

Anticancer Properties of *Caulerpa racemosa*: A Review Study

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ABSTRACT

Introduction: Cancer is the leading cause of death in the world, with approximately 10 million deaths expected by 2020. Several approaches are used in cancer management. However, the cost is one of the main obstacles in cancer therapy as well as side effects in sufferers. *Caulerpa racemosa* is a type of seaweed that is naturally abundant in the Indonesian sea. Recently, there has been much research on the anticancer effects of *Caulerpa sp.* This study aims to find the potency of Sea grapes extract (*Caulerpa racemosa*) in the treatment of cancer and its mechanisms.

Method: A review of the literature was constructed on the potential of the *C. racemosa* extract with the PICOS criteria and the data were extracted from 'PUBMED', 'ScienceDirect' and 'SpringerLink'. The search method was using a boolean operator with the main keywords 'Caulerpa racemosa', 'cancer', and 'Management'.

Results: The main results were 8 articles including in vitro and in vivo experimental studies based on inclusion criteria. Several studies (n=8) revealed the potency of *C. racemosa* extract as an anticancer agent through various activities, such as antiproliferative, apoptotic, antioxidant, cytotoxic activity, and inhibition of tumor progression genes, DNMT, and upregulation of proapoptotic genes, including BAX, P53, Caspase-3, Caspase-8, and Caspase-9.

Discussion: *C. racemosa* possesses several potent antioxidant substances, along with gene regulation activities and in-

hibition of cell line proliferation. Seaweeds has been used widely as functional food and showed minimal or no toxicities against human. With all these benefits, *C. racemosa* has the potential to be commercialized as a promising diet for cancer patients.

Conclusion: Sea grapes extract (*C. racemosa*) has good potential as an anticancer agent through antiproliferation mechanisms, induction of apoptosis, cytotoxic and antioxidant activity.

KEYWORDS

Functional food, *Caulerpa racemosa*, cancer, antiproliferative, cytotoxicity, apoptotic, nutraceutical, bioactive.

INTRODUCTION

Cancer is the leading cause of death in the world, with approximately 10 million deaths expected by 2020¹. The most common in 2020 (in terms of new cases of cancer) were breast (2.26 million cases); lung (2.21 million cases); colon and rectum (1.93 million cases); prostate (1.41 million cases); skin (non-melanoma) (1.20 million cases); and stomach (1.09 million cases). In the same year, the most common deaths caused by cancer were lung (1.80 million deaths); colon and rectum (935 000 deaths); liver (830 000 deaths); stomach (769 000 deaths); and breast (685 000 deaths). The incidence of cancer increases considerably as people age, most likely due to an increase in the risk of certain tumors. The accumulation of risk is compounded by the fact that cellular repair systems become less effective as a person ages¹.

Cancer develops when normal cells are transformed into tumor cells in a multistage process that usually evolves from a precancerous lesion to a malignant tumor². Physical carcinogens, such as ultraviolet and ionizing radiation, chemical car-

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cinogens, such as asbestos, components of tobacco smoke, aflatoxin (a food contaminant), and arsenic (a drinking water contaminant), and biological carcinogens, such as infections from specific viruses, bacteria, or parasites, all interact with a person's genetic factors to cause these changes. The World Health Organization (WHO) maintains a classification of cancer-causing chemicals through its cancer research agency, the International Agency for Research on Cancer (IARC)¹.

The National Cancer Institute states that cancer treatment varies depending on the type and stage of the disease. Currently, several types of therapy are most often used, including surgery, chemotherapy, and radiation therapy, where these therapies can be performed alone or in combination³. Surgery is usually performed on solid tumors located in one area. If cancer has spread, it will be treated with a combination of chemotherapy, radiation, or other treatments. Unfortunately, chemotherapy and radiotherapy are distributed nonspecifically in the patient's body. Not only does this therapy kill cancer cells, but it can also affect healthy cells eventually causing side effects in sufferers².

Cancer treatment is also very expensive. In low- and middle-income nations, late-stage presentation and lack of access to diagnosis and treatment are prevalent. According to reports, comprehensive therapy is available in more than 90% of high-income countries but less than 15% in low-income countries⁴. More than 85 percent of the world's population still uses traditional medicines from natural ingredients as treatment, with about 73% being pharmacological products. The advantage of developing therapeutics from natural ingredients is that they are widely available and the price is relatively cheap⁵.

Caulerpa or commonly called Sea grapes is a genus of marine algae from the *Caulerpaceae* family and belongs to the class *Chlorophyceae* (green algae). *Caulerpa* has many species, and one of them is *Caulerpa racemosa*⁶. *Caulerpa racemosa* is a type of seagrape that lives in Indonesian but is rarely used⁷. Several functional bioactive compounds have been identified in *C. racemosa*, including bisindole alkaloids including caulerpin, caulersin, caulerchlorin, and racemosins A–C. Furthermore, there are terpenoids, sesquiterpenoids, including caulerpenes, and diterpenoids with functional groups of aldehyde and/or enol acetate⁸. The functional potential of the bioactive chemicals found in *C. racemosa* has been discovered and is continuously being studied in various ways. For some of them, *C. racemosa* is known to have antioxidant, antibacterial, and antidiabetic activity^{7,8}. Recently, there has been much research on the anticancer effects of *Caulerpa sp.* The anticancer potential of *Caulerpa sp.* was found in several studies using various cancer cell lines where *Caulerpa sp.* showed anticancer activities such as antiproliferative, proapoptosis, cell cycle arrest, and suppression of cell migration⁹.

Recent experiments show the development of the method using *Caulerpa racemosa* as an anticancer agent^{8,10–13}.

