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by Grace Sanger 2

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Green seaweed *Caulerpa racemosa* - Chemical constituents, cytotoxicity in breast cancer cells and molecular docking simulation

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ABSTRACT

Marine and terrestrial organisms are rich in chemical compounds with medicinal and pharmacological properties, including antitumor agents for chemoprevention. *Caulerpa racemosa*, a marine species, is a potential source of novel compounds with therapeutic agents for human cancer. This study aimed to determine the anticancer activity of *C. racemosa* extracts in breast cancer cells, identify compounds, and determine the mechanism using computational models. Seaweed (*C. racemosa*) was taken from North Sulawesi, Indonesia; Followed by authentication and identification according to the previously published protocol and extracted with three different solvent: hexane, ethyl acetate, and ethanol. *C. racemosa* were evaluated for cytotoxicity against breast cancer MCF-7 cells using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Antioxidant activities were assessed based on free radical scavenging (2,2-diphenyl-1-picrylhydrazyl (DPPH)) and ferric-reducing antioxidant power (FRAP) assays. The phytochemical constituents were identified with a liquid chromatography-electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-QTOF-MS) system. The interaction of the identified compounds with human epidermal growth factor 2 (HER2) protein was achieved by molecular docking with PyRx-vina application and protein-ligand complex visualization. The hexane extract exhibited the highest cytotoxicity against breast cancer cells, followed by the ethyl acetate and the ethanol extract (IC_{50} 23.7 ± 2.0; 66.7 ± 5.8 and 182.7 ± 14.3 μg/mL). The best antioxidant sample for DPPH was the ethyl acetate extract (IC_{50} 21.5 ± 2.0 μg/mL) while the hexane extract was the most active in the FRAP value (14.5 ± 1.3 mg gallic acid equivalent/g). Data from LC-ESI-QTOF-MS allowed the identification of 21 compounds. The molecular docking study showed that 12 of the compounds could prevent tumors in breast cancer by acting as inhibitors of the anti-apoptotic HER2 protein. *C. racemosa* has potential in the chemoprevention of breast cancer through its radical scavenging capacity and inhibition of the HER2 protein. More studies are needed to investigate the efficacy of extracts in different models.

1. Introduction

Cancer is known to cause an increase in oxidative stress in tissue, leading to the production of oxidatively changed nucleotides in DNA [1]. While most of this damage is repaired, a significant mutation can still occur due to the byproducts of DNA base oxidation. Chemoprevention using natural remedies has emerged as a promising approach for preventing, delaying, or reversing the progression of cancer [2]. Several

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studies have demonstrated that seaweed antioxidants can reduce the incidence of cancer in women and may even lower the risk of breast cancer recurrence or death. *Caulerpa racemosa* extract has been shown to improve their oxidative state and suppress *in vitro* cancer [3]. Therefore, the development of new anticancer drugs with minimal toxicity and high efficacy is crucial.

The use of secondary metabolites or phytochemicals from marine resources to mitigate non-transmissible diseases is an ongoing area of research. Amongst the conditions, the rate of cancer has grown faster and now accounts for almost two-third of deaths globally [4,5]). Statistics on cancer showed that in 2020 worldwide, there were an estimated 19.3 million new cases diagnosed while there were about 10 million deaths [5]. Breast cancer was at the first position with 2.3 million (11.7%) new cases and 685 thousand (6.9%) deaths [5]. Diversity treatment, first recommended by Ref. [6], has been comprehensively reviewed as a prod f healing cancer. Presently available synthetic chemotherapeutics are associated with side effects, hence the continuous need for novel synthetic to treat tumors but also natural products to act an chemoprotective [7]. Chemoprevention focuses on a strategy to control cancer by suppressing tumor promoters or by preventing the promoting of tumor cells.

Marine organisms like seaweed or algae are considered sources of bioactive compounds (e.g., antioxidant, ant-inflammation, antitumor) due to their immense biodiversity, safety, and stressors in the quatic environment that allow the production of unique compounds. Seaweed is one of the greatest functional foods and medicinal herbs owing to its excellent biosynthesd polysaccharides, proteins, lipids, and polyphenols [8,9]. Amo their medical properties, seaweed extracts have been demonstrated to reduce the prolif n of human colon cancer cells and lung carcinoma cells [10,11]; to reduce oxidative stress and inflammation *in vivo* in animal models [12]; and to reduce tumor in animal models [13].

Several compounds found in seaweeds have also demonstrated activities that are relevant to cancer prevention. Fucoidan, a sulfated polysaccharide displayed antitumor and antimeta ic effects in cell lines and mice [14,15] while a sterol's rich fraction inhibited the growth of 4T1 cancer cell lines and tumor in 4T1 cell-implanted mice [16]. The polyphenol, diecol induced apoptosis of ovarian cancer cells by modulating the function or expression of caspase-3 activation, cytochrome *c*, and Bcl-2 [17]. Other molecules in seaweeds with antioxidant and antitumor properties include proteins and carotenoids [18–20]. Dietary consumption of seaweed, especially *Caulerpa racemosa*, has been shown to improve cardiometabolic syndrome [21].

Tumor development is complex, and the etiology includes exposure to environmental toxicants but also to dysregulation of metabolisms that occurs with age both of which are linked to the increased production of reactive species and downregulation of the antioxidant mechanism. Phytochemicals are then believed to protect against chronic diseases partly by acting as antioxidants [22,23]. Without sufficient antioxidants, excess reactive oxygen species (ROS) and nitrogen species damage cellular components and nucleotides that can lead for example to mutagenesis and cancer [24]. The ingestion of seaweed for breast cancer therapy has been traced back to the prehistoric Egyptians [25].

Diverse cell lines are used by the scientific community to study tumors and the effectiveness of new agents, plant extracts, and purifying compounds [26,27]. The advantages of cell lines include simple control, unrestricted self-replication, and cellular consistency [28]. MCF-7 cells established in 1973 by Michigan Cancer Foundation (MCF) remain the ultimate standards for breast cancer studies on account of their accurate sensitivity through the expression of estrogen receptors [29]. The mechanism of antitumor compounds is diverse, but a common target is human Epidermal Growth Factor Receptor 2 (HER2) whose amplification or overexpression in breast cancer occurs in about 15–30% of cases [30]. HER2 is then a common valuable target for the development of oncogenic drugs. The alteration of HER-2 (inhibition or reduce expression) is consequently crucial for healthy cell growth and the prevention of mutagenesis [31]. The stimulation of the tyrosine kinase area is a predominant tumorigenesis process and has been related to oncogenic action in breast cancer, and many other types. Tyrosine kinase growth inhibitors and monoclonal antibodies that direct the ligand-binding areas of HER2 to prevent dimerization and, subsequently, the intracellular signaling cascade are two primary treatments for breast cancer [32,33]. Previous works on seaweeds (e.g. *Turbinaria decurrens*, *Gracilaria salicornia*, *Laurencia tronoi, and Halimeda macroloba*) demonstrated the antioxidant and apoptotic properties of their extracts in cervical cells [34]. Another work demonstrated the capacity of an aqueous extract of *C. racemosa* to reduce oxidative stress and inflammation, enhance endothelial function, and reduce gut dysbiosis in metabolic distress mice [21]. The current study builds on our previous works and aimed to determine the cytotoxicity of different extracts of *C. racemosa* against breast cancer cells (MCF-7) coupled with antioxidant activities and characterization of phytochemical compounds. Furthermore, the interaction of the identified compounds with human epidermal growth factor 2 (HER2) protein by molecular docking was investigated as possible mechanisms of action.

