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Lipid extract from Ulva ohnoi algae: chemical characterization, antibacterial potential, and cytotoxicity assessment as an alternative treatment for MDR infections

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Abstract

Antimicrobial resistance is a critical global concern. This study investigated the lipid extract (LE) of *Ulva ohnoi* for its antimicrobial properties. The LE of *U. ohnoi* was characterized by GC-MS, thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC). Antibacterial activity was evaluated using the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) methods, adapted from the CLSI-M07-A24 protocol, using MDR isolates: *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and coagulase-negative *Staphylococcus*. Cytotoxicity assays were performed on murine fibroblast (L929) and bovine renal (MDBK) cell lines using MTT, Neutral Red (NR), Lactate Dehydrogenase (LDH), and hemolytic activity (HA) with sheep erythrocytes. Lipid characterization revealed palmitic acid (63.2%) and oleic acid (21.01%); TGA results indicated mass loss (88%), and DSC showed an absence of amorphous/crystalline domains. The *U. ohnoi* LE demonstrated better MIC results for *S. aureus* and coagulase-negative S. (1.33 mg/mL) and for *A. baumannii* and *E. coli* (2 mg/mL). CBM exhibited bacteriostatic activity against all tested isolates, ranging from 1 to 2 mg/mL for Gram-positive cocci and from 2 to 4 mg/mL for Gram-negative bacilli. LE showed low cytotoxicity toward L929 and MDBK cells, with mean IC_{50} values of 0.9984 mg/mL (MTT), 0.2499 mg/mL (NR), and 0.5816 mg/mL (LDH) for L929; and 0.3604 mg/mL (MTT), 1.950 mg/mL (NR), and 0.2520 mg/mL (LDH) for MDBK. An HA value of 4.705 mg/mL indicates tolerability by blood cells, demonstrating efficacy and a compatible safety profile in cellular models. *U. ohnoi* has shown promise as a source of new antibiotics.

Introduction

Ulva algae are rich in long-chain polyunsaturated fatty acids (PUFAs) (C18), particularly α-linolenic acid (C18:3 n-3), stearidonic acid (C18:4 n-3), and linoleic acid (C18:2 n-6) (Kumari et al. 2013; Moreira et al. 2021). *Ulva* spp., commonly called “sea lettuce,” belongs to the Chlorophyta group and has a higher nutritional composition in carbohydrates, mainly in sulfated heteropolysaccharides (Hmani et al. 2024; Rodríguez-Iglesias et al. 2024). In addition, the species has a diverse composition, rich in proteins, vitamins, minerals, amino acids, and PUFA (Rocha et al. 2023).

Algae of the genus *Ulva* have antibacterial, anti-inflammatory (Kang et al. 2021; Kidgell et al. 2020), antitumor (García-Márquez et al. 2023; Wahlström et al. 2020), anticoagulant, antioxidant (Don et al. 2021; Wahlström et al. 2020), immunomodulatory (Montuori et al. 2022; Pratt et al. 1944), and antihyperlipidemic (Don et al. 2021; Yuan et al. 2018). With a wide variety of applications, algae of the genus *Ulva* are used in various pharmaceutical products, foods, animal feed, and bioremediation (Don et al. 2021; Simon et al. 2024; Tran et al. 2023). In addition to being a raw material in biodegradable polymers for packaging (Guidara et al. 2020; Manikandan and Lens 2023) and biofuels (Biancarosa et al. 2018; Rodríguez-Iglesias et al. 2024; Simon et al. 2022).

Antimicrobial resistance to multiple drugs is a global concern due to the alarming increase in morbidity and mortality rates (Bilal et al. 2024; Laitin et al. 2024; Oliveira et al. 2024). The WHO classifies multidrug-resistant bacteria (MDR) as one of the ten threats to public health worldwide (Oliveira et al. 2024; World Health Organization 2023). Combating this problem is based on the implementation of urgent actions that integrate different sectors, with the aim of fulfilling one of the 17 Sustainable Development Goals (SDGs) related to health and well-being: the eradication by 2030 of epidemics of AIDS, tuberculosis, malaria, tropical diseases, and the promotion of efficient measures for the control of hepatitis, waterborne diseases and other communicable diseases (Ferdinand et al. 2023; ONU Brasil 2015; Organização Pan-Americana de Saúde 2020). In parallel, the antibiotics (ATB) currently in use are ineffective in treating MDR (Alreshidi et al. 2023; Camou et al. 2017; Talbot et al. 2019), as in the case of rifampicin, which in 2017 was identified as having resistance to this antibiotic widely used in the treatment of tuberculosis, which affects 10 million people annually with 1.5 million deaths as a result of the disease (Organização Pan-Americana de Saúde 2020).

Given the urgent need for new antibacterial agents and the versatility of algae, which adapt to diverse environments and can be produced in large quantities, especially during events of high proliferation of *Ulva* spp. known as green tides (Ren et al. 2024; Zeng et al. 2024), there is a growing interest in exploring their potential. These tides have intensified in recent decades around the world (Wahlström et al. 2020; Wang et al. 2022).

Therefore, it is essential to investigate the antibacterial potential of the lipid extract (LE) of *U. ohnoi*, as well as its chemical composition, thermal stability, and cytotoxicity in mammalian cell models, particularly against multidrug-resistant hospital isolates, in order to provide a basis for attracting the pharmaceutical industry and exploiting this raw material, which is abundant in oceans and freshwater, in the search for new sources of effective antibacterial compounds.

Materials and methods

Collection of the macroalga Ulva ohnoi

The algal biomass *U. ohnoi* was collected from an aquaculture facility located at 27° 35' 4.64" S, 48° 26' 31.10" W in Florianópolis-SC, Brazil. The samples were promptly transported to the Laboratory of Phycology at the Federal University of Santa Catarina (LAFIC–UFSC) within one hour, using light-protected thermal containers. Upon arrival, the biomass was rinsed with UV-C sterilized seawater, carefully brushed to remove epiphytes, invertebrates, and sediments, then dried in an oven at 60°C to a constant weight and frozen at -80°C for later analysis. Species-level identification was determined according to Salvi et al. (2021).

Lipid Extraction Procedure

The dried biomass of the algae *U. ohnoi* was ground in a mortar and pestle in the amount of 10g. The lipid was extracted using the Bligh & Dyer method (1959). 60 mL of a chloroform-methanol solution in a 1:2 ratio was added to the sample and homogenized in sealed flasks for 1 hour. After this step, the mixture was centrifuged for 20 minutes at 3000 rpm. The aqueous phase was discarded, and subsequently, the solvent from the oily phase was removed

using a rotary evaporator, leaving only the LE. This extract was resuspended in hexane, and the solvent was again removed in a rotary evaporator to remove impurities. The LE was then stored in light-protected flasks at a temperature of -20°C for future analysis.

Gas Chromatography Coupled to Mass Spectrometry (GC-MS)

The LE of *U. ohnoi* was subjected to derivatization with Boron Trifluoride (BF_3). For this, a 250 mg aliquot of the LE was transferred to a glass flask, where 6 mL of a 2% (m/v) methanolic sodium hydroxide solution was added. The system was refluxed with stirring for 5 minutes. Then, 5 mL of BF_3/methanol (MeOH) was added, and the system was refluxed for another 5 minutes. The solution was then transferred to a separatory funnel, where 20 mL of hexane was added to promote phase separation. After discarding the aqueous phase, the organic phase was filtered with sodium sulfate into a glass flask. The solvent was evaporated in a rotary evaporator, and the remaining solution was eluted in 1 mL of High-Performance Liquid Chromatography (HPLC) grade hexane (≥ 99.9) and transferred to vials for analysis by Gas Chromatography Coupled with Mass Spectrometry (GC-MS).

To determine the composition of fatty acids (FA) present in the LE of *U. ohnoi*, a gas chromatograph (Shimadzu, model GC-2010) coupled to a mass spectrometer (GCMS-QP2020) equipped with a SP 2560 fused silica capillary column (100 m × 0.25 mm × 0.10 µm) was used. The injection volume was 8 µL, in split mode with a ratio of 1:50. The interface and ion source temperatures were set at 280°C and 200°C, respectively, while the injector was maintained at 200°C, with helium carrier gas at a flow rate of 3 mL.min^{-1}. The initial column temperature was 100°C, maintained for 5 minutes, followed by heating at a rate of 5°C.min^{-1} to 250°C, totaling 55 minutes of analysis. The mass spectrometer operated in scan mode, with a detection range of 35–700 m/z, which allowed the identification of the substances. Lipid extraction and GC-MS analyses were performed at the Laboratory of Innovation and Solutions in Chemistry (INNOVASCHEM) at the Federal University of Pelotas (UFPel).

Thermogravimetric analyses (TGA)

Thermogravimetric analyses (TGA) of *U. ohnoi* were performed using a TA Instruments Q5000 instrument from the United States. To ensure measurement accuracy, the instrument was previously calibrated with 99.9% pure calcium oxalate monohydrate ($CaC_2O_4 \cdot H_2O$). Thermal characterization experiments were performed at a constant heating rate of 10°C per minute until a temperature of 650°C was reached. Each analysis used approximately 3 mg of *U. ohnoi*. The atmosphere used was inert, composed of nitrogen (N_2), maintained at a flow rate of 25 mL per minute. No sample drying or isothermal procedure was performed before the start of the analysis.