However, the information about *Caulerpa racemosa* as an anticancer agent is still limited. Therefore, this review of the literature aims to find out the potency of Sea grapes extract (*Caulerpa racemosa*) extract in the treatment of cancer and its mechanisms.

METHODS

This review of the literature was carried out by searching for studies concerning the potential of *Caulerpa racemosa* as an anticancer agent. The literature search utilized PUBMED, ScienceDirect, and SpringerLink using boolean operators with the main keywords "*Caulerpa racemosa*", "Cancer", and "Management" (**Table 1**). The PICOS framework is used as the basis for inclusion criteria consisting of 1) Problem; cancer cells, 2) Intervention; *Caulerpa racemosa* extract, 3) Comparison; no comparison, 4) Outcome; inhibition rate of proliferation, cycle, metastasis, and apoptosis cancer cells, 5) study design; an experimental study.

Table 1. Keywords for Literature Review

Main keywords	Additional keywords
<i>Caulerpa racemosa</i>	<i>Caulerpa sp.</i>
Cancer	<ul style="list-style-type: none"> - Anticancer - Antiproliferative - Antitumor - Tumor - Cytotoxic activity - Metastasis - Apoptosis

As a result, 633 journals were identified (**Figure 1**). Furthermore, many journals were excluded by title screening due to topic discrepancy with inclusion criteria, not discussing the potential of *C. racemosa* as an anticancer (n = 436). Then, many journals are not following the desired study design so they are excluded such as review articles and books (n = 163). Journals that are inaccessible due to paid or on-progress research are also excluded (n=24). Several subsequent studies were excluded due to duplication of studies (n = 2). Through all processes, we obtained a total of 8 studies that were included for data extraction and analysis. We only obtained a few journals, considering the novelty of the type of algae *C. racemosa* and the lack of research on its potential as an anti-cancer.

RESULTS

All studies included in this review are summarized in **Table 2**, and the mechanisms are depicted in **Figure 2** in the Appendix. The experiments were done by testing *C. racemosa* extracts

