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Review Article

A Mini Systematic Review: *Eucheuma cottonii*, a Red Algae, as a Radiosensitizer?

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ABSTRACT

Radiosensitizers assist radiotherapy in providing greater tumor inactivation. Currently there is a search for natural radiosensitizer components which are expected to provide lesser side effects than chemical radiosensitizers. *Eucheuma cottonii* is a plant with antioxidant and anti-tumor effects. This review aims to search for the potential use of *Eucheuma cottonii* as a radiosensitizer. This is a mixed review study, where the main component is a systematic review and then followed by a narrative review. This review suggests that *Eucheuma cottonii* has the potential to become a radiosensitizer, by interfering with the cell cycle control mechanisms and reactivation of p53. Further research is needed to explore the synergistic effect of the combined use of radiotherapy and *Eucheuma cottonii*.

Keywords: *Eucheuma cottonii*, Anti-tumour, Radiosensitizer, Immunomodulator

Introduction

Cancer is among the world's top causes of death. According to the data of GLOBOCAN, in 2018, there were 18.1 million new cancer cases, 9.6 million of which resulted in death. Globally, 1 out of 5 men and 1 out of 6 women suffer from cancer. The data also shows that 1 out of 8 men and 1 out of 11 women died from cancer.[1]

Cancer treatment modalities consist of radiotherapy, surgery, chemotherapy, immunotherapy, and hormone therapy.[2] Either for curative or palliative purpose, 80% of cancer patients require radiotherapy treatment. An effort to optimize radiation therapy is through the use of radiosensitizer. Radiosensitizer is a compound which, when combined with radiation, will provide greater tumour inactivation than the additive effect of each modality.[3,4] The majority of radiosensitizers are synthetic chemical compounds, which have been proven to be too toxic in effective clinical doses. Radiosensitizer made of natural materials is believed to be safer than synthetic materials.[5] *Eucheuma cottonii*, is commonly found in the Indonesian ocean. *Eucheuma cottonii* (*Kappaphycus alvarezi*) is used to produce carrageenan that functions as a stabilizer, a gellant, binder, thickening agent, and supplements in the pharmaceutical and food industries.[6,7] It has high number of proteins, dietary fibers, antioxidants, vitamins, polyphenols, phytochemicals, minerals, and polyunsaturated fatty acids. It also has many medical uses.[8] Many studies have reported the anticancer effect of *Eucheuma cottonii*, either *in vitro* or *in vivo*. However, there have been no studies that observe the combination of radiation and *Eucheuma cottonii* in cancer cell lines. Therefore, the present study aims to investigate the radiosensitization effect of *Eucheuma cottonii*.

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Method

Literature search was conducted on the PubMed, Cochrane, EBSCO, and SCOPUS databases for articles published from 2010 to 2020 on studies on the effects of *Eucheuma cottonii* on cancer cells. Search was performed using search terms (*Kappaphycus alvarezii* OR *Eucheuma cottonii* OR Red seaweed) and (cytotoxic OR antiproliferative OR anticancer OR celldeath) and (tumor OR cancer). Selected references were assessed based on relevance, and suitability for writing purpose. The literature search inclusion criteria included experimental studies that discussed the effects of *Eucheuma cottonii/ Kappaphycus alvarezii* on cancer cells *in vitro* and *in vivo*. The exclusion criteria used were publication with the type of review, used different algae species, or in combination with other substances. The risk of biased *in vivo* studies was conducted using SYRCLE. *In vitro* studies were analyzed using SciRAP. Literature searches and critical reviews were conducted independently by two reviewers. We carried out a narrative review to analyze the role of *Eucheuma cottonii* on the effects of radiation. We made a flow diagram in agreement with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement on systematic review reporting (see Figure 1).

Results and Discussion

Thirteen articles were involved in this systematic review. Eleven articles were *in vitro* studies, and four articles were *in vivo* studies. We made a summary about the type of research, type of cell line, and the results from all studies are presented in Table 1.