Differential Scanning Calorimetry (DSC)

The thermal behavior of *U. ohnoi* was characterized using Differential Scanning Calorimetry (DSC) to investigate its thermal properties, which are directly related to phase transitions and their respective enthalpy changes. The phase transitions were specifically determined using Modulated Temperature Differential Scanning Calorimetry (MTDSC), employing a Q2000 DSC instrument from TA Instruments (USA). The mass of the LE samples from *U. ohnoi* in each analysis ranged from 1 to 5 mg, weighed with an accuracy of ± 0.001 mg on a Sartorius M500P analytical balance. All samples were subjected to a total of three heating and cooling cycles. Measurements were conducted over a temperature range extending from − 80°C to 300°C. The heating rate used was 10°C min^{-1}, and nitrogen (N_2) was used as the inert purge gas at a flow rate of 50 mL min^{-1}.

Multidrug-resistant (MDR) bacterial isolates

The study used Gram-negative bacilli (GNB) and Gram-positive cocci (GPC), with three bacterial isolates from each species: *A. baumannii*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, *S. aureus*, and coagulase-negative *S.* These bacterial isolates were provided by two hospitals in the city of Pelotas-RS, Brazil. The resistance profile of the isolates was analyzed using VITEK 2 - BioMérieux and BD PHOENIX automation and was characterized by resistance to more than three classes of ATB to be considered MDR. These isolates were stored in the Bacteriotheque of the Microbiology and Bioprospecting Laboratory of the Department of Microbiology and Parasitology (DEMP) of the Institute of Biology at UFPel. These isolates were previously approved in the earlier study by Jara et al. (2021) by the Research Ethics Committee (CEP) under numbers 2,961,379 and 2,985,372, as well as by the National Research Ethics Commission (CONEP) under number 2,880,831, registered on the Brazil Platform.

Minimum Inhibitory Concentration (MIC *)*

The antimicrobial activity of *U. ohnoi* LE was evaluated using the Minimum Inhibitory Concentration (MIC) method, according to the guidelines of the Clinical and Laboratory Standards Institute(CLSI 2024) manual M07-A12, adapted for a natural product. For this purpose, a solution with a concentration of 16 mg/mL was prepared by dissolving 100 mg of LE from the algae in 2 mL of pure ethyl alcohol to prepare a solution with the addition of 5.257 mL of Müller-Hinton broth (CMH). In sterile microdilution plates containing 96 wells, previously filled with 100 µL of CMH, 100 µL of the *U. ohnoi* LE dilution (16 mg/mL) was inserted into the second column. Subsequently, ten serial dilutions (ranging from 8.000 to 0.015 mg/mL) were performed from the second to the eleventh column, each with a final volume of 100 µL per well. The first column evaluated the negative control, containing only CMH, while the last evaluated the positive control, combining CMH with the inoculum containing the microorganism of interest. Next, 5 µL of the inoculum was added to the wells of the second to twelfth columns. The plates were incubated at 37°C in an oven for 24 hours. Subsequently, MIC was analyzed using a colorimetric method by adding 40 µL/well of 0.015% 2,3,5-triphenyltetrazolium chloride (TCC) dye, and incubated again in the oven at 37°C for 30 minutes. The reading was made visually, observing the presence or absence of coloration.

Minimum Bactericidal Concentration (MBC)

The Brain Heart Agar (BHA) plating method was used to evaluate the Minimum Bactericidal Concentration (MBC) immediately after the MIC test. A Müller Hinton agar (MHA) plate was used, inoculated with a 5µL aliquot from each well in which the LE of *U. ohnoi* showed antibacterial activity and from the next well in which the EL of the algae showed no bacteriostatic antibacterial activity. The plate was then incubated at 37°C in an oven for 24 hours, and a visual reading was made of the colony growth with bacteriostatic results (inhibition of bacterial growth) or the prevention of bacterial colony growth with bactericidal results (death of bacteria).

Cell viability assessment

Hemolytic activity (HA)

Hemolytic activity (HA) was based on the assay by Vaucher et al. (2010), using defibrinated sheep red blood cells. The red blood cells were washed and resuspended three times in phosphate-buffered saline (PBS) to obtain a concentration of 4% (v/v). Eight mg/mL LE of *U. ohnoi* diluted in 1 mL of the 4% red blood cell suspension was added, and serial dilutions were performed up to a concentration of 0.0625 mg/mL. They were incubated for 2 h at 37°C with agitation in a shaker incubator. The cells were then centrifuged for 10 min at 2000 rpm, and the supernatant was transferred to a 96-well plate. The negative control was a 2.3% EtOH solution in water, and the positive control was TritonX-100. Hemoglobin release was read using a Hitachi U-1100 spectrophotometer (Hitachi), measuring absorbance at 425 to 450 nm. The percentage of hemolysis was calculated using the formula:

[(Abs sample – Abs blank)/(Abs ctrl – Abs blank) × 100]

Where: Abs sample was the absorbance of hemoglobin release in the supernatant, Abs blank was the absorbance of the negative control, and Abs ctrl was the absorbance of the positive control. The analyses were performed in triplicate.

Cell culture

Cell viability was assessed by culturing Madin-Darby-Bovine-Kidney (MDBK) and mouse fibroblast (L929) cells obtained from the Virology and Immunology Laboratory at the UFPel. The cells were propagated in Modified Eagle Medium (MEM) (pH 7.4) enriched with penicillin/streptomycin (100 U/L) and amphotericin B (0.1%), together with 10% (v/v) fetal bovine serum (FBS) at a ratio of 100 µL in 96-well plates and maintained under standard culture conditions (37°C in a humidified incubator with 5% CO2).

Cell treatment

U. ohnoi LE was dissolved in 15% (v/v) ethanol (EtOH) in water to prepare a stock solution of 52 mg/mL. This stock solution was diluted in MEM to obtain an initial concentration of 8 mg/mL with 2.3% (v/v) EtOH in MEM. In a 96-well plate, 100 µL of MEM without SFB was placed in all wells of rows A, B, C, and D (quadruplicate), except for column 1. where 200 µL of the 8 mg/mL LE solution was placed in the first well of column 1. Next, serial dilutions of 1:2 were performed on the plate, transferring 100 µL of this solution from the first column to the next well containing 100 µL of MEM. The final LE concentrations were 8, 4, 2, 1. 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625, and 0.0078125 mg/mL. The EtOH concentration decreased proportionally with each dilution from 2.3% in the first well to 0.00225% in the eleventh well. A control with MEM was included in the last column. For the cell viability assay, cells were seeded at 5×10^3 cells/well and treated with these dilutions for 24 h. The assays were performed in quadruplicate.

Cell viability - MTT

Quantitative assessment of cell viability was performed using the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay, according to the protocol described by Mosmann (1983). This colorimetric technique is based on the ability of functional mitochondria in viable cells to reduce MTT to blue formazan crystals. Briefly, the wells were washed with a balanced saline solution without calcium and magnesium (CMF, pH 7.4) and exposed to a 0.5 mg/mL MTT solution, followed by a 4-hour incubation under standard culture conditions. The resulting formazan was solubilized in 50 µL of absolute EtOH, and the absorbance was measured using a microplate reader (Biotex ELX800) at 570 nm. Concentrations that did not show cytotoxicity were considered capable of preserving cell viability close to 100% relative to the control (untreated cells). The assays were conducted in quadruplicate.

The results were expressed as a percentage relative to the control using the following formula:

Cell viability (%) = (OD_{570} of treated cells/OD_{570} of control) × 100%.

Cell viability EtOH test – MTT

To assess the possible interference of EtOH on cell viability, a parallel assay was performed exposing the MBDK and L929 strains to decreasing concentrations of EtOH p.a. in MEM. The concentrations tested (2.3%; 1.15%; 0.575%; 0.287; 0.143; 0.071; 0.035; 0.017; 0.008; 0.004 and 0.002%) corresponded exactly to those present in the LE dilutions of *U. ohnoi*. In the last column of the plate, a negative control containing only MEM without EtOH was included.

This assay was conducted under the same conditions and simultaneously with the LE assay, using the MTT protocol already described, in order to eliminate inter-experimental variations. All assays were performed in quadruplicate.

Cell viability – Neutral Red (NR)

Cell viability was assessed using the Neutral Red (NR) assay, which is based on the uptake and accumulation of dye in the lysosomes of viable cells (Borenfreund and Puerner 1985). After the cell treatment incubation time, the supernatant was removed and 200 µL of 0.033% NR solution was added to all 96 wells of the plate and incubated at 37°C for 2 h in an incubator with 5% CO_2. The NR was then removed and the cells were washed with 100 µL of MEM

without SFB, which was then removed. The cells were then fixed and decolorized with 100 µL of 1% acetic acid/50% EtOH solution. The plates were shaken for 10 min and the absorbance was read at 540 nm with a microplate reader, against a reference well that did not contain cells.

The cell viability rate was determined by the equation:

Cell viability (%) = (OD_{540} of treated cells/OD_{540} of control) × 100%.

Concentrations that did not show cytotoxicity were considered to maintain cell viability close to 100% compared to the control (untreated cells). The assays were conducted in quadruplicate.

Lactic dehydrogenase (LDH)

The lactate dehydrogenase (LDH) assay was performed by removing the supernatant from dilutions of 0.5 to 0.007 mg/mL after 24 hours of the cell treatment stage of the MTT methods to analyze the plasma integrity of L929 and MDBK cells (Decker and Lohmann-Matthes 1988). The LDH activity in the supernatant was determined using LDH® reagent (Labtest, Diagnostic) in Cobas Miras-Roche® biochemical equipment. The assay is based on a kinetic reaction, where LDH catalyzes the reduction of pyruvate with NADH, producing lactate and NAD + as products. The catalytic concentration is determined by the rate of NADH decomposition, measured by the decrease in absorbance at 340 nm. The analysis was performed in triplicate, and the values were determined as a percentage of LDH released.

