ORIGINAL ARTICLE

Investigation of the Cytotoxic and Molecular Interaction Properties of *Ulva lactuca* for Oral Cancer Applications

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Abstract

Background: Marine seaweeds are rich sources of bioactive compounds with potential anticancer properties. *Ulva Lactuca* (*U. lactuca*), a green seaweed, has gained attention for its diverse phytochemical composition and therapeutic relevance. Objective: This study aimed to evaluate the biochemical composition, cytotoxic activity, and molecular interactions of *U. lactuca* against oral cancer. **Methods:** Proximate analysis was conducted to determine the biochemical composition of the seaweed biomass. Methanol, ethanol, and aqueous extracts were prepared and assessed for cytotoxic effects against KB oral cancer cells. Morphological changes were examined to confirm cytotoxicity. Further, compound–target mapping was performed to identify overlapping genes associated with oral cancer. Protein–protein interaction (PPI) analysis was used to determine key regulatory targets, followed by molecular docking to evaluate ligand–protein binding affinity. **Results:** Proximate analysis revealed high levels of moisture, ash, carbohydrates, lipids, and proteins in *U. lactuca*. Among the extracts, the methanol extract exhibited the highest cytotoxicity against KB cells, accompanied by significant morphological alterations compared to ethanol and aqueous extracts. PPI analysis identified key targets, including TP53, EGFR, CTNNB1, AKT1, TNF, and IL-6. Molecular docking demonstrated strong binding affinity of 5-sulfosalicylic acid and 4-hydroxybenzoic acid with TP53, CTNNB1, and EGFR through stable hydrogen bond interactions. **Conclusion:** The findings demonstrate that *U. lactuca* possesses significant anticancer potential, supported by both experimental and in silico analyses. Its bioactive metabolites may serve as promising therapeutic candidates for oral cancer treatment.

Key words: Cytotoxic activity, hub genes, molecular docking, network pharmacology, oral cancer, *Ulva lactuca*

INTRODUCTION

Marine macroalgae have been known to be an excellent source of natural products with diversity in biological activities. They possess antioxidant, anti-inflammatory, antimicrobial, and cytoprotective properties due to their wide range of chemical components, including polysaccharides, phenolic acids, lipids, and minerals.[1] As a result of this chemical

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diversity, there has been a shift toward seaweed as a source of developing natural agents to promote human health. Green seaweed is widely spread in coastal habitats and is characterized by high mineral content, by ulvan polysaccharides, and small bioactive molecules.[2] These properties have created interest in the exploration of this seaweed as a source of nutritional gains as well as a source of potential therapeutically active compounds.

They are urgent need for natural alternatives in pathologies such as oral squamous cell carcinoma (OSCC) whose response to treatment is limited by toxicity and a mixture of dysregulated signaling pathways.[3] Despite the inherent benefits of the current clinical approaches, the overall effectiveness of these methods is constrained by the complex molecular landscape of OSCC.[4] This has created an interest in the isolation of natural molecules that may influence important cell growth, cell survival, and inflammatory regulators.

Computational methodology can be used to rapidly identify compound-target interactions and to aid in mapping the molecular networks of tumor progression. Target prediction, protein–protein interaction (PPI) analysis, and molecular docking methods would evaluate the potential of natural compounds to bind proteins of interest to OSCC biology.[5] The combination of their application with initial experimental observations presents a multifaceted approach on which to critically assess biological relevance and therapeutic potential.

Ulva lactuca is a green seaweed that belongs to the number of the most widespread species with a series of positive characteristics to use in the exploration of bioactive compounds. It is a sustainable and readily available resource due to its abundance in coastal waters, ease of cultivation, and a thoroughly investigated nutritional profile.[6] The ulvan polysaccharides, phenolic compounds, and bioactive peptides in *U. lactuca* are especially abundant and have been shown to have anti-oxidant, anti-inflammatory, and immunomodulatory activity in a variety of biological systems. Although *U. lactuca* is widely used as a nutritional supplement, its therapeutic potential against oral cancer has not been extensively investigated.[7] Research on the interaction of its bioactive metabolites with molecular targets related to OSCC, other than their effects on oral cancer cell behavior, is suboptimal. Further elucidation of such interaction is highly required for determining whether *U. lactuca* may serve as a potential source of bioactive compounds with considerable anti-cancer properties for OSCC therapy.[8]

The paper analyzed the possible relevance of *U. lactuca* in the treatment of oral cancer by combining biochemical, cytotoxicity, and computational methodologies. Specifically, this work has been focused on the phytochemical profile characterization, the cytotoxic effects of its extracts against KB oral cancer cells, and the computational analysis of the molecular interactions between its bioactive molecules and essential OSCC-related proteins. This complex method has been developed to detect relevant compounds in *U. lactuca* that affect oral cancer and develop an initial strategy to explore them therapeutically.

MATERIALS AND METHODS

Sample collection and preparation

The samples of Fresh *U. lactuca* were collected in the coastal area of Mandapam (Rameswaram, Tamil Nadu, India). Epiphytes and debris were washed in seawater, followed by rinsing in distilled water, room-temperature shade-drying, and reduction to a fine powder. The dried material was kept in airtight containers until it was extracted.[9]

Preparation of extracts

In extraction, 10 g powder of *U. lactuca* was added to 100 mL of distilled water, methanol, or ethanol. The mixtures were maintained under constant conditions of agitation, at a room temperature of 8 h. The extracts were then filtered using Whatman No. 1 filter paper after incubation, concentrated, and stored to be used in further analyses.[9]

Nutritional profiling of *U. lactuca*

Moisture content

The percentage of moisture was analyzed through the oven-drying procedure. Fresh samples were dried in a hot-air oven at 105 ± 2°C until reaching a constant weight. The loss of weight was divided by the original weight to obtain the percentage of moisture.[10]

Ash content

The content of ashes was determined in accordance with the AOAC procedures. A known quantity of a dried sample was put into pre-weighed porcelain crucibles and burnt in a muffle furnace at 550–600°C for 4 h. Crucibles were cooled in a desiccator and reweighed, and ash was determined as a percentage of the dry mass.[10]

Lipid content

The Soxhlet method was used to determine the lipid content.[10] 5 g of the dried seaweed powder was dissolved in acetone over a period of 8 h. The lipid residue was then dried after solvent evaporation to constant weight and reported as a percentage of dry weight.

