



UNRAVELLING THE ANTIOXIDANT AND ANTI-CANCEROUS PROPERTIES OF THE CHEMICAL CONSTITUENTS PRESENT IN METHANOL EXTRACT OF GREEN ALGAE *CHAETOMORPHA ANTENNINA*

Akanksha Dubey¹ and Jayanthi Sivaraman^{2*}

¹Department of Biotechnology, School of Biosciences and Technology, Vellore Institute of Technology, Vellore-632 014, Tamil Nadu, India

²Associate Professor, Computational Drug Design Lab, Department of Biotechnology, School of Biosciences and Technology, Vellore Institute of Technology, Vellore 632014, Tamil Nadu, India

Background: *Chaetomorpha antennina* is a green seaweed with numerous pharmacological properties such as antimicrobial, antimalarial, anti-tumour and antioxidant properties. Thus, it is essential to perform preliminary studies to identify the chemical composition of seaweed extracts and explore the phytoconstituent responsible for pharmacological activities. Cancer is the leading cause of death worldwide. The discovery of novel therapeutic mediators for the treatment of cancer is the main task. Since efficient drug delivery is possible through nanotechnology, biosynthesis of silver nanoparticles was done, followed by molecular docking studies to investigate the anti-cancer activity of seaweed extract. **Methods:** Phytochemical analysis of extracts of *Chaetomorpha antennina* was performed, followed by biosynthesis of silver nanoparticles using these algal extracts. Characterization of silver nanoparticles was done by Scanning Electron Microscopy and X-ray Diffraction. Agar-well diffusion method and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay were used to determine the antibacterial potential and antioxidant potential of algal extracts. Further, the chemical composition of the extract was distinguished by Gas chromatography-mass spectrometry. **Results:** Seaweed extracts possessed numerous phytochemicals and methanol extract showed the best antibacterial and antioxidant activity. Docking studies exposed the occurrence of Carpesterol dehydrate and triazines as an anti-cancer component of *Chaetomorpha antennina*. **Conclusion:** It can be concluded that seaweed is enriched with chemical constituents possessing high antioxidant activity and therefore, can serve as a promising antioxidant and anti-cancer agent.

Keywords: Antibacterial, BRCA1, Breast Cancer, Docking, GC-MS Analysis

INTRODUCTION

Cancer is the foremost basis of death, and breast cancer is one of the most prevalent malignancies and principal grounds of cancer death in women worldwide. Breast cancer is malevolent cancer that initiates in the breast cells. Extensive varieties of carcinogens are accountable for carcinogenicity. Radiation is a well predictable hazard factor for breast cancer and its exposure induces the production of free radicals¹. The features that progress this cancer includes obesity, the deficit of bodily workout,

consumption of liquor, hormone replacement remedy throughout menopause, premature stage at initial menstruation, and older age. BRCA transmutation is a transformation in any BRCA1 and BRCA2 genes, which are the tumour suppressor genes. Destructive alterations in these genes develop the inherited breast-ovarian cancer condition in sickly individuals. The danger of breast and ovarian cancer is advanced for women, which increases the risk of BRCA1 mutation compared to the BRCA2 mutation. BRCA1 are the humanoid genes and comprise a protein product

accountable for the repairing of DNA². The multifactorial BRCA1 gene product is convoluted in DNA repair mechanism, ubiquitination, transcriptional regulation and other functions. In normal cells, these genes assist in preventing cancer by the production of proteins that preserve the cells from growing abnormally¹.

Marine habitat is bestowed with structural and chemical features that are missing in natural substances in terrestrial habitat³. Certain secondary metabolites are secreted in response to environmental transitions⁴. Marine seaweeds are perceived as manufacturers of secondary metabolites including fatty acids, flavonoids, terpenoids, alkaloids, peptides, sterols, glycerol, polysaccharide, peptides, and lipids⁵ possess anti-microbial⁶, anti-fouling⁷, anti-viral⁸ anti-oxidant⁹, anti-cancer¹⁰, anti-allergic¹¹, anti-inflammatory activities¹². In the current era, researchers are exploring certain crude and purified extracts possessing certain bioactive compounds with specific medical significances¹³⁻¹⁹. Thus, it can be stated that seaweeds execute the dominant part of sustainable marine resources. Several dietary fibres extracted from seaweeds implement diverse functions such as antioxidant, anti-mutagenic, anticoagulant, and anti-tumor²⁰. Gas chromatography-mass spectrometry is a trendy technique for determining the composition of algal extracts. Several libraries possess different spectra for a single compound that causes complications in the recognition of individual constituents. GC-MS analysis is the most accepted technique for identifying the composition of different extracts, although this technique should be performed accurately to obtain précised and accurate results²¹. Researchers have a very keen interest in the exploration of medicinal plants with certain therapeutic value²². This analytical technique in the present study was applied to acknowledge the nature of the algal extract. Docking is a procedure by which one can envisage the substantial orientation of one molecule to a second when destined to each other to form a stable complex. Docking is habitually utilized for evaluating the binding between the ligand and the receptor²³. However, the previous studies indicated the antibacterial, antioxidant and anti-cancerous properties of this seaweed in the ethanol extract²⁴⁻²⁶ but the present study

aimed at determining numerous phytochemical compounds responsible for breast cancer through insilico methods. It is well-known that mutated domains are accountable for the cancer progression, and rendering to the Database of Cancer Mutant proteins database(DCMP) BRCA1 C-terminal (BRCT) domains are vital signalling elements in the DNA damage response. As a result, the current study was performed to check the interaction of the chemical constituent present in the seaweed with the Crystal structure of BRCA1 BRCT with doubly phosphorylated Abraxas instead of cell line studies. The present investigation exposed the phytochemicals present in the methanol extract of *Chaetomorpha antennina* by means of Gas chromatography-mass spectrometry. It investigated the anti-cancerous property of the seaweed by molecular docking studies.

METHODS

Collection of sample

Chaetomorpha antennina was collected from Rameswaram, Tamil Nadu. The algal samples were identified and validated by Dr N. Kaliaperumal, Principal Scientist and Scientist in Charge, Mandapam Regional Centre of Central Marine Fisheries Research Institute, Tamilnadu, India. The algal samples were cleaned appropriately with distilled water for the elimination of dust and soil particles. The samples were subjected to drying.

