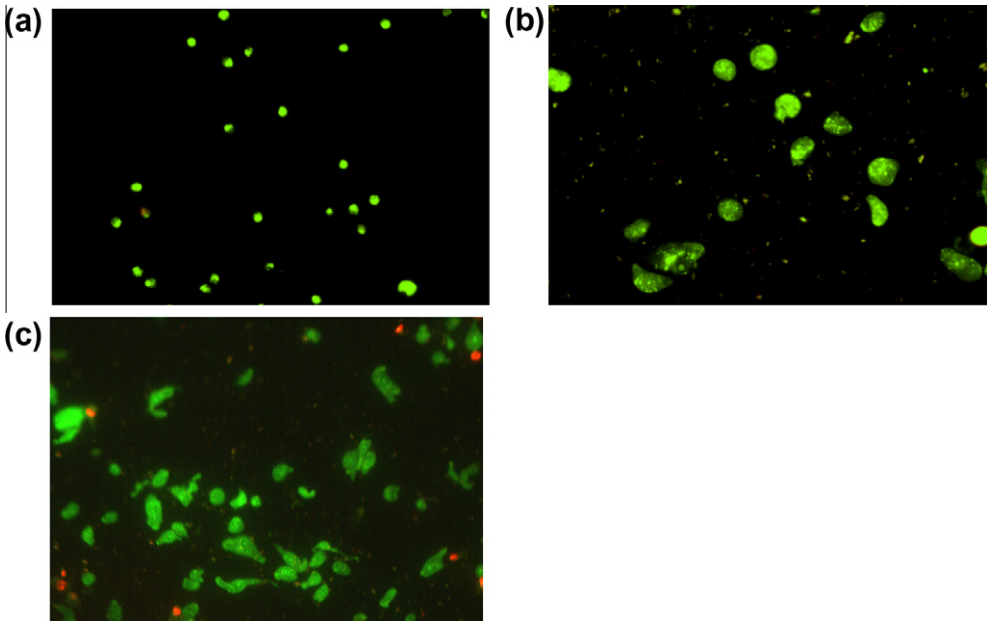


Fig. 2. Morphological changes of MCF-7 cells after treatment by *E. cottonii* extract, followed by AO/PI staining. (a) Fluorescence microscope photographs of control cells treated with 0.1% DMSO; (b) cells treated with 25 μg/ml *E. cottonii* extract after 24 h incubation; (c) cells treated with 25 μg/ml *E. cottonii* extract after 48 h incubation.

response. The cell alleviated oxidative stress either by repairing the damaged nucleotides and lipid peroxidation by-products or by directly reducing the pro-oxidative state via enzymatic and non-enzymatic antioxidants. Oxidative stress and lipid peroxides may subsequently cause cell function loss. Seaweed consumption has been shown to increase the endogenous antioxidant enzymes, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and sometimes catalase activities *in vivo* (Matanjun et al., 2010; Yuan & Walsh, 2006).

The ECME prolonged the oestrus cycle and decreased estradiol levels, which showed similar effects to those of dietary kelp (*Fucus vesiculosus*) which caused an overall 37% increase in the length of the rat estrous cycle in a dose-dependent manner (Skibola et al., 2005). In a human pilot study, the intake of the brown kelp seaweed, *F. vesiculosus*, significantly increased the total number of days of the menstrual cycle, reduced circulating 17 β-estradiol levels, and elevated serum progesterone levels in premenopausal women with abnormal menstrual cycling histories (Skibola et al., 2005). Japanese women have longer menstrual cycles and lower serum estradiol levels than have their western women counterparts, and have the lowest rates of breast, endometrial, and ovarian cancers in the world (Key, Chen, Wang, Pike, & Boreham, 1990).

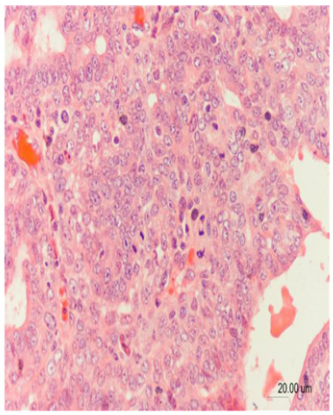
Table 2
In vivo estradiol levels, tumour incidence rate, average tumour volume, apoptotic index, mitotic index and antioxidant biomarkers of experimental rats.