Figure 1. Flow Diagram of Search Algorithm

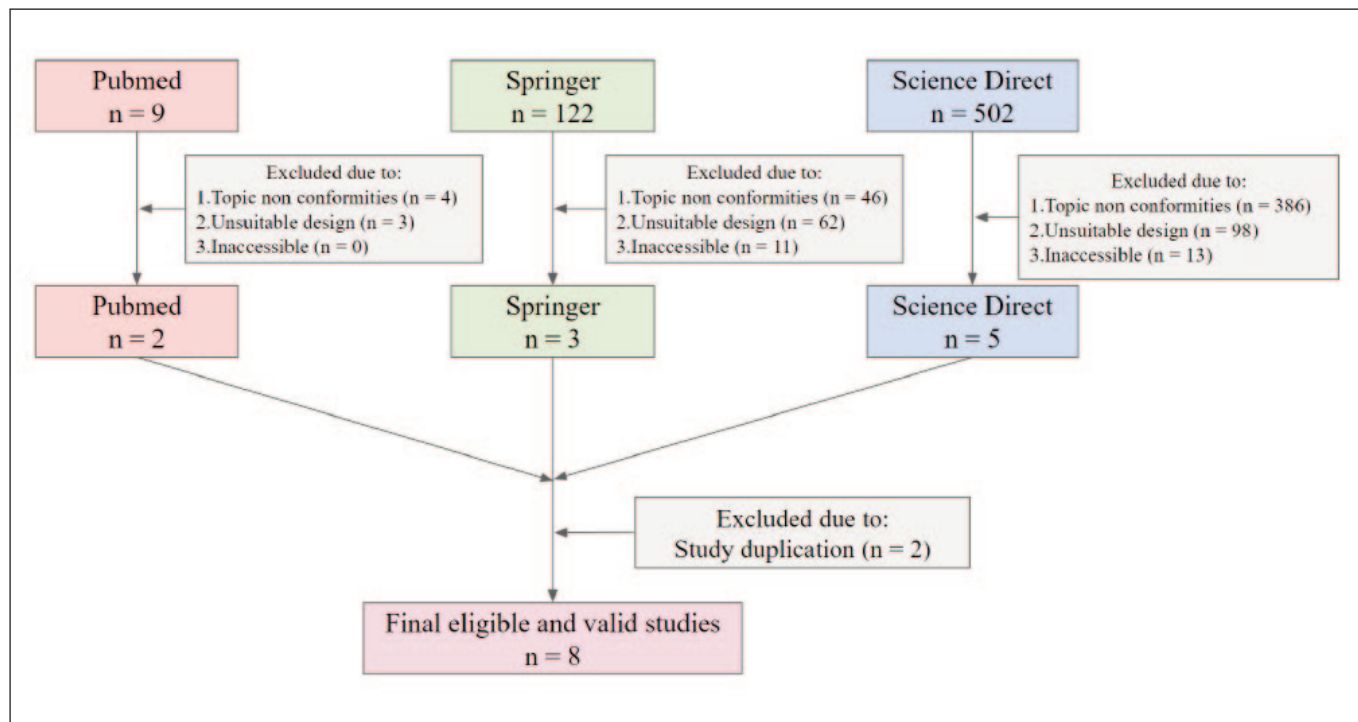


Figure 2. Summary of *C. racemosa* Anti-cancer Mechanism

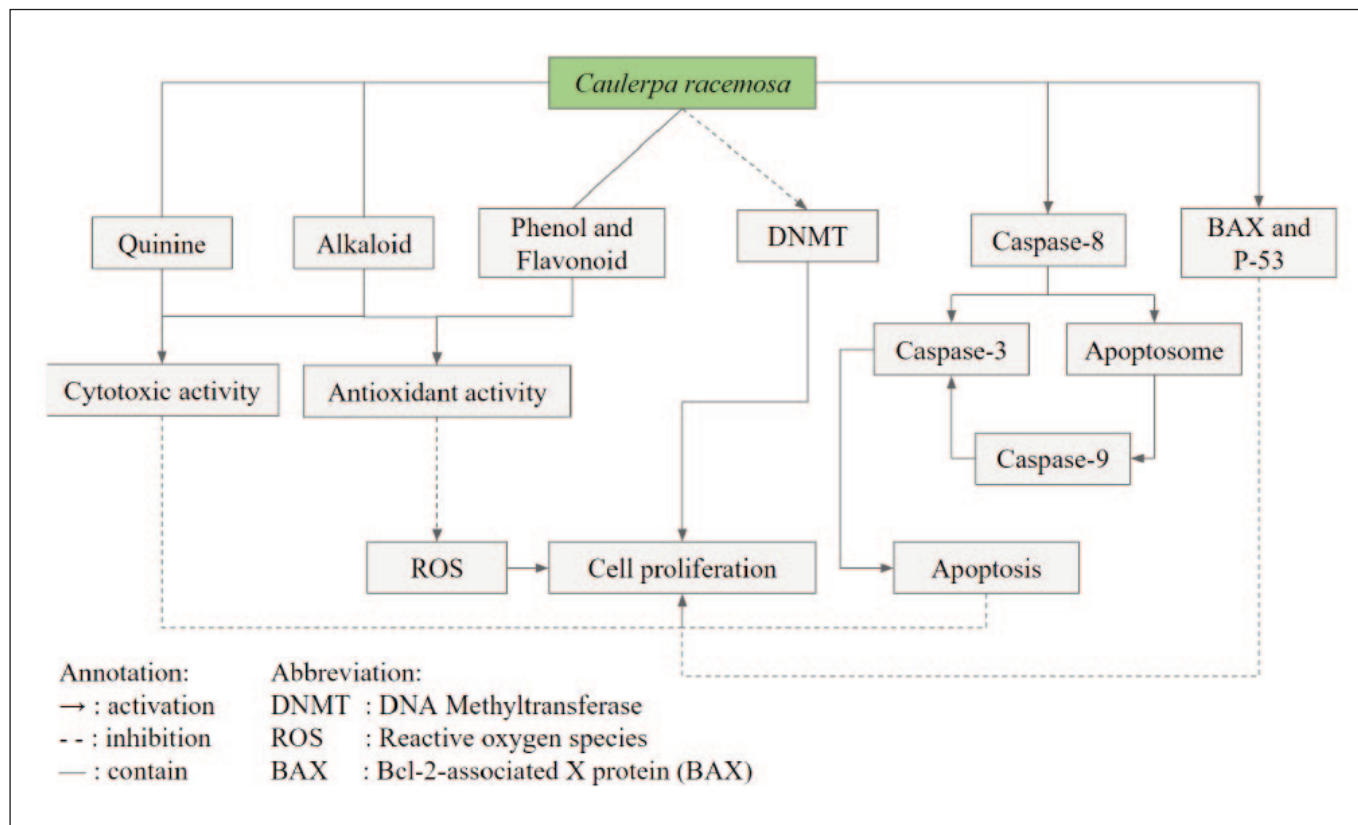


Table 2. Summary of Studies

No.	Authors (Years)	Active Compound	Target Cells	Effect	Measurement
1	Tanna et al., 2020	Five tropical sea grapes <i>C. racemosa</i> , <i>C. scalpelliformis</i> , <i>G. indica</i> , <i>S. linearifolium</i> , <i>S. asperum</i>	HeLa and Huh-7 cells	<p>[Anti-proliferative activities] After 24 hours, the results showed that <i>Caulerpa racemosa</i> had the maximum effective activity with a lower extract concentration compared to the others type of sea grapes (EC50 was 130±30 and 23±1 µg ml⁻¹ for HeLa and Huh-7), respectively.</p> <p>[Apoptosis analysis] The analysis with the EC50 dose of each sea grapes extract showed high fluorescence due to higher binding of the dye to DNA compared to control cell lines. Results revealed that <i>C. racemosa</i> and other types of sea grapes had good activity to induce apoptosis.</p> <p>[ROS inhibitory activity] The greatest ROS inhibition activity was obtained by <i>C. racemosa</i> extract (about 56%) and also ROS scavenging activity was observed whereas about 42% were detected with <i>C. racemosa</i>, followed by other types of sea grapes extracts on Huh-7 and HeLa cells, respectively.</p> <p>[Transcript expression cancer gene analysis] The cancer gene was expressed in HeLa and Huh-7 cells after being treated with an EC50 dose of sea grapes extracts.</p> <ul style="list-style-type: none"> - The expression of CDC2 gene which encoding cell division cycle protein 2 was downregulated in both cells and about two-fold maximum downregulated was observed in HeLa cells treated with <i>C. racemosa</i> extract. - BAX gene encoding apoptosis of BCL2 (B-cell lymphoma 2) was upregulated after treated with an EC50 dose of sea grape extracts. - Caspase-3 genes that were treated with <i>C. racemosa</i> extract in Huh-7 cells were also upregulated. - Tumor suppressor gene (p53 gene) was downregulated in all treatments except HeLa cells treated with <i>C. racemosa</i> extracts. - DNMT2 gene which might induce tumor progression was downregulated in Huh-7 cells after being treated with EC50 sea grape extracts. 	<p>Anti-proliferative activities; MTT assay</p> <p>Apoptosis analysis; DNA-specific fluorescent dye</p> <p>Transcript expression cancer gene analysis; Quantitative Real-time PCR</p>
2	Cavas et al., 2006	<i>C. racemosa</i> extract and Caulerpenyne (CPN)	Neuroblastoma cell lines (SHSY5Y & Kelly)	<p>[Cytotoxic activities] The IC50 values of <i>C. racemosa</i> extract in the Kelly and SHSY5Y cells were 0.49 ± 0.31 & 2.05 ± 0.68 g wet alga/methanol. The XTT assay was repeated after 48 hours and showed that 3 g wet alga/methanol extract, the survival rate of Kelly and SHSY5Y cell were 63.78 ± 3.11% & 52.3 ± 2.93%. The IC50 values of Caulerpenyne (CPN) in the Kelly and SHSY5Y cell were 4.74 ± 0.67 & 5.44 ± 0.34 µM CPN. After 48 hours, the XTT assay was repeated and showed the effect of 4 µM CPN on the percentage of cell survival of Kelly and SHSY5Y were 51.74 ± 1.08% & 69.89 ± 0.90%.</p> <p>[Apoptotic Effect] The percentage rate of apoptosis of Kelly and SHSY5Y cells in 0,01 and 1 µM CPN conditions were (78.00 ± 2.74 & 69.40 ± 3.78) and (49.40 ± 3.78 & 39.60 ± 6.19), respectively. Nuclear morphology also showed the percentages of apoptotic cells of Kelly and SHSY5Y in 1 µM CPN for 48 h were 70% and 80%.</p>	<p>Cytotoxic activities; XTT assay</p> <p>Apoptotic Effect; Hoechst nuclear staining & fluorescence microscopy</p>