2. Materials and methods

2.1. Materials

Analytical-grade solvents and reagents were utilized for the extraction, characterization, cytotoxicity, and antioxidant tests. Penicillin-streptomycin (Invitrog USA), Phosphate Buffer Saline (PBS), Trypsin (Gibco, USA), Dulbecco's Modified Eagle Medium (D-MEM) (Gibco, USA), Fetal Bovine Serum (FBS) (Hy e, USA), DMSO, and Dulbecco's Modified Eagle Medium (D-MEM) 3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals 1,1-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide, $FeCl_3 6H_2O$, formic acid solution, and acetonitrile were also from Sigma-Aldrich (St. Louis, MO, USA). Deionized water (18.2 M/cm) was from a Millipore Milli-Q Gradient Water Purification System (Darmstadt, Germany) (Milli-Q, Darmstadt, Germany). Instruments used were centrifuge (Tommy, Japan), CO_2 incubator (Binder, Germany), flask T25 (Corning, USA), *Inverted Microscope* (Nikon, Japan), *Improved* Neubauer, *Tissue Culture microplate (96 wells)* (ing, USA), and Liquid Chromatography Mass Spectroscopy (LCMS) (Agilent Technologies, Santa Clara, CA, USA).

2.2. Preparation of seaweed Caulerpa racemosa extract

Seaweed (*C. racemosa*) was taken from North Sulawesi, Indonesia; Followed by authentication and id fication according to the previously published protocol [21,35]. The seaweed was washed with tap water to emit salt and debris, then dried breezily. Each of the of sample (500 g) was extracted with 1 L of hexane, 1 L of ethyl acetate, and 1 L of ethanol (EtOH), sequentially using the maceration method 3 times. The solution of each extract w filtered with Whatman paper number 1, then dried with a vacuum rotat vaporator at 45 °C. All extracts were diluted in dimethylsulfoxide at 20 mg/mL and kept at 4 °C until further use.

2.3. Cytotoxicity analysis via MTT assay

Cytotoxicity of *C. racemosa* extract was studied using the MTT test as refe d to prior study [36]. American Type Culture Collection provided the MCF-7 (breast cancer) cells (ATCC; Manassas, VA, USA). The test is based on the reduction of MTT to a purple formazan product in living cells by mitochondrial dehydrogenases. Briefly, MCF-7 cells (ATCC 22) were seeded in culture medium (RPMI 1640) ch has been supplemented with Fetal Bovine Serum (FBS) 5% and Penicillin 100 U/mL and Streptomycin 100 μg/mL). The cells were seeded into 96-well plates at a density of 5×10^3 cells/well incubated at 37 °C, 5% CO_2 for 24 h.

Then the extracts (0–500 μg/mL) were added and incubated for 72 h. After incubation, the diluted sample was added with MTT (5 mg/mL in PBS buffer) amount 10 μL to each well, then incubated for 4 h in humidified CO_2 5% at 37 °C. Blue-purple formazan crystal sediments were diluted in ethanol 100 μL. The microplate reader model 550 measures the absorbance at 590 nm (Bio-Rad, USA). Here is how the inhibition rate is determined: 100% inhibition rate (%) = 1-(absorbance of the treatment group minus absorbance of the control group). The value IC_{50} was calculated from the equation of the dose-response curve [37].

2.4. Morphological analysis

Applying Phase Contrast Microscopy Changes in morphology were monitored to reveal the influence of the extracts in MCF-7. The cells were subjected to various concentrations (0–500 μg/mL) of extracts afterward incubation for 4 h. The pictures were observed using an inverted phase contrast microscope at 100 × magnification.

2.5. Antioxidant activity via DPPH radical scavenging assay

The capacity to scavenge the free radical DPPH of the extract was measured based on the quenching of stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) by antioxidant compounds in seaweed extract according to previous literature [38]. Briefly, 2 mL DPPH 0.93 μM in methanol mixed with 0.5 mL extract in various concentrations. Then the solution was mixed with a vortex and kept in a dark condition for 30 min at room temperature. Absorbance was determined at 517 nm. BHT was used as a control. The antioxidant property was expressed as free radical scavenging activity of the sample compared to the control.

2.6. Ferric-reducing antioxidant power (FRAP)

The FRAP assay was determined, following the (Kumar et al., 2011) procedure [114]. One mL of potassium phosphate buffer 0.2 M (pH 6.6) and 1 mL potassium ferricyanide K_3 [Fe (CN)$_6$] (1%) with added with 1 mL extract of varying dilution. The reaction mixture was incubated at 50 °C for 20 min, after which 1 mL TCA 10% was added and then centrifuged at 3000 rpm for 10 min. The upper layer of solution (1 mL mixed with 1 mL distillate water and 0.5 mL $FeCl_3 6H_2O$ 0.1%. The absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated greater reduction potential. Butylated hydroxytoluene (BHT) served as a positive control. The FRAP value was expressed as uM Fe2+/mg extract.

2.7. Phytochemical screening via LC-MS analysis

LC-MS fingerprint and molecular weight were accomplished by adapting the method of [39]. After diluting each *C. racemosa* extract with ethanol, it was filtered through a 0.45-μm millipore filter. The LC system was Acquity UPLC connected to an ESI and QTOF mass spectrometer, Xevo® G2-S both from Waters Corp. (Milford, MA, USA). Solvents 0.1% formic acid in acetonitrile (B) and 5 mM aqueous ammonium formic (A) while the t flow rate of the solvents was at 0.4 mL/min. The separation was performed on a HSS C18 1.8 m, 2.1 × 150 mm column with a linear gradient of solvent A was from 95% to 5% in 15 min. The QTOF was operated in positive electrospray ionization mode, source voltage +2.9 kV, gas atomizer 50 L/h, source temperature of 41 °C, and capillary temperature 120 °C. Full scan data were collected from *m/z* 100 to 500. By matching the *m/z* from the chromatogram with an exact mass from the database of natural products in the Caulerpa genus, the metabolites in each sample extract were identified.

2.8. In-silico study

2.8.1. Receptor and ligand preparation

Protein Data Bank (PDB) (http://www.rcsb.org) was used to acquire the three-dimensional protein structure of HERS-2, which functions as a receptor. The receptor was set up using the Biovia Discovery Studio program [40]. The goal of this preparation is to create a structure free of water molecules, making molecular docking simpler to accomplish. The receptor has polar hydrogen atoms linked to it. File ligand was accessed from the PubChem database and converted using the Open Babel application [41]. File ligand which was not in the PubChem database was optimized for its three-dimensional structure using the HyperChem application. Optimation used the computation method of semi-empiric AM1 (*Austin Model* 1) and calculate single point and optimate geometry.

2.8.2. Molecular docking and visualization

A docking study was performed using the PyRx-vina application [42]. Receptor and Ligands preparation optimized used the Open Babel option in the PyRx. The target protein was prepared using Biovia Discovery Studio 2020. The results were also assayed using BIOVIA *Discovery Studio Visualizer* 2020 [40]. The arrangement *grid-box* for area molecular docking is around active residue Met 801 with dimension size X: 30 Å, Y: 40 Å, and Z: 30 Å.

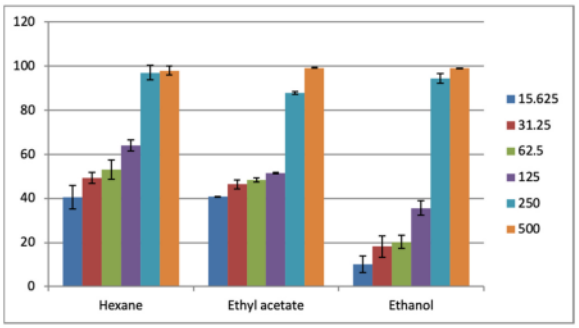


Fig. 1. The cytotoxic (antiproliferative) activities of hexane, ethyl acetate, and ethanol extracts (15.625, 31.25, 62.5, 125, 250, and 500 μg/g) of *C. racemosa* against MCF-7 cells.

2.9. Data management and statistical analysis

Turkey *t*-test and analysis of variance (ANOVA) were employed using IBM® SPSS® Statistics 26.0 software (SPSS Inc, Chicago-USA) macbook version. Statistics consider differences between the values with $p < 0.01$ to be significant.

3. Result and discussion

3.1. Cytotoxicity activity

The cytotoxicity of compounds to cells is often evaluated by determining the capacity of viable mitochondria to metabolize the MTT salt into the formazan product via a reduction reaction catalyzed by succinate dehydrogenase [43]. The data output in the presence of bioactive compounds is interpreted as being cytotoxicity, protective, or neutral. In the case of cancer cells, cytotoxicity is equivalent to antiproliferative or chemoprotective.