Preliminary phytochemical screening

The solvent extraction was done by using the Soxhlet apparatus. The algal extract obtained after Soxhlet extraction was subjected to phytochemical screening using specific chemical tests for the presence of bioactive compounds like steroids, tannins, flavonoids, alkaloids, saponins using standard procedures²⁷.

Antioxidant activity

The antioxidant property of seaweeds was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. DPPH assay was performed using the methanol as the qualitative phytochemical analysis showed the maximum positive results in these extracts. The different concentrations were used from 10 µg/ml-50

µg/ml, and the optical density was recorded at 517 nm against the blank. Results were matched with different concentrations of standard antioxidant Gallic acid²⁸. The ability of DPPH scavenging free radical was calculated using

$$\text{DPPH scavenged (\%)} = \frac{(\text{A control} - \text{A test})}{(\text{A control})} * 100$$

Where A control is the absorbance of the control reaction and A test is the absorbance of the sample of extracts

Synthesis of silver nanoparticles

Biosynthesis of silver nanoparticles was done by adding 99 ml of an aqueous solution of silver nitrate (1 Mm) to 1ml of algal extract of *Chaetomorpha antennina* and was placed in a hot air oven at the temperature of 121°C for 10 minutes. After 10 min. in a hot air oven, the solution was packed with the dark cover and aluminium and incubated in the dark for 24 hrs. After 24 hrs of incubation, the change in colour of the solution will indicate the synthesis of silver nanoparticles and the absorbance was noted by the UV-visible spectrophotometer²⁹.

Characterization of silver nanoparticles

Characterization of synthesized silver nanoparticles was done by UV-Vis spectral analysis, Scanning electron microscopic (SEM) analysis and X-Ray Diffraction analysis (XRD).

Antibacterial activity of algal extract and synthesized nanoparticles

Agar well diffusion method was followed to evaluate the antibacterial potential. Muller Hinton agar plates were swabbed with 24 hrs. old broth culture of corresponding bacteria. Wells were prepared with the aid of a sterile cork borer. Approximately 20 µl of methanol extract and nanoparticles were seeded into wells and permitted to diffuse at room temperature against suitable control. The plates were then subjected to incubation for 24 hrs. at 37°C for 24 hrs. The experiment was repeated twice, and results were noted down³⁰.

GC-MS analysis

The algal sample after extraction were administered to centrifugation at 3000 rpm for 10 min. The supernatant obtained was placed in

Petri plates and was dried completely over a hot air oven followed by scraping the powdered extract. The grated sample was then stored for further GC-MS analysis. The GC-MS analysis was achieved with the help of Perkin Elmer Claurus 680 GC equipment. The capillary column was packed with Elite-5MS, and the constituents were separated by means of carrier gas (Helium) at a constant flow of 1 ml/min. The injection port temperature is 200-300°C for efficient vaporization of the sample. The different factors involved in the process were also standardized. The mass spectrometer functioned in EI mode. The chromatogram and spectrum of the peaks were visualized using Turbo Mass 5.4.2 software. The structural facts of the component of the algal extract were evaluated.

Auto dock

Auto Dock is an assortment of computerized docking tools. The software is accustomed to modelling malleable tiny particles, for instance, molecules bounding to receptor proteins of acknowledged three-dimensional structures. The 3D structure of the protein BRCA1 (PDB ID: 4JLU) was obtained from RCSB-PDB. The ligand and crystallized water molecules were extricated from the protein, and the protein was subjected to modification for missed out hydrogen atoms. Energy minimization was done by adding Kollman charges. The three-dimensional structure of phytochemical compounds was retrieved from the Pub-Chem database. The 3D structure of compounds was downloaded and saved in .sdf format and modified to .pdb format through open babel 2.3.1. The ligand was prepared using MGL tools by the addition of hydrogen. The ligand was minimalized by figuring Gasteiger and Kollman charges and saved in Pdbqt. Docking was carried out using Auto dock tools 4.2 software. The dimensions of the Grid were 17, -8, -3. All over the docking studies, the protein was set aside as rigid and ligand as flexible.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

Marine seaweeds serve as a source of a diverse pool of bioactive compounds and chemical compounds that assist their existence

in tremendously challenging and unfavourable environments. The pharmaceutical industry has gained much attention to the bioactive compounds, which are fruitful outcomes of biosynthesis of secondary metabolites. Due to its diversity in chemical composition and mineral composition depending on its distribution, bioactive compounds from algae are probable candidates for curing numerous illnesses, including cancer. In this study, we made an effort to detect bioactive chemical compounds found in *Chaetomorpha antennina* to study its antimicrobial, antioxidant and anti-cancer activity. Previous studies focused on the elements found in *Chaetomorpha antennina*, including carbon, sulphur, sodium, silicon, potassium, chlorine and oxygen³¹.

In contrast, in this study, we focused on the chemical compounds present in the extracts of *Chaetomorpha antennina*. The existence of these chemical compounds as the main constituents of this alga marks it distinctive due to its therapeutic and pharmaceutical effects. *Chaetomorpha antennina* is known to be rich in polyphenols³². Hydrophilic polyphenols that are bipolar serves as an essential antioxidant that assists algae in fighting oxidative stress³³. Terpenoids are utilized for their aromatic qualities and enact an essential role in classic herbal remedies. Flavonoids are emerging as trendy as they possess health endorsing effects³⁴. The phytochemical analysis displayed the existence of flavonoids, glycosides,

carbohydrates, tannins, quinines, alkaloids, steroids, proteins, terpenoids and saponins, as evident in Table 1. The presence of phenol compounds such as flavonoids, phenolic acids, and tannins is considered the critical donor to the antioxidant capacity of seaweeds³⁵. The phytochemical analysis was performed for all the solvents extracted, and the results drawn comprised slight differences among all the different solvents. The methanol extracts obtained had all the bioactive compounds present in them. Compared to methanol, ethanol extract had little less favourable results for bioactive compounds followed by chloroform, Hexane and DCM extract, as displayed in Table 1.