Anticancer/antiproliferative/cytotoxic activity

Eleven *in vitro* studies were conducted using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test to observe the cytotoxic effect. They are listed in Table 2. Table 2 contains cell types, solvents and IC_{50} values from *in vitro* studies. The inhibitor activity is expressed in Inhibitory Concentration 50% (IC_{50}). IC_{50} is the extract concentration that can inhibit cell growth by 50%. The results of which proved that *Eucheuma cottonii* extract inhibit the growth of cancer cells. However, the IC_{50} values obtained were different with wide variations (IC_{50} = 20 µg/mL to 4 mg/mL).

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This can occur because the procedures, reagents, and passage cells used in each study are different.

Proapoptotic and anti-apoptotic protein regulation

Several main target proteins for radiosensitization were seen in cancer cells treated with *Eucheuma cottonii*. *Eucheuma cottonii* extract was able to restore the apoptotic response of cancer cells by increasing the expression of p53 and Chk1 (Figure 2). This extract inhibited the expression of the antiapoptotic gene BIRC5 (Baculoviral IAP repeat-containing protein) and Bag1 and reduced the expression of MDM2 (mouse double minute 2 homologous).[18] The inhibition of MDM2 which normally binds to p53 will lead to the stabilization of p53 and subsequently activating p21. Activation of p21 will inhibit CDK4 and CDK6 activity resulting in a termination of the cell cycle at the G1 phase.[21,22] In addition to being able to phosphorylate p53, an increase in Chk1 will also phosphorylate CDC25 (cell division cycle 25), thus inhibiting CDK2-cyclin E and CDK1-cyclin B which results in a cessation of the cell cycle at the G1 and G2 phases.[21]

Cell cycle profiles and cell development were seen using FUCCI (fluorescence ubiquitination-based cell-cycle indicator).[16] Cells treated with k-carrageenan (k.CO) showed a rise in the number of cells that stopped in the G2/M phase. Giving k-CO to cells results in a G2 / M phase twice as long as the G1 phase. The cessation of the cell cycle is likely a continuation of the p53 activation response which activates p21 as a cyclin-dependent kinase (CDK) inhibitor that induces the cessation of the cell cycle.[21] This greatly helps the role of radiation, where the G2/M phase is known to be sensitive to radiation. BIRC5 or survivin is an inhibitor of apoptosis (IAP) member which works to inhibit caspase and inhibits cell death. BIRC5 is found in most cancer cells and has been associated with poor clinical outcomes. Survivin expression is very high in the G2/M phase and decreases swiftly in the G1 phase of the cell cycle. Survivin has been proven to inhibit apoptosis via a caspase-dependent and independent pathway. Survivin inhibits caspases 3, 7 and 9 and inhibits apoptosis-inducing factor (AIF) which is released from the mitochondrial intermembrane space into the cytoplasm. Surviving expression can be suppressed by the p53-E2F complex, by directly binding to the surviving promoter. In other words, survivin can also influence p53 activity through regulation of MDM2 and proteosome.[23]

Bag1 (BCL2 associated Athano Gene-1) inhibits cell death by synergistic action with the antiapoptotic gene BCL2. Bag 1 works to regulate the integrity of the mitochondrial membrane and prevent caspase activation.[24] The potential pathways for the radiosensitizer effect of *Eucheuma cottonii* can be seen in Figure 3.

Immunomodulator

The electron microscope imaging shows the presence of macrophage activity, which suggests that the extract modulates the immune response. From the TEM (Transmission Electron Microscopy) examination after administration of the extract, monocytes and macrophages were seen. The macrophages responsible for phagocytosis are the main immune response associated with antigens on the surface of tumor cells. The presence of monocytes activates dendritic cells (DCs), which together with macrophages present CD4 and CD8 T cell antigens, so that CD4 and CD8 are activated. When CD4 and CD8 enter the circulation, it destroys tumour cells.[25,26,27] The extract was also shown to reduce IL4 and increase IFNγ.[18] Cytokines IL4 and IFNγ have a major contribution in the regulation and formation of immune responses. Macrophage activity will increase due to the influence of IFNγ and inhibited by IL4.[28] The immunomodulator pathway of *Eucheuma cottonii* is shown in Figure 4.