Data on the effect of various concentrations of hexane, ethyl acetate, and ethanol extracts of *C. racemosa* on MCF-7 breast cancer viability are presented in Fig. 1. The antiproliferative capacity expressed as percentages of the control cells were dose-dependent with each extract. The reduction of MCF-7 cell growth increased with increasing concentrations of the extract. At the lower concentration range (15.625–125 μg/mL) the hexane extract had the highest antiproliferative activity by inhibiting the growth of MCF-7 cells by 40.5–64.0% compared to 40.7–51.6% for the ethyl acetate extract. The ethanol extract

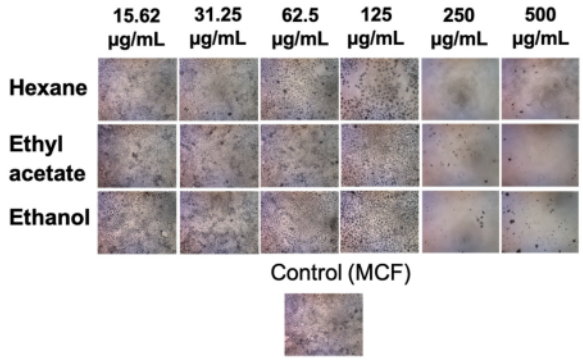


Fig. 2. Morphological changes of MCF-7 cells under an inverted microscope after treatment with hexane, ethyl acetate, and ethanol with different concentrations (15.625, 31.25, 62.5, 125, 250, and 500 µg/mL, for 4 h). The cells were examined using light microscopy after incubation. The data provided a good illustration of redundant testing. 100 times magnification.

antiproliferative activity was however 1.8 to 4-fold less.

The data when converted to IC_{50} translated into anticancer activity of *C. racemosa* against breast cancer cells (MCF-7) being IC_{50} 23.7 ± 2.1 µg/mL for hexane, 66.7 ± 5.8 µg/mL for ethyl acetate and 182.7 ± 14.3 µg/mL for ethanol extract.

Previous work on two seaweed species *U. lactuca* and *E. cotton* reported cytotoxicity values (IC_{50}) of hexane extracts in MCF-7 cells to be 45.1 ± 1.7 and 69.3 ± 1.2 µg/mL, respectively [44]. The hexane extract from C. *racemosa* then appeared to be about 1.9–2.9-fold more chemoprotective. In contrast, as reported by Ref. [45]; ethanol extract of green seaweed *Chaetomorpha* sp inhibited the growth of MDA-MB-231 breast cancer cell line with an IC_{50} of 225.18 ± 0.61 µg/mL while the methanol extract of *Sargassum muticum* exhibited cytotoxicity activity against MCF-7 cells (IC_{50} 20 ± 0.1 µg/mL) and MDA-MB-231 (IC_{50} 55 ± 0.2 µg/mL) [46]. Seaweed extracts then can inhibit the growth of cancer cells at different degrees based on cell types and the nature of marine species. Active compounds may interact with a special receptor of the tumor cells or specific molecules produced by the cells to trigger apoptosis [47]. Possible mechanisms include the prevention of nucleic acid production, DNA damage, and the blocking of RNA or protein synthesis [8]. Additionally, active molecules can inhibit the amplification or overexpression of human epidermal growth factor receptor 2 (HER2) which is upregulated in 15–30% of breast cancers.

3.2. Morphology of cells

Data on the morphology of MCF-7 cells in the presence of investigated *C. racemosa* extracts during a 4 h treatment are presented in Fig. 2. The changes were monitored using a phase contrast inverted microscope. Results showed that the alteration in morphology is concentrations dependent with 15.625 µg/mL having the least effects and 500 µg/mL the greatest. Cells exposed to higher concentrations (250 and 500 µg/mL) of extracts lost their usual morphology which was accompanied by reduced proliferations. The death of the MCF-7 tumor cells at 250 µg/mL hexane and ethanol extract was close to 100% (i.e. 99.8%).

Induction of apoptosis is a beneficial method in cancer treatments. In apoptotic cells, numerous cellular and molecular biological characteristics are shown such as cell reduction, DNA disintegrations, and activation of the caspase cascade [48]. In assessment under an electron microscope, distinctive apoptotic characteristics were obtained, consisting of cell membrane blebbing, microvillus loss or decrease, and detached apoptotic bodies. Moreover, examined MCF-7 cells were detected under Transmission Electron Microscopy (TEM), and the reduction of cells and condensation of chromosomes were revealed.

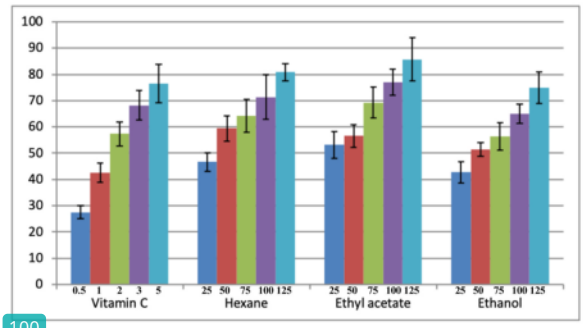


Fig. 3. Radical scavenging activity against DPPH of Vit. C (0.5, 1, 2, 3, and 5 µg/mL), hexane, ethyl acetate, and ethanol extract (25, 50, 75,100, and 125 µg/mL) of *C. racemosa*.

Previous work reported the cytotoxicity of a methanolic extract of *C. racemosa* on HL60 (Human promyelocytic leukemia) cells and showed using fluorescent microscopy and flow cytometric that the mechanism was linked to DNA damage and enlarged sub-G1 DNA [49]. Aqueous extracts of other seaweed species (*U. fasiata, C. antennina*) also cause apoptosis of MCF-7 cells by causing changes in morphology that resulted in their shrinking [50]. Factors that can initiate the formation of tumors include elevated oxidative stress often caused by a greater concentration of reactive oxygen species (ROS). Antioxidant molecules are then considered useful for the chemoprevention of cancer and other conditions associated with elevated ROS [51]. The role of ROS in cancer includes the oxidation of nucleotides such as guanine which can lead to mispair with thymine rather than normal pairing with cytosine, and modification of gene expression via epigenetic mechanisms. The deleterious effects of ROS justify the need to assess the antioxidant of *C. racemosa* extracts.

3.3. Antioxidant DPPH

The scavenging activity against DPPH radical exhibited by the ethyl acetate extract was the highest, followed by hexane and ethanol extracts with respective IC_{50} of 21.5 ± 2.0, 29.9 ± 2.5, and 49.0 ± 4.0 µg/mL. The IC_{50} values were calculated using data from Fig. 3. Seaweed molecules such as polyphenols can quench DPPH radicals through its known mechanism by donating an electron or hydrogen atom [52].

Compare to other works on seaweeds, the activities obtained is this study is lower than ethanol (IC_{50} 9.41 ± 0.54 mg/mL) and aqueous (IC_{50} 15.44 ± 0.98 mg/mL) extracts of *Chaetomorpha* sp. [45]. In the case of *Chaetomorpha* sp., the authors attributed the higher activity of the ethanol extract (relative to water) to the greater amount of total flavonoid and total phenolic compounds. Polyphenolic compounds obtained mainly in plants and seaweeds are natural-derived antioxidants [53]. The existence of hydrophilic polyphenolic compounds for example phlorotannins, which are bipolar in nature could perform as a main antioxidant, which helps the algae struggle with oxidative stress [54]. Many hydrophilic polyphenolics in seaweed compounds are powerful antioxidant components, for example, phlorotannins [55], epicatechin [56], and epigallocatechin gallate [57]. Furthermore, DPPH inhibition (antioxidant activity) of metabolite compounds has been directly linked with antiproliferative activity against cancer cells [45].