Table 2 continuation. Summary of Studies

No.	Authors (Years)	Active Compound	Target Cells	Effect	Measurement
3	Kurt et al., 2009	<i>C. racemosa</i> dry and wet extract	Neuroblastoma cell lines (NA2B)	[Cytotoxic activities] The toxic effects of <i>C. racemosa</i> extracts and independent of methanol extract was 15 µl/ml. During incubation with dry extracts, the doses of 50, 35, 25 µl/ml doses could cause cell death, whereas dry extract of <i>C. racemosa</i> had dose-dependent toxic effects as long as the doses were higher than 15 µl/ml. However, the toxic effects of the dry extract of <i>C. racemosa</i> at 15 µl/ml was higher than the wet extract at all doses. Significant neurite inhibition with wet extracts was shown only at doses 15 and 10 µl/ml, when the dose was lowered, the cells look healthy and had longer neurites.	Cytotoxic activities; MTT assay
4	Chia et al., 2015	<i>P. tetrastromatica</i> , <i>C. racemosa</i> and <i>T. ornata</i> extract	MCF-7 cells	<p>All three seaweed possess potent anticancer activities. <i>C. racemosa</i> extract particularly shows activities as follow:</p> <p>[Cytotoxic activities] Methanol extract of <i>C. racemosa</i> had the lowest IC50 value (60.0 ± 1.47 µg/mL) among five different extracts that have been tested while the partially purified fraction of it exhibited a lower IC50 value (18.0 ± 1.43 µg/mL) compared with its whole extract. Highest TPC was found in methanol-extracted <i>C. racemosa</i>, which is 19.8 ± 2.01 mg GAE/g. TFC was found to be highest in EA-extracted <i>C. racemosa</i>, with 16.0 0.52 mg of catechin equivalents/g dry weight.</p> <p>[Antioxidant activities] The IC50 for DPPH radical scavenging activity (90.00 0 g/mL; hexane extract), nitric oxide scavenging activity (38.33 2.89g/mL; DCM extract), and hydroxyl radical scavenging activity (20.00 + 0 g/mL; hexane and acetone extract) of <i>C. racemosa</i> extract were determined.</p> <p>[Antioxidant enzymes] <i>C. racemosa</i> contains antioxidant enzymes such as SOD, CAT, and GR, the activity of which is reduced over time.</p> <p>[Alkaloid] LC-MS profiling for peaks at m/z 371 revealed the presence of pseudopelletierine eluted at 12.7 min in a partially purified fraction of <i>C. racemosa</i>.</p> <p>[Caspase activity] Partially purified fraction of <i>C. racemosa</i> showed caspase-3 activity (peaked at 24 h treatment by 2.4 folds), caspase-8 activity (peaked at 8 h treatment by 1.2 folds), and caspase-9 activity (peaked at 8 h treatment by 1.2 folds).</p> <p>[DNA fragmentation] When examined using a UV transilluminator, extract-treated MCF-7 cells showed DNA destruction, whereas untreated control cells showed intact genomic DNA.</p>	<p>[Cytotoxic activities] MTT assay and TLC</p> <p>[TPC and TFC] TPC assay (TPC) and aluminum chloride colorimetric method (TFC)</p> <p>[Antioxidant activities] DPPH assay, superoxide anion scavenging assay, nitric oxide scavenging assay, and site-specific hydroxyl radical scavenging assay</p> <p>[Antioxidant enzymes] SOD assay, CAT assay, GR assay</p> <p>[Alkaloid measuring] LC-MS profiling</p> <p>[Caspase activities] Caspase-3, caspase-8, and caspase-9 assay</p> <p>[DNA fragmentation] Electrophoresis with agarose cells</p> <p>[Statistical analysis] One-way ANOVA</p>

