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RESEARCH ARTICLE

Marine-Derived Extracts as Economically Sustainable and Ethical Alternatives to Fetal Bovine Serum in HEK293 Cell Culture

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Abstract: Fetal Bovine Serum (FBS) is a staple supplement in cell culture, providing key growth factors that promote cell health and proliferation. Since 1885, cell culture has been a cornerstone of both basic and applied research, leading to significant discoveries of cell communication and function. However, the use of FBS presents economic and ethical challenges. Millions of bovine fetuses are euthanized annually to produce the volume of FBS necessary for in vitro research. Batch-to-batch inconsistencies often hinder experiment replicability, and FBS can account for over 75% of cell culture costs. It is critical to identify viable FBS alternatives for the sustainability of in vitro research. Marine sources offer an avenue as FBS alternatives because of their abundance of bioactive compounds and sustainable potential. In this study, novel extracts derived from sea urchin roe (known as uni) and the algal species Gracilaria salicornia invasive to Hawai'i, were evaluated as alternatives to FBS. Extracts were prepared at 5%, 10%, and 20% concentrations in Dulbecco's Modified Eagle Medium (DMEM) and exposed to Human Embryonic Kidney (HEK293) cells in culture for 3 days. Cell proliferation, viability, and morphology were assessed through cell quantification, MTT assays, and microscopy. Cells grown in 5% and 10% uni extract and 10% G. salicornia extract treatments showed promising proliferation, with cell counts statistically comparable ($p>0.05$) to the FBS control. Cells grown in the 5% uni treatment exhibited the healthiest morphology, comparable to 84.3% of the cell population in the FBS control group. Despite the proliferative success, the MTT assay showed significantly lower viability across all treatments ($p<0.001$), suggesting the extracts may lack metabolic factors necessary for cellular function. These findings highlight the limited potential of marine-derived extracts as FBS supplements and provide a platform for continued exploration and improvement in the field.

Keywords: Fetal Bovine Serum, Algae, Sea Urchin, Cell Culture

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Introduction

Since its inception in 1885, cell culture has served as an ideal model that has driven significant advancements in biological and biomedical research and industry [1]. From aiding in our understanding of fundamental cellular mechanics, exploring the

effects of drugs and toxic compounds on cells, to biotechnological applications including labgrown meat and tissue engineering, cell culture is an indispensable tool for a wide range of biological endeavors [2, 3].

Fetal sera, derived from the blood of mammalian fetuses such as horses, sheep, goats, cows, and pigs, have served as vital supplements to cell culture media since Montrose T. Burrows' work in 1910 with chicken blood plasma [4, 5]. Sera contain essential growth and attachment factors, nutrients, and bioactive compounds that promote cell growth and proliferation in cell culture models. Among them, Fetal Bovine Serum (FBS) has proven to be the most effective, demonstrating its significant utility and success in cell culture techniques worldwide due to its efficacy across a range of cell lines [6, 7]. Despite its utility, FBS is associated with several ethical, biosafety, scientific reproducibility, and economic concerns. FBS is harvested through the slaughter of pregnant cows and the subsequent collection of serum from the fetus through cardiac puncture [8]. More than two million bovine fetuses are used annually to produce around 800,000 liters of FBS [8, 9]. Due to its mammalian origin, FBS may be contaminated with pathogens and other adventitious agents, with multiple authors reporting pestiviral contamination rates of 22-100% in commercial FBS batches [10]. This contamination frequently disrupts cell culture and renders FBS unusable for certain biotechnology applications, including lab-grown meat. Because FBS contains a wide array of essential biomolecules that support cell growth, its biochemical profile remains poorly characterized. This lack of defined composition, combined with notable batch-to-batch variation, raises concerns about experiment replicability and scientific integrity [9]. In addition to ethical and scientific concerns, the economics of FBS are troubling. Prices have increased by more than 300% since 2017 due to increased demand and limited availability. With FBS constituting upwards of 75% of cell culture costs, general accessibility to biological research is reduced, and upscaling becomes difficult [11, 12]. Due to the critical value of cell culture generated scientific insights, and the myriad of challenges associated with FBS, finding an ethically sound, sustainable, and economically viable FBS alternative is critical to the advancement of biological science.