With dose-dependent cytotoxicity in estrogen-dependent MCF-7 and estrogen-independent MB-MDA-231 human breast cancer cells (IC_{50} values of 20 and 42 g/mL, respectively), a polyphenol-rich extract from *Eucheuma cottonii* has been shown to have anticancer potential. By promoting apoptosis, improving antioxidative conditions, and suppressing the production of endogenous estrogen, polyphenols demonstrated anti-cancer efficacy [58].

Table 1
The activity of DPPH free radical scavenging and FRAP rate reduction.

Samples	DPPH (IC_{50})	FRAP Value (uM Fe^{2+}/mg extract)
Hexane extract	29.91 ± 2.88	14.52 ± 1.28
Ethyl acetate extract	21.52 ± 1.87	12.36 ± 1.25
Ethanol extract	49.03 ± 3.69	9.73 ± 0.85
BHT	2.50 ± 0.11	0.65 ± 0.04

Numerous studies have revealed the anti-cancer properties of seaweeds and the numerous compounds derived from them [3,35,59,60]. These properties have been demonstrated to be effective through a variety of mechanisms, including the inhibition of cancer cell growth, invasiveness, and metastasis as well as the induction of apoptosis in cancer cells. Several of the constituents have been industrialized into drugs for cancer medication [61]. In current years, natural compounds extracted from marine algae have been recommended as valuable in preventing tumor development, adhesion, invasion, and migration [62].

3.4. Ferric-reducing antioxidant power (FRAP)

Ferric-reducing antioxidant power of *C. racemosa* is presented in Table 1. All extracts have high reducing power, so they have the capacity to donate electrons because of their content of bioactive compounds. Therefore, *C. racemosa* could act as an antioxidant primer and secondary antioxidant. Hexane extract (14.5255 ± 1.289 μM Fe^{2+}/mg extract) exhibited the highest ferric-reducing antioxidant power, followed by ethyl acetate (12.3675 ± 1.257) and ethanol (9.7322 ± 0.857 μM Fe^{2+}/mg extract). Prior et al., 2005 reported that *Caulerpa* has antioxidant activity through the transfer of a single electron and the transfer of hydrogen atoms [75].

Considered potential free radical scavengers because of their reducing properties as hydrogen or electron-donating agents, the barbituric acid derivative, BA-5, significantly inhibited HCC and HCC-SR cell viability in a dose- and time-dependent manner. Therefore, compound BA-5 was selected for further experiments. Western blot data revealed that BA-5 treatment decreased the phosphorylation of AKT/p70s6k without affecting the AMPK pathway and increased cleaved PARP and cleaved caspase-7 in both HCC and HCC-SR cells [26,33]. Caulerpin inhibited oxidative phosphorylation (OXPHOS) and facilitated an early intervention of the mitochondrial function, via inhibiting mitochondrial complex I, accompanied by the dissipation of mitochondrial membrane potential and a surge of reactive oxygen species (ROS) generation [3]. Moreover, in response to the increment in AMP/ATP ratio, the energy sensor AMP-activated protein kinase (AMPK) was activated by caulerpin treatment in a calcium/calmodulin-dependent protein kinase 2 (CaMKK2)-dependent manner. Long-term activation of AMPK by caulerpin damaged the glycolysis and glucose metabolism in colorectal cells, finally causing cell death [26]. C25, racemosin B derivative, exerts its anti-cancer activity through inhibition of autophagy, but the underlying mechanism remained unknown. C25 acts as a lysosomotropic agent to induce lysosomal membrane permeabilization and inhibit autophagic flux, resulting in cathepsin release and cell death, polyphenolics (antioxidants) [63]. There is a substantial association between total phenolic content and antioxidant activity (ABTS, DPPH), which is consistent with studies by Refs. [64,65]; and [66]. [67] reports also found that the aqueous extract of seaweed contained higher antioxidant activity than ethanolic extract. Our results revealed that hexane and ethyl extracts are high in antioxidant activity in DPPH and FRAP assay. The variation in the polyphenolic component pattern may be the cause of the enhanced antioxidant activity of hexane extract. Moreover, hexane extract's antioxidant effects were only due to phenolic components. Other hydrophilic substances, such as peptides, fucoidan, and Maillard reaction products, were responsible for the actions [67]. The fact that the ethyl acetate of *H. macroloba*, while having a high phenolic concentration, had relatively little antioxidant action also helped to explain this phenomenon [68]. [69] reported the highest content of TPC in *H. macroloba* (186.80 ± 15.54 μg GAE (gallic acid equivalent) g-1 extract). *T. decurens* exhibited high radical DPPH scavenging activity (IC_{50} 10.01 ± 0.54 mg/mL) and a high value of FRAP of 28.52 ± 1.46 μg GAE g-1 extract.

The low TPC (Total Phenolic Content) of *T. decurrens* suggests that carrageenan may be to blame for its anticancer properties. Moreover, *L. tronoi* demonstrated higher TPC and antioxidant activity in DPPH and FRAP but lower cervical anticancer activity than *T. decurrens* [34]. Because of their antioxidant qualities, seaweed polyphenol compounds can serve as chemopreventive agents. At times of stress, oxidative substances may contribute to the beginning, promoting, and growth of cancer [70,71]. The polyphenol content of seaweed is necessary for the antiproliferative effect [72]. Unfortunately, little is known about the chemical makeup of the bioactive substances found in seaweed, and until recently, there was little and difficult research documentation on these substances.

In the lipid peroxidation pathway, high-polarity molecules could not interact favorably with non- or low-polar free radical molecules [73]. The capacity of the seaweed to do electron donation shows a high reducing power. For the active ingredients in seaweed to function as main and secondary antioxidants, it also showed strong hydrogen atom donating capacity from ABTS and DPPH experiments [83]. Seaweed showed widespread antioxidant properties through both the hydrogen atom transfer system and the single electron transfer mechanism [75].

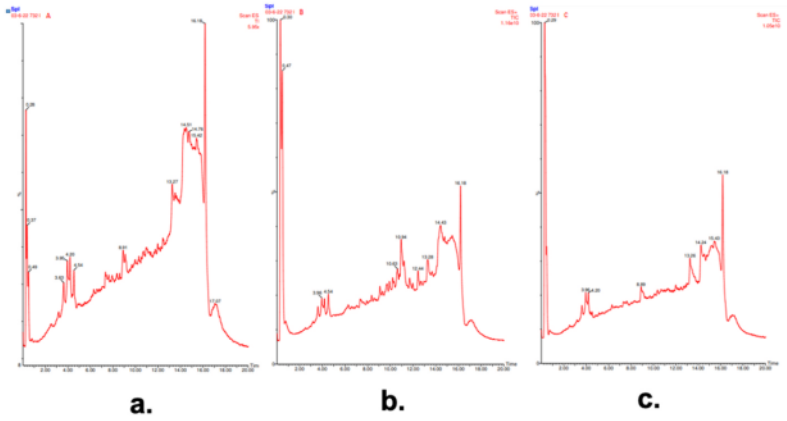
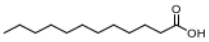
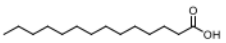
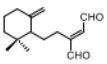
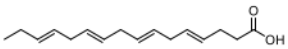
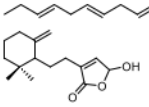
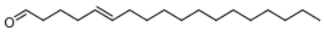
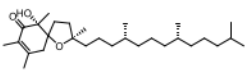
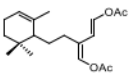
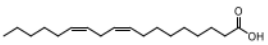
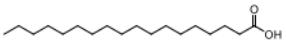
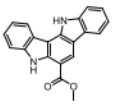
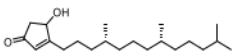
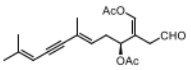
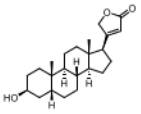
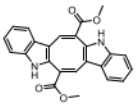
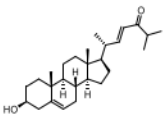
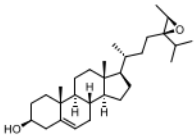
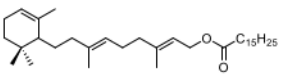


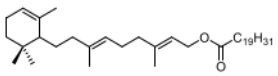
Fig. 4. LC-MS chromatogram of a) hexane, b) ethyl acetate, and c) ethanol extract of *C. racemosa*.

