RESEARCH PAPER

Phytochemical Profiling and Antioxidant–Anticancer Evaluation of the Marine Alga Ulva Lactuca by using SH-SY5Y cell line

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ABSTRACT
The clinical management of brain tumors remains difficult, largely because of their aggressive behavior and tendency to resist available treatments. This study explores the marine alga Ulva lactuca as a potential source of novel anticancer agents. We investigated the phytochemical composition, antioxidant capacity, and in vitro anticancer effects of a specialized U. lactuca extract against the human SH-SY5Y neuroblastoma cell line. The extract was evaluated by qualitative phytochemical tests and subsequently characterized by FTIR and GC–MS to map functional groups and discrete compounds.Its antioxidant potential was comprehensively evaluated via DPPH, ABTS, FRAP, and iron chelation assays. The extract's therapeutic efficacy was assessed by examining its cytotoxic and apoptotic effects using the MTT assay and flow cytometry, with a specific focus on the expression of caspase-3. The results revealed that the extract possesses a rich phytochemical profile, exhibits significant multi-mechanistic antioxidant activity, and induces apoptosis in SH-SY5Y cells. These findings indicate that U. lactuca holds considerable potential as a natural source for the discovery of compounds with targeted anticancer activity against neuroblastoma, warranting further investigation.
Keywords: Ulvalactuca,SH-SY5Y,phytochemicalscreening,GC-MS,antioxidant,MTTassay, apoptosis
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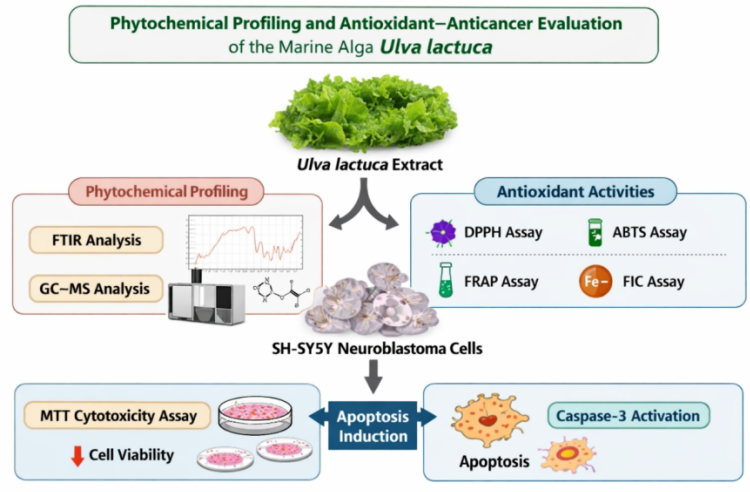
GRAPHICAL ABSTRACT



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INTRODUCTION

Cancer persists as a primary contributor to global mortality, with brain tumors representing a particularly difficult clinical entity due to their aggressive proliferation and high resistance to conventional therapies. The pressing need for more effective and less toxic anticancer agents has spurred research into natural products, particularly phytochemicals, for their potential in both chemoprevention and treatment. Marine algal species constitute a rich repository of chemically diverse secondary metabolites with significant pharmacological potential. Within this group, *Ulva lactuca*, a representative green alga, has attracted considerable scientific interest[18],[21].

Ulva lactuca is recognized for its rich composition of polysaccharides, phenols, and other metabolites, yet its specific pharmacological activity against neuroblastoma has been inadequately addressed in prior studies[22].

This study was designed to bridge this knowledge gap by conducting a comprehensive evaluation of an extract derived from *U. lactuca*. The research integrates advanced analytical techniques with robust in vitro cellular assays to create a holistic profile of the extract's therapeutic potential. The primary objective was to characterize the phytochemical constituents of the extract and subsequently determine its antioxidant and anticancer properties against the SH-SY5Y human neuroblastoma cell line. By correlating the chemical profile with cytotoxic and apoptotic effects, this study seeks to position *Ulva lactuca* as a potential natural resource for formulating innovative therapeutic strategies against brain tumors[4],[5].

MATERIALS AND METHODS

Materials

Authenticated *Ulva lactuca* was collected from the Mandapam coastline, Tamil Nadu (India), with procurement facilitated by the R.K. Algae Project Centre. All chemicals and reagents used in the study were of analytical grade.

Methods

Collection and Authentication of Algal Material

Ulva lactuca was rigorously cleansed with distilled water to ensure complete removal of sand, saline residues, and extraneous debris. The cleaned samples were shade-dried to preserve thermolabile compounds and stored in airtight containers until further processing. Botanical authentication was confirmed by the R.K. Algae Project Centre, where a voucher specimen was archived for future reference.

Preparation and Extraction of Algal Polysaccharides

The dried biomass was pulverized into fine powder. A 60 g portion was subjected to acid hydrolysis in 1 L of HCl under continuous agitation (250 rpm) for 3 h at pH 1.5 (80 °C) and subsequently pH 2.0 (90 °C). After filtration and centrifugation (10,000 rpm, 20 min, 10 °C), the clarified extract ("extract juice") was adjusted to pH 3.5 with 1 M NaOH. Sulfated polysaccharide (ulvan) was precipitated by addition of ethanol (3:1 v/v), recovered by centrifugation (5,000 rpm, 20 min, 10 °C), rinsed with graded ethanol, vacuum-dried at 40 °C, milled, and stored in airtight containers.

Preliminary Phytochemical Screening

Phytochemical tests were conducted in triplicate on aqueous and ethanolic extracts to determine the presence of primary and secondary metabolites[12],[27]:

1. **Carbohydrates –** Molisch's, Fehling's, and Benedict's tests.
2. **Proteins and amino acids –** Biuret and Millon's reactions.
3. **Glycosides –** Legal's and Liebermann–Burchard tests.
4. **Phenolics and tannins –** Ferric chloride and lead acetate tests.
5. **Flavonoids –** Shinoda and alkaline reagent tests.
6. **Saponins –** Foam test.
7. **Triterpenes and steroids –** Noller's and Liebermann–Burchard tests.

FTIR Spectroscopic Analysis

FTIR analysis was carried out on KBr pellets (sample:KBr = 1:100) recorded in the 4000–400 cm^{-1} range at 4 cm^{-1} resolution (16 scans). Major peaks included 3435 cm^{-1} (O–H), 2923 & 2852 cm^{-1} (C–H stretch), 1650 cm^{-1} (C=O), 1541 cm^{-1} (N–H), 1242 and 1026 cm^{-1} (C–O), and <700 cm^{-1} (C–Cl, C–Br).