Survival pathway

Eucheuma cottonii extract can increase NF-κB in cells.[18] NF-κB can increase anti-apoptotic protein expression. NF-κB also induces expression of IAP and several members of the anti-apoptotic family Bcl-2.[29] This raises the question of how *Eucheuma cottonii* can cause cancer cell death, as has been proven from the MTT test results and the visible characteristics of apoptotic cells after administration of *Eucheuma cottonii*. Another thing to keep in mind is that increasing Chk1 and IFNγ results in the upregulation of PDL-1 (Programmed Death Ligand 1).[30] PDL-1 is a PD-1 (Programmed Cell Death 1) ligand. PD1/PDL-1 is an immune checkpoint that causes tumour cells to avoid CD8.[31] It is not known how *Eucheuma cottonii* affects PD-1/PDL-1.

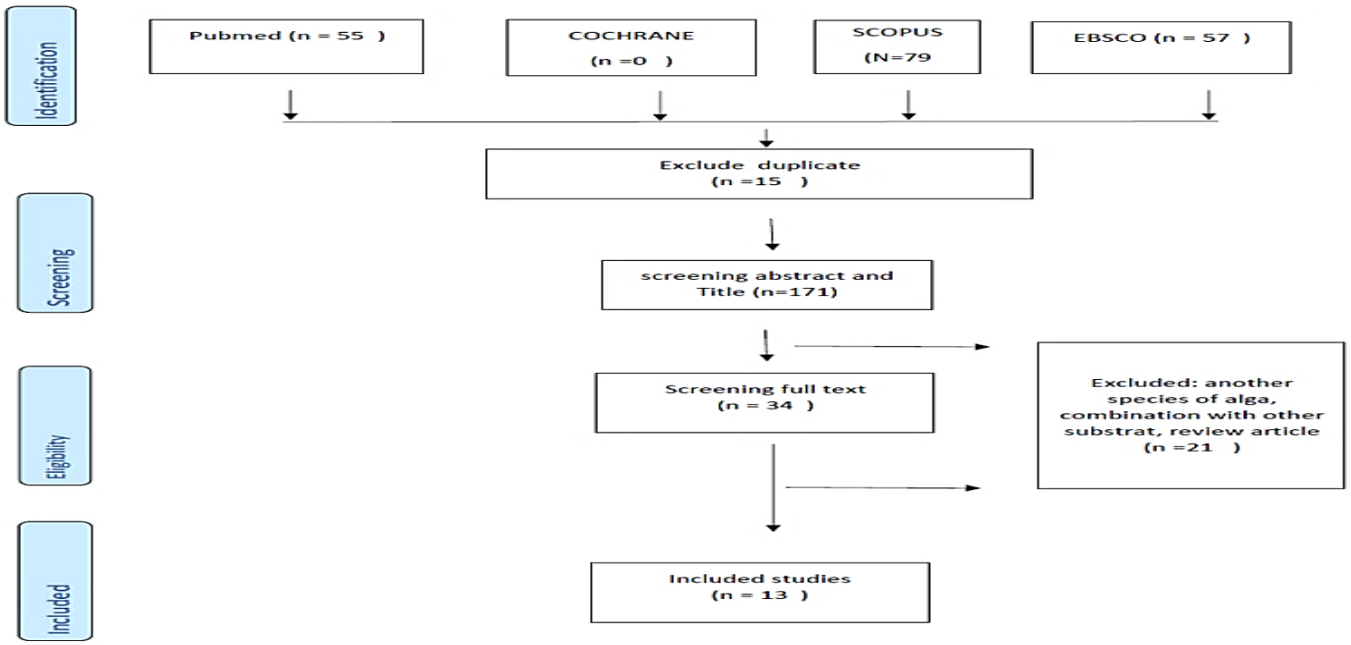

Figure 1: Searching strategy using PRISMA flow diagram

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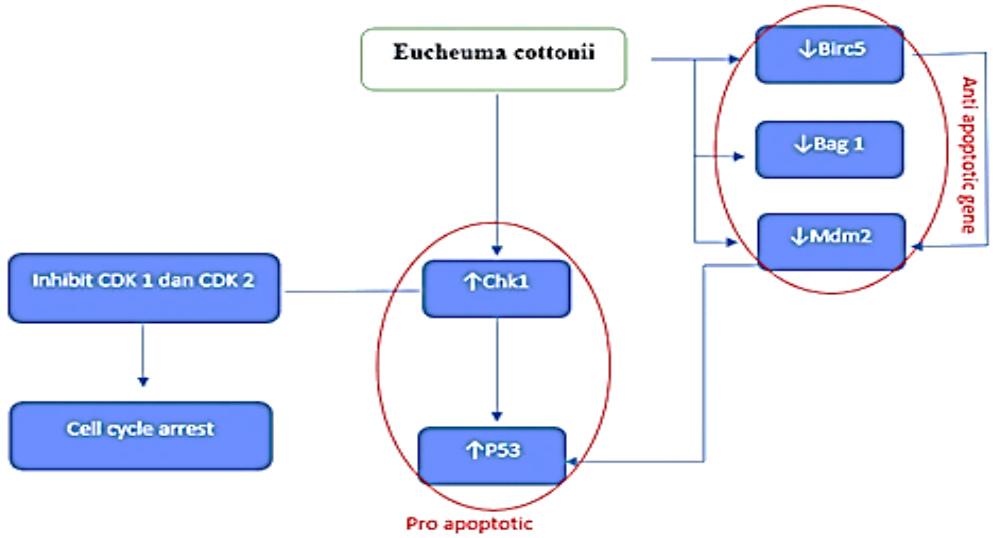

Figure 2: Biomolecular pathway effect of *Eucheuma cottoni*. *Eucheuma cottonii* can decrease the anti-apoptotic gene (Birc5, Bag 1 and MDM2), and increase the proapoptotic gene (Chk1 and p53)[18]

Table 1: The Summary of the reviewed studies

Studies	Type of studies	Cell line	Result