Calculation of the Selectivity Index (SI)

To evaluate the pharmacological safety of *U. ohnoi* LE, the selectivity index (SI) was calculated, which relates cytotoxicity to the antimicrobial properties of the extract. The SI corresponds to the ratio between the concentration that causes 50% inhibition of cell viability (IC_{50}) in eukaryotic cells and the MIC against the microorganisms tested, according to the formula:

$$SI = \frac{IC50}{MIC}$$

A selectivity index equal to or greater than 10 (SI ≥ 10) was considered an indicative parameter of a safe therapeutic margin, ensuring antimicrobial efficacy with minimal cell toxicity. The SI was calculated from the ratio between the IC_{50} (obtained in the MTT, NR, and LDH viability assays in the MDBK and L929 cell lines) and the MIC.

Statistical analysis

The TGA and DSC analysis data were processed using TA Universal Analysis 2000 software (version 4.5, TA Instruments, USA). Lipid extract treatment was performed in triplicate for the HA and LDH tests and in quadruplicate for the MTT and NR tests. The results were analyzed using two-way analysis of variance (ANOVA) using GraphPad Prism software version 8.0 (Prism GraphPad Software, San Diego, USA). The results were expressed as mean ± standard error of the standard error (SEM), and the means were compared using the Sídak test. Differences were considered significant when $P < 0.05$.

Results

Lipid extract composition

The major fatty acid composition of *U. ohnoi* is shown in Table 1. Gas chromatography analysis of fatty acids (FAMEs) showed a significant predominance of palmitic acid, corresponding to 63.62% of the total relative area. Saturated fatty acids (SFA) such as myristic acid (C14:0; 1.24%), stearic acid (C18:0; 1.52%), and behenic acid (C22:0; 0.72%) were identified, with a total SFA content of 67.10%. The fraction of monounsaturated fatty acids (MUFA) represented 25.85%, consisting mainly of oleic acid (C18:1; 21.01%) and palmitoleic acid (C16:1; 4.84%). Polyunsaturated fatty acids (Σ PUFA) accounted for 7.05%, including α-linolenic acid (C18:3; 3.93%), linoleic acid (C18:2; 1.77%), stearidonic acid (C18:4; 0.97%), and hexadecatetraenoic acid (C16:4; 0.37%).

Table 1
Fatty acid composition of the lipid extract from *Ulva ohnoi* analyzed by GC-MS. The table presents the identified fatty acids, their chemical formulas, and their relative abundances (%)

Chemical Formula (C:n)	Relative Area (%)	Fatty Acid
C14:0	1.24	Myristic acid (methyl myristate)
C16:4	0.37	Hexadecatetraenoic acid (methyl ester)
C16:1	4.84	Palmitoleic acid (methyl palmitoleate)
C16:0	63.62	Palmitic acid (methyl palmitate)
C18:4	0.97	Stearidonic acid (methyl stearidonate)
C18:2	1.77	Linoleic acid (methyl linoleate)
C18:3	3.93	α-Linolenic acid (methyl α-linolenate)
C18:1	21.01	Oleic acid (methyl oleate; E isomer indicated)
C18:0	1.52	Stearic acid (methyl stearate)
C22:0	0.72	Behenic acid (methyl behenate)
Σ SFA	**67.10**	
Σ MUFA	**25.85**	
Σ PUFA	**7.05**	

The lipid profile observed shows a composition dominated by medium-chain SFA, with a significant contribution from MUFA, particularly oleic acid, and the presence of PUFA of biotechnological interest. The qualitative distribution shows a well-defined lipid pattern, with a predominance of C16 and C18.

Thermal analyses

Thermogravimetric analyses (TGA)

The results of the TGA of the *U. ohnoi* LE are illustrated in Fig. 1. and its respective thermal parameters are detailed in Table 2. An initial mass decrease of approximately 4% was observed in the *U. ohnoi* extract, attributed to the evaporation of volatile components, such as residual solvents and/or water. The thermal decomposition of the material occurred in a complex manner, evidencing four distinct degradation events. These events occurred at 198°C (Td_1), 334°C (Td_2), 447°C (Td_3), and 477°C (Td_4), culminating in a cumulative mass loss of approximately 88%.

Table 2
Thermogravimetric analysis (TGA) results for the of *Ulva ohnoi* lipid extract

Lipid extract	Volatile (mass loss) (%)	T_{d1} (°C)	T_{d2} (°C)	T_{d3} (°C)	T_{d4} (°C)	Mass loss (%)
Ulva ohnoi	4	198	334	447	477	88%

Differential Scanning Calorimetry (DSC)

The *U. ohnoi* LE displayed no significant thermal transitions when subjected to DSC between − 80 and 300°C (Fig. 2). This finding indicates the non-occurrence of processes like glass transition, crystallization, or melting, thereby suggesting an absence of measurable amorphous or crystalline domains within the extract under the conditions tested.

Susceptibility of MDR hospital isolates

The bacterial samples revealed resistance to at least three groups of antibiotics, as evidenced by the susceptibility of the 16 classes evaluated (Table 3).

Table 3
Susceptibility profile of the three bacterial isolates of each species for each class of antimicro

Bacteria (Nº Isolates)	Classes of Antibiotics							
	Aminoglycosides	B-Lactamase Inhibitors	Carbapenems	Cephalosporins	Glucopeptides	Glycylcyclines	Lincosamides	Lipopeptíde
Acinetobacter baumannii (I)	S	R	R	R	99	99	99	99
Acinetobacter baumannii (II)	R	R	R	R	99	S	99	99
Acinetobacter baumannii (III)	R	R	S	R	99	99	99	99
Escherichia coli (I)	S	R	R	R	99	99	99	99
Escherichia coli (II)	R	R	S	R	99	S	99	99
Escherichia coli (III)	S	R	S	R	99	99	99	99
Klebsiella pneumoniae (I)	S	R	R	R	99	S	99	99
Klebsiella pneumoniae (II)	R	R	R	R	99	99	99	99
Klebsiella pneumoniae (III)	R	R	R	R	99	99	99	99

(Continue)

Table 3
Continuation.

Bacteria (Nº Isolates)	Classes of Antibiotics							
	Aminoglycosides	B-Lactamase Inhibitors	Carbapenems	Cephalosporins	Glucopeptides	Glycylcyclines	Lincosamides	Lipopeptíc
Pseudomonas aeruginosa (I)	S	R	R	R	99	99	99	99
Pseudomonas aeruginosa (II)	S	S	S	R	99	99	99	99
Pseudomonas aeruginosa (III)	R	R	R	R	99	99	99	99
Staphylococcus aureus (I)	S	99	S	S	S	S	R	S
Staphylococcus aureus (II)	S	99	99	S	S	S	R	88
Staphylococcus aureus (III)	S	99	99	S	S	S	R	88
Coagulase-negative Staphylococcus (I)	R	99	99	88	R	S	R	S
Coagulase-negative Staphylococcus (II)	88	99	99	R	S	88	R	88
Coagulase-negative Staphylococcus (III)	88	99	99	R	S	88	R	88

R: resistant; S: sensitive; I: Sensitive, increasing exposure; 88: not tested; 99: not applicable

Antibacterial activity

MICs and MBCs are recorded for each bacterial species in Table 4. The MBC test had bacteriostatic action at the same concentrations identified in the MIC that were evaluated in the LE concentrations of *U. ohnoi*.

Table 4
Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *U. ohnoi* against hospital-acquired bacteria resistant to more than three classes of antimicrobials

Bacterial Species	MIC of *Ulva ohnoi* (mg/mL)				
(Isolates)	**Repetitions**			**Mean per isolate**	**Mean**
	1[a]	**2[a]**	**3[a]**		
Acinetobacter baumannii (I)	2.00	2.00	2.00	2.00	2.00
Acinetobacter baumannii (II)	2.00	2.00	2.00	2.00	
Acinetobacter baumannii (III)	2.00	2.00	2.00	2.00	
Escherichia coli (I)	2.00	2.00	2.00	2.00	2.00
Escherichia coli (II)	2.00	2.00	2.00	2.00	
Escherichia coli (III)	2.00	2.00	2.00	2.00	
Klebsiella pneumoniae (I)	4.00	4.00	4.00	4.00	4.00
Klebsiella pneumoniae (II)	4.00	4.00	4.00	4.00	
Klebsiella pneumoniae (III)	4.00	4.00	4.00	4.00	
Pseudomonas aeruginosa (I)	4.00	4.00	4.00	4.00	4.00
Pseudomonas aeruginosa (II)	4.00	4.00	4.00	4.00	
Pseudomonas aeruginosa (III)	4.00	4.00	4.00	4.00	
Staphylococcus aureus (I)	2.00	1.00	1.00	1.33	1.33
Staphylococcus aureus (II)	2.00	1.00	1.00	1.33	
Staphylococcus aureus (III)	2.00	1.00	1.00	1.33	
Coagulase-negative Staphylococcus (I)	2.00	1.00	1.00	1.33	1.33
Coagulase-negativeStaphylococcus (II)	2.00	1.00	1.00	1.33	
Coagulase-negative Staphylococcus (III)	2.00	1.00	1.00	1.33	

Cell viability assay

To assess the safety of LE from *U. ohnoi* in systemic routes, the hemolytic potential against red blood cells was evaluated to estimate the selective toxicity of LE in blood cells. LE from *U. ohnoi* demonstrated a promising safety profile in erythrocytes. As illustrated in Fig. 3, it did not promote significant lysis of red blood cells at concentrations of 0.0625 to 4 mg/mL. It was observed that at a concentration of 8 mg/mL, there was a significant increase in the percentage of HA, which can be attributed to analytical interference, probably due to the concentration of pigments contained in the algae, such as chlorophylls and fucoxanthin, which can cause spectral overlap and affect absorbance in a nonspecific manner. At concentrations with antibacterial activity ranging from 1.00 to 4.00. LE demonstrated low toxicity, showing a drastic reduction in hemolysis at lower concentrations. Additionally, the IC_{50} value calculated for LE was 4.705 mg/mL (Table 5), reinforcing the low cytotoxicity of LE against red blood cells and indicating a suitable profile for potential systemic applications.