Table 2
Observed compounds in hexane, ethyl acetate, and ethanolic extract of *C. racemosa* via LC-ESI-QTOF-MS screening.

No	Proposed Compounds	Molecular formula	RT (min)	Molecule weight	Observed (*m/z*)	Structural molecule
1.	Barbituric acid	$C_4H_4N_2O_3$	0.30	128	128.3181	
2.	Lauric acid	$C_{12}H_{24}O_2$	17.07	200.1776	200.4260	
3.	Myristic acid	$C_{14}H_{28}O_2$	3.95	228.2089	229.0142	
4.	Sesquiterpene (2-(2-(2,2-dimethyl-6 methylenecyclohexyl) ethyl)fumaraldehyde) $C_{15}H_{22}O_2$	$C_{15}H_{22}O_2$	4.54	234.1620	233.6164	
5.	Hexadeca-4,7,10,13-tetraenoic acid	$C_{16}H_{24}O_2$	10.63	248.1776	248.1768	
6.	Sesquiterpene (3-(2-(2,2-dimethyl-6-methylenecyclohexyl) ethyl)-5-hydroxyfuran-2(5H)-one)	$C_{15}H_{22}O_3$	10.63	250.1559	249.6250	
7.	5-Octadecenal	$C_{18}H_{34}O$	0.28	266.261	265.4956	
8.	(2R,6R)-6-hydroxy-3,7,9-tetramethyl-2-((4R,8R)-4,8,12-trimethyltridecyl)-1-oxaspiro[4.5]dec-8-en-7-one.	$C_{29}H_{52}O_3$	0.28	448.318	448.2999	
9.	Sesquiterpene ((1Z,3E)-2-(2-(2,6,6-trimethylcyclohex-2-en-1-yl)ethyl)buta-1,3-diene-1,4-diyl diacetate)	$C_{19}H_{28}O_4$	12.44	320.1882	320.7102	
10.	Linoleic acid	$C_{18}H_{32}O_2$	12.44	280.2402	280.7452	
11.	Stearic acid	$C_{18}H_{36}O_2$	14.24	284.2715	284.8854	
12.	Racemosins B	$C_{20}H_{14}N_2O_2$	13.26	314.1055	313.8666	
13.	4-hydroxy-3-((4R,8R)-4,8,12-trimethyltridecyl)cyclopent-2-en-1-one	$C_{21}H_{38}O_2$	4.20	324.2664	322.8041	
14.	Sesquiterpene ((S,1Z,5E)-6,10-dimethyl-2-(2-oxoethyl) undeca-1,5,9-trien-7-yne-1,3-diyl diacetate))	$C_{19}H_{24}O_5$	3.96	332.1624	331.8073	
15.	1-tricosanol	$C_{23}H_{48}O$	3.96	340.3705	340.8466	
16.	Digitoxigenin	$C_{23}H_{34}O_3$	3.96	358.2508	358.8564	
17.	Caulerpin	$C_{24}H_{18}N_2O4$	4.20	398.1267	397.4289	
18.	Oxygenated sterols 1	$C_{27}H_{42}O_2$	8.91	398.3185	398.7400	
19.	Oxygenated sterols 2	$C_{29}H_{48}O_2$	5.67	428.3654	427.9972	
20	Diterpene ((2E,6E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-2-en-1-yl)nona-2,6-dien-1-yl icosa-2,4,6,8,10,12,14,16,18-nonaynoate compound with dihydrogen (1:14))	$C_{36}H_{58}O_2$	14.24	522.4437	523.3590	

(continued on next page)

Table 2 (*continued*)

No	Proposed Compounds	Molecular formula	RT (min)	Molecule weight	Observed (*m/z*)	Structural molecule
21	Diterpene ((2E,6E)-3,7-dimethyl-9-(2,6,6-trimethylcyclohex-2-en-1-yl)nona-2,6-dien-1-yl hexadeca-2,4,6,8,10,12,14-heptaynoate compound with dihydrogen (1:11))	$C_{40}H_{64}O_2$	14.51	576.4906	576. 7671	

RT: Retention Time (Minutes).

Oxidative stress and the prevalence of cancer are strongly correlated. Exogenous antioxidant therapy may lessen the chance of developing cancer by preventing free radical generation and damage to DNA and proteins, according to several in-vivo and in-vitro studies which showed that the methanol extract of *Sargassum ilicifolium* with TPC 55.95 ± 4.33 mg GAE/100 g extract and antioxidant activity using the FRAP method of 37.05 mmol Fe^{2+}/100 g dried extract can stop the growth of cervical cancer (HeLa cells) and breast cancer MCF-7 cells, whose IC_{50} values were 45 ± 0.9 and 37 ± 0.2 g/mL, respectively after 72 h of treatment [46,76,77]. Phlorotannins and their derivatives work principal functions as anticancer metabolites, performing in numerous cancer properties such as proliferative modulator, cell cycle, metastasis, endurance to cell death, prevention angiogenesis, and growth suppressors [70,78].

A great way to stop tumor growth is to use naturally occurring antioxidants either alone or in conjunction with current treatment. Much research on the cytotoxic properties of different macroalgae and their ability to inhibit the proliferation of cancer cells has been published [77, 79,113]. Impacting factors of antioxidant seaweed in the inhibition of carcinogenesis such as activating the immune system [80,81], inhibiting angiogenesis [82], apoptosis induction, [83]. Inhibition raised cells in the G1 phase and generated terminal differentiation [9], and increased natural killer cell activity [80].

3.5. Characterization of compounds

The characterization of metabolites in extract is important to understand their mechanism of actions and standardization purposes. Liquid chromatography (LC) coupled to a mass spectrometer (MS) is the furthermost general method to investigate metabolites in plant extracts and other type of matrixes [84]. Fig. 4 shows the chromatogram of each of the three bioactive extracts of *C. racemosa*.

There are 13 high peaks in hexane extract in retention times 0.28–17.07 min; In Ethyl the acetate extract there are 10 detected peaks between 0.30 and 16.18 min; and 8 peaks (0.29–16.18 min) in the ethanol extract. The compounds were identified accordingly to their retention time (RT), molecular formula, molecular weight (MW), and mass to charge ratio (*m/z*) as presented in Table 2. In the present work, by matching experimental data with those in the database of natural products in the Caulerpa genus, 21 compounds were identified. They consist of fatty acids, sesquiterpenes (terpenes), sterols, alcohol, and aldehydes/ketones.

They identified molecules may have contributed to the activity of the extracts, specifically in the MCF-7 cells. A literature attributed the highest cytotoxicity in MCF-7 cells of an lipid extract of the red alga, *A. utricularis* in comparison to related species *C. racovitzae* and *G. confluens* to its high content polyunsaturated fatty acids including linoleic acid which is present in the extract of *C. racemosa* from this work [85].

Our research identified some sterol compounds in *C. racemosa* like digitoxigenin (compound 16 *m/z* 358.8564, 358.8224) in all three extracts; oxygenated sterols 1 (compound 18, *m/z* 398.7400 and 398.8090) in in hexane and ethanol; and oxygenated sterol 2 (compound 19, *m/z* 427.9972) in ethanol extract. These compounds are known to occur in *C. racemosa* [86]. Like fatty acids, sterols can be cytotoxicity to tumor cells as reported in a previous work where, a sterol-rcih fraction of the red alga, *P. dentata* fraction *in vitro* significantly inhibited growth and induced apoptosis in 4T1 cancer cells [16,87].