Table 2 continuation. Summary of Studies

No.	Authors (Years)	Active Compound	Target Cells	Effect	Measurement
5	Xiao et al., 2018	Racemosin B derivatives from <i>C. racemosa</i>	MCF-7 cells and MDA-MB-231 cells	Some alkylamide compounds inhibited the proliferation of human breast cancer cell lines in a moderate to significant way. In the MDA-MB-231 cell line, they induced G2/M cell cycle arrest and apoptosis. Among 26 derivatives synthesized, compound 25 with the lowest IC50 ($1.06 \pm 0.10 \mu\text{M}$ for MDA-MB-231 cells and $2.00 \pm 0.24 \mu\text{M}$ for MCF-7 cells) triggered cell death by suppressing autophagy. This was supported by inhibition of autophagic flux and accumulation of autophagy protein 1 light chain 3, LC3II, and p62.	Biological activities; MTT assay (cell proliferation), apoptosis and cell cycle analysis, western blotting, immunofluorescence staining Statistical analysis; Two-tailed Students t-test
6	Yang et al., 2015	16 compounds extracted from <i>C. racemosa</i>	Human promyelocytic leukemia cells (HL-60), human lung adenocarcinoma cells (A-549)	[Cytotoxicity assay] With IC50 values of 49.3, 67.4, and 45.0 μM , respectively, the samples containing compound racemobutenolids A (1) and B (2), 7, and 9 demonstrated moderate cytotoxicity against HL-60, whereas compound 9 showed mild cytotoxicity with an IC50 value of 85.3 μM to A-549 cell lines. The other compounds were inert (IC50 >100 μM)	Cytotoxicity assay; Sulforhodamine B (SRB) method
7	Tanna et al., 2018	Sea grapes extract	HeLa and Huh-7 cells	[Total antioxidant, scavenging, and reducing activities] The most potent antioxidant activity (more than 50%) possessed by <i>C. racemosa</i> var. <i>macrophysa</i> (CRM) extracts (400 μg) and <i>C. racemosa</i> var. <i>macrophysa</i> (CRM), with approximately 75% inhibition was observed. Highest scavenging ability as high as 70% was obtained from 1000 μg CRM extract. Another species, <i>C. scalpelliformis</i> (CS), and <i>C. racemosa</i> var. <i>occidentalis</i> (CRO) showed approximately 66% and 60% scavenging activity, respectively. The reducing capability was positively correlated with the concentration of seaweed extract. The highest reducing capacity was observed from CRM extract (85%), followed by CS, UF, and CRC with a reducing capability up to 78%, 72%, and 62%, respectively. <i>Caulerpa</i> spp., especially CRM, showed maximum biochemical activities due to the lowest half-maximal effective concentration (EC50) and the maximum capacity of antioxidants, scavenging, and reducing activities. [Total Phenolic and Flavonoid Content] CRC, CRM, and CS exhibited the highest phenolic amount followed by remaining seaweed species. These results were similar to flavonoid content, except for the highest amount of flavonoid which was found in CS. Overall, phenolic and flavonoid amounts were increasing concomitantly with antioxidant activity. However, CRC showed negative correlation between flavonoid/phenolic content and antioxidant activities, which was statistically non-significant. [Anti-proliferative Activity] CRM and CRO extracts showed about 55% and 38% anti-proliferative activity on Huh-7 cells, and 30% proliferation inhibition on HeLa cells, respectively.	Total antioxidant, scavenging, and reducing activities; comparing free radical scavenging ability with standard Trolox and represented as percent inhibition Radical scavenging activity: spectrophotometry read at 517 nm Reducing power: spectrophotometry read at 700 nm Total Phenolic and Flavonoid Content; total Phenolic spectrophotometry read at 760 nm and 510 nm for phenolic and flavonoid, respectively Antiproliferative activity; treatment of HeLa and Huh-7 cell lines with seaweed extract, bioactivity was measured using MTT based In vitro Toxicology Assay Kit

Table 2 continuation. Summary of Studies

No.	Authors (Years)	Active Compound	Target Cells	Effect	Measurement
8	Manikan et al., 2019	Gold nanoparticle from <i>C. racemosa</i> extracts (Cr@AuNPs)	HT-29 cells	<p>[Cell viability assay] Cr@AuNPs showed dose-related activities against HT-29 cells lines. The decline of the viability of the HT-29 cells was observed after 24 hours treatment of the cells with Cr@AuNPs. About 50% of (IC₅₀) of cell viability was noticed at 20.84 µg/ml.</p> <p>[Cytomorphological analysis] Treatment of the HT-29 cells with Cr@AuNPs at IC₅₀ or above results in morphological change which is typical for apoptosis, such as cell shrinkage, oxidative stress, cytoplasmic condensation while untreated control cells showed the morphology of a healthy cell.</p> <p>[Acridine orange/ethidium bromide staining] Treatment of the HT-29 cells with Cr@AuNPs at the IC₅₀ or above showed yellow and reddish or orange fluorescence, which represented an early and late cells apoptosis, compared to the untreated control cells which showed green fluorescence color.</p> <p>[DNA Fragmentation Assay] The cells treated with IC₅₀ (20.84 µg/ml) and a higher concentration (100 µg/ml) showed significant apoptotic fragmentation. Therefore, these findings suggested that changes in HT-29 cells related to apoptosis were caused by Cr@AuNP.</p>	MTT assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]

against various types of cell lines, including HeLa cells, Huh-7 cells, neuroblastoma cell lines, MCF-7 cells, MDA-MB-231 cells, human promyelocytic leukemia cells, human lung adenocarcinoma cells, and HT-29 cells. The potencies identified from *C. racemosa* include antiproliferative activities, apoptotic activities, antioxidants (such as 1,1-diphenyl-2-picrylhydrazyl [DPPH], superoxide dismutase [SOD], catalase [CAT], and glutathione reductase [GR], phenolics, and flavonoids), regulation of cancer-related gene (CDC2, DNMTs, BAX, p53, and Caspase-3), and cytotoxic activities. We also found some bioactive compounds derived from *C. racemosa*, including caulerpin, racemosin B, and Caulerpenyne with its anti-cancer potencies.