The aqueous extract comprised bioactive substances such as terpenoids, steroids, flavonoids, and coumarins, whereas hexane extracts showed steroids and terpenoids. DCM and chloroform extract also paved the way for bioactive substances such as alkaloids, terpenoids, steroids etc. Lastly, it was brought into notice that methanol extract comprised of all the bioactive substances. From Table 1, we have chosen the extracts with the maximum number of bioactive compounds. It was found that methanol extract possessed all the bioactive compounds compared to the other solvents. Thus, methanol extract was used for further classification and characterization studies.

Table 1: The phytochemical analysis of different extracts in *Chaetomorpha antennina*

Phytochemicals	Hexane	DCM	Chloroform	Methanol	Ethanol	Water
Alkaloids	Negative	Positive	Positive	Positive	Positive	Negative
Terpenoids	Positive	Negative	Positive	Positive	Positive	Positive
Steroids	Positive	Negative	Positive	Positive	Positive	Positive
Tannins	Negative	Positive	Negative	Positive	Positive	Negative
Saponins	Positive	Negative	Negative	Positive	Positive	Negative
Flavonoids	Negative	Positive	Negative	Positive	Negative	Positive
Phenols	Negative	Negative	Positive	Positive	Positive	Negative
Coumarins	Negative	Negative	Positive	Positive	Positive	Positive
Quinones	Negative	Negative	Negative	Positive	Negative	Negative
Glycosides	Negative	Negative	Negative	Positive	Positive	Negative

Antioxidant activity

The antioxidant potential of algal extracts was evaluated using a DPPH assay. The methanol extract of algae was exposed to DPPH assay to detect the scavenging potential of the algal extract. Different concentrations from 0 µg/ml - 50 µg/ml of methanol extract were taken. It was found that the radical scavenging potential increased at each concentration, as displayed in Table 2. Antioxidant activity of standard Gallic acid showed an increase in the absorbance and likewise algal extract. This antioxidant activity of algal extract initiated at a minimal concentration of 10µg/ml and was found to increase linearly from 10µg/ml to 50 µg/ml. Similar to the standard Gallic acid, methanol extract also revealed increased absorbance. The percentage inhibition of methanol extract of *Chaetomorpha antennina* was found to increase from 11.21% at 10µg/ml to 32.54% at 50µg/ml. The IC₅₀ noted for methanol extract was 25.075 µg/ml. In the present study, the methanol extracts revealed a promising DPPH scavenging capacity compared to standard as there was an increase in percentage inhibition. Thus, it can be stated that in this study, the methanol extract of *Chaetomorpha antennina* showed a higher scavenging activity when compared to standard.

Synthesis of silver nanoparticles

Synthesis of silver nanoparticles displayed the change in colour of solution and presence of brown colour in a solution confirmed the synthesis as displayed in Fig. 1. Further, absorbance was noted using UV-visible Spectrophotometer. It reflected a gradual increase of absorbance, and the absorbance at 420nm indicated the synthesis of silver nanoparticles in methanol extract, chloroform, and dichloromethane extract. Further, the synthesized nanoparticles from methanol extracts were subjected to characterization studies, including SEM and XRD analysis

Characterization of silver nanoparticles

Scanning electron microscopy (SEM) has provided further insight into the morphological details of the synthesized nanoparticles. Fig. 2 displayed the SEM image of methanol extract mediated silver nanoparticles, and it was observed that the sample contained several dispersive nanoparticles. It was also reported that the nanoparticles synthesized were relatively uniform X-ray diffraction analysis further characterized the synthesized nanoparticles, and the diffraction peaks were recorded at different 2θ degrees ranging from 0-100° as displayed in Fig. 3.

Table 2: Inhibition % of DPPH radical scavenging activity of methanol extracts of *Chaetomorpha antennina*

Extracts	Inhibition in percentage at different concentrations (%) (DPPH free radical scavenging activity)				
	10 µg/ml	20 µg/ml	30 µg/ml	40µg/ml	50µg/ml
Standard (Gallic acid)	12.11± 0.2	13.52± 0.1	13.86±0.2	14.25± 0.4	16.44±0.8
Methanol extract	11.21± 0.1	13.21± 0.1	22.35±0.2	23.51± 0.4	32.54±0.8