Protein content

The protein content was identified using the Kjeldahl method. The samples were digested using a mixture of copper sulfate,

potassium sulfate, and concentrated H_2SO_4. The solution was digested and then neutralized with NaOH, and ammonia was distilled into boric acid. Total nitrogen was determined by titration using standardized HCl, and a conversion factor was used to determine the amount of crude protein.[11]

Carbohydrate content

The composition of moisture, ash, lipid, and protein in percentages was added and deducted from 100 to get the overall carbohydrate composition.[11]

MTT cytotoxicity assay

The MTT assay was used to determine the cytotoxic effects of the aqueous, methanolic, and ethanolic extracts.[12] The cells were seeded in 96-well plates and allowed to adhere for 24 h. Then, the cells were incubated with a determined concentration of each extract for 24 h. After that, 20 µL of MTT solution (5 mg/mL) was placed in each well and allowed to incubate the cells for 4 h. The medium was discarded and formazan crystals were dissolved in 150 µL dimethyl sulfoxide. The absorbance was measured at 570 nm, and cell viability was calculated as compared to the untreated controls.

In silico molecular docking analysis

Oral cancer-associated target retrieval

Oral cancer-related genes were collected from the GeneCards database (https://www.genecards.org/) using keywords such as oral cancer, OSCC, and head-and-neck carcinoma. Genes with high relevance scores were selected, and redundant entries were removed manually.[13]

Comparative target mapping and Venn analysis

Predicted compound targets were compared with the oral cancer gene list. Overlapping genes were identified using an online Venn tool (https://bioinformatics.psb.ugent.be/webtools/Venn/). These common targets represent oral cancer–associated proteins potentially modulated by *U. lactuca* metabolites.[14]

PPI network construction

The overlapping genes were submitted to the STRING database (https://string-db.org/) with the organism set to Homo sapiens and a confidence score of 0.7. The interaction network was exported and visualized in Cytoscape v3.5.1.

Network topology was analyzed using CytoHubba, and hub genes were ranked using five centrality metrics: Degree, Betweenness, Radiality, Bottleneck, and Maximum clique centrality. The highest-scoring genes were selected for docking.[14]

Molecular docking of *U. lactuca* bioactive

Docking studies were carried out for four bioactive compounds – 5-sulfosalicylic acid (CID: 7322), 4-hydroxybenzoic acid (CID: 135), hexadecasphinganine (CID: 656816), and azelaic acid (CID: 2266) – against key oral cancer regulatory proteins. Crystal structures of CTNNB1 (β-catenin; PDB ID: 1JDH), tumor protein p53 (TP53) (PDB ID: 3DCY), and epidermal growth factor receptor (EGFR) (PDB ID: 7AEI) were retrieved from the RCSB Protein Data Bank. Water molecules, heteroatoms, and co-crystallized ligands were removed. Polar hydrogens and Gasteiger charges were added using AutoDock Tools to optimize protein geometry.[15]

Ligand structures were obtained from PubChem and converted to 3D PDB format using Open Babel. Geometry optimization and energy minimization were performed using PyRx. Docking simulations were executed in PyRx 0.8 using AutoDock Vina. A 90 Å × 90 Å × 90 Å grid box was applied to each protein to allow unrestricted binding-site exploration. Docking was performed in triplicate, and the lowest binding-energy pose from each run was selected. The docked complexes were visualized in BIOVIA Discovery Studio Visualizer 2021. Hydrogen bonding, hydrophobic contacts, π–π stacking, van der Waals forces, and electrostatic interactions were evaluated. Particular focus was placed on residues relevant to β-catenin, TP53, and EGFR signaling pathways. Both 2D and 3D interaction maps were generated.

RESULTS

Compound–target overlap analysis

The comparative analysis of target proteins across the four selected compounds revealed both shared and unique interaction profiles. The major phytochemicals identified through GC–MS analysis of *Ulva lactuca* extract were selected based on their relative abundance and reported biological activity. Among these, four compounds—4-hydroxybenzoic acid, 5-sulfosalicylic acid, azelaic acid, and hexadecasphinganine—were chosen for further target prediction and molecular interaction analysis. As illustrated in Figure 1a, 4-hydroxybenzoic acid, hexadecasphinganine, and 5-sulfosalicylic acid commonly targeted 59 proteins, while 22 proteins were shared among all four compounds. Despite this overlap, each compound exhibited a distinct target profile, with 5-sulfosalicylic acid showing the highest number of unique targets (79 proteins), followed by azelaic acid (54 proteins), hexadecasphinganine (28 proteins), and 4-hydroxybenzoic acid with only 3 unique targets.