ECME (mg/kg)	Control (0)	150	300
Estradiol level (pg/ml)			
Week 0	20.6 ± 5.03	21.4 ± 4.53	21.5 ± 2.88
Week 2	21.5 ± 6.23	19.0 ± 3.2[a]	15.4 ± 6.17[b]
Week 4	22.2 ± 3.5	15.2 ± 6.07[b]	12.1 ± 8.8[c]
% tumour incidence	87.5 (7/8)	37.5[a] (3/8)	12.5[a] (1/8)
Avg tumour vol. (cm^3)	10.7 ± 1.2	2.5 ± 0.8[a]	0.95 ± 0.7[a]
Apoptotic. I(Count)	1(2 ± 1)	2(6 ± 0.5)	3(11 ± 0.9)
Mitotic. I(count)	1(15 ± 3)	1(4 ± 0.8)	1(2 ± 1)
Malondialdehyde MDA (nmol/ml)	8.44 ± 0.1	5.07 ± 0.7[a]	4.8 ± 0.6[a]
Reduced glutathione GSH (mg/dl)	27.1 ± 4.8	31.1 ± 1.9	31.1 ± 3.0
Superoxide dismutase SOD (U/ml)	192 ± 3.8	190 ± 1.3	192 ± 2.0
Catalase CAT (kU/ml)	1.02 ± 0.13	1.03 ± 0.37	1.03 ± 0.28

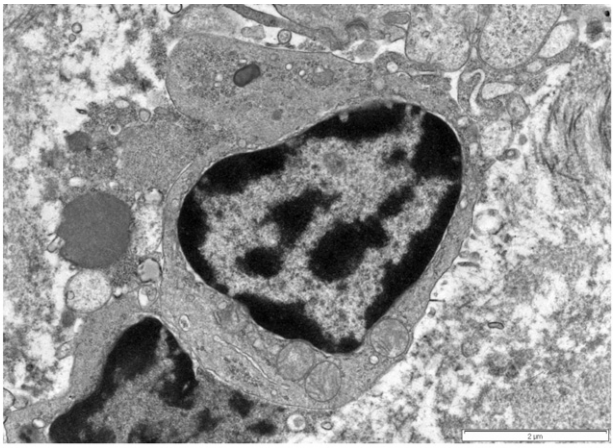
Each value is the mean ± SD.
[a] Significantly different at $p < 0.05$ compared to control group.
[b] Very significantly different at $p < 0.001$ compared to control group.
[c] Very high significant difference at $p < 0.001$ compared to control group.

Exposure to estrogens and an imbalance in the oestrogen: progesterone ratio is an important determinant of oestrogen-dependent disease risk. Serum estradiol levels are associated with oestrogen-dependent cancers (Shimizu, Ross, Bernstein, Pike, & Henderson, 1990), and the reduced incidence among Asian populations has been partly attributed to the soy-rich diets. However, dietary soy or genistein showed either no effects or only a modest 10% increase in cycle length on the rat estrous cycle, suggesting that seaweed has a greater effect in increasing the cycle length than has soy intake.

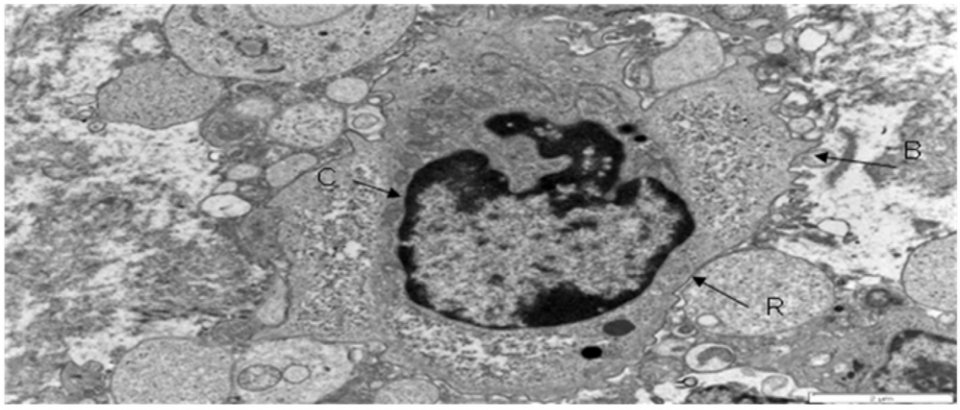
Low doses of oestrogenic compounds induce pseudopregnancy and irregular oestrus in females (Gilmore & Mcdonald, 1996). Thus, a smear of the oestrogen-treated female will initially show leucocyte persistence because oestrogen affects prolactin secretion, which rescues and maintains a functional corpora lutea. Apparently, the endogenous progesterone in such animals prevents the action of exogenous estrogens on the vaginal epithelium. After prolonged treatment, however, the smear will become cornified. This generally occurs when the ovaries have atrophied as a result of the low gonadotropin (Luteinizing hormone LH) and follicle-stimulating hormone (FSH) secretions, attributable to the effect on the brain and pituitary glands of the oestrogen or oestrogenic compounds. Thus, when intact young-adult females are treated, the rapid development of persistent vaginal cornification is not the best indication that the compound is an oestrogen. However, when regularly cycling females were injected subcutaneously with environmental oestrogen (4-tert-octylphenol), a persistent oestrus was observed 3 days after the treatment (Blake & Ashiru, 1997).



Tumour area of control group shows mitotic figures (H and E) (200X)



Tumour area from treated rat (low dose ECME) under TEM shows early apoptotic characteristics, including cell membrane blebbing (B) (6000X).



Tumour area from treated rat (High dose ECME) under TEM shows more apoptotic characteristics, including cell membrane blebbing (B), microvillus disappearance or reduction(R), and condensation of chromosomes (C) (6000X).