While the search for an effective FBS alternative remains ongoing, much of the current work is focused on terrestrially derived and chemically defined extracts, such as human platelet lysate, earthworm coelomic fluid, and insect and plant-based supplements. [13, 14]. Each of these alternatives aims to address one or more of the drawbacks of FBS, with the goal of identifying a universal supplement as effective at promoting cell health. By testing a wide range of natural and synthetic sources, researchers can develop an archive of potential alternatives, each one more viable than the next. Marine sources present an exciting avenue for research, as they are known to contain unique bioactive compounds that often have biotechnological applications [15]. Several studies have shown extracts from microalgae, chum salmon eggs, and fish serum improve certain cell functions, including growth, protein expression, cell differentiation, and viability across various cell lines, showcasing the potential for marine extracts as alternative candidates of FBS [16-19].

Marine algae is a rich source of biologically active substances, including proteins, fats, carbohydrates, and macro and micronutrients, many of which are essential to cell health [20]. A previous study conducted by [21] found that the presence of bioactive polysaccharides led to increased cell proliferation through gene induction pathways. Gracilaria salicornia, commonly known as gorilla ogo, is an invasive species of macroalgae that grows rampant on Hawai'i's reef flats. Its overgrowth can smother native algae and coral species, alter the natural landscape, and reduce overall biodiversity. Due to its abundance and ecological invasiveness, utilizing G. salicornia biomass would be economically beneficial for the Hawaiian ecosystem, while promoting research within the local community. The presence of bioactive components in G. salicornia, including proteins, polysaccharides, and nutrients, parallels many of the growth-promoting factors found in FBS, suggesting its potential as a sustainable and functional alternative in cell culture [20].

Sea urchin roe, referred to as uni in Japanese, also contains an abundance of biological components, including vitamins, minerals, proteins, unsaturated fatty acids, and polysaccharides, many of which are known to have nutritional value and medicinal properties [22]. Due to its fetal origin, FBS contains an abundance of growth-promoting factors that support cell viability in vitro. Given the gametic nature of uni, which contains similar developmental and regulatory molecules essential for embryogenesis, it was hypothesized that uni may contain bioactive compounds similar to those found in FBS. Studies by [23] have extensively covered the pharmacological properties of sea urchin gonads, spines, intestines, and full bodies, highlighting their diverse physiological applications. However, the utility and efficacy of sea urchin extract in cell culture applications remain unknown. Sea urchins are easily farmed in ocean pens and in tanks on land, with established vendors across continents from Canada to Japan [24]. In Hawai'i, native urchin species are farmed and released to combat invasive algae overgrowth. Given their known bioactive properties and ease of propagation, sea urchins present an intriguing and sustainable option as an FBS alternative.

FBS dependency in cell culture poses significant ethical, scientific, and economic challenges that threaten the sustainability of cell culture research. By investigating the capacity of macroalgae and sea urchin roe extracts on cell growth, morphology, and metabolic health in vitro, this novel study explored the potential utility of ecologically and economically sustainable marine-derived alternatives for FBS.

Materials and Methodology

Experimental Design

Marine alternatives (algae and uni) were collected, and extracts were processed, sterilized, and supplemented as 5%, 10%, and 20% sera concentrations in culture media. Human Embryonic Kidney (HEK) 293 cells (ATCC, Manassas, VA) were cultured in various concentrations of experimental sera for 72 hours and then evaluated for cell growth, cell morphology, and metabolic activity (Fig. 1).

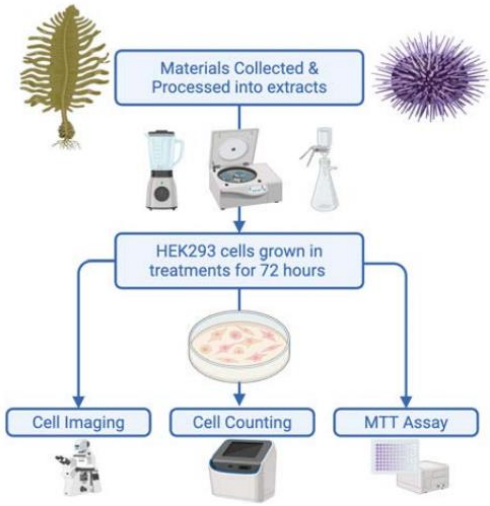

Fig. 1: Schematic Overview of Experimental Workflow

Raw materials (G. salicornia and sea urchin roe) were collected and processed into extracts via homogenization, centrifugation, and filtration. HEK293 cells were cultured in treatment groups for 72 hours. Cell health was assessed through microscopy, cell counting, and metabolic activity assays.