Further comparison between compound-predicted targets and oral cancer-associated genes [Figure 1b] demonstrated a substantial overlap of 100 proteins, indicating strong relevance

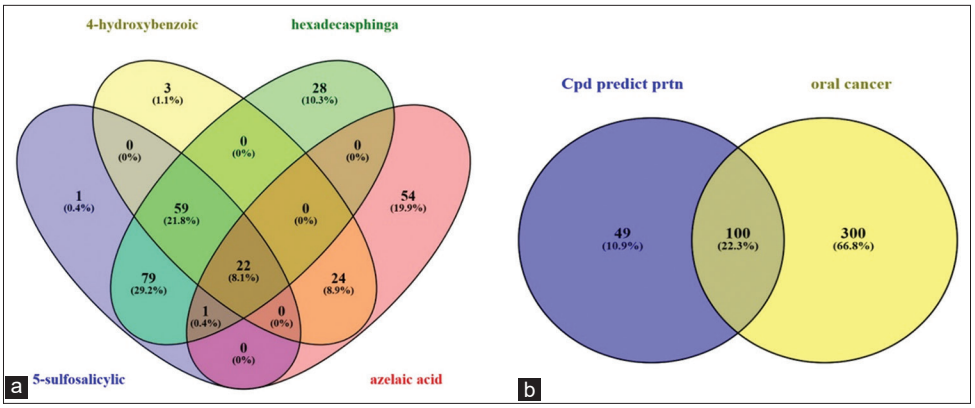

Figure 1: Venn diagrams of predicted compound targets and disease associations. (a) Overlap of predicted protein targets for the four major compounds identified in *Ulva lactuca*: 4-hydroxybenzoic acid, hexadecasphinganine, 5-sulfosalicylic acid, and azelaic acid. (b) Intersection of compound-predicted protein targets with oral cancer-associated genes

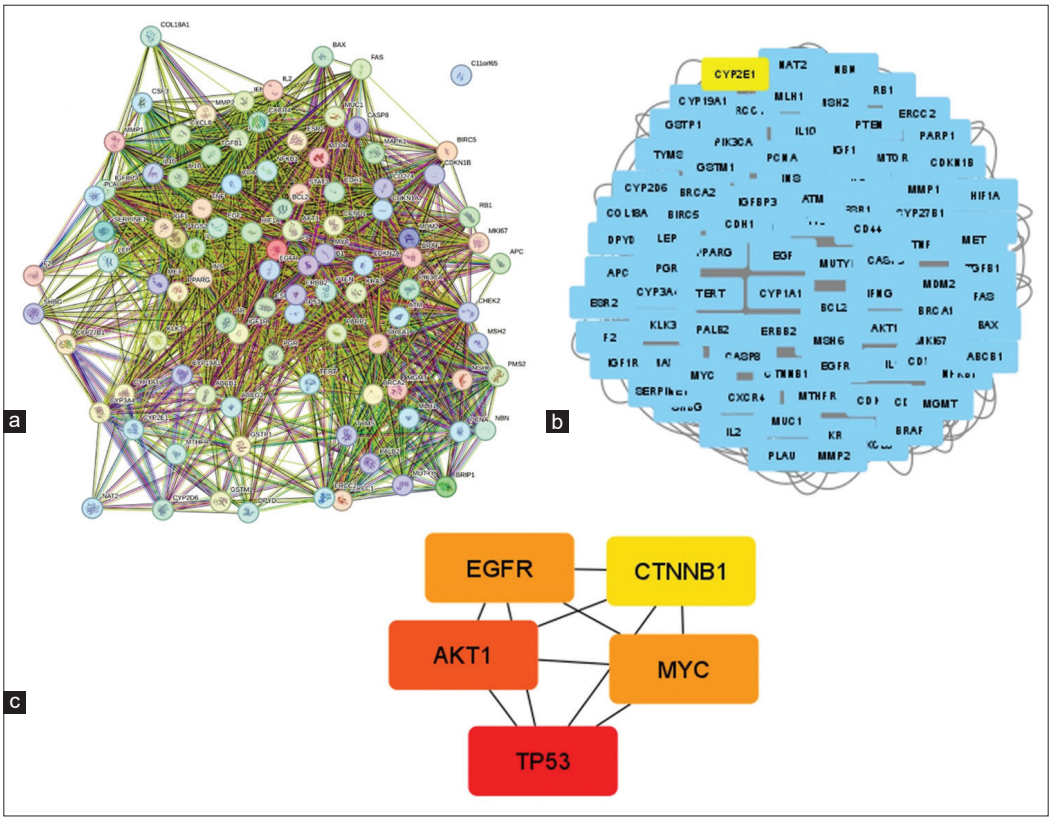

Figure 2: Network construction and hub gene identification. (a) Protein–protein interaction network of predicted compound-associated targets visualized using STRING. (b) Expanded interaction network showing all mapped compound-associated and oral cancer-related targets. (c) Hub gene module highlighting key regulatory nodes identified through topological analysis

of these targets to oral cancer biology. In addition, 49 proteins were exclusively associated with the compounds, whereas 300 proteins were unique to the oral cancer gene dataset.

Analysis of protein–protein interaction network

The integrated network of PPIs [Figure 2a] displays a densely connected structure symbolizing the complex relations between the overlapped targets. Major regulators comprising TP53, EGFR, CTNNB1, tumor necrosis factor (TNF), IL6, AKT1, and MYC were found to be major hubs with very high interaction density, pointing toward their central role in the whole network. One of the highest-ranked nodes in the degree-based visualization was CYP2E1, which is highly connected in this network [Figure 2b]. Similarly, many of the cancer-related proteins clustered together in close proximity, reflecting strong functional associations. Focused

core-network extraction [Figure 2c] highlighted TP53, EGFR, CTNNB1, AKT1, and MYC as central hub genes forming a tightly connected module. These genes represent major regulatory axes commonly implicated in oral cancer signaling and therefore provide key points of interaction for the selected compounds.

Proximate composition of *U. lactuca*

Table 1 presents the proximate analysis of *U. lactuca* with moisture content of 83.76 ± 1.3%, an expected result due to the hydrated nature of fresh seaweed biomass. A very high ash content of 43.4 ± 1.3% was found, which is indicative of the high mineral fraction characteristic of marine macroalgae. The nutritional composition on a dry weight basis was mainly carbohydrate (67.7 ± 4.9%), followed by lipids (21.9 ± 0.6%) and proteins (15.4 ± 0.9%).

