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EXPLORING THE DUAL EFFECTS OF SEAWEED EXTRACTS: ANTICANCER POTENTIAL AND ANTIOXIDATIVE ACTIVITY AGAINST HORMONE-DEPENDENT AND INDEPENDENT BREAST CANCER CELL LINES

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Abstract

Seaweeds represent a vast and renewable source of medicinal compounds, including polysaccharides, lipids, phenolic compounds, alkaloids, and proteins, and renowned for their pronounced anticancer effects across various cancer types. Notably, the composition of these bioactive molecules varies significantly depending on seaweed species, diversity, and geographic distribution. Minimal resistance to these compounds is observed due to the activation of multiple pathways engaged during their anticancer action. In this study, twelve seaweeds were collected from Kovalam, Tamilnadu, India, and their methanolic extracts were evaluated for anticancer and antioxidative activities using MCF-7 (hormone-dependent) and MDA MB 231 (hormone-independent) cell lines, along with 2,2-diphenyl-1-picrylhydrazyl and lipid peroxidation assays. *Ulva lactuca* exhibited the most effective anticancer activity against both cell lines, while Sargassum terrinum showed lesser efficacy against MCF-7 cells compared to MDA MB 231 cells. The antioxidant activity of the seaweed extracts was found to correlate with their anticancer properties. Our conclusions are supported by existing research and highlight the potential of green algae to inhibit MCF-7 cells and brown algae against MDA MB 231 cells. Furthermore, this study underscores the importance of further research to identify active biomolecules and elucidate their specific toxicity on mammalian cell lines derived from seaweed extracts. These findings contribute to expanding our understanding of the anticancer potential of seaweed-derived compounds and underscore the importance of exploring their therapeutic applications in cancer treatment.

Keywords: Seaweeds, Anticancer Activity, Antioxidant Activity, Algae Extracts, *Ulva Lactuca*

INTRODUCTION

Breast cancer remains one of the most prevalent and deadly cancers affecting women worldwide. Despite advances in treatment modalities, the search for novel therapeutic agents with improved efficacy and reduced side effects continues. In recent years, natural products, particularly those derived from marine sources like seaweeds, have garnered significant attention for their potential anticancer properties. Seaweeds, abundant in oceans and seas, harbor a diverse array of bioactive compounds, including polysaccharides, lipids, proteins, phenolic compounds, and alkaloids, which have demonstrated promising anticancer and antioxidative activities. The development of breast cancer is influenced by various factors, including hormonal status. Hormone-dependent breast cancer, characterized by the expression of estrogen and progesterone receptors, responds to hormonal therapies targeting these

receptors. In contrast, hormone-independent breast cancer, which lacks hormone receptor expression, presents challenges in treatment due to its aggressive nature and resistance to hormonal therapies. Therefore, there is a critical need for alternative therapeutic approaches to effectively combat hormone-dependent and independent breast cancers. Seaweeds have emerged as promising sources of bioactive compounds with potential anticancer and antioxidative effects. The composition and bioactivity of seaweed-derived compounds vary significantly depending on factors such as species, geographic location, and environmental conditions. Polysaccharides, particularly sulfated polysaccharides like fucoidan and carrageenan, have shown notable anticancer properties by modulating various cellular processes, including apoptosis, cell cycle arrest, and angiogenesis inhibition. Additionally, phenolic compounds, such as phlorotannins, exhibit potent antioxidative and anticancer activities through their ability to scavenge free radicals and regulate signaling pathways involved in cancer progression.