Table 5
Mean and median IC_{50} values of *Ulva ohnoi* lipid extract evaluated in red blood cell, L929, and MDBK cell lines

Assays	Cell IC_{50} (mg/mL)		Mean IC_{50} (mg/mL)	Median IC_{50} (mg/mL)	Cell IC_{50} (mg/mL)		Mean IC_{50} (mg/mL)	Median IC_{50} (mg/mL)
	MBDK	**Red blood cells**			L929	Red blood cells		
HA		4,705	**1,8168**	**1,1552**		4,705	**1,6338**	**0,79015**
MTT	0,3604				0,9984			
NR	1,950				0,2499			
LDH	0,252				0,5819			

MTT: [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], NR: Neutral Red, LDH: Lactate Dehydrogenase, HA: Hemolytic Activity; L929: mouse fibroblast cell line; MDBK: bovine kidney cell line

The results of cell viability by MTT assay showed that exposure of MDBK and L929 cells (Fig. 4a and 4b) to decreasing concentrations of EtOH (0.575% to 0.002%) did not cause a significant reduction in cell viability at any of the concentrations tested. Viability remained close to 100% in all dilutions, comparable

to the control (untreated cells). This indicates that EtOH, at the concentrations used to convey *U. ohnoi* LE, is not cytotoxic to the MDBK and L929 cell lines under the experimental conditions adopted.

The MTT assay evaluates cell viability based on mitochondrial activity, indicating the ability of cells to reduce the MTT dye. *U. ohnoi* LE caused a significant reduction in MDBK cell viability (Fig. 5a) at concentrations of 2.0 to 0.125 mg/mL, however, an increase in cell viability can be observed from 0.063 to 0.008 mg/mL. In the L929 cell line (Fig. 5b), a significant reduction in cell viability was observed only at concentrations of 2 and 1 mg/mL. At lower concentrations, an increase in cell viability was observed between 0.5 and 0.008 mg/mL. Table 4 shows the estimated IC_{50} values, which were 0.3604 for MDBK and 0.9984 mg/mL for L929. The highest concentrations of 8 and 4 mg/mL were not reported in the graphs (Fig. 3a and 3b) due to the probable interference of pigments present in LE, which impair the reading of optical absorbance. Similarly, in the NR and LDH methods, the highest concentrations were also excluded from the graphical results.

The NR method test for lysosomal integrity in MDBK cell lines against *U. ohnoi* LE showed a significant reduction in concentrations of 1.0 and 0.5 mg/mL, and an increase and maintenance in cell viability from 0.25 to 0.08 mg/mL was observed, with no statistical difference from the cell control, as shown in the graphs in Fig. 6a. However, in L929 cells (Fig. 6b), there was a significant reduction in cell viability at all concentrations. The estimated IC_{50} in this experiment was 1.950 mg/mL for MDBK cells and 0.2499 mg/mL for the L929 cell line. MDBK cells were more resistant than L929 cells in the NR assay. The NR test was effective in identifying changes in lysosome integrity and is a reliable method for assessing cytotoxicity.

The LDH assay showed a significant reduction in concentrations from 0.25 to 0.007 mg/mL, whereas at 0.5 no significant reduction was observed. In L929 cells, a significant reduction was observed at all concentrations from 0.5 to 0.007 mg/mL (Fig. 7a and 7b). Table 4 shows the IC_{50} values found to be 0.2520 mg/mL for MDBK and 0.5819 for the L929 cell line. The rupture of the MDBK and L929 cell membranes verified by the LDH supernatant assay using the MTT assay showed greater resistance of the L929 strain compared to MDBK when treated with LE from *U. ohnoi*.

Table 5 shows the overall IC_{50} determined from cytotoxicity and hemolytic activity assays. The mean and the median IC_{50} cell viability were also calculated, and it was observed that L929 cells were more resistant in the MTT and LDH tests than MDBK cells in these tests, while in the NR test, MDBK cells showed greater resistance and also had higher mean and median IC_{50} values than those calculated with all L929 cell tests. Thus, it was concluded that IC_{50} values varied depending on the type of test and cells, ranging from 0.2499 to 4.705 mg/mL. In addition, the HA test was performed, which showed that the LE of *U. ohnoi* did not cause significant hemolysis at the concentrations evaluated. Furthermore, it was observed that, in general, L929 cells were more resistant to LE than MDBK cells.

SI assessment, which relates cytotoxicity (IC_{50}) to antimicrobial activity (MIC), revealed that, for all bacterial strains tested, SI values were less than 10. which is the established parameter for a safe therapeutic margin (Table 6). In fact, SI values ranged from 0.41 (for *K. pneumoniae* and *P. aeruginosa* in L929 cells) to 1.37 (for *S. aureus* and coagulase-negative *S.* in MDBK cells). This indicates that the LE concentrations of *U. ohnoi* required to exhibit antimicrobial activity are close to or, in many cases, higher than the concentrations that cause cytotoxicity in mammalian cells. The greater resistance observed in L929 cells compared to MDBK cells for some bacterial species, as well as the low hemolysis observed in additional assays, suggest the possibility of a more favorable therapeutic window under specific conditions or with strategies for delivery and chemical modification of the extract.

Table 6
Evaluation of the Selectivity Index of *Ulva ohnoi* Lipid Extract: Cytotoxicity in MDBK and L929 Cells with Antibacterial Activity

Bacterial species (isolates)	Mean Cell MDBK IC_{50} (mg/mL)	Mean Cell L929 IC_{50} (mg/mL)	Mean CIM bacterial species (mg/mL)	SI Cell MDBK	SI Cell L929
Acinetobacter baumannii	1.82	1.63	2.00	0.91	0.82
Escherichia coli			2.00	0.91	1.80
Klebsiella pneumoniae			4.00	0.45	0.41
Pseudomonas aeruginosa			4.00	0.45	0.41
Staphylococcus aureus			1.33	1.37	1.20
Coagulase-negative *Staphylococcus*			1.33	1.37	1.20
SI: Selectivity Index					

Discussion

The present study with *U. ohnoi* presented the following results: SFAs were the main components, with palmitic acid (C16:0) being the most abundant (63.62%), followed by stearic acid (C18:0–1.52%), myristic acid (C14:0–1.24%) and behenic acid (C22:0–0.72%). Total MUFAs accounted for 25.85%, with a predominance of oleic acid (C18:1–21.01%) and palmitoleic acid (C16:1–4.84%). PUFAs were detected in lower concentrations, characterized by α-linolenic acid (C18:3, 3.93), linoleic acid (C18:2–1.77%), stearidonic acid (C18:4, 0.97%), and hexadecatetraenoic acid (C16:4–0.37%), totaling 7.05% of PUFAs.

Several studies indicate that palmitic acid can induce cytotoxicity in different eukaryotic cells by promoting oxidative stress and activating apoptotic pathways, among others. However, unsaturated fatty acids, especially oleic acid, can neutralize or attenuate the toxic effects of palmitic acid (Gómez-Lechón et al. 2007; Moravcová et al. 2015; Urso and Zhou 2021). Our study has a palmitic:oleic ratio of about 3:1. where oleic acid can exert a significant protective effect.

The thermal decomposition profile of the LE from *U. ohnoi*, obtained by TGA, revealed a behavior characterized by successive episodes of mass loss. The initial stage, corresponding to a mass reduction attributable to the release of volatile constituents—such as solvents remaining from the extraction or residual moisture—provides relevant information about the nature and degree of purity of the analyzed sample. As the temperature rose, subsequent stages of thermal degradation were observed, indicating the presence of different organic fractions in the extract, each with specific thermal stability and a characteristic decomposition temperature. This multi-stage degradation pattern reflects the chemical complexity of the material, whose cumulative mass loss at the end of the process highlights the extensive decomposition of the organic components present.

Regarding phase transitions, DSC analyses did not identify significant thermal events within the investigated temperature range. The absence of endothermic or exothermic peaks associated with processes such as melting, crystallization, or glass transition suggests that the lipid extract of *U. ohnoi* behaves as an amorphous or disordered system, without the formation of structured domains on a measurable scale. This characteristic indicates that, under the experimental conditions adopted, the material does not exhibit crystalline organization or detectable phase separation, which is consistent with complex lipid mixtures where interactions between components can suppress well-defined thermal transitions.

The data presented, which include thermal decomposition characteristics, mass loss profiles, and the absence of phase transitions, are unprecedented for LE from *U. ohnoi*. With regard to *U. ohnoi*, to date, no reports have been found in the literature on the thermal characterization by TGA and DSC of LE from algae of the genus *Ulva*, especially the species *U. ohnoi*. Most available studies focus on polysaccharide extracts or microalgal oils for biotechnological purposes. Therefore, the thermal parameters described here—such as the decomposition stages, the characteristics of mass loss, and the absence of observed phase transitions—constitute a set of previously unreported data that significantly contributes to the understanding of the thermal stability and physicochemical properties of these extracts. Such information is fundamental for future biotechnological applications, aiding in the definition of temperature limits for processing and storage, as well as providing insights into the behavior of these materials in pharmaceutical or cosmetic formulations.