Fig. 1: Biosynthesis of silver nanoparticles using algal extracts

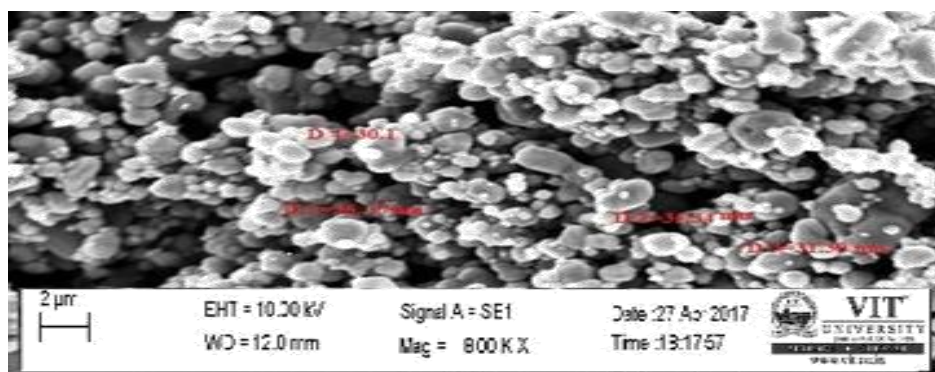


Fig. 2: SEM images of methanol extract mediated synthesized silver nanoparticles

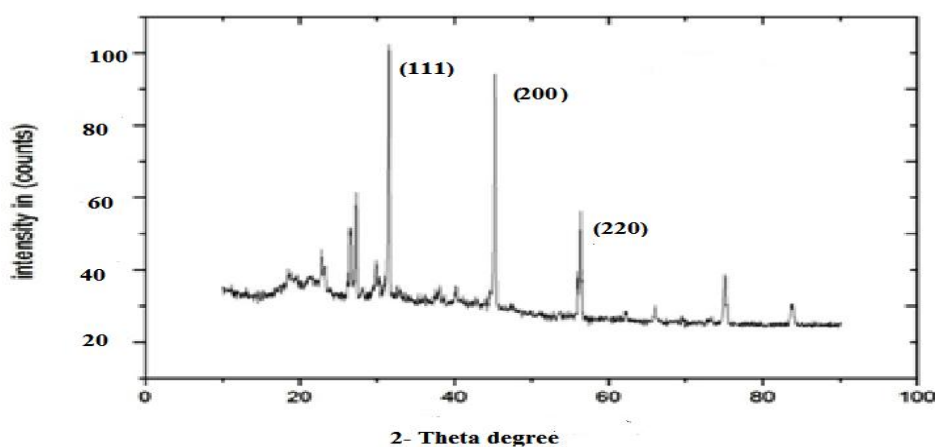


Fig. 3: XRD graph of methanol extract mediated synthesized silver nanoparticles

Three prominent peaks were observed at the 2θ degree of 32.3° , 43.4° , and 58.8° that corresponds to (111) (200) and (220) planes, respectively. Entire peaks obtained in the XRD pattern could be indexed to the cubic structure of silver (face-centred) as accessible in literature (JCPDS, File No. 4-0783). The crystal size of the silver nanoparticles was estimated from the FWHMs of the diffraction peaks using the Scherrer equation. The crystallite size in different planes of silver was determined as 23.3 nm, 20.1 nm, and 14.5 nm, with the mean value of all peaks as 19.3 nm.

Antibacterial activity

In the present study, the antibacterial activity was tested for methanol extract and silver nanoparticles using Agar well diffusion method. The results obtained revealed that the methanol extract and silver nanoparticles synthesized using methanol extracts possessed antibacterial potential. The test was done against the three pathogenic microorganisms, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. The inhibition was measured as an inhibition zone in millimetres. The results are displayed below in Table 3.

Table 3: Antibacterial activity of methanol extract and silver nanoparticles of *Chaetomorpha antennina*

S. no	Solvents	Zone of clearance(in mm)		
		<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
1	Control (Deionised H ₂ O)	0	0	0
2	Methanol extract	15	14	13
3	Silver nanoparticles	13	15	12

GC-MS analysis

The complete chromatogram for the methanol extract of *Chaetomorpha antennina* was displayed in Fig. 4, revealing mixtures of several chemical constituents. The peaks observed in the chromatogram were incorporated and equated with the database of spectrum stored in the GC-MS library. The GC-MS analysis of the methanol extract of *Chaetomorpha antennina* displayed 8 peaks in the chromatogram, which was further processed, and it was observed that each peak was further integrated. Henceforth, 35 identified compounds were identified with the help of the NIST library.

The highest peak was observed at the retention time of 19.16 min. The highest peak obtained at 19.16 min was further processed, and 20 peaks were obtained, indicating the presence of 20 different compounds. The chromatogram obtained after further processing and integration of the highest peak is represented below in Fig. 5. This peak displayed the presence of several chemical compounds such as tridecanoic acid, octadecanoic acid, pentadecanoic acid, tetradecanoic acid, tridecanoic acid, undecanoic acid, dodecanoic acid, eicosanoic acid, carpesterol dehydrate and n-hexadecanoic acid.

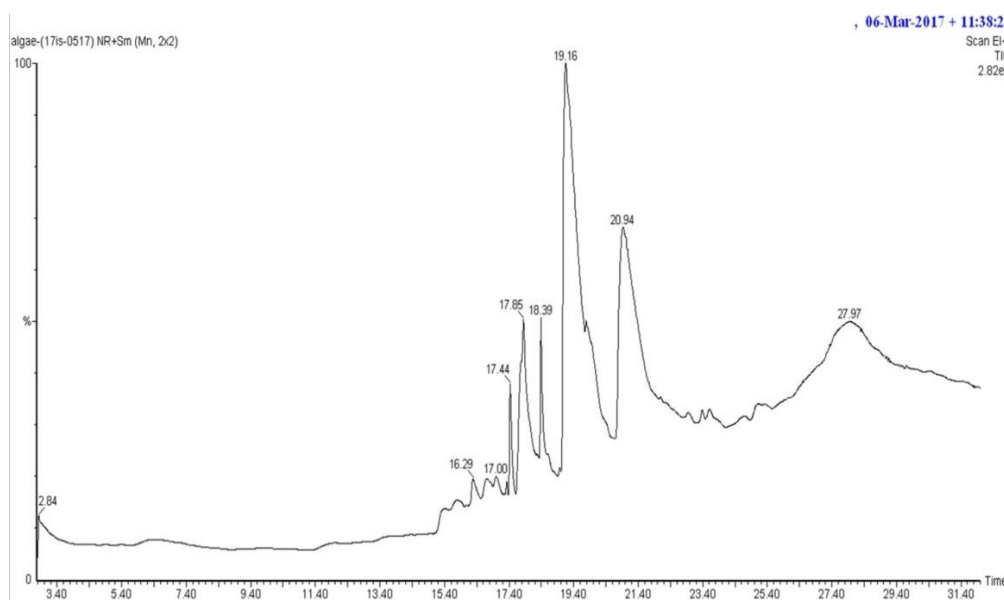


Fig. 4: Complete GC-MS Chromatogram for methanol extract of *Chaetomorpha antennina*