Several studies have investigated the anticancer potential of seaweed extracts on various cancer cell lines, including breast cancer. However, there is a paucity of research specifically focusing on the effects of seaweed extracts on hormone-dependent and independent breast cancer cell lines. Understanding the differential response of these cell lines to seaweed extracts could provide valuable insights into their therapeutic potential and contribute to the development of targeted treatment strategies. In this study, we aim to evaluate the anticancer and antioxidative activities of seaweed extracts on hormone-dependent (MCF-7) and hormone-independent (MDA MB 231) breast cancer cell lines. By elucidating the effects of seaweed extracts on these distinct breast cancer subtypes, we seek to identify potential candidates for future development as novel therapeutic agents for breast cancer management. Cancer is becoming a major public health concern worldwide, and it continues to elude us despite extensive pharmacological and molecular research. Despite discovering important medicines, issues such as medication resistance among cancer patients create a gap in treatment efficiency. Alternative treatments, such as natural medicines, effectively address numerous pathways in malignancies and, as a result, provide better outcomes worldwide [1]. On the other hand, Seaweeds include several substances that have been recognized for their extraordinary biological activities in current therapeutic disciplines, such as polyphenols, terpenes, tannins, polysaccharides, and sterols. Many studies have shown that those chemicals target a group of pathways that are generally over-expressed or unregulated in cancer cells. Rhodophycophyta (red marine algae) are typically found in subtropical areas, whilst Phaophycophyta (brown marine algae) and Chlorophycophyta (green marine algae) are found in colder seas [2]. There are 271 genera and 1153 species of marine algae in the Indian subcontinent [3]. This study aimed to assess anticancer and antioxidant activity from twelve marine algal extracts collected from in Kovalam, Tamilnadu, India. The isolation and discovery of novel anticancer agents are paramount in the quest for more effective cancer treatments, and seaweeds offer a vast reservoir of chemically active metabolites with valuable cytotoxic properties. Seaweeds, being one of the largest producers of bioactive compounds in marine ecosystems, present a promising avenue for the development of new chemotherapeutic agents or as a source of inspiration for the synthesis of novel ones. The identification of potent and selective anticancer components isolated from various types of seaweeds, including brown, green, and red algae, represents a significant advancement in pharmacological research. Numerous studies have been conducted to isolate and characterize

bioactive compounds from seaweeds, with a focus on their anticancer properties. Researchers have employed both in vitro and in vivo models, utilizing various cancer cell lines to evaluate the cytotoxic and antiproliferative effects of seaweed-derived metabolites. Among the bioactive compounds identified, terpenoids have garnered considerable attention due to their diverse chemical structures and potent biological activities. Within the realm of terpenoids, carotenoids, polyphenols, and alkaloids stand out as particularly promising candidates for anticancer drug development.

Carotenoids, well-known for their antioxidant properties, have also been shown to exhibit cytotoxic effects against cancer cells, with potential applications in cancer prevention and treatment. Polyphenols, abundant in seaweeds, possess a wide range of biological activities, including anticancer properties attributed to their ability to modulate signaling pathways involved in cell proliferation and apoptosis. Alkaloids, another class of bioactive compounds found in seaweeds, have demonstrated potent cytotoxic effects against cancer cells by interfering with essential cellular processes. In addition to elucidating the cytotoxic or antiproliferative effects of seaweed-derived metabolites, researchers have endeavored to unravel their mode of action, structure-activity relationships, and selectivity towards cancer cells. Understanding the mechanisms by which these bioactive compounds exert their anticancer effects is crucial for the rational design and optimization of therapeutic agents targeting specific cancer types. Overall, the exploration of seaweeds as a source of novel anticancer agents holds great promise for advancing cancer research and therapy. By harnessing the rich chemical diversity of seaweed metabolites and elucidating their biological activities, researchers can contribute to the development of innovative treatments that offer improved efficacy and reduced side effects for cancer patients.

MATERIALS AND METHODS

Collection of plant leaves and storage

This study tested methanolic extract of 12 seaweeds, collected from Kovalam (12.7898° N, 80.2542° E) in Tamilnadu, India (Table. 1). The abundance of species and accessibility of access to these areas were factors in their selection. The seaweeds were carefully carried to the laboratory and rinsed with running tap water. After that, they were sun-dried, pulverized, and extracted with 80% methanol. The alcoholic extract was vacuum evaporated and stored at four °C for further analysis.

Table 1: Details of the collected seaweeds from the Tamilnadu coastal area

S. No	Name of the seaweed	Code	Place of collection
1.	*Ulva latuca*	UL	Kovalam, Tamilnadu
2.	*Caulerpa taxifolia*	CL	Kovalam, Tamilnadu
3.	*U. rigida*	UR	Kovalam, Tamilnadu
4.	*Entreomorpha compressa*	EC	Kovalam, Tamilnadu
5.	*Codium geppsiorum*	CG	Kovalam, Tamilnadu
6.	*Pedina pavonica*	PP	Kovalam, Tamilnadu
7.	*Sargassum terrinum*	SAR	Kovalam, Tamilnadu
8.	*Cystoseira trinodis*	CyS	Kovalam, Tamilnadu
9.	*Dictyota dichotama*	DIC	Kovalam, Tamilnadu
10.	*Turbinaria canoides*	TR	Kovalam, Tamilnadu
11.	*Gracilaria cortiata*	GCT	Kovalam, Tamilnadu
12.	*Rhodomela confervoides*	RC	Kovalam, Tamilnadu