Extract Preparation

Autoclaving was performed as a sterilization step to eliminate potential microbial contaminants and ensure biosafety prior to exposure of extracts to mammalian cell cultures. To maintain consistency, this procedure was adapted from published protocols of marine biomass processing [17, 20].

Seaweed Extract

Five pounds of G. salicornia biomass, an invasive species to Hawai'i, was collected by hand at a depth of two feet along Paiko Beach on O'ahu, Hawai'i, a site known to be overrun with G. salicornia. Specimens were rinsed in distilled water and hand-processed thoroughly to remove debris and hitchhiker organisms, such as crustaceans, mollusks, and other algae species (Fig. 2A). Biomass was air dried in a mesh cage for 24 hours (Fig. 2B) and transferred to a dehydrator (Nesco, Two Rivers, WI) for one hour at 60˚C.

Dried biomass was transferred to a laboratory, where it was ground into a powder using a portable blender (Magic Bullet, Los Angeles, CA). Powdered algae was suspended in Milli-Q ultrapure water at a concentration of 10% w/v, mixed thoroughly, and placed on a hot plate for 20 min at 100˚C to solubilize bioactive components [17]. After boiling, the mixture was pulse-blended again for one minute to further homogenize the sample and filtered through a cheesecloth to separate out solids. Next, the solution was centrifuged at room temperature at 8,000 g for 30 minutes, and water-soluble components were harvested by collecting the supernatant with a micropipette. Next, the extract was autoclaved and sterilized for 30 min. After

sterilization, the extract was again centrifuged at room temperature at 8,000 g for 30 min and then vacuum-filtered through a 0.22 µm filter to remove particulates until the solution was clear. The extract was used as processed with no pH adjustment.

Fig. 2: Algae Collection Site & Processing in Dehydration Cage

(A) Photograph of G. salicornia abundant on the shores of sample collection site, Paiko Beach (east shore of O'ahu); (B) Photograph of G. salicornia drying in mesh cage for 24 hours prior. Dried samples were then transferred to an electronic dehydrator for additional drying.

Sea Urchin Roe Extract

Uni (sea urchin roe) from the purple sea urchin (Heliocidaris crassispina) was sourced commercially from a Nijiya Market (1009 University Ave, Honolulu, HI). Commercial sourcing was selected over hand collection due to ease of access and assurance of consistent source material. Sixty grams of sea urchin roe, a visually comparable biomass to the amount used in the algae extract, was suspended in 350 mL of Milli-Q ultrapure water and blended for 45 seconds in the Magic Bullet blender until the sample was homogenized. Next, the solution was centrifuged at 12,000 rpm for 20 minutes, and the water-soluble components were harvested by collecting the supernatant. Following collection, the extract was sterilized by steam autoclave for 30 minutes. After sterilization, the extract was again centrifuged at room temperature at 12,000 rpm for 30 minutes and then vacuum-filtered through a 0.22 µm filter to remove large particulates. The extract was used as processed with no pH adjustment.

Cell Culture

HEK293 cells were purchased from the American Type Culture Collection (Manassas, VA) and passaged prior to experimentation. Cells were subcultured in Dulbecco's Modified Eagle Medium (DMEM, Thermo Fisher Scientific, Waltham, MA) supplemented with 10% FBS and 1% Penicillin/Streptomycin (P/S) and incubated (Thermo Fisher Scientific, Waltham, MA) at 37˚C and 5% CO2 for 72 hours following standard lab protocols.

To assess the utility of seaweed and uni as potential sera substitutes, cells were grown in culture media consisting of DMEM supplemented with either the algae or uni extract. To assess cell growth at various concentrations, extracts were prepared in low (5%), medium (10%) and high (20%) concentrations. Identical concentrations of FBS (5%, 10%, and 20%) served as positive controls, while DMEM-only (serumfree media or SFM) was used as the negative control. The 5%, 10%, and 20% concentrations were chosen to cover the serum range used in typical cell culture media, which for FBS is generally 10%. All media was supplemented with 1% P/S. Cells were seeded in 75 cm2 flasks using control media, and cells were allowed to adhere for 24 hours. Media was then aspirated and washed with PBS, and cells were exposed to experimental media concentrations for 72 hours at 37ºC and 5% CO2 incubation.