The fatty acids (FA) have been extensively studied as antimicrobial agents capable of penetrating bacterial walls, destabilizing their cell membranes (Yoon et al. 2018). The absence of an outer membrane and the porous structure of the peptidoglycan cell wall in GBP facilitate the entry of these molecules. In contrast, in GNBs, the presence of a thinner layer of peptidoglycan together with lipoproteins (proteins and lipopolysaccharides) and lipid A hinders the absorption of hydrophobic molecules (Barák and Muchová 2013; Epand and Epand 2009; Heesterbeek et al. 2019). However, in our study, the action described in the cited works was not verified when using *U. ohnoi*. This may have occurred due to the composition of the LE tested, probably because of the structure-activity of lipids by long, medium, or short chains; saturated or unsaturated; number of double chains and cis or trans orientation, in the same terms as the authors' position(Epand and Epand 2009; McGaw et al. 2002) on FA structures and antimicrobial action. This divergence in results suggests the need for new specific studies of the components found in LE.

Nazzaro et al. (2013) reference other studies (Burt 2004; Chorianopoulos et al. 2008; Gutierrez et al. 2008; Marino et al. 2001)) that corroborate these observations. As a result, phytochemicals can penetrate damaged bacterial membranes and interfere with fundamental cellular processes, leading to the death or inactivation of bacterial cells (Berneira et al. 2021; Lopez-Romero et al. 2015).

Given this, our research used LE from *U. onhoi*, which has been little explored to date and has shown antimicrobial properties. The three bacterial isolates evaluated showed similar results in both MIC and MBC and demonstrated bacteriostatic activity in all species and concentrations tested for antibacterial activity. The best MIC results were observed in *S. aureus* and coagulase-negative *S.*, with 1.33 mg/mL, as well as in *A. baumannii* and *E. coli*, which presented 2 mg/mL.

To date, no studies have been found on the antimicrobial action of *U. ohnoi* LE using the MIC method; however, we found only one article by Kang et al. (2021) that used *U. ohnoi* oil collected on an island in South Korea, which showed antimicrobial activity conducted using the Ultrasensitive Radical Diffusion Assay method. The authors used three oils (A, B, and C) at a concentration of 10 mg/mL against *Bacillus subtilis* (KCTC1021), with a result of 10.36 mm for B and 7.35 mm for A, *E. coli* (isolate-D31) with a halo greater than 10 mm in all oil samples, and the fungus *Candida albicans* (KCTC76965) did not show halo formation. The LE of the biomass of *U. ohnoi* from the island of Florianópolis-SC, Brazil, in this experiment obtained excellent antibacterial results in *E. coli* (2 mg/mL) and, when compared to the antibacterial activity of the oil from *E. coli* (isolate-D31) showed that in both studies, despite being evaluated by different methodologies, they complemented each other, as the oil from the experiment by Kang et al. (2021) and our LE from *U. ohnoi* presented positive antibacterial properties against the *E. coli* isolate. However, the antibacterial and antifungal effects of the Bacillus subtilis and *Candida albicans* strains from the study by Kang et al. (2021) were not tested in our research.

Our study evaluated the IC_{50} in four methods: LDH (membrane damage), MTT (metabolic activity), NR (lysosomal integrity), and HA (red blood cell toxicity) assays in different cell cultures: L929 and MDBK cell lines. The NR assay indicated greater sensitivity in L929 cells (IC_{50} = 0.2499 mg/mL) compared to MDBK cells (IC_{50} = 1.950 mg/mL). On the other hand, the MTT assay showed more pronounced toxicity in L929 (IC_{50} = 0.9984 mg/mL) than in MDBK (IC_{50} = 0.3604 mg/mL), suggesting a possible difference in cellular metabolic response to LE.

The results of the MTT test with EtOH confirmed that the vehicle (EtOH at 15% v/v) used to dissolve the LE from *U. ohnoi* did not interfere with the viability of MDBK and L929 cells at the initial and final concentrations applied in EtOH (2.3% to 0.002%). Thus, it is concluded that any changes in cell viability observed during treatment with LE can be attributed exclusively to the compounds present in the algae, and not to the solvent. This is particularly relevant for interpreting the dose-response curve of LE, since the absence of EtOH toxicity at these concentrations validates the reliability of the model and the conclusions about the biological activity of the extract.

The LDH assay, which assesses damage to the plasma membrane, also showed distinct sensitivity between strains, with IC_{50} values of 0.5819 mg/mL (L929) and 0.2520 mg/mL (MDBK). The hemolysis assay, performed on red blood cells, showed a considerably higher IC_{50} value (4.705 mg/mL), suggesting good tolerability of blood cells with low hemolytic potential of *U. ohnoi* LE compared to its cytotoxicity in cell cultures. Although most studies on *Ulva* spp.

involve traditional viability methods, the multidimensional approach of this work offers a more complete assessment of the effects of LE from *U. ohnoi*, contributing to the development of protocols and potentially expanding its application in pharmacological and biotechnological areas, reinforcing the importance of detailed characterizations in macroalgae research.

Despite the promising antibacterial properties demonstrated by LE from *U. ohnoi* against MDR isolates, the SI assessment revealed a crucial limitation in terms of pharmacological safety. According to the methodology adopted, an SI ≥ 10 is indicative of a safe therapeutic margin. However, our results in Table 5 show SI values significantly below this threshold, ranging from 0.41 to 1.37. This suggests that *U. ohnoi* LE may exhibit cytotoxicity at concentrations that are equal to or even lower than those required to inhibit bacterial growth, especially for *K. pneumoniae* and *P. aeruginosa*, where the SI was below 0.5.

It is important to consider that the intense greenish coloration of the extract, observed at higher concentrations (8 and 4 mg/mL), represented a methodological limitation in colorimetric assays such as MTT and NR. This pigmentation may have compromised the accuracy of the IC_{50} determination in these ranges, due to the potential to mask optical readings and, consequently, affect the calculated SI values. This reinforces the need for complementary approaches or purification of the extract for a more accurate assessment of its intrinsic cytotoxicity.

Conclusion

The LE from *U. ohnoi* demonstrated promising antimicrobial activity against all MDR strains tested, with the best results observed against *S. aureus* and coagulase-negative *S.*

To better understand its properties, chemical characterization of the LE revealed a composition predominantly consisting of palmitic acid and oleic acid, key elements for future investigations. In parallel, thermogravimetric analyses revealed that the extract undergoes complex degradation in multiple events, indicating the presence of different organic fractions with distinct thermal stabilities. Additionally, Differential Scanning Calorimetry did not identify significant phase transitions, suggesting that the extract behaves as an amorphous and thermally stable system, without measurable structural reorganizations within the evaluated temperature range.

Regarding safety, cytotoxicity assays (MTT, NR, and LDH) in MDBK and L929 cell lines, along with the hemolysis assay (HA), indicated a favorable initial safety profile. The LE exhibited low systemic cytotoxicity and did not induce significant red blood cell lysis. It is important to note that the ethanol solvent control confirmed that the vehicle did not interfere with cell viability, ensuring the reliability of the results obtained.

However, the comprehensive pharmacological safety evaluation, which included the SI, revealed values below the threshold for a safe therapeutic margin (SI ≥ 10), suggesting the need for optimization. Furthermore, the intense greenish coloration of the LE at higher concentrations represented a significant methodological limitation in the colorimetric assays (MTT and NR), which may have affected the accuracy of the cytotoxicity measurements and the calculated SI values. In this regard, formulation strategies for the *U. ohnoi* LE show promise in overcoming the interference caused by pigments in the tests, as well as enhancing the extract's biological activity; however, further studies are needed to ensure its efficacy and safety.

Therefore, the observed biological effects are attributable to the bioactive compounds in the *U. ohnoi* LE. The use of this biomass for the production of antimicrobials constitutes an innovative and sustainable strategy, with potential environmental and economic benefits associated with the management of green tides and the development of new drugs.

Limitations

The *U. ohnoi* LE exhibited an intense greenish coloration, which directly interfered with the evaluation of cell viability, especially at the higher concentrations tested (8 and 4 mg/mL). This pigmentation made it difficult to interpret the results in assays based on colorimetric techniques, such as MTT and NR, due to the possible masking of the optical reading. Despite attempts to minimize this effect through washing and the use of buffered solutions, the persistence of the coloration compromised the correction of the data and the accuracy in determining the IC_{50} in the higher ranges of the extract. This factor represents an important methodological limitation and highlights the need to consider complementary approaches or alternative techniques for a more precise quantitative evaluation of the cytotoxic activity of LE in future research. Another advantageous approach could be the encapsulation of *U. ohnoi* LE in nanoparticles to overcome pigment interference in the tests and enhance the biological action of the extract.