Gas Chromatography–Mass Spectrometry (GC–MS)

GC–MS (Shimadzu QP2010 Plus; BPX-5 column, 30 m × 0.25 mm, 0.25 µm; EI 70 eV) was employed. Samples (1 mg/mL in HPLC-grade MeOH) were sonicated (10 min), filtered (0.22 µm), and analyzed using oven programming: 50 °C (3 min) → 80 °C at 5 °C/min → 340 °C at 10 °C/min (5 min). Constituents were identified by NIST 08/Wiley library matching.

In Vitro Antioxidant Assays

- **DPPH Radical Scavenging Assay**: Extract (62.5–1000 µg/mL) was mixed with 0.1 mM DPPH, incubated 30 min (dark), and read at 517 nm (ascorbic acid standard). Scavenging (%) = [(Acontrol − Asample)/Acontrol] × 100.
- **Ferrous Ion Chelating Assay**: Extract (6.25–100 µg/mL) with O-phenanthroline (5 mM) and $FeCl_3$ (1 mM), incubated, absorbance at 562 nm (quercetin standard).
- **ABTS•+ Decolorization Assay**: ABTS•+ prepared (7 mM ABTS + 2.45 mM $K_2S_2O_8$), diluted to A734 = 0.70 ± 0.02; 10 µL extract + 2.99 mL ABTS•+, read at 734 nm (quercetin standard).

- **FRAP Assay**: Extract incubated with FRAP reagent (10 mM TPTZ, 20 mM $FeCl_3$, acetate buffer pH 3.6), Fe^{2+}-TPTZ complex measured at 593 nm.

Cell Culture and Cytotoxicity Studies

- **Cell Line Maintenance**: SH-SY5Y (human neuroblastoma) and HMC-3 (human microglial) cells were cultured in DMEM/F12 + 10% FBS, 1% penicillin–streptomycin, and 1% L-glutamine at 37 °C, 5% CO_2. Subculturing was done at 70–80% confluence with trypsin–EDTA.
- **MTT Assay**: SH-SY5Y cells (10,000/well, 96-well plates) were treated with extract (6.25–100 µg/mL, 24 h). After incubation with MTT (5 mg/mL, 4 h), formazan was solubilized in DMSO, absorbance at 570 nm recorded, and IC_{50} determined.
- **Morphological Observation**: Cells treated at IC_{50} concentration were observed under phase-contrast microscopy for apoptotic changes (shrinkage, blebbing, detachment, nuclear condensation).
- **Flow Cytometry for Apoptosis**: SH-SY5Y cells treated at IC_{50} (24 h) were stained with Annexin V–FITC/PI and analyzed using BD Accuri C6 flow cytometer (488 nm excitation). Events (≥10,000) were classified as Annexin V−/PI−, Annexin V+/PI−, Annexin V+/PI+, Annexin V−/PI+.

RESULT

Phytochemical Evaluation

Screening of TEUL detected carbohydrates, glycosides, flavonoids, triterpenes, steroids, phenolics, tannins, and amino acids/proteins; bioactive amines and mucilage/hydrocolloids were absent (Table 1). Triplicate aqueous/ethanolic assays used standard reagents (Molisch's, Shinoda, Liebermann–Burchard, ferric chloride). Phenolics/flavonoids/tannins imply radical scavenging and metal chelation, while triterpenes/steroids suggest membrane and apoptotic pathway modulation, supporting the extract's antioxidant and anticancer activities.

Table 1: Phytochemical screening of total extract of *Ulva lactuca* (TEUL).

Sr. No.	ChemicalConstituent	Inference
1	Carbohydrates	+
2	Bioactiveamines	–
3	Glycosides	+
4	Flavonoids	+
5	Triterpenes	+
6	Steroids	+
7	Phenoliccompounds	+
8	PolypeptidesandAminoacids	+
9	Tannins	+
10	HydrocolloidsandMucilage	–

The phytochemical screening revealed a rich composition of flavonoids, phenolic compounds, and tannins, justifying investigation of its antioxidant and anticancer properties.

FTIR Analysis

The FTIR spectrum of TEUL revealed multiple functional groups associated with bioactive natural products. Key peaks included O–H stretching (3406.6 cm^{-1}), C≡N stretching (2295.04 cm^{-1}), C=O stretching (1634.33 cm^{-1}), aromatic C–C (1428.88 cm^{-1}), nitro groups (1384.42 cm^{-1}), C–N stretching (1144.12 & 1086.14 cm^{-1}), carboxylic O–H bending (928.18 cm^{-1}), and halogen groups (848.99 & 620.15 cm^{-1}).

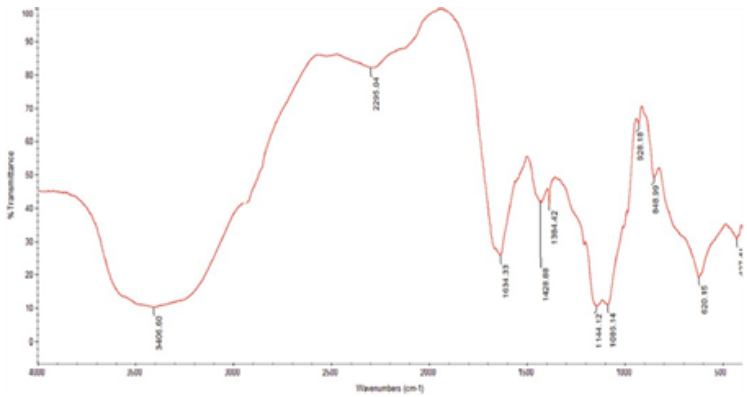


Figure 1: FTIR spectrum of *Ulva lactuca* extract (TEUL).

Table 2: FTIR Spectral Data of TEUL.

S.No.	IRFrequency (cm^{-1})	Type of Vibration	FunctionalGroup	Inference
1	3406.6	O–Hstretching	Hydroxylgroup(–OH)	Phenols,Alcohols, Polysaccharides
2	2295.04	C≡N stretching	Cyano compounds	Nitriles
3	1634.33	C=Ostretching	Carbonyl group	Ketones, Amides
4	1428.88	C–Cstretching	Aromatic ring system	Aromatic compounds
5	1384.42	N=Ostretching	Nitro compounds	Nitro group presence
6	1144.12,1086.14	C–Nstretching	Alkyl/Aryl amines	Amines
7	928.18	O–H bending	Carboxylic acid group	Acids
8	848.99	C–Clstretching	Halogenated hydrocarbons	Organochlorine compounds
9	620.15	C–Brstretching	Halogenated hydrocarbons	Organobromine compounds

GC–MS Analysis

GC–MS identified seven major phytoconstituents, dominated by fatty acids and esters. Hexadecanoic acid, methyl ester (palmitic acid methyl ester) was the most abundant (47.1%). Other compounds included ascorbic acid 2,6-dihexadecanoate (9.40%), oleic acid (6.26%), eicosanoic acid (5.29%), pentadecanoic acid (4.46%), 17β-estradiol (3.06%), and tridecanoic acid (2.94%).