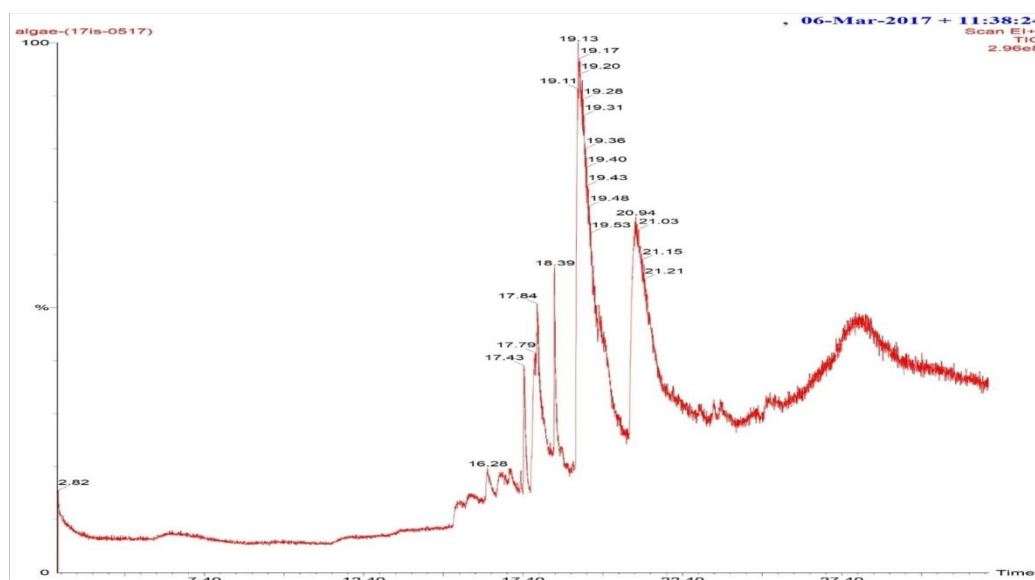


Fig.5: The chromatogram obtained from GC-MS analysis when processed at 19.16 min

The compound octadecanoic acid is acknowledged as an antioxidant whereas, carpesterol dehydrate is also reported to have anti-tumour activity. Anti-inflammatory compound hexadecanoic acid and seasoning agent such as pentadecanoic acid was also recognized. Similarly, when the other peaks were further processed yielded different peaks indicating the presence of the remaining 15 compounds. The outcomes of the integrated peaks of the chromatogram, i.e. 35 compounds obtained from GC-MS analysis, are mentioned in table 4. The second highest peak at the retention time of 20.94 min revealed the presence of certain compounds such as 1-hexyl-2-nitrocyclohexane, oleic acid, 9-hexadecenoic acid, 6-octadecenoic acid, (z), 1-hexyl-1-nitrocyclohexane, 9-octadecenal, (z), 1,19-eicosadiene, cis-9-hexadecenoic acid, 10-undecenyl ester, oleic acid. Oleic acid is accountable for the hypotensive effects. This peak also comprises of 8-hexadecenal, 14-methyl-, (z), cis-10-heptadecenoic acid, 2-methyl-z, z-3,13-octadecadienol, 2-(1-amino-3-methyl) butyl-4-amino-6-dimethylamino-s-triazine, cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl), oleyl alcohol, z-8-methyl-9-tetradecenoic acid, e-9-tetradecenoic acid, oxirane, tetradecyl,9-hexadecenoic acid, 9-hexadecenyl ester. Triazine is an anti-cancer compound. Constant exposure to Ethylene oxide (Oxirane) is also mutagenic. However, extracting individual phytochemical substances and subjecting them to the biological activity will certainly give a prolific outcome. The results could be concluded that this methanol extract of *Chaetomorpha antennina* comprises many important chemical substances with anti-cancer properties.

Docking results

BRCA1 receptor plays a crucial part in the expansion of breast cancer. To explore the anti-cancerous potential of the algal extract against the BRCA1 receptor, docking studies were performed using Auto dock. Docking stands for a computational endeavour to envisage the interaction between macromolecule and the phytochemical compound. Auto dock utilized

for docking studies is technologically advanced software that displays additional accuracy than other software's. In the current study, methanol extract of *Chaetomorpha antennina* was exposed to GC-MS investigation to categorize the budding phytochemical elements. These compounds obtained from GC-MS analysis were docked against the receptor BRCA1 (4JLU) employing Auto dock to envisage the phytochemical compounds' affinity and binding alignment. Table 5 displayed the top five compounds with higher binding affinity with the target protein. The investigation was completed grounded on higher binding energy. The more negative binding energy values were deliberated as the strong binding affinity value of the compound (ligand) for each docking. A summary of hydrogen bond interaction with BRCA1 is displayed in Fig. 6. Carpesterol dehydrate and 2-(1-amino-3-methyl) butyl-4-amino-6-dimethylamino-s-triazine was found highest in binding energy.

Carpesterol dehydrate (b) 2-(1-amino-3-methyl) butyl-4-amino-6-dimethylamino-s-triazine

The phytochemical compound Carpesterol Dehydrate exhibited a negative binding affinity value of -5.79 kcal/mol. It exposed single hydrogen bond interaction with Lysine 1750 that facilitated the composite to accomplish the conventional conformation of the complex structure. 2-(1-amino-3-methyl) butyl-4-amino-6-dimethylamino-s-triazine was found second higher in binding energy with a binding affinity value of -5.18 kcal/mol with hydrogen bond attractions at ASP1733, ASN1730, and HIS1732. Cyclohexanol,1-ethyl-2,2-dimethyl-6-methylene was found to have a binding energy of -4.29 kcal/mol followed by Cyclohexanol,1-ethyl-2,2-dimethyl-6-methylene with a binding energy of -4.18kcal/mol. Therefore, it was noted that compounds present in the methanol extract of *Chaetomorpha antennina* were found effective against breast cancer receptor target BRCA 1, thus indicating this marine alga as a promising candidate for designing novel drugs against breast cancer.