Cytotoxicity assay using MTT

The MTT test, based on the previous approach [4], was used to screen the cancer cell inhibitory characteristics of the chosen seaweed extracts. MCF 7 and MDA MB 231 cells were grown in DMEM medium (2mM L-glutamine, 1.5 $g.L^{-1}$ sodium bicarbonate, 90% fetal calf serum), 37°C, and 5% CO_2 condition). The extract was diluted in sterile dimethylsulfoxide (DMSO, 1 ml), filtered through a membrane (20-100l), an aliquot at various concentrations (10, 20, 50, 100, and 200 $g.mL^{-1}$). The aliquots were mixed with $1*10^6$ cancer cells plated in a 96-well microtiter plate and incubated for 24 hours at 37°C. Each well-received 20µl of MTT [(3, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] was maintained at 37°C for 4 hours. Finally, isopropanol (100µl) was added, and its optical absorbance was measured at 595nm using a multi-well spectrophotometer plate reader (Epoch 2 microplate reader, Biotec) and the formulae was as follows, Cell Viability (%) = (Optical density of samples - Optical density of control) / Optical density of control X 100. The findings were tabulated and compared to the control (adriamycin) in triplicates at doses 1, 2, 5, 7.5, 10, and 15 $g.mL^{-1}$.

Lipid peroxidation assays

The crude extracts were tested for lipid peroxidation using the prior approach [4]. For peroxidation induction, liver tissue was ground in tissue (10% w/v) in cooled tris buffer (10 mM, pH 7.4) and treated with Iron-ADP complex in the presence of ascorbic acid. 0.5 ml liver homogenate (tris HCl (20 mM, pH 7.0), potassium chloride (150 mM), ferrous ammonium sulphate (0.16 mM), and ascorbate (0.06 mM) were used, and the samples were maintained at 37°C for an hour. The degree of peroxidation was determined by measuring malondialdehyde (MDA). 0.2 mL sodium dodecyl sulfate (0.2 mL), 1.5 mL acetic acid (20% v/v, pH 3.5), and 0.2 mL TBA were used in the MDA reagent (0.8%, 1.5ml). The OD was measured at 532 nm. Ascorbic acid, butylated hydroxytoluene (BHT), Vitamin B12 (α- tocopherol), and commercial tea powder were employed as standards (on 10–50 $g.mL^{-1}$). The decrease in MDA content was calculated by Inhibitory activity (%) = ($A_{control}$ - A_{sample}) * 100/ $A_{control}$. The results were tabulated and compared with the seaweed extracts.

DPPH assay

The radical scavenging ability of the chosen seaweed extracts was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical test [5]. 0.2 mL of DPPH (0.02 mM) in ethanol was added to 1 ml of extract solution, with concentrations ranging from 100 to 500 $g.mL^{-1}$. At 20°C, the combination was held in the dark for 40 minutes before being measured at 517 nm. The inhibitory activity was estimated using the following formula: DPPH radical scavenging activity (%) = [(A0 A1)/A0] 100, where A0 was the absorbance of the control and A1 was the absorbance of the sample. The standards used were ascorbic acid, butylated hydroxytoluene (BHT), Vitamin B12 (- tocopherol), and commercial tea powder (on 10–50 $g.mL^{-1}$).

Statistical Analysis

Triplicattive examinations were carried out for each assay, and the findings were analyzed using the Graphpad prism statistical tool as mean SE (Standard Error). The statistical significance of the samples was determined using the Student T-test (p 0.05).

RESULTS

Two kinds of breast cancer cell lines, MCF -7 and MDA MB 231 cells, were used to test the anticancer efficacy of the seaweed extracts. All extracts demonstrated stronger inhibitory action on the oestrogen dependent and independent human cancer cell lines MCF 7 and MDA MB 231. The standard error was calculated using triplicates in the experiments. Figure 1 shows the anticancer activity of the obtained seaweeds on the MCF – 7.