Declarations

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

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Author Contribution

MCJ: Conceptualization, Investigation, Methodology, Writing-original draft, Writing-review and editing. **DSA:** Methodology, Formal analysis, Writing-review & editing. **LW:** Visualization. **EB:** Methodology. **LAA:** Methodology. **NSP** : Methodology. **VPG:** Methodology, Visualization. **MPA:** Methodology, Formal analysis, Data curation, Software, Visualization. **CPF** : Methodology, Formal analysis. **MN** : Methodology, Formal analysis. **WRS:** Methodology, Formal analysis, Data curation *.* **SOH:** Supervision *,* Resources **RAV:** Resources, Writing-review and editing *.* **CMPP:** Resources. **PSN:** Project administration, Funding acquisition, Writing- reviewing and editing.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Figures

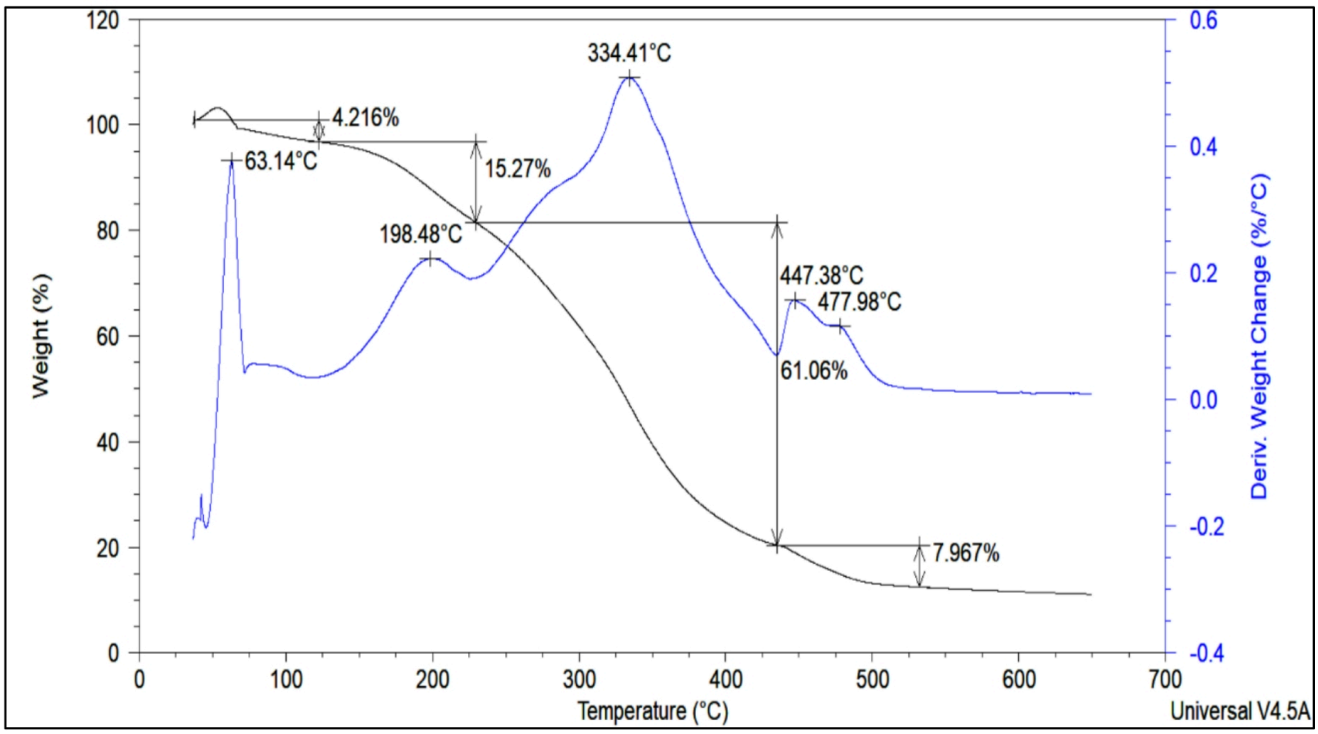


Figure 1

Thermogravimetric analyses (TGA) curve of *Ulva ohnoi*lipid extract.

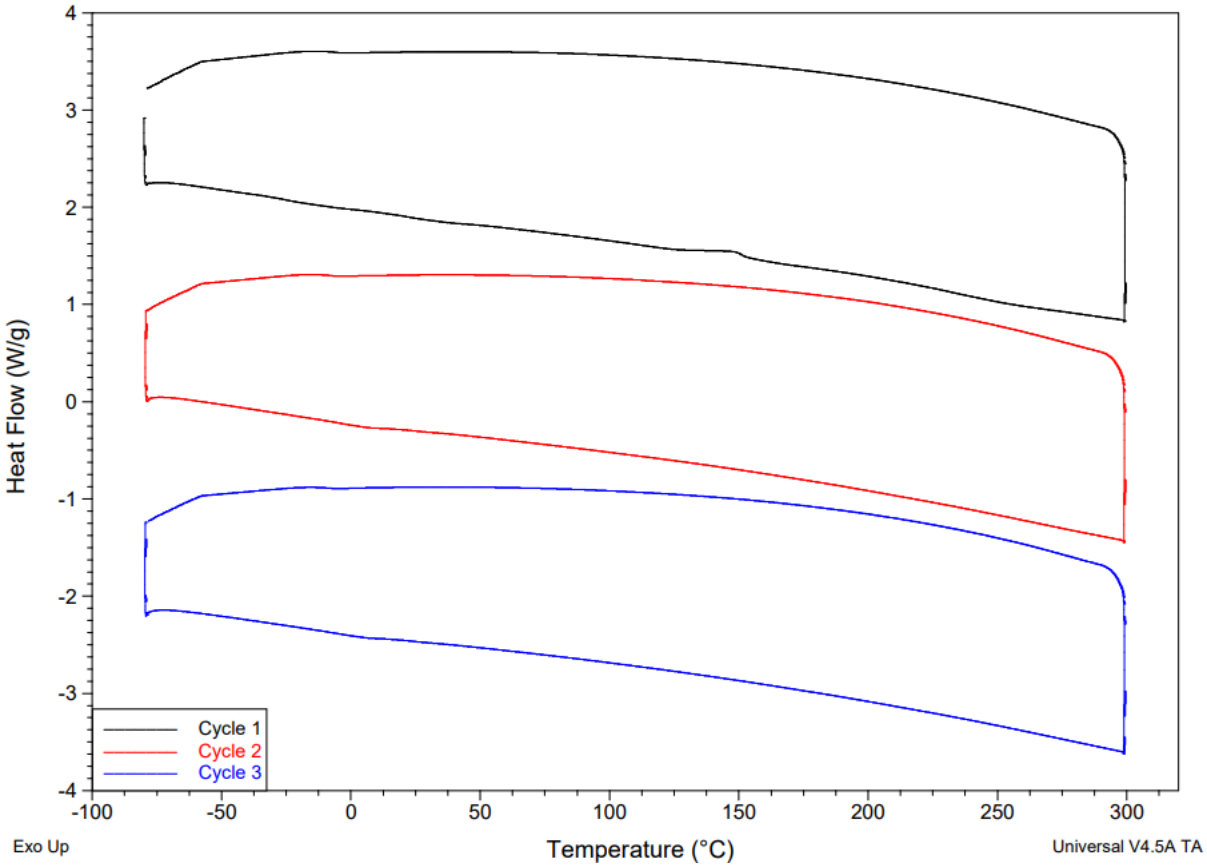


Figure 2

Differential Scanning Calorimetry (DSC) curve of *Ulva ohnoi* lipid extract.

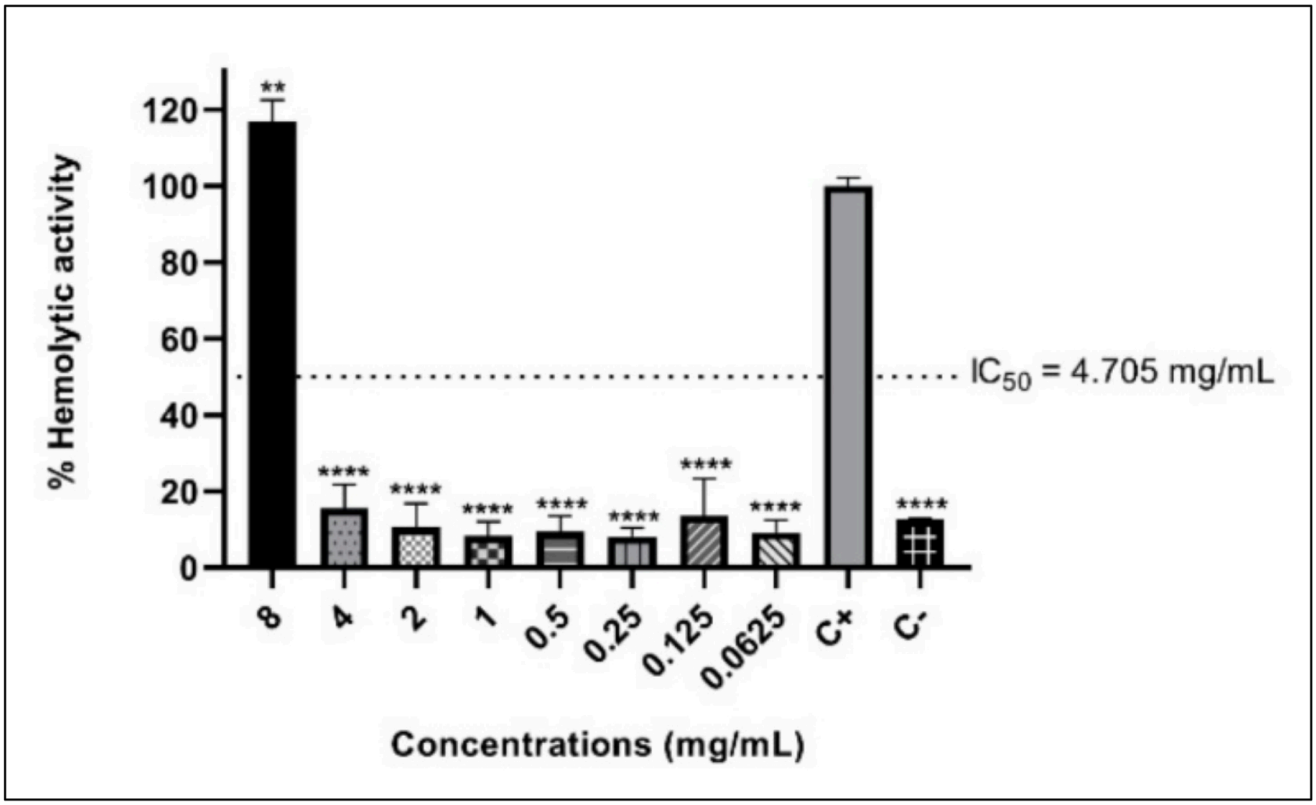
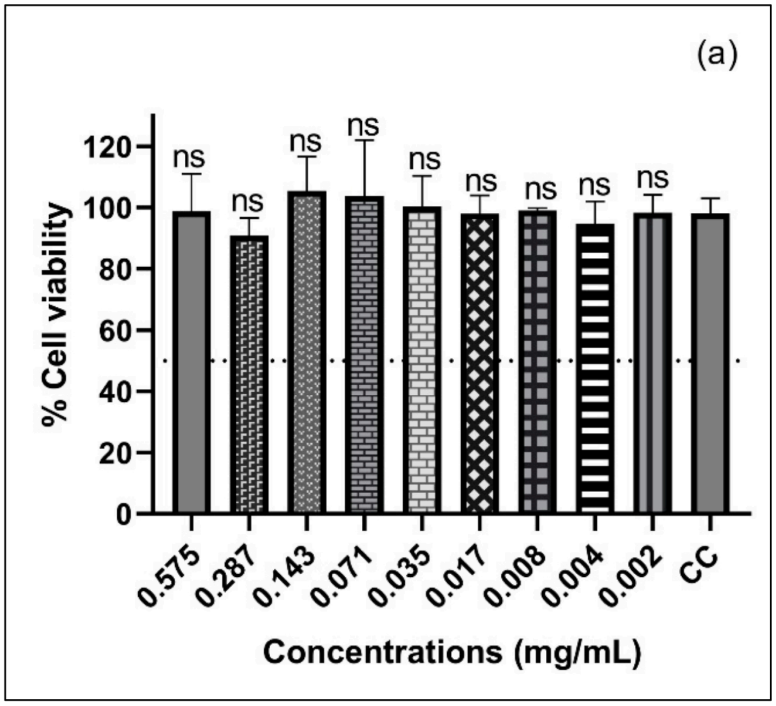