Table 4: Identified bioactive compounds of methanol extract of algae by GC-MS

S.no.	Compound Name	Mol. Formula	Mol. weight	Retention time
1	2-(1-amino-3-methyl)butyl-4-amino-6-dimethylamino-s-triazine	C ₃ H ₆ N ₆	126.12 g/mol	19.31min
2	6,10-dodecadien-1-yn-3-ol	C ₁₂ H ₁₈ O	178.27 g/mol	16.28 min
3	4-methyl-dodec-3-en-1-ol	C ₁₃ H ₂₆ O	198.34 g/mol	12.9min
4	4-methyl-z-4-hexadecen-1-ol	C ₁₇ H ₃₄ O	254.5 g/mol	12.59min
5	9-hexadecenoic acid	C ₁₆ H ₃₀ O ₂	254.41 g/mol	19.36 min
6	octadecanal,2-bromo	C ₁₈ H ₃₅ OBr	347.4 g/mol	24.24min
7	Carpesterol dehydrate	C ₃₇ H ₅₂ O ₃	544.821g/mol	19.40min
8	Cyclohexanol, 1-ethyl-2,2-dimethyl-6-methylene	C ₁₁ H ₂₀ O	168.28 g/mol	19.53min
9	1-(1,5-dimethylhexyl)-4-(4-methylpentyl)cyclohexane	C ₂₀ H ₄₀	280.5 g/mol	12.97min
10	dodecanoic acid	C ₁₂ H ₂₄ O ₂	201.31 g/mol	19.17min
11	e-2-tetradecen-1-ol	C ₁₄ H ₂₈ O	212.37 g/mol	19.13min
12	1,19-eicosadiene	C ₂₀ H ₃₈	278.5 g/mol	27.26min
13	1b,4a-epoxy-2h-cyclopenta[3,4]cyclopropa[8,9]cycloundec[1,2-b]oxir	C ₂₈ H ₃₈ O ₁₁	550.6 g/mol	19.48min
14	ethyl iso-allocholate	C ₂₆ H ₄₄ O ₅	436.6 g/mol	24.78min
15	heptadecanoic acid	C ₁₇ H ₃₄ O ₂	272.44 g/mol	20.94min
16	n-hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256.42 g/mol	19.20min
17	oxirane, hexadecyl	C ₁₈ H ₃₆ O	268.5 g/mol	24.23min
18	4-methyl-z-4-hexadecen-1-ol	C ₁₇ H ₃₄ O	254.5 g/mol	17.85min
19	2-methyl-z,z-3,13-octadecadienol	C ₁₉ H ₃₆ O	280.5 g/mol	17.79min
20	octadecanoic acid	C ₁₈ H ₃₆ O ₂	284.5 g/mol	19.28min
21	z-2-octadecen-1-ol	C ₁₈ H ₃₆ O	268.5 g/mol	21.03min
22	2-octadecyl-propane-1,3-diol	C ₂₁ H ₄₀ O ₃	340.5 g/mol	32.6min
23	oleic acid	C ₁₈ H ₃₄ O ₂	282.5 g/mol	27.83 min
24	oleyl alcohol	C ₁₈ H ₃₆ O	268.5 g/mol	20.92min
25	9-oxononanoic acid	C ₉ H ₁₆ O ₃	172.22 g/mol	31.82 min
26	pentadecanoic acid	C ₁₅ H ₃₀ O ₂	242.4 g/mol	19.13 min
27	e-3-pentadecen-2-ol	C ₁₅ H ₃₀ O	226.4 g/mol	24.3 min
28	pentanoic acid	C ₁₆ H ₃₀ O ₂	254.41 g/mol	21.21min
29	phytol	C ₂₀ H ₄₀ O	296.5 g/mol	20.61min
30	tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228.37 g/mol	18.39min
31	1b,5,5,6a-tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	C ₁₃ H ₂₀ O ₂	208.3 g/mol	19.48min
32	tridecanoic acid	C ₁₃ H ₂₆ O ₂	239.5 g/mol	17.43min
33	11-tridecen-1-ol	C ₁₃ H ₂₆ O	198.34 g/mol	32.7min
34	cyclopentane,	C ₂₆ H ₅₀	362.7 g/mol	21.15min
35	4-methyl-dodec-3-en-1-ol	C ₁₃ H ₂₆ O	100.16 g/mol	31.9min

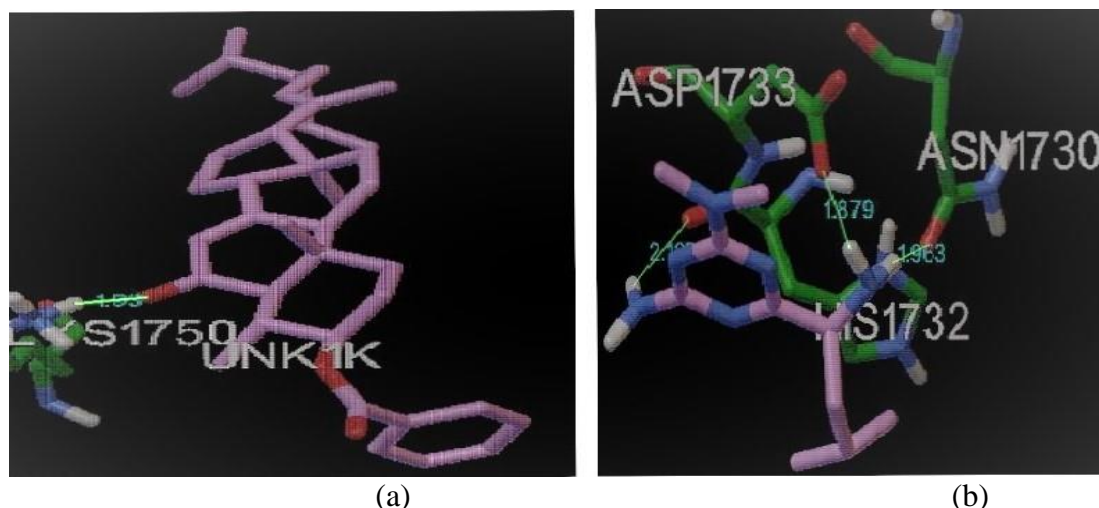


Fig. 6: Summary of the docking hydrogen bond interaction with BRCA1 of compounds (a) Carpesterol dehydrate (b) 2-(1-amino-3-methyl) butyl-4-amino-6-dimethylamino-s-triazine

Table 5: Docking results for top five compounds with highest binding energies

S. No.	Compound	Binding Energy	Ligand Efficiency	No. of H-Bond	Details Of H- Bond
1	Carpesterol dehydrate	-5.79	-0.14	1	4JLU:A: LYS:1750
2	2-(1-Amino-3-Methyl)Butyl-4-Amino-6-Dimethylamino-S-Triazine	-5.18	-0.32	3	UNK: 1K: H27:ASP1733 UNK: 1K:H28: ASN1730 UNK:1K:H35:HIS1732
3	1b,5,5,6a-tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	-4.41	-0.29	1	UNK:1K:H35:ASP1733
4	1b,4a-epoxy-2h-cyclopenta[3,4]cyclopropa[8,9]cycloundec[1,2-b]oxir	-4.35	-0.11	1	4JLU:A:ARG1747
5	Cyclohexanol,1-Ethyl-2,2-Dimethyl-6-Methylene	-4.23	-0.35	1	UNK:1K: H27:GLN1747

Conclusions

This study displayed several phytochemical compounds in methanol extract of marine algae *Chaetomorpha antennina*, and the occurrence of these chemical compounds makes the algal sample pharmacologically significant. It was noted that the methanol extract of this seaweed possessed higher antioxidant, antibacterial and anti-cancer activity when compared with suitable controls. The silver nanoparticles synthesized using the methanol extract also confirmed the antibacterial actions of the phytochemicals present in the extract. The chemical constituents budding from the seaweed extract were analyzed. They were checked for anti-cancer activity employing molecular docking studies against the foremost contributing protein breast cancer susceptibility protein 1

(BRCA1) receptor. Further, when the chemical constituents were screened for anti-cancer activity, the BRCA1 receptor was considerably affected by the chemical constituents present in the methanol extracts of *Chaetomorpha antennina*. This study demonstrated the anti-cancer activity of methanol extract of *Chaetomorpha antennina* because of numerous anti-cancer compounds present in the extract, such as Carpesterol dehydrate and Triazines with the highest binding energies. Thus, it can be stated that this seaweed has a broad spectrum of secondary metabolites with different potentialities that can serve as a promising antioxidant and can be utilized as an enhanced drug in the treatment of breast cancer.