Cytotoxicity analysis

Table 1: Proximate analysis of *Ulva lactuca*

S. No.	Content	*Ulva lactuca* (%)
1	Moisture	83.76±1.3
2	Ash	29.3±1.3
3	Carbohydrate	45.6±4.9
4	Protein	10.4±0.9
5	Lipid	14.8±0.6

Values are articulated as mean±SD (*n*=3). SD: Standard deviation

The cytotoxic activities of *U. lactuca* extracts against KB oral cancer cells using the MTT assay are presented in Figure 3a. All the extracts clearly decreased cell viability dose-dependently after 48 h. An IC_{50} of 132.74 mg/mL was exhibited for the methanol extract, which had the highest cytotoxicity, followed by the ethanol and water extracts with IC_{50} values of 187.49 and 224.47 mg/mL, respectively. Microscopic examination [Figure 3b] revealed significant morphological changes in treated cells, which included loss of confluence, rounding off, and loss of adhesion. Maximal morphological disruption was caused by the methanol extract, which corroborated its higher cytotoxic activity than that of the other tested extracts.

Molecular docking

Molecular docking analysis was done to determine the binding affinity and interaction profiles of the identified four compounds, including 5-sulfosalicylic acid, 4-hydroxybenzoic acid, hexadecasphinganine, and azelaic acid, against the selected key oral cancer-related proteins CTNNB1 [Figure 4], TP53 [Figure 5], and EGFR [Figure 6]. All the compounds bound stably within the active sites of the selected proteins, showing hydrogen bonding with key amino acid residues. The docking results are summarized in Table 2, which presents the binding energies and hydrogen-bond interactions of the four *U. lactuca* compounds with TP53, CTNNB1, and EGFR.

The binding of 5-sulfosalicylic acid was highest among the interactions of TP53 complexes with a binding energy of –7.1 kcal/moL. This ligand interacted through six hydrogen bonds, including LYS162, ALA161, LYS118, GLY17, ASP120, and GLY17, showing stable anchoring within the binding

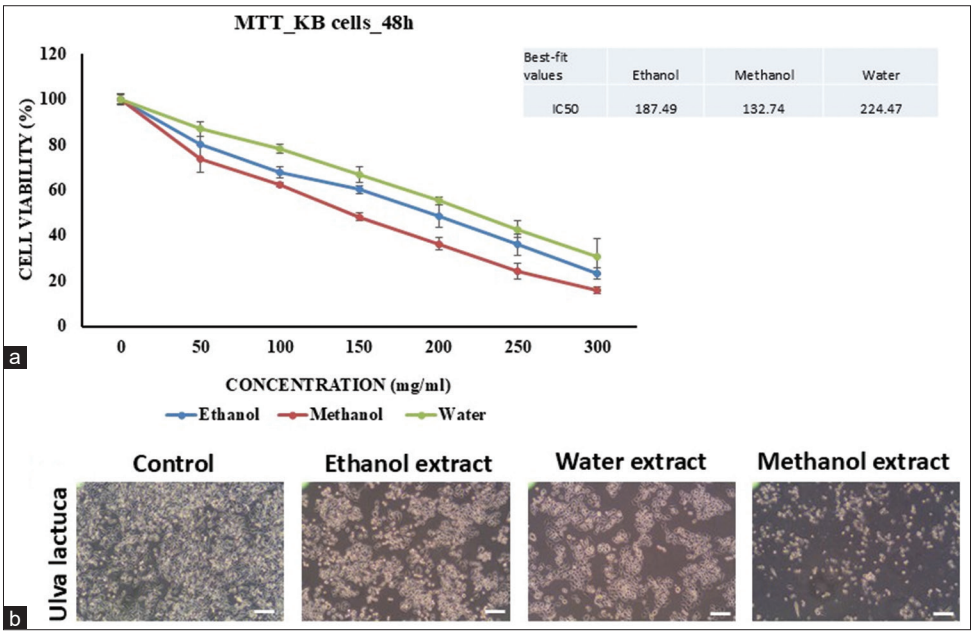

Figure 3: Cytotoxic activity of *Ulva lactuca* extracts against KB cells. (a) Dose–response curve showing cell viability (%) of KB cells treated with ethanol, methanol, and water extracts of *U. lactuca* for 48 h, measured by the MTT assay. IC_{50} values for each extract are shown in the table. (b) Phase-contrast micrographs of KB cells after 48 h exposure to ethanol, methanol, and water extracts compared with untreated control cells. Scale bars indicate 100 μm

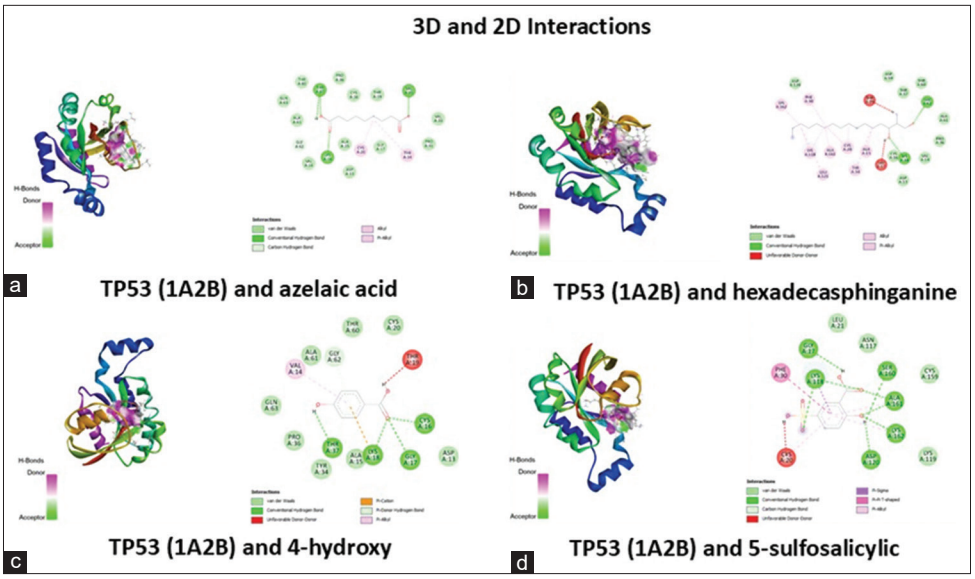

Figure 4: Molecular docking interactions of CTNNB1 (1JDH) with Ulva-derived compounds (a) 3D and 2D interaction profiles of CTNNB1 with 4-hydroxybenzoic acid. (b) 3D and 2D interaction profiles of CTNNB1 with 5-sulfosalicylic acid. (c) 3D and 2D interaction profiles of CTNNB1 with azelaic acid. (d) 3D and 2D interaction profiles of CTNNB1 with hexadecasphinganine