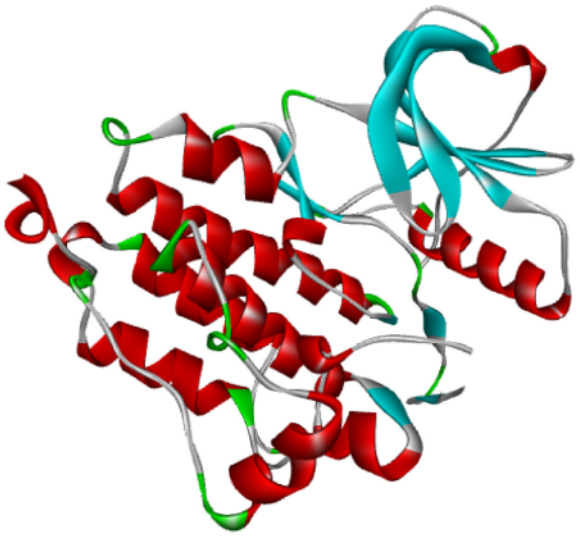


Fig. 5. Target protein breast cancer's three-dimensional structure HERS-2 (PDB ID 3PP0N).

Monoterpene, diterpene, and sesquiterpene are isolated from many *Caulerpa* genera [88]. Our study detected two hexane extract are detected diterpenes (compounds 20 and 21) in the hexane extract as well as the sesquiterpene (compound 4) in both hexane and ethyl acetate extracts. Additionally, the sesquiterpenes, compounds 9 and 14 were found in the ethyl acetate and ethanol extract, respectively. Terpenes like fatty acids and sterols can affect cell membrane integrity of tumor cells and contribute to the cytotoxicity of the extract.

The cytotoxicity activity of *C. racemosa* extracts against MCF-7 breast cancer cells was tested. The hexane extract was found to have the highest antiproliferative activity, with an IC_{50} of 23.7 ± 2.1 μg/mL, followed by the ethyl acetate extract with an IC_{50} of 66.7 ± 5.8 μg/mL, and the ethanol extract with an IC_{50} of 182.7 ± 14.3 μg/mL. These values indicate that the hexane extract was approximately 1.9–2.8-fold more chemoprotective than those of the two other extracts. Active compounds in seaweeds, such as polyphenols, can inhibit the growth of cancer cells at different degrees based on the cell type and the nature of the marine species. They may interact with a special receptor of the tumor cells or specific molecules produced by the cells to trigger apoptosis. As for the antioxidant activity, the scavenging activity against the DPPH radical exhibited by the ethyl acetate extract was found to be the highest, followed by the hexane and ethanol extracts. The IC_{50} values of these three extracts were 21.5 ± 2.0, 29.9 ± 2.5, and 49.0 ± 4.0 μg/mL, respectively.

Polyphenolic compounds obtained mainly in plants and seaweeds are natural-derived antioxidants, and their existence, such as phlorotannins, could act as a primary antioxidant that helps the cell reduce oxidative stress. Although Racemocin B, Caulerpin, and 5-Octadecenal were not tested in this study, they are known to possess cytotoxic and

Table 3
Binding free energy of selected compounds of hexane, ethyl acetate, and ethanol extract in *C. racemosa* with target protein breast cancer (HER-2/3PPO), retrieved by *website* PubChem.

Molecule Mass	PubChem CID	Ligands	Docking with *Receptor* 3PP0
			Binding free energy (Kcal/mol)
128.0000	6211	Barbituric acid	−6.1
200.1776	3893	Lauric acid	−6.3
228.2089	11005	Myristic acid	−6.7
248.1776	71358585	Hexadeca-4,7,10,13-tetraenoic acid	−7.9
266.2610	5365016	5-Octadecenal	−7.3
280.2402	5280450	Linoleic acid	−7.4
284.2715	5281	Stearic acid	−7.0
340.3705	18431	1-tricosanol	−7.2
358.2508	4369270	Digitoxigenin	−5.4
398.1267	5326018	Caulerpin	−7.2
543.5000	31703	Doxorubicin (Syntetic drug)	−12.5

Molecule Massa Molekul	Ligand	Docking with receptor 3PP0
		Binding free energy (Kcal/mol)
314.1055	Racemosins B	−11.3
234.1620	Sesquiterpene (2-(2,2-dimethyl-6-methylenecyclohexyl)ethyl)fumaraldehyde)	−5.7
250.1559	Sesquiterpene (3-(2-(2,2-dimethyl-6-methylenecyclohexyl)ethyl)-5-hydroxyfuran-2(5H)-one)	−2.3
324.2664	4-hydroxy-3-((4R,8R)-4,8,12-trimethyltridecyl)cyclopent-2-en-1-one	−1.1

Binding free energy ligands and receptors were Optimated by HyperChem.

antioxidant properties, and their presence in seaweeds may contribute to the observed activities. Racemocin B, in another study, was found to inhibit the proliferation of MCF-7 cells [3], while Caulerpin was found to inhibit tumor angiogenesis, and 5-Octadecenal was found to be one of the biologically important compounds in antiproliferative activity against various cancer cell lines [115]. Therefore, further studies on these compounds in *C. racemosa* extracts are recommended to better understand the mechanisms behind the observed cytotoxic and antioxidant activities.

3.6. Molecular docking

We have selected Human Epidermal Growth Factor 2 (HER-2) proteins as the target receptors for our docking approach because of their significance in breast cancer pathophysiology, especially in the regulation of proliferation, differentiation, and migration of breast cells [16,87]. Our goal was to determine the binding behavior in terms of docking scores here of phytochemicals characterized by *C. racemosa* against target receptors HER2 (PDB ID: 3PP0N). The target protein breast cancer type 1 susceptibility protein's 3D structure is shown in Fig. 5.

HER2, as a 185-kDa protein, has a transmembrane domain, an extracellular (ligand-binding) domain, and a cytoplasmic tyrosine kinase domain. Four domains make up the extracellular region's ligand-binding domain (I–IV). Upon ligand interaction, receptors get activated, which causes them to form homo- and/or heterodimers. Due to downstream signaling and ligand-stimulated tyrosine phosphorylation, the HER-2 and HER-3 heterodimer is regarded as a particularly powerful oncogenic component [89].

The molecular docking in our study used PyRx-vina application [42] results from energy binding, interaction ligand, and residue. Validation of molecular docking using RMSD value. A total of 21 phytocompounds were identified from–the analysis of the *C. racemosa*, in which 14 compounds were docked with breast cancer target protein. The binding affinity of the compounds ranges from −1,1–11.3 kcal/mol, respectively summarized in Table 3. Among these 3 compounds showed good binding affinity with the target protein. which are racemosins B; hexadeca-4,7,10,13-tetraenoic acid, and linoleic acid.

The first three most effective phytocompounds (compound identified in *C. racemosa*) and the synthetic drug doxorubicin's (2D and 3D) interactions with the target protein are shown in Figs. 6–9. Also, the modeling outcome with the best conformation was selected based on the optimized potential's lowest energy indication for effective structure prediction (sOPEP). For a substructure to engage with the target receptor and provide high affinity, this sOPEP energy defines the modeling structure of the substructure near its original form [90]. The chemical was then docked with the receptor protein. The strength of the binding affinity was evaluated using the binding-free energy (BFE) value, which shows how strong a reversible connection may form between two or more molecules [91]. This is because to trigger the immunological response, the drug candidate has to be able to engage effectively with the receptor [92]. The interaction resulted in a BFE value of racemosins B (−11.3 kcal/mol; Hexadeca-4,7,10,13-tetraenoic acid and (−7,9 kcal/mol and Linoleic acid (- 7.4 kcal/mol). Every time a ligand binds with a receptor, BFE is released because the total energy of the complex is reduced.

Moreover, any changes in the ligand's minimal energy to its bound conformation with the receptor are made up for by the release of BFE. At any time throughout the procedure, this transition may take place. As a

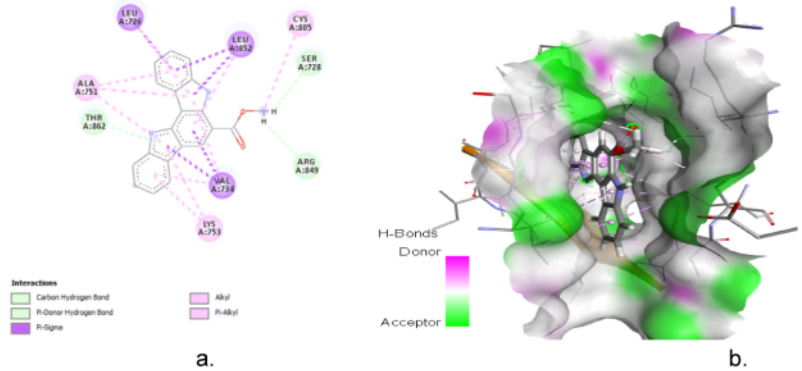


Fig. 6. a 2D interaction of phytocompound Racemosins B with the target protein (HERS-2).
b 3D interaction of phytocompound Racemosins B with the target protein (HERS-2).