Figure 3

Hemolytic activity of lipid extract from *Ulva ohnoi*. Values represented as mean ± SD. * $p < 0.05$ ** $p < 0.01$ ***$p < 0.001$ **** $p < 0.0001$ compared to the positive control.



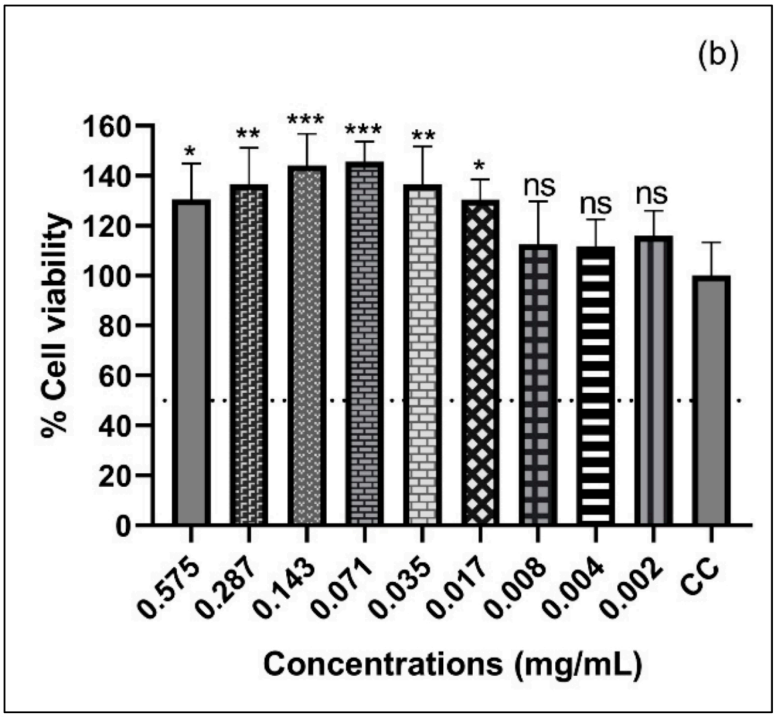
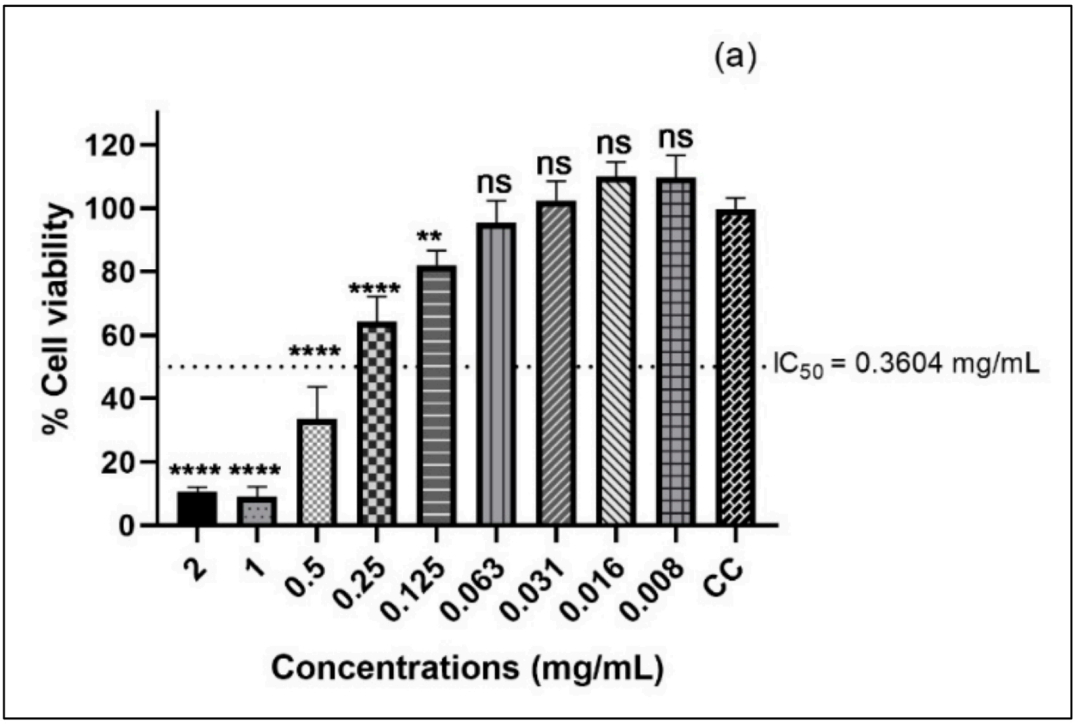


Figure 4

(a) MDBK and (b) L929 Cytotoxic activity of ethanol in the MTT assay. Values represented as mean ± SD. * $p < 0.05$ ** $p < 0.01$ ***$p < 0.001$ **** $p < 0.0001$ compared to the cell control.



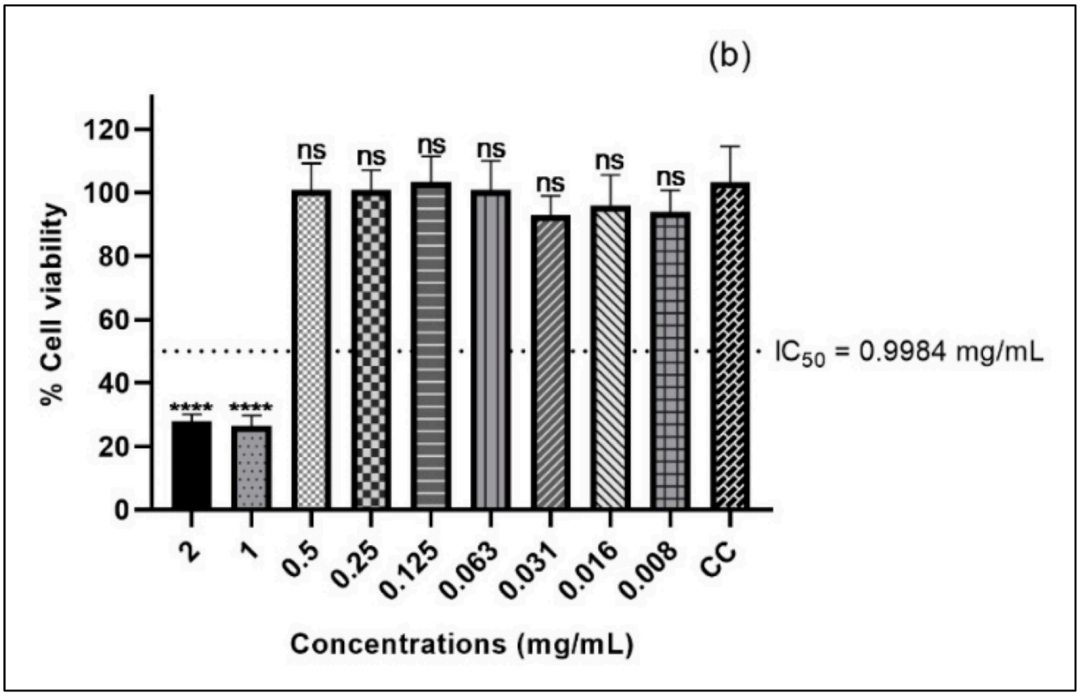
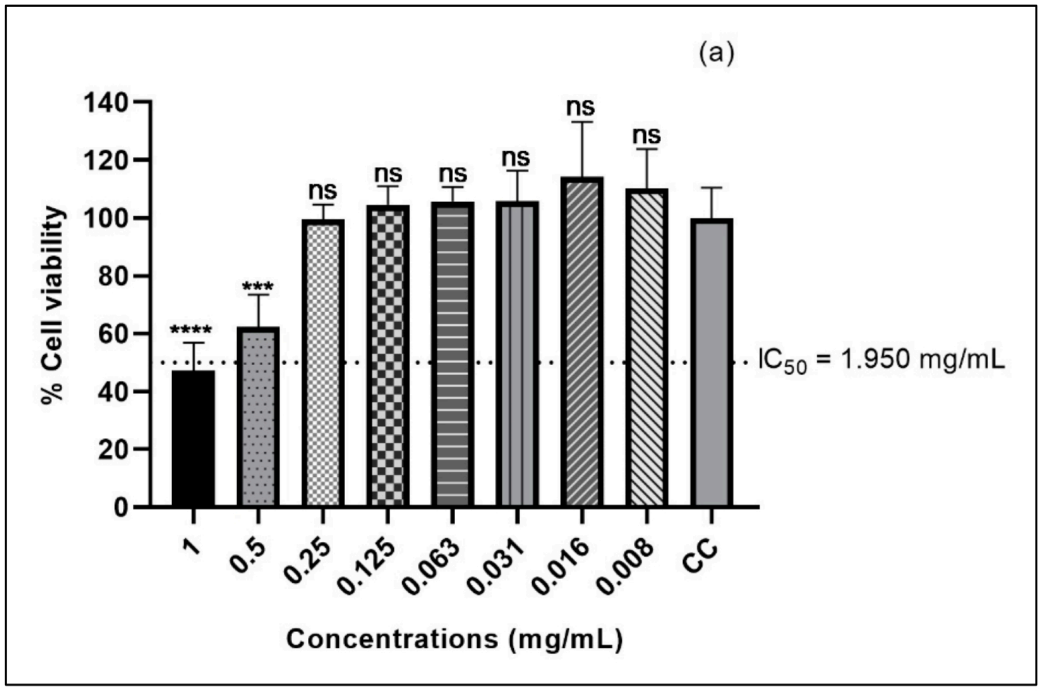