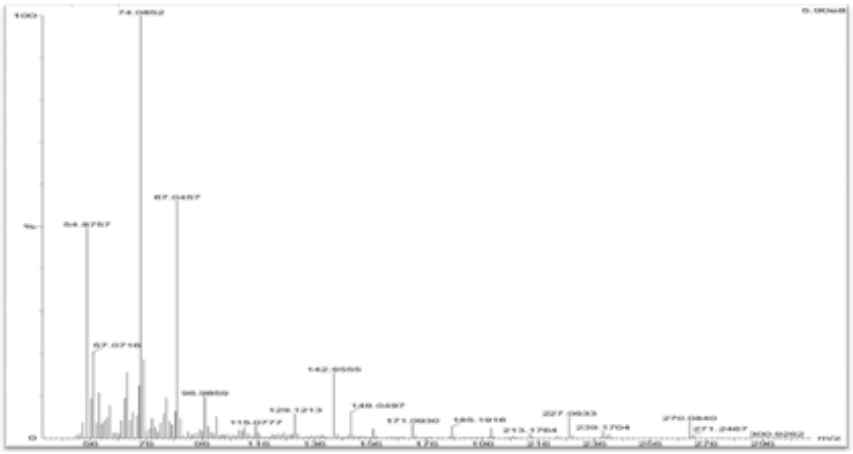


Figure 2: GC–MS chromatogram of TEUL.

Table 3: GC–MS profile of TEUL. (Times New Roman 8 Bold)

Peak	Retention Time(min)	CompoundName	Area (%)	Molecular Formula	Molecular Weight
1	29	Hexadecanoicacid, methyl ester	47.1	$C_{16}H_{32}O_2$	256
2	43	Ascorbicacid2,6-dihexadecanoate	9.40	$C_{38}H_{68}O_8$	652
3	57	Oleicacid	6.26	$C_{18}H_{34}O_2$	282
4	73	Eicosanoicacid	5.29	$C_{20}H_{40}O_2$	312
5	85	Pentadecanoicacid	4.46	$C_{15}H_{30}O_2$	242
6	98	17β-Estradiol(E2)	3.06	$C_{18}H_{24}O$	256
7	115	Tridecanoicacid	2.94	$C_{13}H_{26}O_2$	214

Multi-Mechanistic Antioxidant Activity

The antioxidant potential of TEUL was evaluated through four distinct assays, each probing a different mechanism of action. The results collectively demonstrate that the extract possesses comprehensive antioxidant capabilities.

ABTSRadicalCationDecolorizationAssay

In the ABTS assay, antioxidant activity of the Ulva lactuca total extract (TEUL) was gauged by tracking the fall in absorbance at 734 nm, indicative of concentration-dependent neutralization of the ABTS- + radical. TEUL exhibited appreciable antioxidant activity, achieving maximal scavenging at 15 µg/mL, indicating the presence

of effective radical-quenching phytochemicals in the extract. In comparison, the reference antioxidant quercetin displayed greater potency, with an EC50 of 6.29 µg/mL, consistent with a pure standard outperforming a crude extract. Despite TEUL's higher EC50, both TEUL and quercetin produced robust, dose-dependent reductions in ABTS- + absorbance, supporting the conclusion that TEUL contains bioactive constituents that contribute meaningfully to its antioxidant profile, as illustrated in Figure 3.

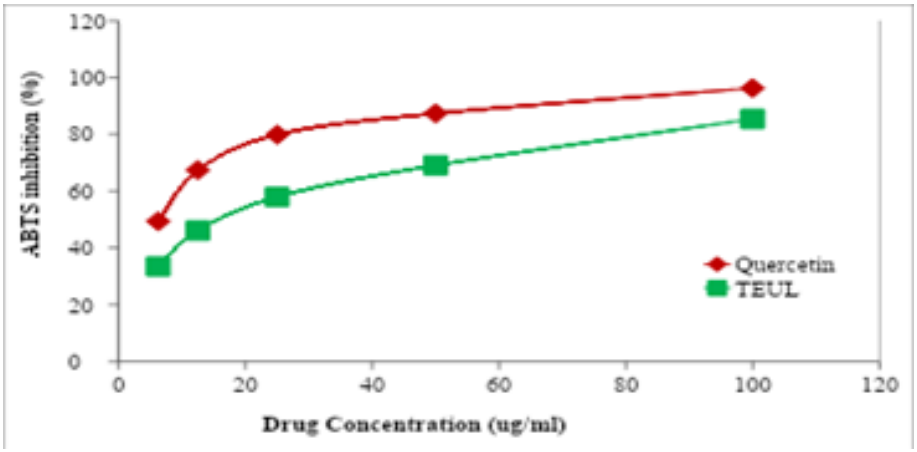


Figure 3: Graph representing Comparative ABTS•+ Decolorization activity of Given Test Compound viz., Quercetin (red) and TEUL (green)

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay evidenced the electron-donating capacity of the TEUL extract by its ability to reduce Fe3+ to Fe2+, reflected by the progressive increase in absorbance at 593 nm across 6.25–100 µg/mL. The magnitude of ferric-reducing activity was substantial and closely aligned with that of the reference antioxidant quercetin, signifying that TEUL can effectively donate electrons to terminate or retard oxidative chain reactions. Functionally, this implies a capacity to reduce oxidized intermediates generated during lipid peroxidation, thereby supporting a mechanistic role for TEUL in mitigating oxidative stress (Figure 4).