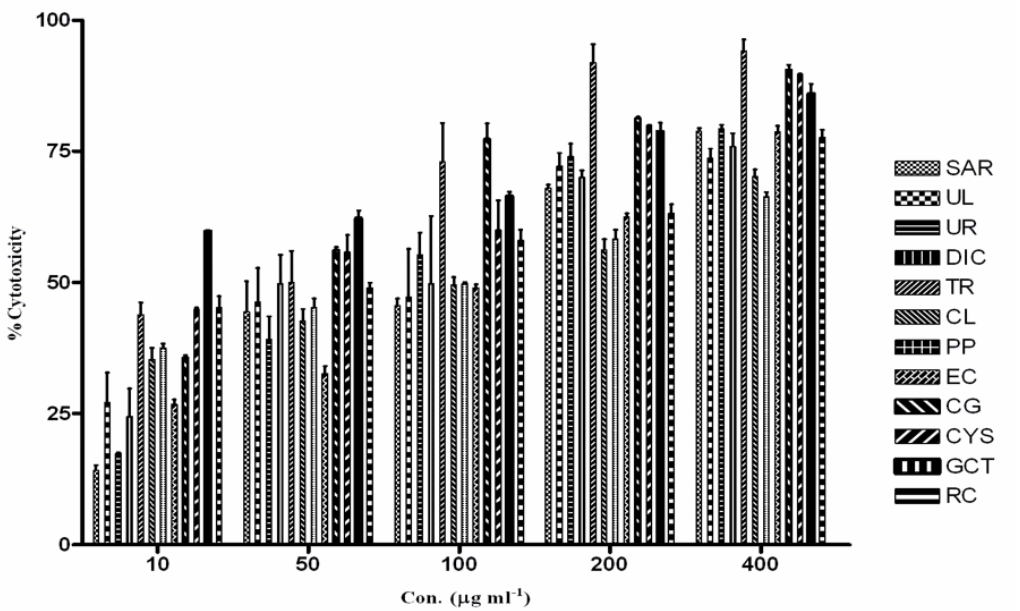

Figure 1: Anticancer activity of the collected extracts on MCF – 7 Cells

The methanolic extracts of the collected seaweeds were tested in triplicates against cultured MCF-7 cells and MDA MB 232 cells at varying doses (10, 50, 100, 200, and 200 g.mL^{-1}), and the findings were provided as the mean, standard error.

Figures 1 and 2 showed the effects of methanolic extracts of seaweeds on MCF-7 and MDA MB 231 cell lines, respectively. The positive control, adriamycin (doxorubicin) (as 1, 2, 5, 7.5, 10, and 15 g.mL^{-1}), has an IC_{50} of 2.4 against MCF-7 cell lines. The IC_{50} values of all the extracts were lower than the positive control (Table. 3). On MCF – 7 cell lines (34.64 g.mL^{-1}) and MDA MB 232 cells (35.76 g.mL^{-1}), *U. lactuca* had an IC_{50} value of 34.64 g.mL^{-1} and slightly higher on MDA MB 232 cells (35.76 g.mL^{-1}). On MDA MB 231 cells, *U. lactuca* had a two-fold higher antiproliferative activity (%) than MCF–7 cells at 100 g.mL^{-1} (22.611.51 and 45.029.74 g.mL^{-1}, respectively). On both cancer cell lines, *U. rigida* demonstrated somewhat stronger activity than *U. lactuca*. On MDA MB 231 cell lines, *D. dichotoma*, *P. pavonica*, and *G. cortiana* had more anticancer activity than MCF-7 cells. The green algae showed more inhibition on MCF-7 cell lines than MDA MB 231 cell lines.

Table 2: IC_{50} value of the collected seaweed methanolic extracts on hormone-dependent and hormone-independent cell lines, MCF-7 and MDA MB 231 cancer cell lines

S. No	Seaweed	IC $_{50}$ (µg.mL^{-1})	
		MCF 7	MDA MB 232
1.	CG	149.52±5.6	170.54±3.4
2.	CL	70.35±1.7	105.56±2.5
3.	CYS	85.32±4.5	150.24±6.3
4.	DIC	127.75±2.7	84.53±4.3
5.	EC	145.35±3.6	95.34±1.7
6.	GCT	75.26±9.3	56.83±4.6
7.	PP	72.04±3.6	30.48±1.8
8.	RC	138.35±7.8	105.67±7.5
9.	SAR	150.55±3.6	110.55±1.4
10.	TR	154.57±3.6	144.54±2.6
11.	UL	22.61±1.51	45.02±9.74
12.	UR	70.23±3.7	78.45±2.7