Namvar,2012[7]	*In vitro*/non randomized	• MCF-7 (breast cancer cell estrogen dependent) • MB-MDA-231(breast cancer cell estrogen independent) • A normal African green monkey kidney Vero cell line	*In vitro* test found that ECME (*Eucheuma cottonii* polyphenol-rich extract) was anti-proliferative against estrogen-dependent MCF-7 and estrogen-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.
	In vivo/ randomized	Rat mammary tumour was induced with LA7 cells	*Eucheuma cottonii* hindered tumor growth and erythrocyte lipid peroxidation in the cancer-induced rats, dose-dependently. The histopathology observation and electron microscopy affirmed apoptosis: cell shrinkage, DNA (deoxyribonucleic acid) fragmentations, cell membrane blebbing, microvillus disappearance or reduction, condensation of chromosomes and apoptotic bodies with complete membrane and activation of the caspase cascade, in the rat mammary tumours.
Lee, 2013[9]	*In vitro*/ non randomized	HeLa (Human Cervix Adeno carcinoma)	MTT (3-(4,5-Dimethylthiazol-2,5-diphenyltetrazoliumbromide) cell viability inspection displayed that various concentrations of the crude extracts from *Eucheuma cottonii* hindered the HeLa cells for development after 24 hours and 48 hours being incubated with crude extracts from *Eucheuma cottonii*. The concentration of extracts from *Eucheuma cottonii* 0.5, 0.7, 0.9, and 1.0 mg/mL for 24 hours indicated that HeLa cells have experienced significant DNA fragmentation. The HeLa cells induced by a concentration of 20 mg/mL 24 hours did not