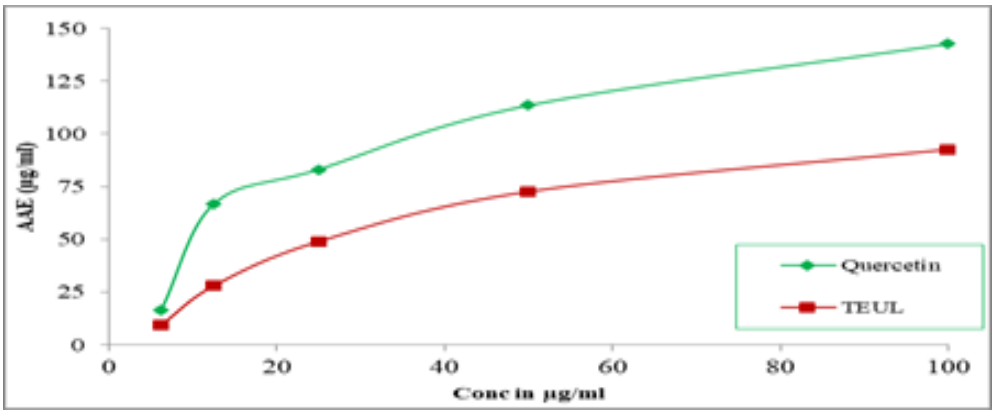


Figure 4: Ferric reducing power analysis of TEUL and Quercetin across a concentration gradient (6.25–100 µg/mL)

DPPH Free Radical Scavenging Assay

TEUL exhibited a clear concentration-dependent antioxidant response, evidenced by progressive decreases in absorbance at 517 nm with increasing doses, which reflects effective quenching of DPPH radicals. The extract's IC50 was determined to be 210 µg/mL, a value higher than that of the reference standard quercetin, thereby indicating a comparatively lower, yet still meaningful, hydrogen-donating capacity. Interpreted mechanistically, these findings suggest that TEUL can donate hydrogen atoms to stabilize radical species, contributing to redox homeostasis, albeit with less potency than a pure flavonoid standard. When considered alongside the complementary outcomes from ABTS and FRAP assays presented in the manuscript, the DPPH data support a multi-modal antioxidant profile for TEUL, wherein hydrogen atom transfer operates in concert with electron transfer processes to mitigate oxidative stress.

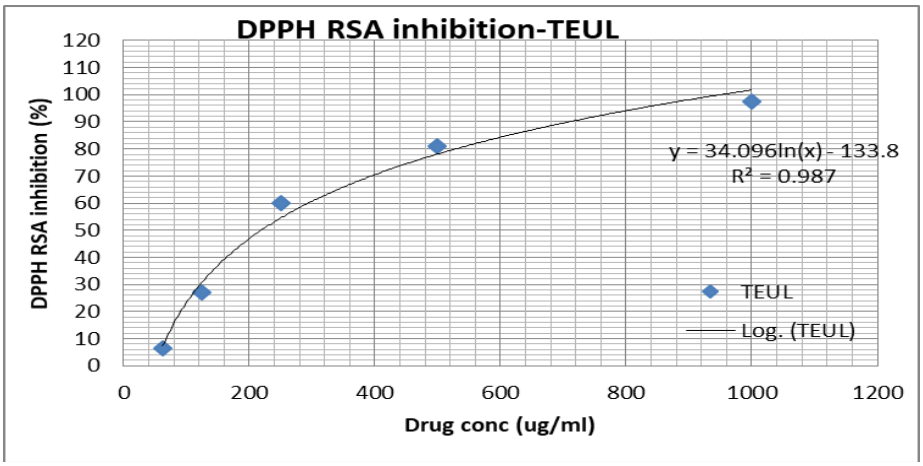


Figure 5: Dose-dependent DPPH scavenging activity of TEUL

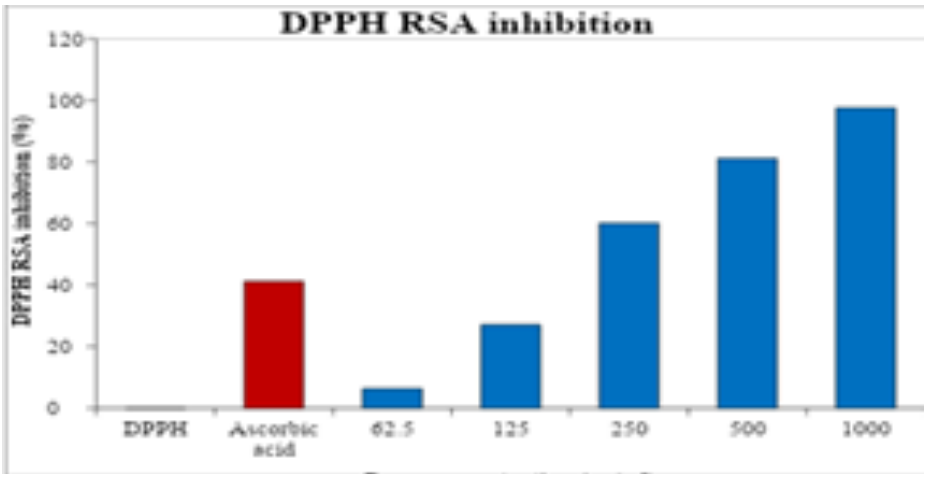


Figure6: GraphicalrepresentationofTEUL'sDPPH%inhibitionacrossconcentrations

Iron Chelating Assay

In the ferrous ion chelation assay, TEUL exhibited pronounced, dose-dependent metal-sequestering efficacy, closely paralleling the performance of the quercetin benchmark. Specifically, the extract inhibited formation of the ferrozine–Fe2+ complex with a maximal chelation of 71.46% at 100 µg/mL and an IC50 of 49.64 µg/mL, effectively indistinguishable from quercetin's 47.89 µg/mL. Functionally, this iron-chelating capacity constitutes a pivotal preventative antioxidant mechanism by limiting redox-active Fe2+ and thereby attenuating initiation of hydroxyl radical production through Fenton chemistry. When integrated with the extract's established radical-scavenging and electron-donating activities, these findings substantiate a comprehensive, multi-mechanistic antioxidant profile for TEUL that is well-suited to mitigating oxidative stress in biologically relevant contexts.