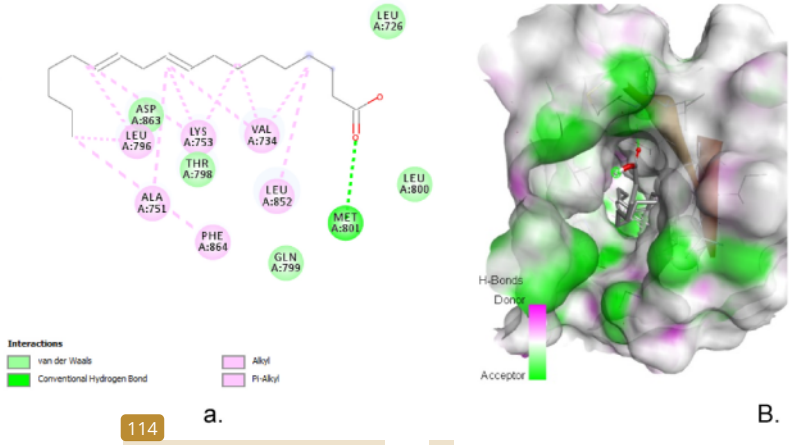


Fig. 7. a 2D interaction of phytocompound Hexadeca-4,7,10,13-tetraenoic acid with the target protein (HERS-2). b 3D interaction of phytocompound Hexadeca-4,7,10,13 tetraenoic acid with target protein (HERS-2).

result, the quantity of energy released result of a ligand binding to a certain receptor causes an increase in the affinity of that ligand for that receptor. The outcomes of the encounter with the lowest BFE value are the best [93]. It's better to be negative. With this BFE value, it is anticipated that the molecule serving as a candidate therapy would bind with the receptor extremely strongly, giving it great potential for preventing breast cancer. The affinity of protein-ligan is influenced by the interaction of noncovalent intermolecular between two molecules, such as hydrogen bonding, electrostatic interaction, hydrophobic, and Van der Waals [91,94].

The interaction receptor and ligan Racemosins B (Fig. 6) formed Pi-Alkyl and Alkyl binding, included in hydrophobic interaction. Interaction hydrophobic is the interaction characterized avoid water environments and tends to group inside globular structural proteins [95]. Hydrophobic interaction can form Pi-Sigma and Alkil/Pi-Alkil binding. Alkyl and Pi-alkyl binding were in residue LYS A 753; ALA A: 751 and LYS 805. There are binding interactions between C–H and Pi-Donor hydrogen in residue THR A:862, SER A:728, and ARG A:849, and Pi-Sigma interaction in residue LEU A: 726, LEU A: 852, and VAL A:734 [96,97]. also identified pi-sigma interaction functioned in protein HER-2 binding in residue VAL A:734 [98]. reported Leu726, Ala751, Arg 849, Leu 852, and Thr 862, the significant interacting amino acid residues with the catalytic site ZINC 000014780728 and ZINC 000014762512.

Visualization molecular docking (Fig. 7) shows the interaction between protein HER-2 and Hexadeca-4,7,10,13-tetraenoic acid. There was hidrogen interaction at residu of MET A: 810 and ligan Hexadeca-4,7,10,13-tetraenoic acid. Hydrophobic interaction form and Alkil/Pi-Alkil binding were in residue LEU A: 796, LYS A:753; VAL A:734, ALA A:752, PHE A:864, and LEU A:852; Interaction Van der waals are at residue ASP A:863, THR A:798, LEU A:726, LEU A:800, and GLU A:799 [98]. also reported the docking result Val734, Lys753, Leu796, Leu800, and Phe864 including the catalytic side of HERs-2 with ZINC 000014780728 and ZINC 000014762512.

[96,97] reported the side activities of protein HER-2 at residue VAL 734 and ASP 863 of the binding site together with hydrophobic interactions with LEU 726 and LEU 852. To stabilize protein-ligand complexes, the hydroxyl group of the ligand attaches to the ASP 863 and acts as an H donor. Moreover, the delocalized aromatic ring pi electrons interact indestructibly with VAL 734 to provide a compound-favorable inhibitor. The chemical has significant contact with the binding site residue MET 801, as well as additional interactions with LEU 726, VAL734 LEU 752, and ASP 863. The interaction has better interaction detail and may limit the function of the protein [98].

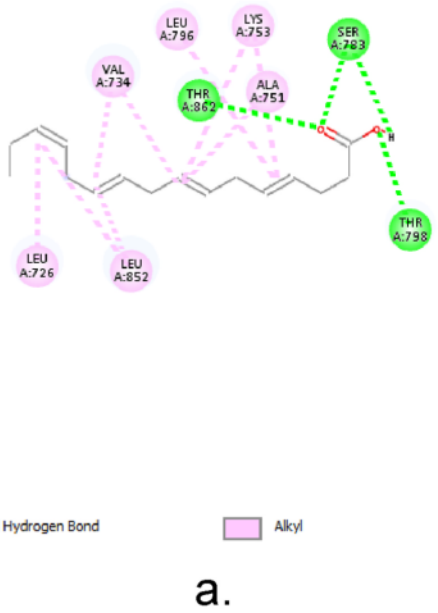


Fig. 8a. 2D interaction of phytocompound linoleic acid with the target protein (HERS-2).

Visualization of molecular docking of linoleic acid and protein HER-2 (Fig. 8) resulted in conventional hydrogen bonding interaction to residue at SER A: 783, THR A:862, and A:798. Alkil interaction at residue VAL A:734, LEU A:796, LYS A:743, ALA A:751, LEU A:706, and LEU A:852. Residu [99]. reported that residue THR A:798 is an active site of amino acid in protein HER-2.

Visualization molecular docking of synthetic drug, Doxorubicin with HER-2 (Fig. 9) resulted from hydrogen bonding to residue ASP A:863, SER A:728, MET A:801 dan GLN A:799, ARG A:849. This synthetic drug in the treatment of cancer has chemical binding as same as with Racemosins B; Hexadeca-4,7,10,13-tetraenoic acid dan Linoleic acid Table 4.

The stability of the complex's structure depends on hydrogen bonding [100]. The more hydrogen bond interaction in amino acids, the more the binding interaction and the lesser the energy score and stability

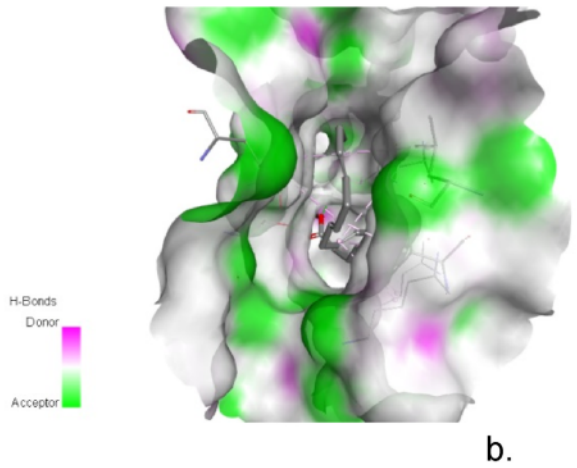
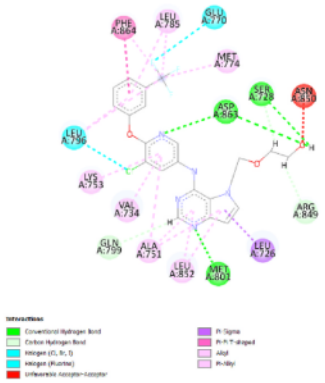


Fig. 8b. 3D interaction of phytocompound linoleic acid with the target protein (HERS-2).