			undergo apoptosis. The induction of apoptosis by a concentration of 0.1 mg/mL for 48 hours has degraded.
Shamsabadi, 2013[10]	*In vivo*/ randomized	LA7	The oral administration 100 mg/kg body-weight of ECE (*Eucheuma cottonii* ethanol extract) was correlated with tamoxifen (10 mg/kg body-weight). Subcutaneous injection of LA-7 cells (6×10^6 cells/rat) was performed on the rat to develop mammary tumor. The ECE was proven to have better effectiveness than tamoxifen in tumor development suppression (27%), tissues improvement (plasma, liver, and kidney) malondialdehyde concentrations, superoxide dismutase activity and erythrocyte glutathione concentrations ($P < 0.05$). Unlike tamoxifen, the ECE showed little toxicity to the liver and kidneys.
Tan, 2014[11]	*In vitro*/non randomized	MCF7	The cytotoxic effect of the extract was decided through the MTT test. Cell apoptosis was detected using GeneTex Enhanced Apoptotic DNA Ladder Detection Kit. The Result of the MTT test shows that MCF-7 cancer cells growth was suppressed by the crude extracts of *Eucheuma cottonii* in a dose-dependent manner of IC_{50} at 3.5mg/mL (24h) and 0.85mg/mL (48h). The presence of fragmented DNA of cells treated with the crude extract indicated cytotoxic effect is through apoptosis.
Lau, 2014[12]	*In Vitro*	HeLa	The extract's cytotoxic effect was tested by the MTT test. *Kappaphycus alvarezii* was extracted with 90% methanol, 70% acetone, and aqua. The 90% methanol extract showed an anti-proliferative effect at 200-500 µg/mL. The aqua extract showed no anti-proliferative effect.
Lee, 2015[13]	*In vitro*/non randomized	HeLa (Human Cervix Adeno carcinoma) Human lung carcinoma cell line (SKLU-1) Human colon carcinoma cell line (HCT-116) Fibroblastwere	By using the maximum dose of the extract (20.0mg/mL) for 24 hours incubation, a complete cessation in cell proliferation was observed, showing a significant cytotoxic effect of *Eucheuma cottonii* extract. The extracts of *Eucheuma cottonii* showed no effect on fibroblast, a human normal cell line.
Suganya, 2016[14]	*In vitro*/non randomized	Breast cancer (MCF7), colon (HT-29), liver (Hep G2) and osteosarcoma (MG63).	The pharmacological properties of native carrageenan (k) that was extracted from *Kappaphycus alvarezii* and commercial carrageenan (Sigma-Aldrich) were then evaluated. Native carrageenan exhibited an excellent anticancer activity on colon carcinoma cell lines (67.66 ± 0.168%) with the IC_{50} value of 73.87 mg/ml, and commercial carrageenan possessed a potent hindrance on breast cancer cell lines growth (67.33 ± 0.077%) with the IC_{50} value of 123.8 mg/mL, liver cancer cell line (Hep G2) with the IC_{50} values of both native and commercial carrageenans determined as 56.71 and 125 mg/mL, respectively. Colon cancer cell line (HT-29) with IC_{50} value was observed as 73.87 and 123.8mg/mL for native and commercial carrageenans, respectively. Osteosarcoma cancer cell line, with IC_{50} of 47.85 and 55.48 mg/mL for native and commercial carrageenans, respectively.
Arsianti, 2016[15]	*In vitro*	MCF-7 and HCT-116	*Eucheuma cottonii* extract was found to hinder the proliferation of MCF-7 and HCT-116 cells
Prasedya, 2016[16]	*In vitro*/non randomized	human cervical carcinoma cells (HeLa) cells as and	Decreased the cell viability of HeLa cells exposed with k-CO and λ-CO over a 72 h period. Both k-CO and λ-CO showed IC_{50} values of