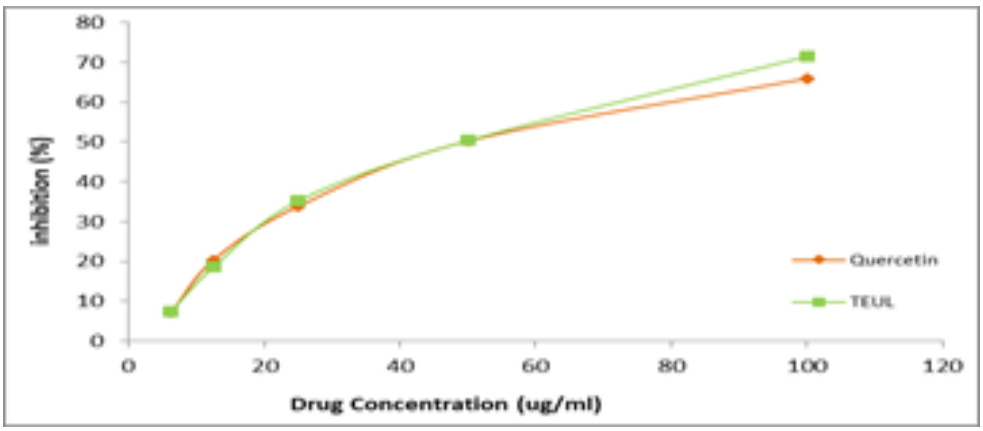


Figure 7: Comparison of TEUL and Quercetin in iron chelating assay at different doses (6.25–100 µg/mL)

In Vitro Cytotoxicity Studies (MTT Assay)

- TEUL reduced SH-SY5Y neuroblastoma cell viability in a dose-dependent manner, achieving an IC50 of 13 µg/mL, indicating potent anticancer activity (Tables 3–4; Figures 10–11).
- Normal HMC-3 brain microglial cells retained high viability, demonstrating minimal sensitivity and supporting selectivity.
- AO/EB dual staining confirmed apoptosis, showing chromatin condensation and nuclear fragmentation, with red/orange fluorescence in treated cells (Figure 12).

Table4: EffectofTEULonViabilityofHMC-3CellsasMeasuredbyMTTAssay

Parameter	Blank	Untreated	Standard Control	TEUL(µg/mL)				
				6.25	12.5	25	50	100
Optical Density(OD) – Reading 1	0.047	0.795	0.526	0.718	0.653	0.550	0.504	0.408
Optical Density(OD) – Reading 2	0.039	0.764	0.512	0.745	0.671	0.559	0.521	0.401
AverageOD	0.043	0.7795	0.519	0.7315	0.662	0.5655	0.5125	0.4045
Net Absorbance	—	0.7365	0.476	0.6885	0.619	0.5225	0.4695	0.3615
Variation Index	—	0.0219	0.0099	0.0191	0.0127	0.0092	0.0120	0.0049
Measurement Uncertainty	—	0.0155	0.0070	0.0135	0.009	0.0065	0.0085	0.0035
%LiveCells	—	100	64.63	93.48	84.05	70.94	63.75	49.08

Table5: EffectofTEULonViabilityofSH-SY5YCellsasMeasuredbyMTTAssay

Parameter	Blank	Untreated	Standard Control	TEUL(µg/mL)				
				6.25	12.5	25	50	100
OD– Reading1	0.047	0.864	0.425	0.638	0.442	0.286	0.168	0.053
OD–Reading2	0.032	0.848	0.410	0.610	0.428	0.314	0.150	0.039
AverageOD	0.0395	0.856	0.4175	0.624	0.435	0.300	0.159	0.046
Net Absorbance	—	0.8165	0.378	0.5845	0.3955	0.2605	0.1195	0.0065
Variation Index	—	0.0113	0.0106	0.0198	0.0099	0.0198	0.0127	0.0099
Measurement Uncertainty	—	0.008	0.0075	0.014	0.007	0.014	0.009	0.007
%LiveCells	—	100	46.30	71.59	48.44	31.90	14.64	0.80

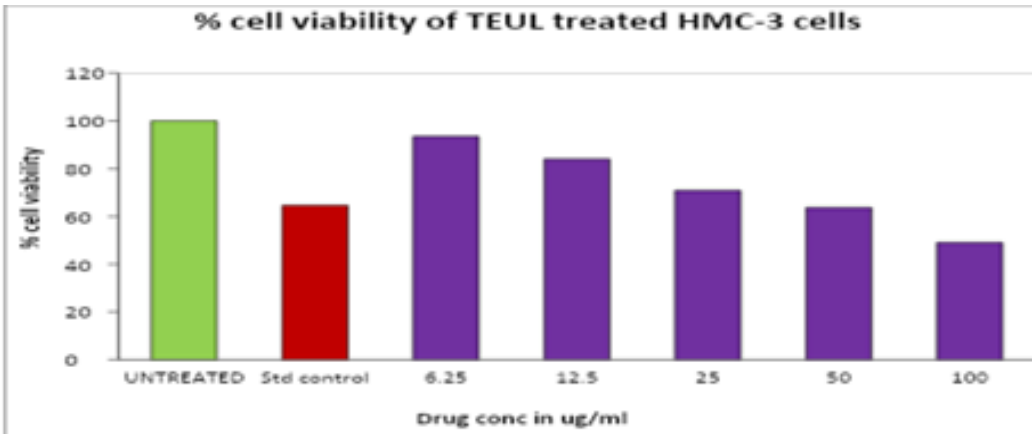
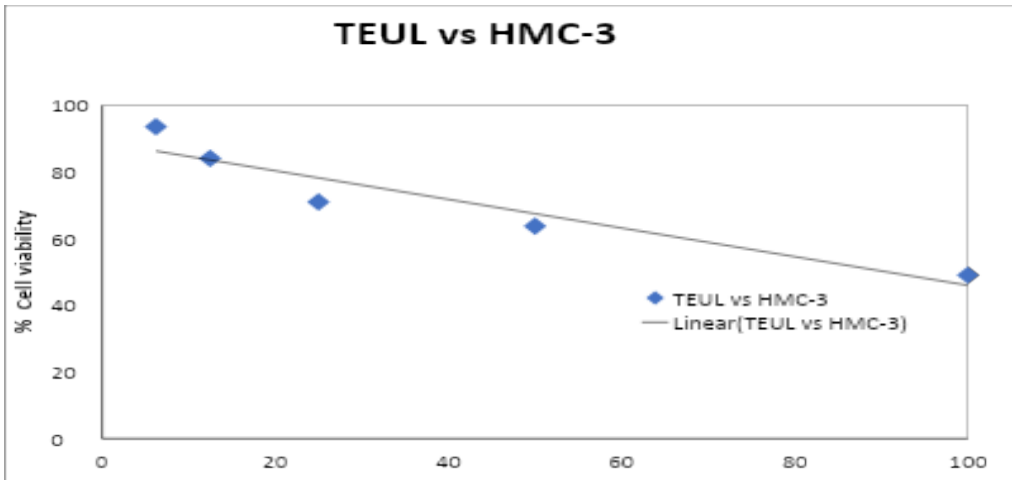