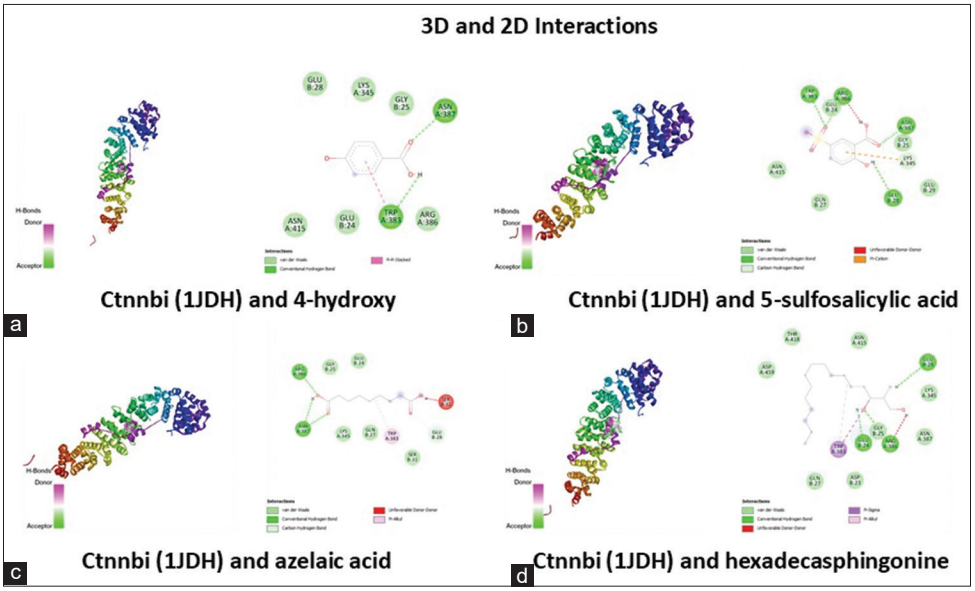

Figure 5: Molecular docking interactions of tumor protein p53 (TP53) (1A2B) with Ulva-derived compounds. (a) 3D and 2D interaction profiles of TP53 with azelaic acid. (b) 3D and 2D interaction profiles of TP53 with hexadecasphinganine. (c) 3D and 2D interaction profiles of TP53 with 4-hydroxybenzoic acid. (d) 3D and 2D interaction profiles of TP53 with 5-sulfosalicylic acid

pocket. 4-Hydroxybenzoic acid showed a binding energy of –6.4 kcal/moL, supported by four hydrogen bonds with LYS18, CYS16, GLY17, and THR37. Hexadecasphinganine exhibited interaction with TP53 at –5.8 kcal/mol, forming two hydrogen bonds with GLY62 and LYS18. Azelaic acid recorded a binding energy of –5.3 kcal/moL, forming three hydrogen bonds with VAL831, THR37, and LYS18.

In the case of CTNNB1, 5-sulfosalicylic acid again had the most favorable binding, at –6.0 kcal/moL, forming four hydrogen bonds with ARG386, TRP386, ASN387, and GLU28. 4-Hydroxybenzoic acid showed binding at –5.0 kcal/mol, forming two hydrogen bonds with ASN387 and TRP383. Azelaic acid had binding interaction at –4.7 kcal/moL, forming two hydrogen bonds with ARG386 and ASN387. And finally, hexadecasphinganine showed a binding energy of –4.0 kcal/mol, forming three hydrogen bonds with GLU28, GLU24, and ARG386. Among these, 4-hydroxybenzoic acid showed the most stable binding with EGFR, with a binding energy of –6.1 kcal/moL, forming four hydrogen bonds with LYS721, THR766, ASP831, and ALA719. 5-Sulfosalicylic acid was found to bind at –5.9 kcal/moL, stabilized by a single hydrogen bond with MET769. Hexadecasphinganine showed binding of –5.1 kcal/moL, forming three hydrogen bonds with MET74,