		human umbilical vein endothelial cells (HUVEC)	550.8μg/mL and 475 ± 12 μg/mL. Both carrageenans had no significant cytotoxic effect on HUVEC. Cell cycle profiles of k-CO treated cells showed increased arrest in the G2/M phase. Cells treated with λ-CO needed a longer time to finish one cycle, around 59 ± 4.6 hours and with k-CO treatment which was 50.2 ± 2.9 hours. Most cells treated with λ-CO were unable to perform cell division. The cell cycle in cells treated with λ-CO, the progress continues as FUCCI cells change color, except the cells cannot divide and would later die. Cells treated with k-CO were able to divide at least once before cell death.
Chang, 2017[17]	*In vitro*/non randomized *In vivo*/ randomized	Breast cancer cell line (MCF-7) Investigated toxicity effect of high dosage *Kappaphicus alvarezii* extracts in rats and determined the effect of *Kappaphicus alvarezii* on 7, 12-dimethylbenz[a] anthracene (DMBA) mammary carcinogenesis in rats	Crude *Kappaphicus alvarezii* extract reduced the cell viability of MCF-7 from 84.91% to 0.81% and the IC_{50} value was 4.1 ± 0.69 mg/mL. For sub-chronic and heavy metal toxicity studies, there was no significant difference in hematological and biochemical values between the control group and experimental group. The tumor growth rate in the untreated group of mice was found necessarily higher than the experimental group of rats. The specific tumor growth rate for untreated group was 1.580 ± 0.270 mm^3/t and *Kappaphicus alvarezii* extract treated group is 0.097 ± 0.060 mm^3/t.
Bakar, 2017[18]	*In vivo*/ randomized	Mammary tumor was induced by subcutaneously injecting LA7 cells in female rat mammary pads	After 2 weeks of cancer development, the mice were orally-administered with either SECE (Seaweed *Eucheuma cottonii* ethanol extract) 150 mg/kg body weight (BW) and 300 mg/kg (BW) or tamoxifen. The Electron microscopy-imaging results affirmed the presence of macrophage activity. Hematoxylin and eosin staining showed that the seaweed extract restored the tumor histopathological alterations to normal. The extract hindered tumor growth and regulated the immune responses. This was proven by the microscopic observations, the increased spleen weight, size, spleen CD19 B cells, and blood immunoglobulin G (IgG) levels. The extract also raised the circulating total white blood cells, lymphocytes, segmented neutrophils count, T cells (CD3), T-helper cells (CD4), cytotoxic T cell (CD8), and nuclear factor-kappa beta expressions. The extract raised cancer cell death, by upregulating the Birc5, Chk1, and p53 levels and downregulated the tumor development cellular Mdm2 (transformed mouse 3T3 cell double minute 2) messenger RNA (mRNA) expression. The extract did not show toxicity at 150 mg/kg BW in rats. The lectin-rich SECE displayed suppression of tumor by developing immune responses and upregulating the canver cell apoptosis mRNA expressions.
Arsianti, 2018[19]	*In vitro*	HeLa	Ethanol, n-hexane, chloroform, and ethyl acetate extracts of *Eucheuma cottonii* displayed strong cytotoxic activity against cervical HeLa cells with IC_{50} of 7.54 μg/mL, 5.73 μg/mL, 4.82 μg/mL and 4.34 μg/mL
Arsianti, 2020[20]	*In Vitro*	A-549	The cytotoxic effect of *Eucheuma cottonii* was tested by MTT, controlled

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by cisplatin. *Eucheuma cottonii* extract with ethanol, ethyl acetate, and n-hexane solvents was shown to inhibit proliferation, with IC_{50} of 251.73 µg/mL, 261.41 µg/mL, 3508 µg/mL.
The antioxidant effect was tested by comparing it with ascorbic acid. The ethanol extract reduced DDDH free radicals with IC_{50} of 559.76 µg/mL.