Figure 5

(a) MDBK and (b) L929 Cytotoxic activity of *Ulva ohnoi* lipid extract in the MTT assay. Values represented as mean ± SD. * $p < 0.05$ ** $p < 0.01$ ***$p < 0.001$ **** $p < 0.0001$ compared to the cell control.



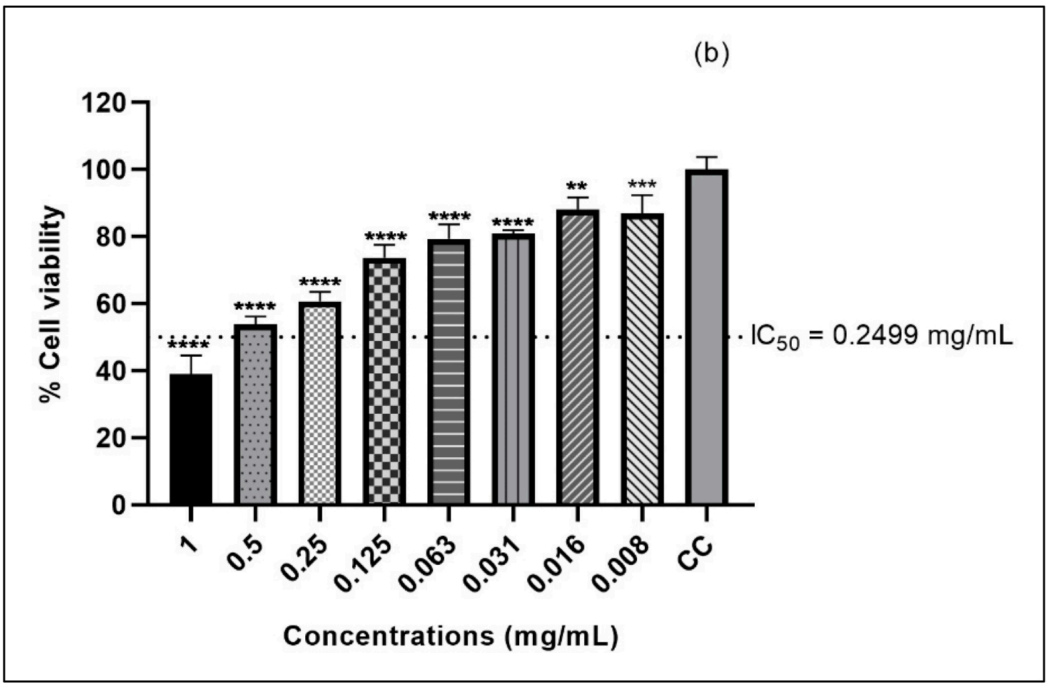
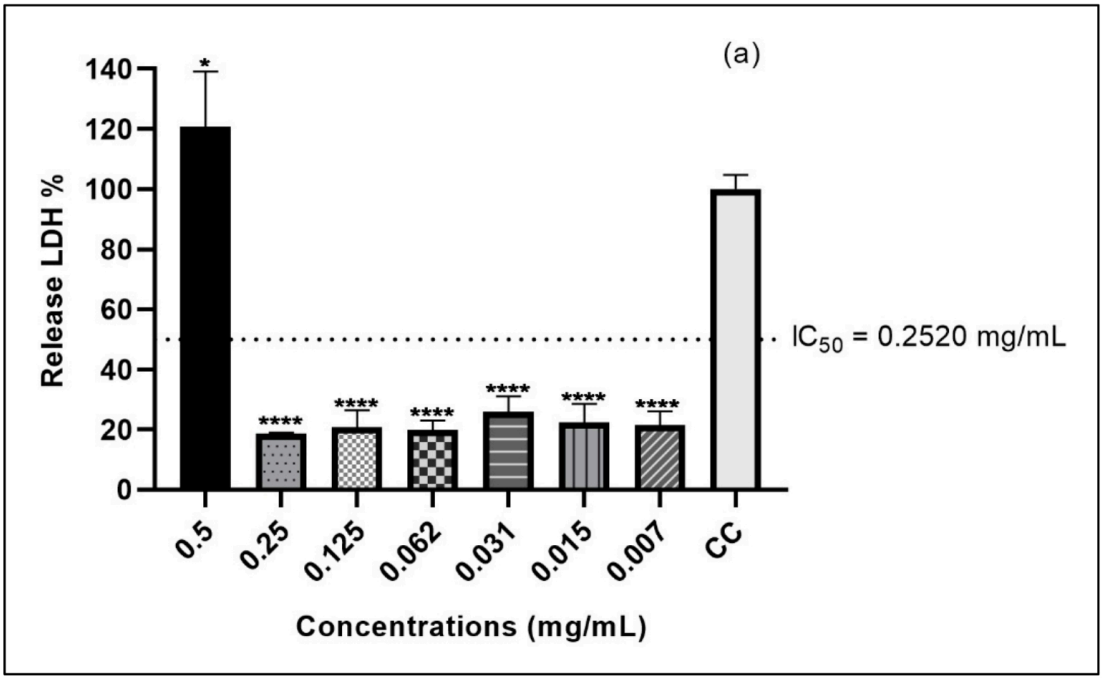


Figure 6

(a) MDBK and (b) L929 Cytotoxic activity of *Ulva ohnoi*lipid extract in the NR assay. Values represented as mean ± SD. * $p < 0.05$ ** $p < 0.01$ ***$p < 0.001$ **** $p < 0.0001$ compared to the cell control.



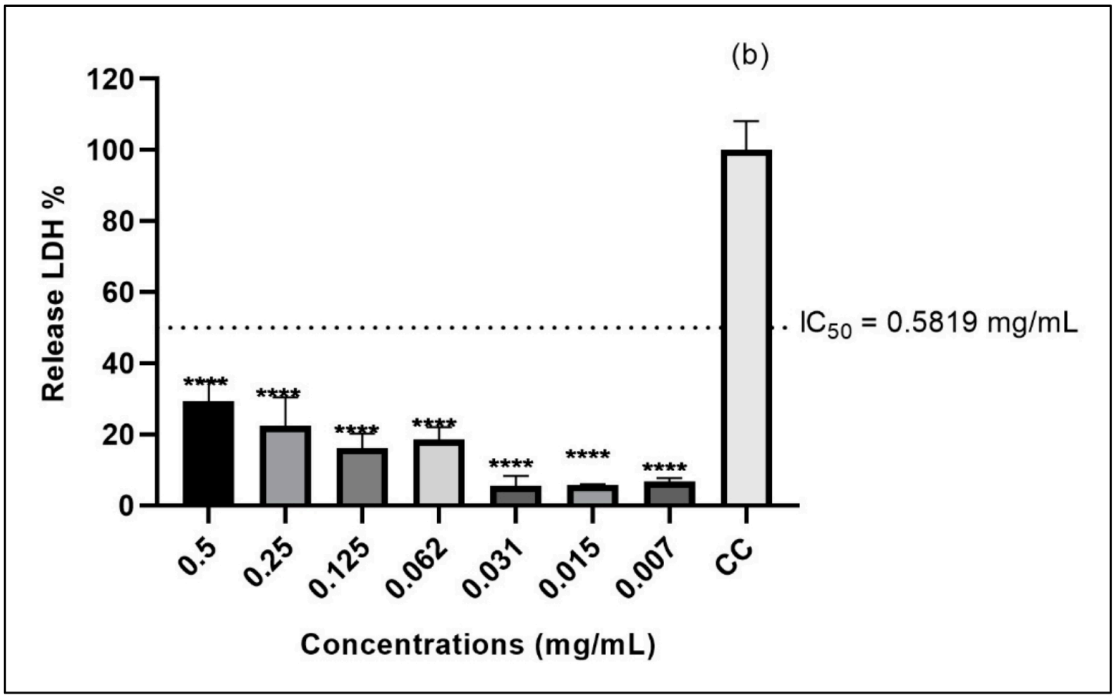


Figure 7

(a) MDBK and (b) L929 Cytotoxic activity of *Ulva ohnoi* lipid extractin the LDH assay. Values represented as mean ± SD. * $p < 0.05$ ** $p < 0.01$ ***$p < 0.001$ **** $p < 0.0001$ compared to the cell control.

Supplementary Files

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