Fig. 3. Electron micrographs of tumours from experimental rats.

Chemopreventive agents help to interrupt or reverse the progresses of cancer to prevent carcinogenesis and reverse precancerosis. This study confirmed that the ECME showed breast tumour protective activity by its ability to induce apoptosis, improve oxidative status and downregulate the endogenous active oestrogen biosynthesis. Mechanisms reported for the anticarcinogenic effects of other seaweeds include (i) cancer induction inhibition (Reddy, Sharma, & Mathews, 1984), (ii) hyaluronidase activity inhibition (Shibata, Fujimoto, Nagayama, Yamaguchi, & Nakamura, 2002), (iii) anti-inflammatory (Berge, Debiton, Dumay, Durand, & Barthomeuf, 2002), (iv) anti-proliferative activities (Ellouali, Boisson-Vidal, & Jozefonvicz, 1993), (v) regulation of mammary gland integrity (Aceves, Anguiano, & Delgado, 2005), (vi) antioxidative (Eskin, Grotkowski, & Connolly, 1995), (vii) apoptosis induction (Yamasaki-Miyamoto, Yamasaki, Tachibana, & Yamada, 2009) through the expression of transforming growth factor (TGF-β) (Funahashi et al., 2001), (viii) suppression of tumour initiation (Lee & Sung, 2003), (ix) anti-angiogenic properties, that inhibited VEGF165 (vascular endothelial growth factor 165) binding to its cell surface receptors (Ye et al., 2005), (x) invasion by tumour cell inhibition (Ye et al., 2005), (xi) immune responses enhancement (Itoh, Noda, Amano, & Ito, 1995), (xii) SOD activity inhibition (Funahashi et al., 2001), and (xiii) oestrogen production suppression.

The active compounds in ECME include iodine (*E. cottonii* iodine content: 9 μg/g dry weight) which is alcohol-soluble, antioxidative and antiproliferative against mammary gland tumour growth (Aceves et al., 2005; Funahashi et al., 2001; Matanjun et al., 2009), but is most likely not the only candidate. *E. cottonii* is a rich source of natural antioxidants (Matanjun et al., 2008), which include flavonoids and possibly phlorotannins. Catechol, quercetin, rutin and myricetin have previously been found in seaweeds (Yoshie, Hsieh, & Suzuki, 2003). Flavonoids have three interconnected rings, while seaweed phlorotannins have up to eight interconnected rings. Phlorotannins are therefore 10–100 times more potent and more stable antioxidants than are other polyphenols. The half-life of the phlorotannins in the body, is up to 12 h, compared to 30–180 min for terrestrial polyphenols (Kang et al., 2003).

Sulfolipids from the other red seaweeds have been shown to possess anti-inflammatory and anti-proliferative properties (Berge et al., 2002). Water-soluble polysaccharides, such as laminarans and fucoidans, represent some anticancer substances from seaweeds (Yamasaki-Miyamoto et al., 2009). Oversulfation of fucoidan enhances its anti-angiogenic and antitumor activities (Koyanagi, Tanigawa, Nakagawa, Soeda, & Shimeno, 2003), which is naturally effective against human breast cancer (Yamasaki-Miyamoto et al., 2009) with anti-angiogenic (Koyanagi et al., 2003; Ye et al., 2005), anti-tumour cell invasion (Itoh et al., 1995; Ye et al., 2005), and immune-enhancing properties (Itoh et al., 1995). Porphyran, the polysaccharide from the red algae *Porphyra* sp. also has antioxidant and antitumor properties (Kwon & Nam, 2006). Fucoxanthin, the major 'anti-obesity' carotenoid in brown seaweeds, which showed antiproliferative effects on human leukemia and colon cancer cell lines (Hosokawa et al., 2004), is not present in ECME.

Daily intake of seaweeds was associated with lower risk of breast cancer with suppressive effects on the development and size of both benign and cancer neoplasis (Eskin et al., 1995). The active compounds in this study were in the alcoholic extract. This rules out large molecular weight polysaccharides but there may be synergistic effects between iodine and oligosaccharides, peptides, phlorotannins, carotenoids, other phenolic compounds or combinations of them. Seaweeds are noted to contain, not only labile antioxidants (i.e. ascorbate, glutathione) when fresh, but also more stable molecules, such as carotenoids, mycosporin-like amino acids and a variety of polyphenols.

5. Conclusions

E. cottonii is an underutilised, abundant source of potential complementary and alternative therapeutic functional food, for the prevention and management of breast cancer and possibly other cancers, in general. This study provides some evidence that ECME prevents breast cancer via hormonal modulation, apoptosis induction, and oxidative status modulation. The present study is the first report on the *in vitro* antiproliferative and apoptotic effects of *E. cottonii* alcohol extracts on the growth of oestrogen-dependent and oestrogen-independent human breast cancer cell lines, cell cycle analysis and *in vivo* examinations to determine its modes of action on breast cancer induced by LA7 cells.

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