Table 2: The IC_{50} value of *Eucheuma cottonii's* extract in the included studies

Studies	Cell line	Solvent	Time Incubated (hours)	IC_{50}
Namvar, 2012[7]	MCF7	Methanol	24	25 ± 0.1µg/ml
			48	22 ± 0.3µg/ml
			72	20 ± 0.2µg/ml
	MB-MDA231		24	50 ± 0.4µg/ml
			48	50 ± 0.6µg/ml
			72	42 ± 0.3µg/ml
Lee, 2013[9]	HeLa	Methanol	24	NA
			48	
Tan,2014[11]	MCF7	Methanol	24	3.5mg/ml
			48	0.85mg/ml
Lau, 2014[12]	HeLa	Methanol	24	NA
		Aceton	24	
		Aqua	24	
Lee,2015[13]	HeLa	Methanol	24	± 0.5mg/ml
	SK-Lu1			± 0.5mg/ml
	HCT116			± 0.5mg/ml
Suganya, 2016[14]	MCF7	NA	NA	103.2 µg/ml
	HT29			73.87µg/ml
	HepG2			56.71µg/ml
	MG63			47.85µg/ml
Prasedya, 2016[16]	HeLa	NA	72	550.8 ± 7.6 µg/ml
Arsianti, 2016[15]	MCF7	DT-ethanol	4	149,5 ± 2.8 µg/mL
		DU-Ethanol		75.7 ± 1.3 µg/mL
		Chloroform		189.0 ± 2.2 µg/mL
		DT Hexane		111.5 ± 1.9 µg/mL
		Ethyl acetate		259.0 ± 2.6 µg/mL
	HCT 116	DT-ethanol		65.3 ± 1.8 µg/mL
		DU-Ethanol		419.1 ± 2.7 µg/mL
		Chloroform		99.3 ± 1.8 µg/mL
		DT Hexane		43.0 ± 1.3 µg/mL
		Ethyl acetate		21.4 ± 1.4 µg/mL
Chang, 2017[17]	MCF7	Methanol	24	4.1 ± 0.69

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				mg/mL
Arsianti, 2018[19]	HeLa	Etanol	48	7.54 µg/ml
		n-Hexana		5.73 µg/ml
		Chloroform		4.82 µg/ml
		Etil asetat		4.34 µg/ml
Arsianti, 2020[20]	A-549	Etanol	4	251.73µg /mL
		Ethil asetat		261.41µg / mL
		n-Hexana		3508µg / mL