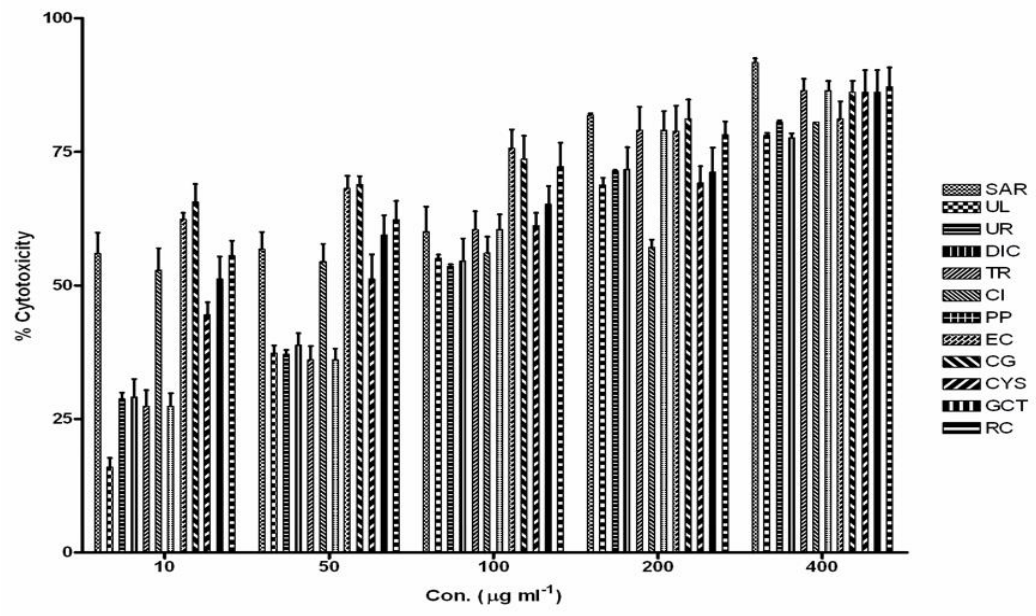

Figure 2: Anticancer activity of the collected extracts on MDA MB 231cell lines

Antioxidant activity of seaweed extracts evaluated using DPPH assay.

Twelve seaweed species were gathered from the Indian subcontinent's coastlines in Kovalam, Tamilnadu. Their crude methanolic extracts showed a range of antioxidative potential in the present study. We employed two well-known assays in this study: DPPH and lipid peroxidation tests (Figure. 3 and 4). In the DPPH technique, all seaweed extracts demonstrated antioxidant activity to varying degrees. A lower IC50 value indicates higher antioxidant activity. In each of the experiments, vitamin B12 had the strongest antioxidative activity. The EC_{50} values were 18.6 g.mL^{-1} and 26.5 g.mL^{-1}, respectively. In both experiments, BHT had somewhat greater activity than ascorbic acid. The study investigated the antioxidative activity of commercially available tea powder as one of the standards with other positive controls.*U. lactuca* had the highest antioxidative activity among the seaweed extracts, with IC_{50} values of 122.9 and 140.5

g.mL^{-1} against lipid peroxidation and DPPH tests, respectively, and was comparably low. In the DPPH experiment, *T. canoides* had the lowest activity of the extracts (EC_{50}– 354.76 g.mL^{-1}). The computed EC_{50} of the various extracts was displayed in table 3. We performed a statistical T-test to compare our EC_{50} values from the Lipid Peroxidation and DPPH tests. As demonstrated by the p-value (0.016), we discovered that their means varied significantly (Table. 3).

Table 3: Free radical scavenging activity (EC_{50}) of positive controls and collected seaweed methanolic extracts evaluated by two different antioxidative assays.