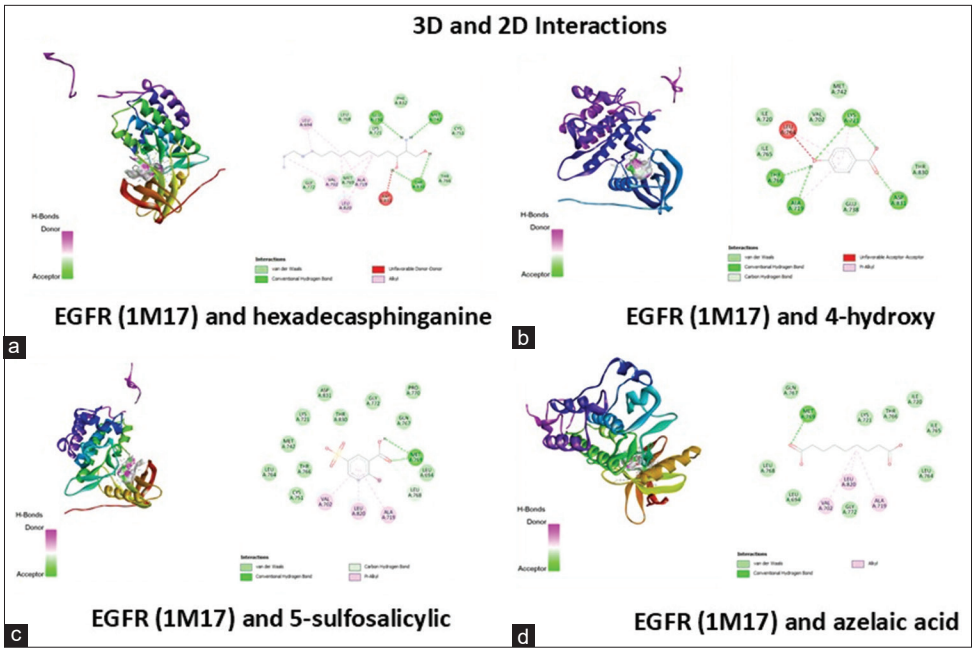

Figure 6: Molecular docking interactions of epidermal growth factor receptor (EGFR) (1M17) with Ulva-derived compounds. (a) 3D and 2D interaction profiles of EGFR with hexadecasphinganine. (b) 3D and 2D interaction profiles of EGFR with 4-hydroxybenzoic acid. (c) 3D and 2D interaction profiles of EGFR with 5-sulfosalicylic acid. (d) 3D and 2D interaction profiles of EGFR with azelaic acid

Table 2: Binding energies and hydrogen-bond interactions between key *Ulva lactuca* compounds and oral cancer-related target proteins

Complex	Binding energies	H-bond interactions
TP53 and 5-sulfosalicylic acid	−7.1	6 (LYS A: 162, ALA A: 161, LYS A: 118, GLY A: 17, ASP A: 120)
TP53 and 4-hydroxybenzoic	−6.4	4 (LYS A: 18, CYS A: 16, GLY A: 17, THR A: 37)
TP53 and hexadecasphinganine	−5.8	2 (GLY A: 62, LYS A: 18)
TP53 and azelaic acid	−5.3	3 (VAL A: 831, THR A: 37, LYS A: 18)
CTNNBI and 5-sulfosalicylic acid	−6.0	4 (ARG A: 386, TRP A: 386, ASN A: 387, GLU B: 28)
CTNNBI and 4-hydroxybenzoic	−5.0	2 (ASN A: 387, TRP A: 383)
CTNNBI and hexadecasphinganine	−4.0	3 (GLU B: 28, GLU B: 24, ARG A: 386)
CTNNBI and azelaic acid	−4.7	2 bonds (ARG A: 386, ASN A: 387)
EGFR and 5-sulfo salicylic acid	−5.9	1 (MET A: 769)
EGFR and 4- hydroxy benzoic acid	−6.1	4 (LYS A: 721, THR A: 766, ASP A: 831, ALA A: 719)
EGFR and hexadecasphinganine	−5.1	3 (MET A: 74, THR A: 830, GLU A: 738)
EGFR and azelaic acid	−5.0	1 (MET A: 769)

TP53: Tumor protein p53, EGFR: Epidermal growth factor receptor

THR830, and GLU738. Azelaic acid showed a binding energy of −5.0 kcal/moL, forming one hydrogen bond with MET769.

DISCUSSION

The current study focused on the biochemical profile, cytotoxic potential, molecular targets, and docking interactions of *U. lactuca* and its associated compounds in the context of oral cancer. The proximal composition contained high moisture and ash contents and had large quantities of carbohydrates, lipids, and proteins. This profile is consistent with the overall biochemical characteristics of green marine macroalgae, which naturally store minerals and intricate polysaccharides.[16] High carbohydrate fraction shows the presence of polysaccharides such as ulvan, and the protein and lipid levels are typical of the balanced nutrient constitution usually observed in marine algal biomasses.[17] Such types of compositions are commonly related to antioxidant and cytoprotective properties and hence provide a biochemical

rationale for the study of the composition of *U. lactuca* in a therapeutic context.[18]

Of the various extracts, methanol exhibited the greatest cytotoxicity against KB oral cancer cells, then ethanol extracts and subsequently water extracts. This sequence concurs with the broad pattern of medium polarity marine extracts which tend to be concentrated in phenolics, sulfated polysaccharides, and other bioactive compounds that have been previously reported to have antiproliferative effects.[19] The determined IC_{50} values are within the range that is usually obtained in models of epithelial cancer cells when using the macroalgal extracts. Rounding and shrinkage with detachment of the substratum are morphological alterations indicative of cytotoxic stress and indicative of cells in membrane instability or in early apoptotic responses.[20] The MTT assay results demonstrated that the ethanol, methanol, and aqueous extracts exhibited cytotoxic activity against KB oral cancer cells. This study used a literature-based computational approach to identify potential bioactive compounds responsible for the observed anticancer effects.[21] The chemical constituents of our extracts were not directly characterized using analytical techniques such as GC–MS. Instead, compounds previously reported in *U. lactuca* and related species were catalogued through a literature survey. These literature-derived metabolites were subsequently screened in relevant databases and subjected to molecular docking analyses to predict their interactions with key oral cancer-associated protein targets. This approach enabled the formulation of hypotheses regarding potential mechanisms underlying the cytotoxic effects observed in our assays, although the actual composition of our extracts was not experimentally determined.[22]

There was a significant overlap between the oral cancer-related genes and the predicted molecular targets, as indicated by the compound-target overlap. The PPI network created key regulatory proteins as central nodes, such as TP53, EGFR, CTNNB1, TNF, IL6, AKT1, and MYC. These genes are key control nodes of cell survival, proliferation, inflammatory signaling, and carcinogenic transformation. High levels of inflammatory and inflammation-induced and stress-response proteins, including TNF, IL6, and CYP2E1, indicate the biological environment that oral cancer is characterized by, where the pathways of inflammation and xenobiotic metabolism play a significant role in tumor progression. The central module retrieved from the network showed signaling axes widely accepted as central to oral cancer biology to support the relevance of the predicted interactions of compounds.[23-25]