[101]. Hydrogen bonds are stronger than hydrophobic interaction and electrostatic interaction stronger than hydrophobic interaction [102].

H-bonds that cling to a protein's active site residues. H-bonds play a vital role in ligand-receptor interactions and help keep the complex stable [103]. Another crucial aspect to take into account is the angle at which the connection is established. The degree of accuracy in its geometry directly correlates with the strength of the hydrogen bond [104]. Salt bridges frequently serve as the structural driving factors that promote interaction stability [105]. The more bonds formed between phytocompounds and the active site of the receptor, the connection will be steady, and more negative energy will result in a stronger potential activity of an anticancer drug.

High binding affinity between the ligand and the protein is shown by the hydrogen bond, and a high negative score implies good binding affinity with the target protein [93,106]. The binding affinity of the phytocompounds from *C. racemosa* is close to the synthetic drug Doxorubicin and Cianidanol. Based on the molecular docking of Doxorubicin with protein BRCA1, protein plays a role in the differentiation of breast epithelial cells have a binding affinity of −7.2 kcal/mol [94] and molecular docking of Cianidanol with HER-2 was discovered by the binding energy of −8.2 kcal/mol [107].



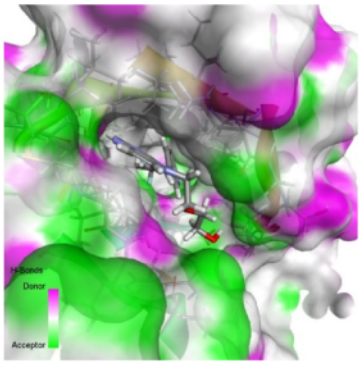


Fig. 9. a 2D interaction of Doxorubicin d with target protein (HERS-2).
b 3D interaction of Doxorubicin d with target protein (HERS-2).

3.7. Pharmacokinetic properties

ADMET physicochemical pharmacokinetic properties were tested for the fourteen best-interacted phytocompounds of the extracts of *C. racemosa* and the synthetic drug doxorubicin (Table 5). The identified compounds had molecular weights that varied from less than 500 g/mol to 543.5 g/mol for doxorubicin. The range of 14 compounds' X Log P values was 0.053101–6.990874 (Table 5).

For a molecule to demonstrate its drug-likeness, it must exhibit many key physicochemical characteristics, including molecular weight, lipophilicity, polarity, solubility, a saturation of carbon fractions, and flexibility, which is represented by rotatable bonds [108]. It is possible for substances with molecular weights of 500 g/mol or less to be readily absorbed, diffused, and transported [109]. Based on the interaction resulted in a BFE value and Visualization molecular docking of interaction with protein HER-2, racemosins B; Hexadeca-4,7,10, 13-tetraenoic acid and Linoleic acid of *C. racemosa* are potential to be anti-breast cancer drug.

Lipinski's Role of Five (RO5) functions to predict the ability of chemical compounds or drugs while replicated orally to humans. The receptor location became the target of is in the cells so the ligand can enter membrane cells and bond with the receptor target. In designing an active drug when applicated orally have characterization such as molecular weight of < 500, hydrogen donor of < 5, acceptor donor < 10, log p value < 5, and molar refractivity between 40 and 130 [110].

Ro5 is a tool to determine compounds that have the highest possibility of actually being studied as a prospective drug. Medicines must be quickly absorbed, digested, and removed from the bloodstream without having any negative side effects [111]. These medications are positioned at the body's intended location of action where they interact with receptor molecules. These facts suggest that these substances are

Table 4
Hydrogen binding interaction of B; Hexadeca-4,7,10,13-tetraenoic acid, linoleic acid, and Doxorubicin with Protein HER-2 (PDB ID: 3PP0).

Ligand	Amino acid interaction		
	Conventional H-bond	Carbon H-bond	Pi-donor H-bond
Racemosins B	–	SER A:728, ARG A:849	THR A:862
Hexadeca-4,7,10,13-tetraenoic acid	THR A: 862, SER A: 783, THR A:798	–	–
Linoleic acid	MET A: 801		
Doxorubicin (syntetic drug)	ASP A:863, SER A:728, MET A:801	GLN A:799, ARG A:849	–

Table 5
Pharmacokinetic properties of identified compounds from the hexane, ethyl acetate and ethanol extracts of *C. racemosa*.

No	Ligand	Molecular weight <500	H-Donor <5	H-Aceptor <10	Log P < 5	Refraction Molar 40-130	Deviation
1	Barbituric acid	128.000000	2	5	−1.257500	26.298399	0
2	Lauric acid	200.000000	1	2	3.991899	59.479782	0
3	Myristic acid	228.000000	1	2	4.772099	68.713783	0
4	Hexadeca-4,7,10,13-tetraenoic acid	248.000000	1	2	4.656299	77.571785	0
5	5-Octadecenal	266.000000	0	1	6.222801	85.515968	1
6	Linoleic acid	280.000000	1	2	5.884500	86.993774	0
7	Stearic acid	284.000000	1	2	4.937631	99.194283	0
8	1-tricosanol	340.000000	1	1	6.990874	128.733765	1
9	Digitoxigenin	374.000000	2	4	3.604298	101.725555	0
10	Caulerpin	398.000000	2	4	3.940978	114.137383	0
11	Racemosins B	312.000000	5	6	−0.053101	77.145782	0
12	Sesquiterpene (2-(2-(2,2-dimethyl-6- methylenecyclohexyl)ethyl) fumaraldehyde)	312.000000	5	6	−0.053101	77.145782	0
13	Sesquiterpene (3-(2-(2,2-dimethyl-6-methylenecyclohexyl)ethyl)-5-hydroxyfuran-2(5H)-one)	312.000000	5	6	−0.053101	77.145782	0
14	4-hydroxy-3-((4R,8R)-4,8,12-trimethyltridecyl)cyclopent-2-en-1-one	312.000000	5	6	−0.053101	77.145782	0

providing medicine with a new chance to treat metabolic and degenerative illnesses. The pharmacokinetic properties analyzed were fitted well within the suitable range for human usage [112].

4. Conclusion

According to this study's findings, green seaweed *Caulerpa racemosa* can be used as a source of bioactive substances or chemical constituents for breast cancer therapy and prevention. The IC_{50} anticancer activity of *C. racemosa* against breast cancer cells (MCF-7) being IC_{50} 23.7 ± 2.1 μg/mL for hexane, 66.7 ± 5.8 μg/mL for ethyl acetate and 182.7 ± 14.3 μg/mL for ethanol extract. Furthermore, the scavenging activity against DPPH radical exhibited by the ethyl acetate extract was the highest, followed by hexane and ethanol extracts with respective IC_{50} of 21.5 ± 2.0, 29.9 ± 2.5, and 49.0 ± 4.0 μg/mL, and ferric-reducing antioxidant power of *C. racemosa* has also been observed. Interestingly, Racemosins B (−11.3 kcal/mol); Hexadeca-4,7,10,13-tetraenoic acid and (−7,9 kcal/mol) and Linoleic acid (−7.4 kcal/mol) has the potential to be a candidate for breast cancer drug with its binding-free energy. This value indicates that the phytocompound and receptor protein HERS-2 form a stable bond. "Rule of 5" (Ro5) attributes were developed by Lipinski along with chemical descriptions of the compounds. The rule is useful while creating new medications. According to the current investigation, there was no violation of Lipinski's limit range for any of the phytocompounds examined. Thus, green seaweed *C. racemosa* has great potential as an anticancer functional food and drug candidate. To fully evaluate the safety and immunogenicity profile of these phytocompounds and determine their potential, experimental assessment is still necessary.

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Author contributions

Conceptualization and methodology: G.S, D.W, and F.N; software, investigation, data curation, and visualization: F.N, and A.A.S; validation: B.K, A.T, and H.K.P; formal analysis and supervision: B.K and H.K.P; writing, original draft preparation, review and editing: G.S, D.W, N.T, V.D, A.A.S, F.N, B.K, S.M, A.T and H.K.P. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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