Acknowledgements

The authors gratefully acknowledge VIT, Vellore, for the support through Seed Grant for Research.

REFERENCES

- 1- M. Schapira, B.M. Raaka, H.H. Samuels and R. Abagyan, "In silico discovery of novel retinoic acid receptor agonist structures", *BMC Struct Biol*, 1(1),1-7(2001).
- 2- S. Pal, "An insilico drug designing approach to target the BRCA1 protein involved in breast cancer", *Helix*, 6(1),761-765(2016).
- 3- B. K. Carte, "Biomedical potential of marine natural products", *Bioscience*, 46(4),271-286(1996).
- 4- C. M. Ireland, B. R. Copp, M. P. Foster, L. A. McDonald, D. C. Radisky and J. C. Swersey, "Bioactive compounds from the sea", *In Pharmaceutical and bioactive natural products*,143(1993).
- 5- S.S. Al-Saif, N Abdel-Raouf, H.A. El-Wazanani and I.A. Aref, "Antibacterial substances from marine algae isolated from Jeddah coast of Red sea, Saudi Arabia", *Saudi J Biol Sci*, 21(1),57-64(2014).
- 6- H. Zbakh, H. Chiheb, H. Bouziane, V.M. Sánchez and H. Riadi, "Antibacterial activity of benthic marine algae extracts from the Mediterranean coast of Morocco", *J Microbiol Biotechnol Food Sci*, 2021, 219-228(2021).
- 7- P. Bhadury and P. C. Wright, "Exploitation of marine algae: biogenic compounds for potential antifouling applications", *Planta*, 219(4), 561-578(2004).
- 8- R. Bouhlal, C. Haslin, J.C. Chermann, S. Collic-Jouault, C. Siquin *et.al*, "Antiviral activities of sulfated polysaccharides isolated from *Sphaerococcus coronopifolius* (Rhodophyta, Gigartinales) and *Boergeseniella thuyoides* (Rhodophyta, Ceramiales)", *Mar Drugs*, 9(7),1187-1209(2011).
- 9- K.P. Devi, N. Suganthy, P. Kesika and S.K. Pandian, "Bioprotective properties of seaweeds: in vitro evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content", *BMC Complement Altern Med*, 8(1),1-1(2008).
- 10- S.K. Kim, N.V. Thomas and X. Li, "Anti-cancer compounds from marine macroalgae and their application as medicinal foods", *Adv Food Nutr Res*, 64,213-24(2011).
- 11- H.J. Na, P. D. Moon, H. J. Lee, H.R. Kim, H.J. Chae, T. Shin *et al.*, "Regulatory effect of atopic allergic reaction by *Carpopeltis affinis*", *J Ethnopharmacol*, 101(1-3),43-48(2005).
- 12- H.A. Monsur, "Anti-inflammatory compounds of macro algae origin: A review", *J Med Plant Res*, 5(33),7146-7154(2011).
- 13- L. Mhadhebi, K. Chaieb and A. Bouraoui, "Evaluation of antimicrobial activity of organic fractions of six marine algae from Tunisian Mediterranean coasts", *Int J Pharm Pharm Sci*, 4(1),534-537(2012).
- 14- M. Balamurugan, G.G. Selvam, T. Thinakaran and K. Sivakumar, "Biochemical study and GC-MS analysis of *Hypnea musciformis* (Wulf.) Lamouroux", *Am Eurasian J Agric Environ Sci*, 8(3),117-123(2013).
- 15- M. Barbosa, P. Valentão and P.B. Andrade, "Bioactive compounds from macroalgae in the new millennium: implications for neurodegenerative diseases", *Mar Drugs*, 12(9),4934-4972(2014).
- 16- F.A. Torres, T.G. Passalacqua, A. Velásquez, R.A. Souza, P. Colepicolo and M.A. Graminha, "New drugs with antiprotozoal activity from marine algae: a review", *Rev bras farmacogn*, 24, 265-276(2014).
- 17- K. Kolanjinathan, P. Ganesh and P. Saranraj, "Pharmacological importance of seaweeds: a review", *World Journal of Fish and Marine Sciences (WJFMS)*,6(1),1-5(2014).
- 18- I. Hamed, F. Özogul, Y. Özogul and J.M. Regenstein, "Marine bioactive compounds and their health benefits: a review", *Compr Rev Food Sci Food Saf*, 14(4),446-465(2015).