The docking analysis results indicated that 5-sulfosalicylic acid and 4-hydroxy benzoic acid had the best binding affinities with the TP53, CTNNB1, and EGFR. Both compounds may have multiple hydrogen bonds with functionally significant residues in structural regions required to maintain the activity or stability of the proteins. There is a high affinity between 5-sulfosalicylic acid and TP53, which indicates the possibility of the compound influencing major regulatory domains of this protein.[26] Likewise, the stable docking of EGFR and CTNNB1 with 4-hydroxybenzoic acid, which indicates that a small phenolic structure is compatible with binding pockets of receptors and signaling proteins.[27] Overall, lower affinity was also observed to be high with hexadecasphinganine and azelaic acid. Their binding behavior conforms with the general behavior of fatty acids and long-chain aliphatic compounds that are usually dependent on hydrophobic contacts and few polar interactions. Collectively, the docking outcomes can indicate the potential of these compounds to regulate pathways important in oral cancer development, including p53 signaling, EGFR-mediated proliferation, and β-catenin-mediated transcriptional regulation.

While this study provides valuable insights into the potential anticancer properties of *U. lactuca* extracts, several limitations must be acknowledged. The extracts were not subjected to direct chemical characterization using techniques such as GC-MS or related analytical methods, and thus, the precise composition remains undetermined. Instead, the compounds employed in molecular docking analyses were derived exclusively from literature reports of previously identified constituents of *U. lactuca*.[28] The chemical composition of our extracts is presumed to be broadly comparable to that reported in previous studies, although such profiles are known to vary depending on environmental conditions, geographical origin, harvest time, and extraction methodology. Furthermore, the molecular docking and network pharmacology analyses presented here represent computational predictions that require experimental validation.[29] Although cytotoxicity was studied with MTT assay and morphological studies, no detailed mechanistic studies such as apoptosis studies, cell cycle studies, and western blotting of target protein expression. The study further discussed the use of only KB cancerous oral cells, which may not reflect the heterogeneity of oral cancer since the various subtypes of oral cancer might react differently to the extracts. The selectivity of the extracts between cancer cells and healthy oral epithelial cells was also not assessed which is essential in finding the possible therapeutic potential.[30] Depending on the results and the limitations of the current study, it is possible to suggest a number of future research lines to confirm and expand on these initial results. The bioactive compounds in the methanol, ethanol, and aqueous extracts of *U. lactuca* should be identified and quantified through comprehensive chemical profiling by use of GC-MS, LC-MS, and NMR. This will give conclusive findings on the true chemical composition that causes the observed cytotoxic effects.

CONCLUSION

The combined biochemical, cytotoxic, and computational analyses demonstrate that *U. lactuca* exhibits significant bioactive potential relevant to oral cancer. This macroalga has a nutrient-rich composition, and the methanolic extract

of this plant has high cytotoxic effects against KB cancer cells. The presence of overlapping oncogenic targets and key regulatory hubs in the interaction network, including TP53, EGFR, and CTNNB1, highlights the biological significance of the predicted protein-ligand interaction. The potential of 5-sulfosalicylic acid, 4-hydroxybenzoic acid, hexadecasphinganine, and azelaic acid to interact with proteins involved in cancer development was evaluated. In conclusion, these findings suggest that the compounds and constituent metabolites of the genus *U. lactuca* represent a promising natural therapeutic candidate that warrants further investigation to establish its potential utility in the treatment of oral cancer.