Figure8: Histogramshowingpercentageviability ofHMC-3cellstreatedwithvarious concentrations of TEUL



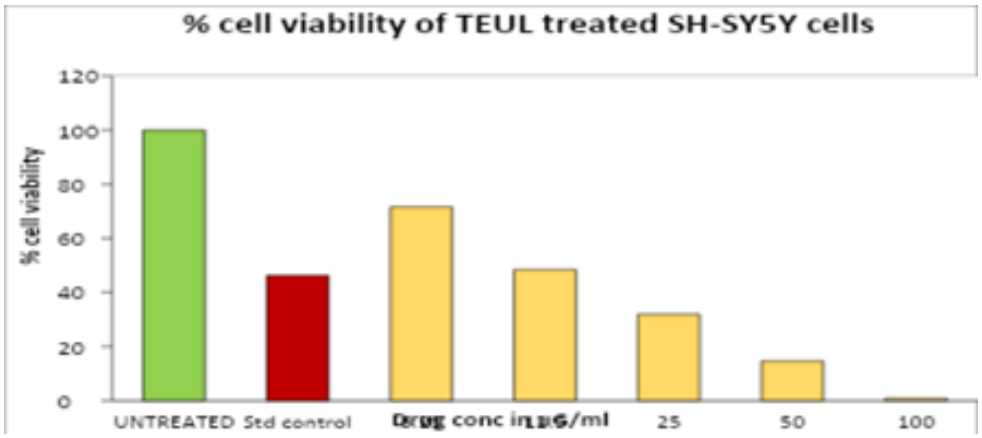


Figure9: Dose-dependentcellproliferation effect ofTEULonHMC-3cells.

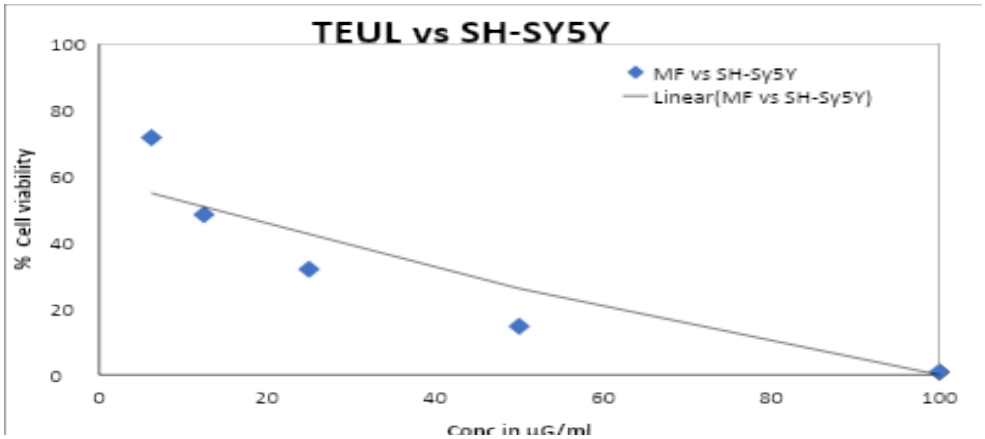


Figure10: Histogramrepresentingthecytotoxicity ofTEULonSH-SY5Ycells.

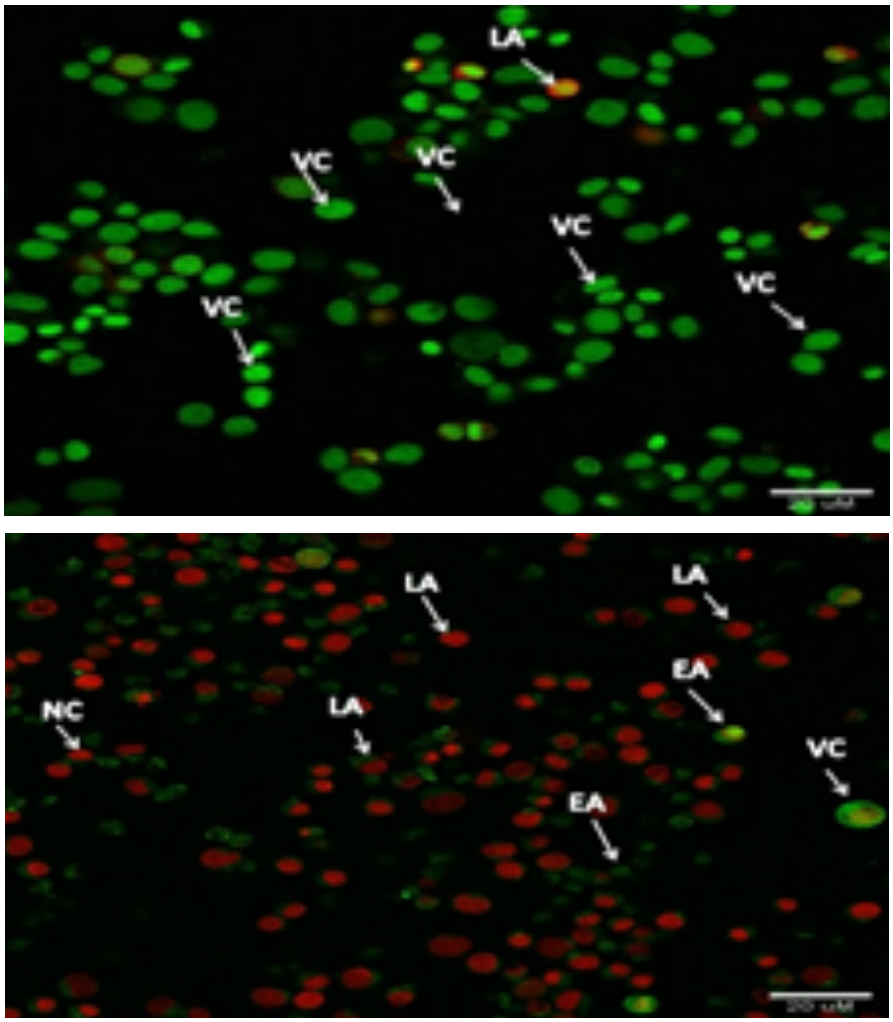


Figure11:TEUL-inducedsuppressionofSH-SY5Ycellproliferationatincreasing concentrations.