- 19- J. W. Blunt, B. R. Copp, R. A. Keyzers, M.H. Munroa and M. R. Prinsepd, "Natural product reports", *Nat Prod Rep.*, 33,382-431(2016).
- 20- A.H. Shobier, S.A. Ghani and K.M. Barakat,"GC/MS spectroscopic approach and antifungal potential of bioactive extracts produced by marine macroalgae", *Egypt J Aquat Res*, 42(3),289-299(2016).
- 21- R. Shellie, L. Mondello, P. Marriott and G. Dugo, "Characterization of lavender essential oils by using gas chromatography–mass spectrometry with correlation of linear retention indices and comparison with comprehensive two-dimensional gas chromatography", *J Chromatogr A*, 970(1-2), 225-234(2002).
- 22- A. Periyasamy and K. Mahalingam, "Phytochemical screening and antimicrobial activity from five Indian medicinal plants against human pathogens", *Middle East J Sci Res*, 5(6),477-482(2010).
- 23- C. M. Venkatachalam, X. Jiang, T. Oldfield and M. Waldman, "LigandFit: a novel method for the shape-directed rapid docking of ligands to protein active sites", *J Mol Graph Model*, 21(4), 289-307(2003).
- 24- S. Thanigaivel, S. Vijayakumar, A. Mukherjee, N. Chandrasekaran, J. Thomas, "Antioxidant and antibacterial activity of *Chaetomorpha antennina* against shrimp pathogen *Vibrio parahaemolyticus*", *Aquaculture*, 433,467–475 (2014).
- 25- S.H. Haq, G. Al-Ruwaished, M.A. Al-Mutlaq, S.A. Naji, M. Al-Mogren *et.al*, "Antioxidant, anti-cancer activity and phytochemical analysis of green algae, *Chaetomorpha* collected from the Arabian Gulf", *Sci Rep*, 9(1),1-7(2019).
- 26- T. H. Ranahewa, A. D. Premarathna, R. M. Wijesundara, V. Wijewardana, A.P. Jayasooriya *et.al*, "Biochemical composition and anti-cancer effect of different seaweed species (In-vitro and in-vivo studies)", *Sustain MAr Struct*, 2(2019).
- 27- J. B. Harborne, "Methods of extraction and isolation", *Phytochem Anal*, 3,60-66(1998).
- 28- K. P. Devi, N. Suganthy, P. Kesika, S.K. Pandian, "Bioprotective properties of seaweeds: in vitro evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content", *BMC complement med ther*, 8(1),1(2008).
- 29- S. Murugesan, S.U. Bhuvanewari, V. A. Sivamurugan, "Green synthesis, characterization of silver nanoparticles of a marine red alga *Spyridia fusiformis* and their antibacterial activity", *Int J Pharm Pharm Sci*, 9(5),192-197(2017).
- 30- A. Sen and A. Batra, "Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach* L.", *Int J Curr Pharm Res*, 4(2),67-73(2012).
- 31- K.R. Martin, "Silicon: the health benefits of a metalloid", *Interrelations between essential metal ions and human diseases.*, 451-473(2013).
- 32- Q. Wu, L. Liu, A. Miron, B. Klímová, D. Wan and K. Kuča, "The antioxidant, immunomodulatory, and anti-inflammatory activities of *Spirulina*: an overview", *Arch Toxicol*, 90(8),1817-1840(2016).
- 33- P. Senthilkumar and S. Sudha, "Antioxidant and antibacterial properties of methanolic extract of green seaweed *Chaetomorpha linum* from Gulf of Mannar: Southeast coast of India", *Jundishapur J Microbiol*, 5(2),411-415(2014).
- 34- K. Subathraa and T.V. Poonguzhali, "Effect of different extracts of *Chaetomorpha antennina* and their phytochemical screening", *Int J Curr Res*, 6,35-39(2013).
- 35- K.E. Washburn and J.E. Birdwell, "Multivariate analysis of ATR-FTIR spectra for assessment of oil shale organic geochemical properties", *Org Geochem*, 63,1-7(2013).



نشرة العلوم الصيدلانية جامعة أسيوط



كشف الخصائص المضادة للأكسدة والمضادة للسرطان للمكونات الكيميائية الموجودة في مستخلص الميثانول من الطحالب الخضراء كيتومورفا انتينينا

أكانكشا دوبي^١ - جاياتني سيفارامان^٢

^١ باحث ، قسم التكنولوجيا الحيوية ، كلية العلوم البيولوجية والتكنولوجيا ، معهد فيلور للتكنولوجيا ، فيلور ٦٣٢٠١٤ ، تاميل نادو ، الهند

^٢ أستاذ مشارك ، قسم التكنولوجيا الحيوية ، كلية العلوم الحيوية والتكنولوجيا ، معهد فيلور للتكنولوجيا ، فيلور ٦٣٢٠١٤ ، تاميل نادو ، الهند

الخلفية: كيتومورفا انتينينا هو عشب بحري أخضر له العديد من الخصائص الدوائية مثل مضادات الميكروبات ، ومضادة للملاريا ، ومضادة للأورام ومضادة للأكسدة. وبالتالي ، من الضروري إجراء دراسات أولية لتحديد التركيب الكيميائي لمستخلصات الأعشاب البحرية واستكشاف المكونات المسؤولة عن الأنشطة الدوائية. السرطان هو السبب الرئيسي للوفاة في جميع أنحاء العالم. إن اكتشاف أدوية جديدة لعلاج السرطان هو المهمة الرئيسية. نظرًا لأن توصيل الأدوية بكفاءة أمر ممكن من خلال تقنية النانو ، فقد تم إجراء التخليق الحيوي لجسيمات الفضة النانوية ، تليها دراسات الالتحام الجزيئي للتحقيق في النشاط المضاد للسرطان لمستخلص الأعشاب البحرية.

الطرق: تم إجراء التحليل الكيميائي لمستخلصات كيتومورفا انتينينا ، تلاها التخليق الحيوي لجزيئات الفضة النانوية باستخدام مستخلصات الطحالب. تم توصيف جسيمات الفضة النانوية عن طريق المسح المجهر الإلكتروني وحيود الأشعة السينية. تم استخدام طريقة آجار ويل ديفيوشن باستخدام ٢،٢-ثنائي فينيل -١ بيكريل هيدرازيل (DPPH) لتحديد إمكانات مضادات الميكروبات وإمكانات مضادات الأكسدة لمستخلصات الطحالب. علاوة على ذلك ، تم تمييز التركيب الكيميائي للمستخلص بواسطة مقياس الطيف الكتلي اللوني للغاز.

النتائج: احتوت مستخلصات الأعشاب البحرية على العديد من المواد الكيميائية وأظهر مستخلص الميثانول أفضل فعالية كمضاد للبكتيريا والأكسدة. كشفت دراسات عن وجود كاربستيرول ديهيدرات والتريازينات كعنصر مضاد للسرطان في كيتومورفا انتينينا

الخلاصة: يمكن أن نستنتج أن الأعشاب البحرية غنية بالمكونات الكيميائية التي لها نشاط مضاد للأكسدة ، وبالتالي يمكن أن تستخدم بمثابة مضاد للأكسدة ومضاد للسرطان.