REFERENCES

1. Carpena M, Garcia-Perez P, Garcia-Oliveira P, Chamorro F, Otero P, Lourenço-Lopes C, *et al*. Biological properties and potential of compounds extracted from red seaweeds. Phytochem Rev 2023;22:1509-40.
2. Akter A, Sobuj MK, Islam MD, Chakroborty K, Tasnim N, Ayon MH, *et al*. Seaweed polysaccharides: Sources, structure and biomedical applications with special emphasis on antiviral potentials. Future Foods 2024;10:100440.
3. Kumar M, Jha AK. Exploring the potential of dietary factors and plant extracts as chemopreventive agents in oral squamous cell carcinoma treatment. Front Oral Health 2023;4:1246873.
4. Aswathy M, Banik K, Parama D, Sasikumar P, Harsha C, Joseph AG, *et al*. Exploring the cytotoxic effects of the extracts and bioactive triterpenoids from *Dillenia indica* against oral squamous cell carcinoma: A scientific interpretation and validation of indigenous knowledge. ACS Pharmacol Transl Sci 2021;4:834-47.
5. Rehman AU, Khurshid B, Ali Y, Rasheed S, Wadood A, Ng HL, *et al*. Computational approaches for the design of modulators targeting protein-protein interactions. Expert Opin Drug Discov 2023;18:315-33.
6. Pappou S, Dardavila MM, Savvidou MG, Louli V, Magoulas K, Voutsas E. Extraction of bioactive compounds from *Ulva lactuca*. Appl Sci 2022;12:2117.
7. Hofmann LC, Strauss S, Shpigel M, Guttman L, Stengel DB, Rebours C, *et al*. The green seaweed Ulva: Tomorrow's "wheat of the sea" in foods, feeds, nutrition, and biomaterials. Crit Rev Food Sci Nutr 2025;65:3728-63.
8. Pradhan B, Bhuyan PP, Ki JS. Immunomodulatory, antioxidant, anticancer, and pharmacokinetic activity of ulvan, a seaweed-derived sulfated polysaccharide: An updated comprehensive review. Mar Drugs 2023;21:300.
9. Sivakumar SR, Pandian J, Arumugam R, Ramalingam S, Elanchezhian B. Molecular structure and bioactivity analysis of *Ulva lactuca* using spectroscopic and computational methods. Discov Chem 2025;2:222.
10. Bokhtiar SM, Sarker D, Akter A, Salam MA, Ahmed KU, Anwar MM, *et al*. Nutritional profiling, phytochemical screening, cytotoxicity, and antioxidant content analysis for different crude extracts of *Ulva lactuca* from coast of Bangladesh. Future Foods 2024;10:100513.
11. Rasyid A. Evaluation of nutritional composition of the dried seaweed *Ulva lactuca* from Pameungpeuk waters, Indonesia. Trop Life Sci Res 2017;28:119-25.
12. Premarathna AD, Ranahewa TH, Wijesekera SK, Harishchandra DL, Karunathilake KJ, Waduge RN, *et al*. Preliminary screening of the aqueous extracts of twenty-three different seaweed species in Sri Lanka with *in-vitro* and *in-vivo* assays. Heliyon 2020;6:e03918.
13. Natarajan SR, Krishnamoorthy R, Alshuniaber MA, Alsulami TS, Gatasheh MK, Rajagopal P, *et al*. ABCE1 facilitates tumour progression via aerobic glycolysis and inhibits cell death in human colorectal cancer cells through the p53 signalling pathway. Sci Rep 2025;15:24674.
14. Gatasheh MK, Natarajan SR, Krishnamoorthy R, Alsulami TS, Rajagopal P, Palanisamy CP, *et al*. Molecular analysis to identify novel potential biomarkers as drug targets in colorectal cancer therapy: An integrated bioinformatics analysis. Mol Cell Oncol 2024;11:2326699.
15. Velmurugan Y, Natarajan SR, Chakkarapani N, Jayaraman S, Madhukar H, Venkatachalam R. *In silico* and *in vitro* studies for the identification of small molecular inhibitors from *Euphorbia hirta* Linn for rheumatoid arthritis: Targeting TNF-α-mediated inflammation. Mol Divers 2025;29:1189-206.
16. Rahman S, Mamun MZ, Noor F, Islam MS, Khondker M, Hasan M, *et al*. Marine algae from coastal Bangladesh: Insights into nutritional potential and dietary implications. Reg Stud Mar Sci 2026;93:104650.
17. Hemavathy RV, Ragini YP, Shruthi S, Ranjani S, Subhashini S, Thamarai P. Biofuel production from marine macroalgae: Pathways, technologies, and sustainable energy solutions. Ind Crops Prod 2025;224:120282.
18. Ouahabi S, Daoudi NE, Loukili EH, Asmae H, Merzouki M, Bnouham M, *et al*. Investigation into the phytochemical composition, antioxidant properties, and *in-vitro* anti-diabetic efficacy of *Ulva lactuca* extracts. Mar Drugs 2024;22:240.
19. Liu W, Hsu YY, Tang JY, Cheng YB, Chuang YT, Jeng JH, *et al*. Methanol extract of *Commelina* plant inhibits oral cancer cell proliferation. Antioxidants (Basel) 2022;11:1813.
20. Abalos N, Ebajo VD Jr., Camacho DH, Jacinto SD. Cytotoxic and apoptotic activity of aglaforbesin derivative isolated from *Aglaia loheri* Merr. On HCT116 human colorectal cancer cells. Asian Pac J Cancer Prev 2021;22:53-60.
21. Kamaria P, Tiwari P, Prabhakaran P, Bhardwaj S, Kolachi K, Thapa S. Evaluation of *Quercus infectoria* phytoconstituents against oral cancer: Network pharmacology, docking simulation, and *in vitro* cytotoxicity assay. Food Sci Nutr 2025;13:e70857.

22. Mazri R, Ouassaf M, Zekri A, Khan SU, Rengasamy KR, Alhatlani BY. *In silico* network pharmacology, molecular docking, and molecular dynamics analysis of rosemary-derived compounds as potential HSP90 inhibitors for cancer therapy. Curr Issues Mol Biol 2025;47:860.
23. Kumar R, Samal SK, Routray S, Dash R, Dixit A. Identification of oral cancer related candidate genes by integrating protein-protein interactions, gene ontology, pathway analysis and immunohistochemistry. Sci Rep 2017;7:2472.
24. Choi S, Myers JN. Molecular pathogenesis of oral squamous cell carcinoma: Implications for therapy. J Dent Res 2008;87:14-32.
25. Tanaka T, Ishigamori R. Understanding carcinogenesis for fighting oral cancer. J Oncol 2011;2011:603740.
26. Dunjic M, Turini S, Nejkovic L, Sulovic N, Cvetkovic S, Dunjic M, *et al*. Comparative molecular docking of apigenin and luteolin versus conventional ligands for TP-53, pRb, APOBEC3H, and HPV-16 E6: Potential clinical applications in preventing gynecological malignancies. Curr Issues Mol Biol 2024;46:11136-55.
27. Merecz-Sadowska A, Isca VM, Sitarek P, Kowalczyk T, Małecka M, Zajdel K, *et al*. Exploring the anticancer potential of semisynthetic derivatives of 7α-acetoxy-6β-hydroxyroyleanone from *Plectranthus* sp.: An *in silico* approach. Int J Mol Sci 2024;25:4529.
28. Putra NR, Fajriah S, Qomariyah L, Dewi AS, Rizkiyah DN, Irianto I, *et al*. Exploring the potential of *Ulva lactuca*: Emerging extraction methods, bioactive compounds, and health applications - A perspective review. South Afr J Chem Eng 2024;47:233-45.
29. Belahcene S, Kebsa W, Akingbade TV, Umar HI, Omoboyowa DA, Alshihri AA, *et al*. Chemical composition antioxidant and anti-inflammatory activities of *Myrtus communis* L. Leaf extract: Forecasting ADMET profiling and anti-inflammatory targets using molecular docking tools. Molecules 2024;29:849.
30. Raafat SN, El Wahed SA, Badawi NM, Saber MM, Abdollah MR. Enhancing the anticancer potential of metformin: Fabrication of efficient nanospanlastics, *in vitro* cytotoxic studies on HEP-2 cells and reactome enhanced pathway analysis. Int J Pharm X 2023;6:100215.

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