S. No.	Sample name	EC_{50} (µg.mL^{-1})	
		Lipid Peroxidation	DPPH
1.	Ascorbic acid	59.35	78.5
2.	BHT	23.14	27.8
3.	Vitamin B_{12}	18.6	26.5
4.	Tea powder (Commercial)	26.2	49.47
5.	CG	235.89	326.67
6.	CL	178.89	168.45
7.	CYS	257.9	268.97
8.	DIC	158.36	265.76
9.	EC	265.89	300.35
10.	GCT	275.90	234.87
11.	PP	245.68	256.80
12.	RC	190.45	167.98
13.	SAR	156.74	188.76
14.	TR	245.8	354.76
15.	UL	122.9	140.5
16.	UR	145.8	158.67
T test (p-value ≤ 0.05 is significant)			0.0155

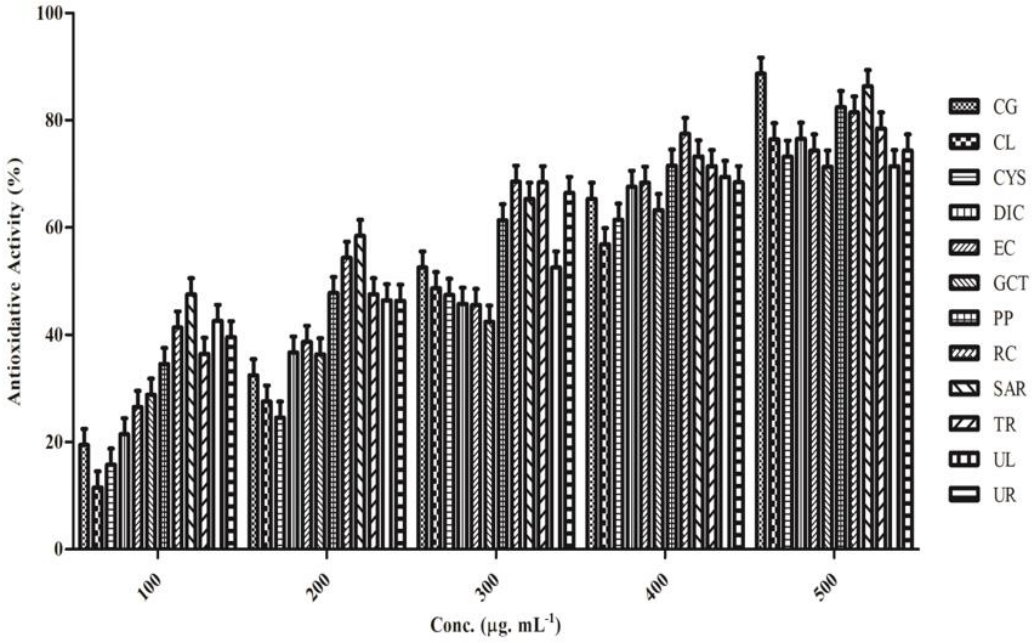

Figure 3: Antioxidative activity of selected seaweed methanolic extracts on DPPH based assay