Antiproliferative activity

Several studies showed antiproliferative activity of *C. racemosa* against various cell lines, mainly HeLa and Huh-7 cells, and also other cell lines, including breast cancer (MCF7 and MDA-MB-231), leukemia (HEL and K562), and melanoma cell lines (WM9 and WM35)^{13,24,25}. This is true, especially for *C. racemosa* var *macrophysa* which exhibited a maximum growth inhibition effect with a lower half-maximum effective concentration (EC₅₀) for HeLa and Huh-7 cell lines than for the other seaweed species^{13,24,25}. The study by Xiao et al. (2018) provided evidence of growth inhibition

through the accumulation of autolysosomes and blockage of autophagy flux for breast cancer, leukemia, and melanoma cell lines²⁵. Interestingly, the level of cell inhibition (IC₅₀) of caulerpenyne (CPN) extracted from *C. racemosa* was found to be similar to that of the etoposide. Etoposide is an anti-cancer drug that works with antiproliferative effects (Cavas et al., 2006)¹⁰.

Apoptotic activity

The study conducted by Chia et al. evaluated the presence of DNA degradation in three kinds of extract-treated MCF-7 cells, including the extract of *C. racemosa*. Based on this study, DNA degradation was found in all three types of extract-treated MCF-7 cells, while untreated control cells exhibited intact genomic DNA when observed with a UV transilluminator²⁸. These results are in line with the study of Tana et al. which showed that the EC₅₀ dose of Sea grapes extract of *C. racemosa* caused significant cell apoptosis (HeLa and Huh-7) marked by high DNA fluorescence²⁴. Another study by Cavas et al. that also used fluorescence microscopy found that SHSY5Y and Kelly cells that were treated with caulerpenyne extracted from *C. racemosa* experienced apoptotic cell percentages up to 70 - 80%¹⁰.

Antioxidant activity

The antioxidant activities of the extract of *C. racemosa* are proven by several studies^{10,13,24,28}. According to a study conducted by Chia *et al.*, the extract of *C. racemosa* demonstrated IC₅₀ for the radical scavenging activity of DPPH; (90.00 ± 0 g/mL; hexane extract), nitric oxide scavenging activity (38.33 ± 2.89 g/mL; DCM extract), and the hydroxyl radical scavenging activity (20.00 ± 0 g/mL; hexane and acetone extract). This study also looked at antioxidant enzymes found in the extract of *C. racemosa*, including superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR). Since phenolic compounds are well known for their antioxidant properties, Chia *et al.* investigated the total phenolic content (TPC) and total flavonoid content (TFC) of three types of extracted seaweed, including extract of *P. tetrastrumatica*, *C. racemosa*, and *T. ornata*²⁸. The extract of *C. racemosa* had strong TPC, up to 19.8 ± 2.01 mg GAE/g in the extraction of methanol, and TFC, up to 16.0 ± 0.52 mg of catechin equivalents / g of dried weight in the extraction of EA²⁸. Tanna *et al.* (2018) also stated that the main metabolites of *C. racemosa*, phenolics, and flavonoids are considered antioxidants. Their concentration is positively correlated with antioxidant activity¹³. Another antioxidant activity is the highest inhibition of reactive oxygen species (ROS) by *C. racemosa* extract compared to other types of Sea grapes up to 56% and also the maximum ROS scavenging activity of up to 42%. Targeting the ROS signaling pathway involved in cancer progression is a new potential strategy to prevent cancer²⁴.

Analysis of cancer gene transcription expression

The study by Tanna *et al.* evaluated the expression of 5 cancer genes in HeLa and Huh-7 cells using qRT-PCR after being treated with EC₅₀ Sea grapes extract. The extract of *C. racemosa* affected the expression of the CDC2 gene, which was significantly down-regulated. This gene is highly expressed in osteosarcoma tumor cells and its expression can promote the process of tumor development²⁴. Chia *et al.* discovered caspase-3 activity in a partially purified fraction of *C. racemosa* (peaked at 24 h of treatment by 2.4 folds). It also indicated caspase-8 (1.2 folds peak at 8 h treatment) and caspase-9 (1.2 folds peak at 8 h treatment) activity, both of which are initiators of apoptosis²⁸.

Cytotoxic Activity

Several investigations have shown that the *C. racemosa* extract has in vitro cytotoxic action against specific cancer cell lines^{8,10,11,28}. A study by Chia *et al.* showed that methanol-extracted *C. racemosa* has cytotoxic activity against MCF-7 cells. *C. racemosa* revealed the lowest IC₅₀ value (60.0 ± 1.47 µg/mL) among five different extracts tested (acetone, ethyl acetate [EA], dichloromethane [DCM] and hexane) while the partially purified fraction form exhibited a lower IC₅₀ value

(18.0 ± 1.43 µg/mL) compared to its entire extract²⁸. A study by Kurt compared the neurotoxicity effects between dry and wet extracts of *C. racemosa* in terms of neurite inhibition. Based on the results, it is known that the effective dose that causes significant neurite inhibition is a 15 µl/ml dose in both dry and wet extracts of *C. racemosa*. However, the dry extract of *C. racemosa* presented a more toxic effect compared to the wet extract¹¹. In line with the study by Cavas on neuroblastoma cell lines, which showed that caulerpenyne has a lower IC₅₀ value than the *C. racemosa* extract, which could be explained by its interaction that can convert caulerpenyne to oxytoxin-1 and 2 derivatives¹⁰. Researchers are also interested in alkaloids because of their physiological activity, which includes anticancer action and can lead to cytotoxic activity. The work of Chia *et al.* revealed the liquid chromatography-mass spectrometry (LC-MS) profiling of *C. racemosa* extract to investigate the alkaloids within it²⁸.