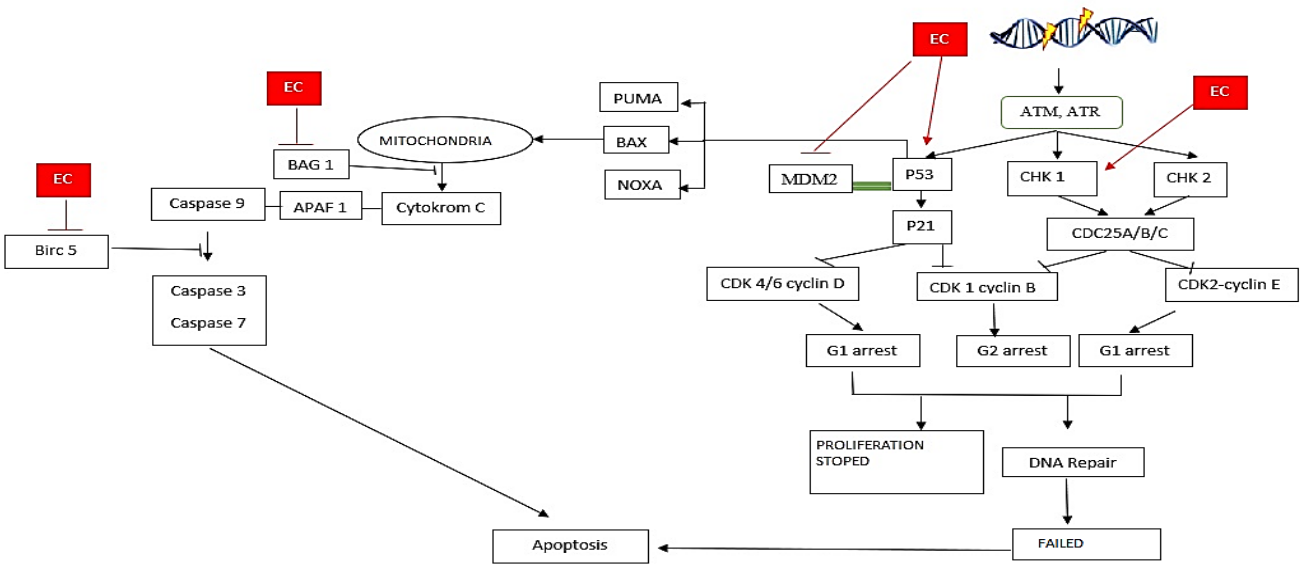

Figure 3: Radiosensitizer mechanism of *Eucheuma cottonii* (EC = *Eucheuma cottonii*, black line shows radiation effect, red line shows *Eucheuma cottonii* effect, arrow shows activating effect, T model shows inhibiting effect)[18,21-24]

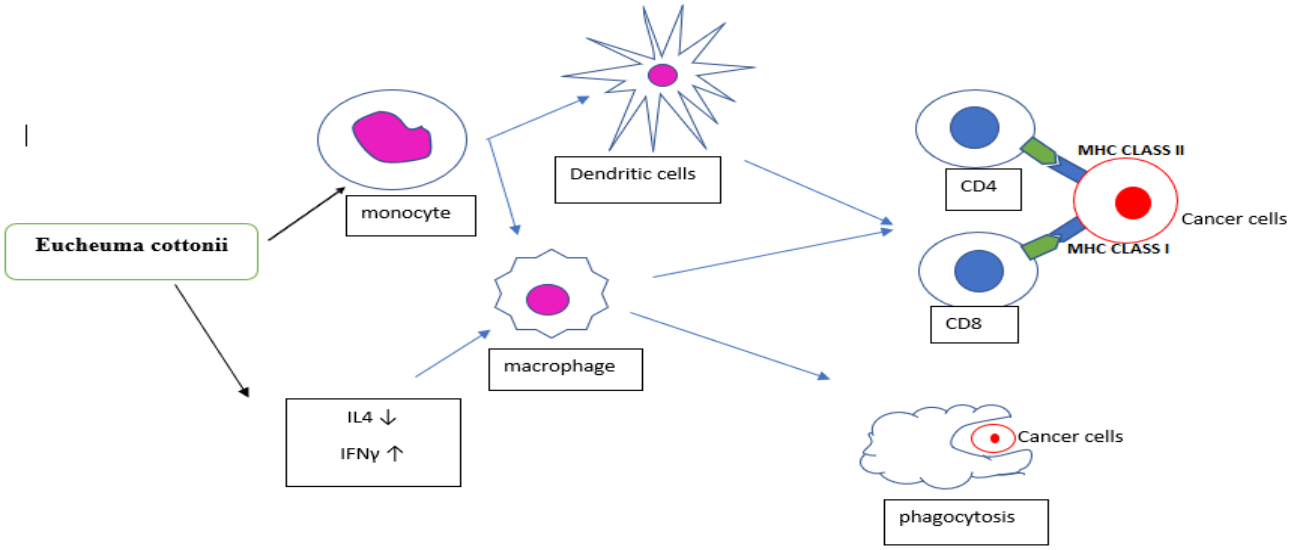

Figure 4: Immunomodulator effect of *Eucheuma cottonii* by increasing monocytes and regulating IL4 and IFNγ.[18,24,25]

Conclusion

Our systematic review and further analysis of several selected pathway found that *Eucheuma cottonii* is able to suppress the proliferation of various cancer cells and cause cancer cell death in the form of apoptosis through regulation of proapoptotic proteins (Chk1 and p53) and anti-apoptosis (Birc5, Bag 1 and MDM 2) and also interfere with the cell cycle control mechanisms by a cessation of the cell cycle at the G2/M phase. We discovered the future potential of *Eucheuma cottonii* as a promising radiosensitizer. All journals in this systematic review are still preclinical trials of the effects of *Eucheuma cottonii* extract on cancer cells. Further studies are needed to determine whether the extract can synergize with radiation as a radiosensitizer.

Conflict of Interest Disclosure

Authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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