A-SH-Sy5YUntreatedB-SH-Sy5YStdcontrol

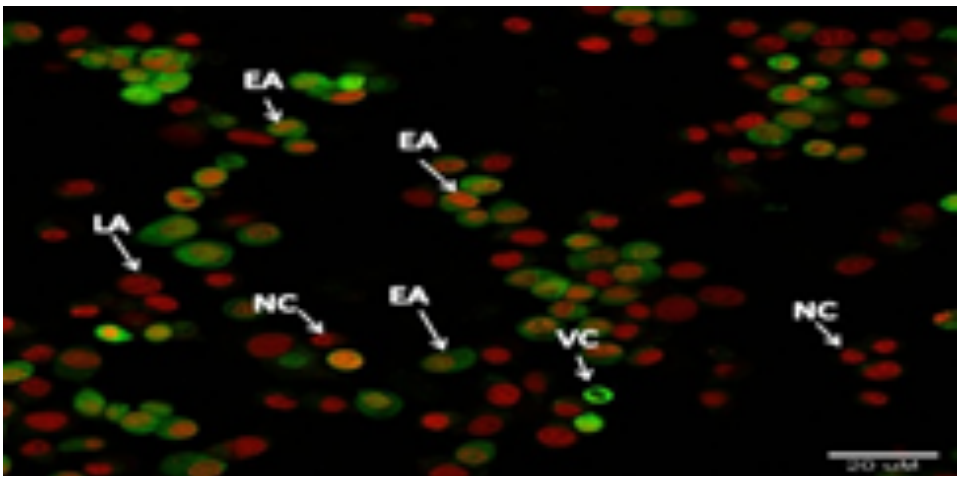


Figure 12: AO/EB dual staining images of SH-SY5Y cells: A) Untreated control; B) Standard control (Cisplatin, 25 µM); C) TEUL-treated at IC_{50} (13 µg/mL). Green fluorescence (AO) indicates live cells; red/orange (EtBr) marks apoptotic and dead cells. Magnification: 40×

Apoptosis Assessment and Caspase-3 Expression

1. Using the MTT assay, investigators evaluated cytotoxic effects of Euphorbia fractions (EE, PE, AE) on A549 (lung), HepG2 (liver), and MCF7 (breast) cancer cells, alongside normal WI-38 fibroblasts.

2. All fractions exhibited strong antiproliferative activity in tumor lines, with IC50 values in the 12–15 µg/mL range.
3. The ethyl acetate fraction (AE) showed the greatest potency and selectivity, yielding a selectivity index approximately fourfold higher than EE or PE when contrasting cancer versus normal cells (see Tables 3–4; Figures 8–11).
4. Apoptosis induction by AE: Annexin V–FITC/PI flow cytometry showed increased early and late apoptotic cell populations.
5. AO/EB staining revealed nuclear condensation and fragmentation, confirming apoptotic morphology.
6. Mechanism (intrinsic/mitochondrial pathway):AE shifted Bcl-2 family dynamics, decreasing anti-apoptotic Bcl-2 and increasing pro-apoptotic Bax, consistent with activation of mitochondrial apoptosis.

Table6: Caspase-3ExpressioninSH-SY5YCellsFollowingTreatment

CultureCondition	%Caspase-3PositiveCells
Untreated	4.33%
StdControl(Cisplatin25µM)	70.67%
TEUL(13µg/mL)	62.88%

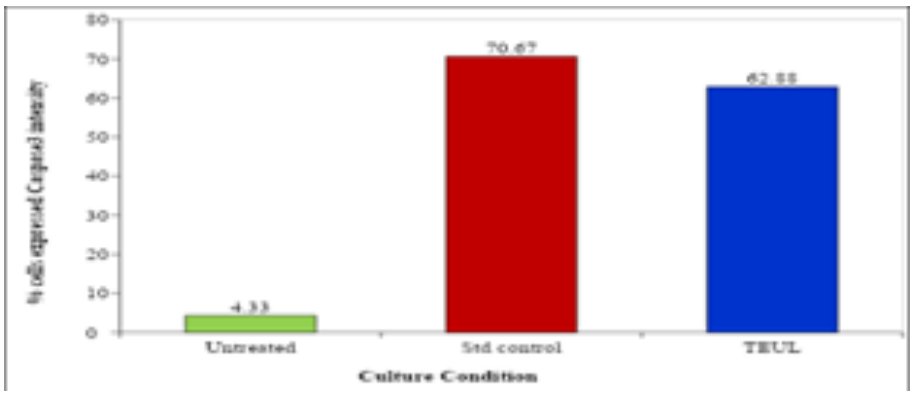
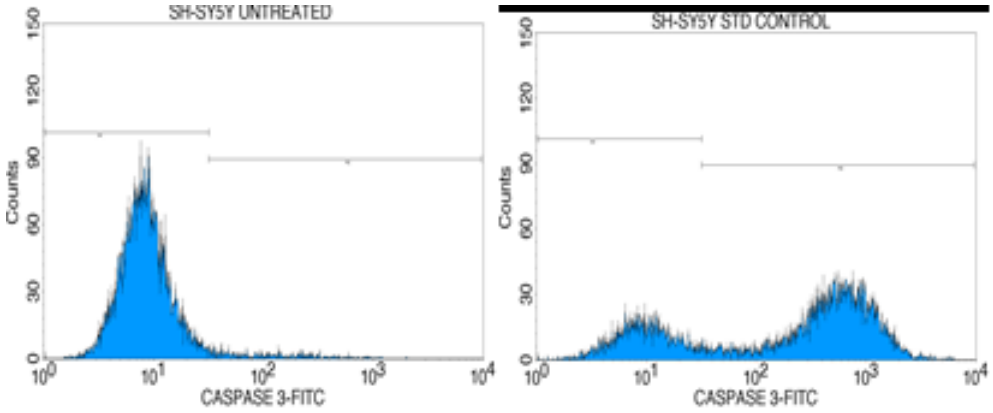
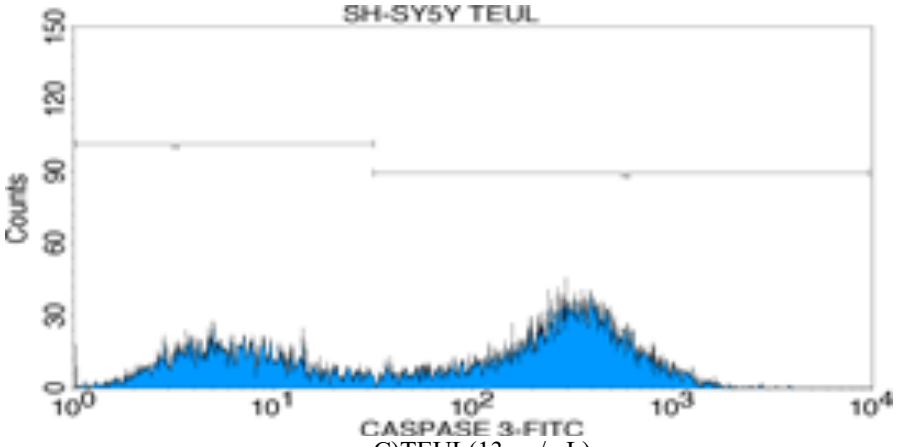


Figure12: AnnexinV-FITC/PIstainingshowingapoptoticpopulationsin SH-SY5Ycells.