DISCUSSION

Individual cells can develop cancer in a variety of ways, both genetically and epigenetically. Normal cells that have damaged their cellular genome or have altered gene expression may experience disruptions in their normal functioning. Viruses, mutagenic chemicals, and radiation are among the factors that might cause it¹⁴.

Inflammation is the body's reaction to tissue damage, which can be caused by physical injury, ischemia injury, infection, toxins, or other types of trauma. The inflammatory response of the body causes cellular alterations and immunological responses, which result in the healing of damaged tissue and cell proliferation at the injury site. If the cause of inflammation remains or specific control mechanisms responsible for shutting down the process fail, inflammation can become chronic¹⁶. When these inflammatory reactions become persistent, they can lead to cell mutation and proliferation, which can often lead to the development of cancer. Chronic inflammation has been associated with cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis, among other stages of cancer. Cancer patients face an enormous difficulty known as the "perfect storm." This is true at the beginning of cancer, but it is even more critical as the disease progresses. Various signaling pathways play a critical role in bringing epigenetic modifications to the cell's surface and turning on internal mutations. As a result, it is critical to address inflammatory causes whenever possible^{16,17}.

Reactive oxygen species (ROS) are important regulators of carcinogenesis and the cellular response to anticancer treatments¹⁸. Free radicals, on the other hand, can be harmful to the body in excessive concentrations, causing damage to all major components of cells, including DNA, proteins, and cell membranes. Cell-free radical damage, particularly DNA damage, can play a role in the development of cancer and other

health problems^{15,18,19}. ROS, for example, can serve as both an upstream activator of p53 and a downstream effector of p53 that mediates apoptosis¹⁸.

Antioxidants are substances that interact with free radicals and neutralize them, preventing them from harming. Increased levels of exogenous antioxidants have been shown in laboratory and animal experiments to inhibit free radical damage forms that have been linked to cancer formation. As a result, researchers investigated whether consuming dietary antioxidant supplements can help humans reduce their chance of getting or dying from cancer²⁰.

Flavonoid and phenolic is one of the main antioxidants featured in various seaweeds, including *C. racemosa*²⁸. Flavonoid has radical scavenging properties due to presences of phenolic hydroxyl groups on its structure. Besides the ability of scavenging free radicals, flavonoid has been proven for xanthine oxidase inhibition properties, one of the enzymes that are responsible for free radicals formation, mainly after ischemia reperfusion^{13,28}. Meanwhile phenolic compounds possess the radical scavenging and metal chelating properties which later stabilize the free radicals and prevent them from reacting with the cell components^{22,23}. One of the examples is phlorotannins, which possess multiple phenolic groups as antioxidant properties that help algae in preventing oxidative stress from their surroundings²⁸.

Superoxide dismutase (SOD), glutathione reductase (GR) and catalase (CAT) are the enzymes involved in free radical defense in cells requiring aerobic metabolisms²¹. SOD converts superoxide into H₂O₂, which is later converted to H₂O by involving the role of GR for scavenging GSSH and providing GSH. Meanwhile CAT is able to convert H₂O₂ into H₂O directly²¹.

Autophagy is essential for the survival of cancer cells to regulate proliferation, progression, and response to anticancer drugs¹⁷. Some anticancer drugs showed effectiveness in targeting cancer lysosomes and thus inhibiting autophagy^{25,26}. Supported by Barbier et al. who showed that CPN and the two anticancer drugs fotemustine and cisplatin had similar levels of IC₅₀ value. All these studies indicate that *C. racemosa* extract may be a potential anticancer therapeutic agent²⁷.

Cellular genes, namely proto-oncogenes, influence the proliferation and maturation of these cells by encoding proteins that engage in signal transduction pathways relevant to cell proliferation regulation. Changes in gene structure or expression can cause a proto-oncogene's function to be disrupted, resulting in aberrant cell proliferation and, eventually, tumor development. The proto-oncogene becomes an oncogene after this change takes place. Different types of cancer generally have different types of oncogenes^{2,14}. In this review, two studies by Chia et al, and Tanna et al, discussed the role of protein or genetic expression induced by sea grape interven-

tion in cancer cells. The protein or genetic roles mentioned include CDC2, DNMT, BAX, tumor suppressor gene (p53), and Caspase 3,8,9^{24,28}. Related mechanisms such as DNMT (DNA methyltransferases) which normally function in embryonic development, cell differentiation, and gene transcription are known to play a role in tumorigenesis²⁴. Disturbances in DNMT can affect tumor development such as hypermethylated suppressor genes and genomic instability, thereby increasing tumor malignancy and worsening patient prognosis. The role of sea grapes here as a DNMT inhibitor is similar to the available class of anticancer drugs²⁴. The way it works is by reactivation of the genes aberrantly silenced via methylation by DNMTs and restoration to normal function. Furthermore, the mechanism of apoptosis induction by sea grapes in cancer cells is through the executor protein, Caspase-3²⁸. Caspase-3 protein can trigger apoptosis when exposed to internal and external stimuli such as carcinogens or ROS. Initially this stimulus will activate the proteins caspase-8 and caspase-9 until finally the activation of caspase-3 which triggers apoptosis through the release of cytochrome c from the mitochondria which will fill the cells and burst²⁸.