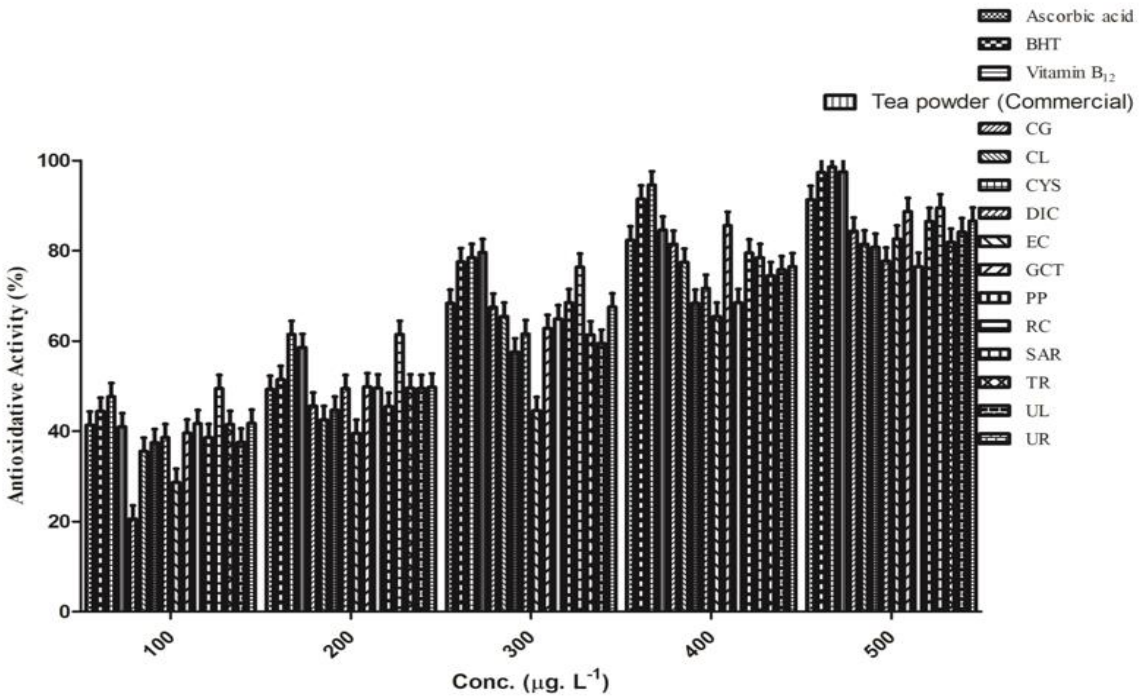

Figure 4: Antioxidative activity of selected seaweed methanolic extracts on Lipid Peroxidation inhibitory assay

DİSCUSSION

As significant works have been revealed every day in the pharmaceutical environment, seaweeds remain an eternal source for new bioactive compounds. Due to estrogens and phytoestrogens supplementation, dietary seaweeds have a key role in the chemoprevention of a wide spectrum of malignancies, particularly breast cancer in women [7-8].

On a biochemical or molecular level, the evaluation of phytoextracts in various models aids the scientific community in anticipating potential anticancer medications [9]. The methanolic seaweed extracts revealed (i) antiproliferative activity against MCF 7 and MDA MB 232 cells at different doses and (ii) equivalent antioxidative activity in free radical scavenging experiments, according to the findings.

Although the present study did not investigate the specific roles of seaweed phytochemicals, it has previously been reported that saponins, tannins, alkaloids, flavonoids, and phenols make up the majority of active ingredients in plants and vegetables and that these may be responsible for many of the pharmacological actions of such plants. Saponins are steroidal glycosides that have foaming properties used to treat viral infections.

Tannins are bitter polyphenols found in plants that bind, precipitate, and shrink proteins and other chemical molecules. Tannins contain antiviral, antitumor, anti-inflammatory, and wound healing effects, among other things. Cardiac glycosides are helpful in the therapy of heart problems and can help with cardiac arrhythmias [10]. Phenolic chemicals, in particular, play as important antioxidants with a wide variety of therapeutic characteristics, including anticancer, anti-inflammatory, and diabetic [11].

Flavonoids are among the most varied and common groups of natural chemicals, with a wide range of chemical and biological activity [12-13], including radical scavenging, anti-allergenic, antiviral, anti-inflammatory, and vasodilating effects. The presence of alkaloids, saponins, tannins, steroids, terpenoids, flavonoids, and glycosides in the collected extracts of the individual seaweeds employed in this study was shown by component analysis (results not shown). These findings are consistent with earlier research [14-15].

Antioxidative ability tests confirmed that such seaweeds might be a useful option for treating disorders linked to excessive free radical production and changing physiological activity as medications [16-17]. Though the present study did not identify any particular chemical constituents in the algal extract were not identified in this study, the literature can give some useful information for future research. Polymers of the brown algae are well known for their anticancer activates [18].

According to Teas (1981) [19], eating seaweeds may help to slow the spread of breast cancer. They later revealed that uPAR has a role in preventing breast cancer [20]. In this study, we also observed that the inhibition ratio of seaweed extracts varied amongst cell types.

MCF-7 and MDA-MB-232 cell lines were used to represent low and high invasive breast carcinoma, as well as hormone-dependent and hormone-independent cancer types, in the hunt for novel cancer treatment targets [21- 27]. As a conclusion, green algae are more effective on MCF-7 cells than MDA MB 231 cells. Further study of these extracts might lead to developing a valuable anticancer bioactive medication in the future.

CONCLUSIONS

According to our findings, the brown algae *D. dichotoma, T. canoides*, and G. cortiata have stronger inhibitory action on MDA MB 231 cell lines. According to the current study, the antioxidative activity of the respective seaweed extracts might be a rationale for their anticancer effect against MCF-7 and MDA MB231 cancer cell lines.

Conflict of interest

The authors declare no conflict of interest.

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