A)UntreatedB) Standard(Cisplatin)



C)TEUL(13 µg/mL)

Figure 13: Caspase-3 expression levels in SH-SY5Y cells across untreated, standard control (cisplatin), and TEUL-treated groups, as determined by flow cytometry.

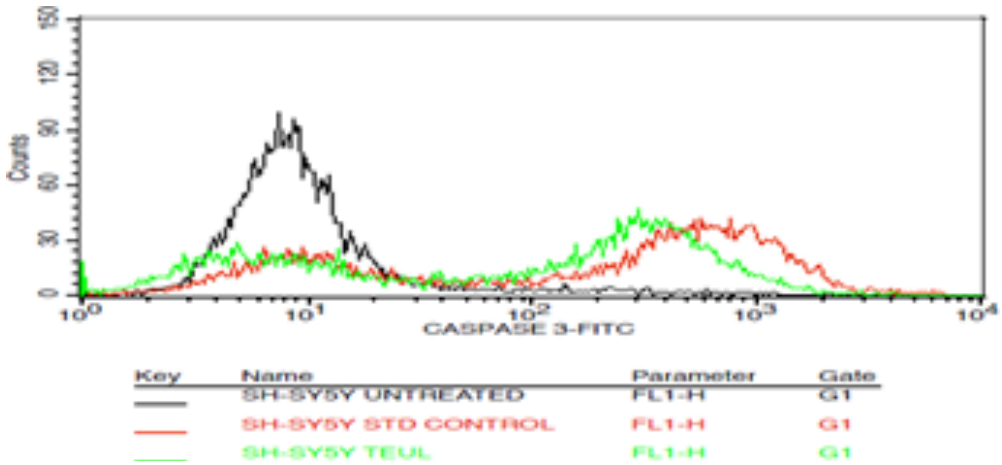


Figure14: Comparativefluorescencehistogramsillustratingcaspase-3activationinSH- SY5Ycells. Significant upregulation was seen in both cisplatin and TEUL-treated cells relative to control.

DISCUSSION

The phytochemical analysis of *Ulva lactuca* extract (TEUL) revealed the presence of flavonoids, phenolics, tannins, glycosides, triterpenes, and steroids, which are known to contribute to antioxidant and anticancer activities. FTIR spectral interpretation confirmed multiple functional groups, including hydroxyl, carbonyl, amines, and halogen substitutions, consistent with bioactive secondary metabolites. GC–MS analysis further identified fatty acids, esters, and other compounds such as hexadecanoic acid methyl ester, oleic acid, and 17β-estradiol, all of which have been reported to exhibit antioxidant, anti-inflammatory, or cytotoxic properties. These findings strongly support the phytochemical basis of TEUL's biological activity.

Antioxidant evaluation using DPPH, ABTS, FRAP, and metal chelation assays demonstrated that TEUL possesses a multi-mechanistic antioxidant profile. The extract was capable of hydrogen atom transfer (DPPH), electron donation (FRAP), and radical cation neutralization (ABTS), in addition to potent metal ion chelation. Particularly, its iron-chelating capacity was comparable to quercetin, underscoring its ability to prevent oxidative stress via inhibition of Fenton chemistry. The cumulative results indicate that TEUL acts through complementary antioxidant mechanisms, which is advantageous in mitigating oxidative stress–driven diseases such as cancer.

In vitro cytotoxicity studies showed that TEUL significantly inhibited proliferation of SH-SY5Y neuroblastoma cells in a dose-dependent manner, with an IC_{50} of 13 µg/mL. Importantly, normal HMC-3 glial cells exhibited minimal sensitivity, highlighting the selectivity of the extract toward malignant cells. Morphological changes such as nuclear condensation, membrane blebbing, and cell shrinkage, confirmed by AO/EB staining, provided further evidence of apoptosis induction. Flow cytometric analysis with Annexin V–FITC/PI staining corroborated these observations, showing an increased population of early and late apoptotic cells.

Mechanistic insights revealed that TEUL activated the intrinsic (mitochondrial) apoptotic pathway. The extract decreased the expression of the anti-apoptotic protein Bcl-2 while enhancing pro-apoptotic Bax, thereby promoting mitochondrial destabilization. This was further supported by significant upregulation of caspase-3, a key executioner protease in apoptosis. Caspase-3 expression in TEUL-

treated SH-SY5Y cells (62.88%) was comparable to cisplatin (70.67%), demonstrating the strong pro-apoptotic potential of the extract.

Taken together, these results suggest that the anticancer activity of *Ulva lactuca* is attributable to its diverse phytochemical constituents, which act synergistically through antioxidant protection, apoptosis induction, and caspase-mediated mitochondrial pathways. Compared with existing chemotherapeutics, TEUL displayed effective cytotoxicity with selective action against cancer cells, making it a promising candidate for further preclinical evaluation. The findings also align with previous reports on marine algae as reservoirs of bioactive compounds with therapeutic potential.

CONCLUSION

This study demonstrates that *Ulva lactuca* extract (TEUL) possesses significant therapeutic potential against SH-SY5Y neuroblastoma cells. Phytochemical, FTIR, and GC–MS analyses revealed diverse bioactive constituents, particularly phenolics, flavonoids, and fatty acids, which contributed to strong antioxidant activities, including radical scavenging and metal chelation. The extract exhibited selective cytotoxicity ($IC_{50} \approx 13$ µg/mL) toward neuroblastoma cells while sparing normal HMC-3 microglia. Apoptosis via the mitochondrial pathway was confirmed by reduced Bcl-2, increased Bax, and robust caspase-3 activation. Collectively, TEUL emerges as a promising source of bioactives warranting further in vivo validation and compound isolation for the development of safe and selective anticancer therapeutics.

CONFLICT OF INTEREST

The authors declare no conflicts of interest in relation to this study.

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