Another pro-apoptotic gene that plays a role is p53 (tumor suppressor genes). The p53 protein is strong in the induction of apoptosis by damaging cell DNA thereby controlling cancer cell progression^{24,28}. The p53 protein is involved in the defense mechanism of cancer cell development by retarding cell growth, DNA activation for cell repair, and activation of apoptosis if cell damage is irreparable. Studies prove that sea grapes are capable of synthesis of high amount of p53 protein which inhibits the growth of cancer cells (anti-proliferative activity)²⁴. Meanwhile, another process that was mentioned was that the CDC2 gene which encodes cell division cycle protein 2 appeared to be decreasing²⁸. The CDC2 gene is needed in the initiation process of cell division, both mitotic S-phase and M-phase which is crucial in order to proceed to the DNA synthesis stage and meiosis II. Sea grapes in fact also have antimitotic activity as seen from the lowest CDC2 expression in the *Caulerpa racemosa* intervention in HeLa cancer cells²⁸.

Cytotoxic effect of *C. racemosa* extract have also been elaborated in this review. Toxicities cause cell degeneration, cell death, inhibition of proliferation, and apoptosis. Study conducted by Cavas et al. (2006) and Kurt et al. (2009) revealed that the toxicity comes from the substance caulerpenyne, which is later converted to oxytoxin-1 and oxytoxin-2. Oxytoxin-2 has been proved for its interaction with nucleophilic amino acids¹⁰. Another explanation of the cytotoxic effect of *C. racemosa* is due to active phytochemicals compounds, including quinine and alkaloids. Quinine possesses cytotoxic effects by interfering with the DNA and RNA replication, as well as mitochondrial pathway²⁸. Quinine cytotoxic effects also come from the formation of radicals, such as peroxide, superoxide and hydroxyl. Meanwhile, the alkaloids re-

sult in cytotoxicities by blocking the microtubule spindle formation which is required for cell division²⁸.

Caulerpa racemosa or Sea grapes is commonly served as food and cultivation³². It is classified as green algae mainly distributed in tropical and subtropical coastal regions. Sea grapes are well known for their various physiological and biological activities, including anticancer potential through many kinds of mechanisms, as explained above³². This finding is also supported by the study conducted by Kung et al. (2021). This study proved that caulerpin, an alkaloid rich in *C. racemosa*, naturally plays a role in chemical protection against herbivores, is considered to possess the ability to suppress inflammation, tumor angiogenesis, and food pathogen growth^{33,36,40}.

Seaweeds have been used widely as a functional food in several countries, for example, Japan, Thailand, Fiji, and the Philippines. In Japan, several edible seaweeds are used as a food additive and green seaweeds (*Caulerpa sp.*) are used as umi budo cuisine. Meanwhile, in Thailand, it is commonly used as spice sauce. In Indonesia, *C. racemosa* is commonly used as fresh vegetables (such as salad), although it's still limited to the coastal population or fishermen. It is also known by people in Japan, China, and Korea as a beauty food, and either consumed in a fresh form or processed into soup. Recent studies have also attempted to composite seaweed into a more standardized functional food form to increase its health benefits. According to an in vivo study conducted by Permatasari et al., *C. racemosa* kombucha drink has potential as a functional anti-aging food, by reducing blood glucose and cholesterol level. Since *C. racemosa* contains antioxidant properties, the favorable nutritional benefit of including *C. racemosa* in a biscuit and kombucha drink is also supported^{34,35,37,38,39}. However, there are some challenges to develop seaweeds into functional food, including the identification of seaweeds, as well as standardization of the product, because seaweeds grown in different locations will produce different nutrient compositions.

There is currently no proof of any negative effects of *C. racemosa* consumption on people. However, animal experiments have been done to test the safety of *C. racemosa*¹². *C. racemosa* has been proven for its safety profile through the in vivo study conducted by Manikandakhrisnan et al. (2019) using *Artemia* nauplii species¹². Administration of *C. racemosa* extract did not affect the survival rate of *Artemia* nauplii compared to the control, which showed that *C. racemosa* has little or no known toxicity for living organisms. Another in vivo study using mice samples showed that *C. racemosa* did not cause samples death or organ damage compared to controls²⁶.

From various studies on the effect of *C. racemosa* on cancer cells, it is known that *C. racemosa* has different mechanisms to work as an anti-cancer. However, *C. racemosa* was generally shown to have antioxidant, cytotoxic, and antipro-

liferative effects in every cancer cell. Further investigation is required to identify which cancer kinds are most impacted by *C. racemosa*'s anticancer effect because not all forms of cancer cells have been taken into account in the study that has been conducted thus far.

We identified the strengths of this review because we have combined several experimental studies, both in vivo and in vitro, that explored the anticancer potential of *C. racemosa* against various cancer cells. Although many different mechanisms are identified, the experiments showed statistically significant effects in inhibiting or killing cancer cells. However, there are some weaknesses of this review, as our study did not include any clinical trials or human studies, due to the lack of data. As a result, we urge more research on the clinical application of *C. racemosa* products as cancer therapeutic agents, as well as their safety profile, formulation, optimal dose, and efficacy.

CONCLUSION AND PRACTICAL IMPLICATIONS

Sea grapes extract (*Caulerpa racemosa*) is potentially effective as an anticancer agent through antiproliferation mechanisms, apoptosis induction, cytotoxic, and antioxidant activity. The extract of *C. racemosa* has many potential benefits as an anticancer agent derived from herbs so that it can attract public consumption, and it's practical for long-term consumption. Further research and review are needed regarding the potential of *C. racemosa* extract as a cancer therapeutic agent.

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