For consistency in data presentation, initial seeding density was consistent across all treatment groups at 1.0×10^4 cells per well in a defined working volume. Cell counts obtained during the assessment were converted to cells per milliliter to

facilitate statistical comparison across treatment conditions. All conversions were performed using standardized volume calculations.

Cell Morphology and Proliferation Assessments

Cell growth and morphology were monitored longitudinally throughout the experimental period. Measurements were collected at 0, 48, and 72 hours to evaluate temporal changes in proliferation and cell behavior. The 72-hour time point represents the predefined experimental endpoint for comparative analysis across treatment groups.

Cell morphology and proliferation were assessed by cell counts and light microscopy over the course of three days. HEK-293 cells were seeded at 1.0x104 cells/well in individual 35mm plates at 2 mL working volume per well, with each treatment and control group performed in quadruplicate (n = 4).

Each plate was imaged at time (t) = 0, 48, 72 hours using an Olympus IX50 inverted microscope at 10x magnification. Cell growth and morphology were examined by observational analysis, and experimental groups were compared to controls. Cell counts were obtained using an Invitrogen Countless 3 Automated Cell Counter (Waltham, MA), following standard operating procedures.

MTT Assay

Cell viability and metabolic activity was examined using the MTT Cell Viability Assay Kit (Biotium, Fremont, CA). The MTT assay utilizes the reduction of 3- (4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) into purple-colored formazan crystals via mitochondrial dehydrogenases, which can then be analyzed colorimetrically [25].

Cells were seeded in 96-well plates at 1.0x104 cells/well, with each group performed in quadruplicate. Cells were allowed to adhere to plates for 24 hours, spent media was then aspirated, and cells were washed with PBS. Cells were then exposed to the different media treatments at a 100 µL working volume per well. After 72 hours of exposure, 10 µL of MTT solution was added to each well, and plates were incubated at 37˚C for 4 hours. Following incubation, 200 µL of Dimethyl Sulfoxide (DMSO) was added into each well to dissolve formazan salts. Absorbance was then measured on a Spectra Max Mini Multi-Mode Microplate Reader (Molecular Devices, San Jose, CA) at 570 nm and 670 nm wavelengths.

Statistical Analysis

Data analysis was performed using the GraphPad Prism 10 software (La Jolla, CA). Average cell counts from t = 0 to t = 72 were graphed separately for each condition to visualize general trends. All experiments were performed in quadruplicate and expressed as the mean ± standard deviation. Data for both the cell counts and MTT was graphed multiple times, with one-way ANOVA and Tukey's post-hoc tests conducted to compare extract groups to one another (FBS, algae, uni) and to the FBS controls. For the MTT assay, percent viability was calculated by normalizing the absorbance of each treatment to the average absorbance of the FBS control. An unpaired t-test was conducted to compare the negative control (serum-free media) to the positive control (10% FBS). Significance was determined by an alpha value of 0.05.

Results

Observations of Sera Preparation

Following dehydration and pulverization, the algae extract appeared cloudy dark green with a sludge-like texture, and visible particulates were incorporated into the mixture. After centrifugation and filtration, all visible particulates were removed, and the extract "algae sera" appeared a transparent amber color. The uni sera appeared a solid opaque yellow with a thin texture after initial homogenization. After centrifugation and filtration, the "uni sera" appeared more transparent and off-white. Both sera became significantly clearer and lighter throughout the extraction process, eventually having reached a comparable transparency and consistency to FBS.

Cell Images and Qualitative Analysis

The Serum Free Media (SFM) treatment showed a sharp decline in cell numbers, with few viable cells remaining after 72 hours (Fig. 3-5). Cells were sparse and displayed minimal clustering and spreading, suggesting poor cell adhesion and proliferation. Most cells appeared dead as they floated on the surface. Cells cultured in FBS showed a marked increase in

cell density after 72 hours. Cells exhibited distinct cell clustering, adherence to the plate surface, and extensive spreading, suggesting formation of a healthy monolayer of cells.

5% Sera Treatments

Cells cultured in 5% algae sera exhibited minimal cell growth, demonstrating marginal spreading and formation of small clusters. Some areas showed more densely populated regions, but overall confluence was much less than seen in FBS treatments. The 5% uni treatment displayed a notable increase in cell density after 72 hours, with cells exhibiting elongated shapes and moderate clustering. Confluency resembled the 5% FBS treatment, though cells appeared sparser and more dispersed (Fig. 3).

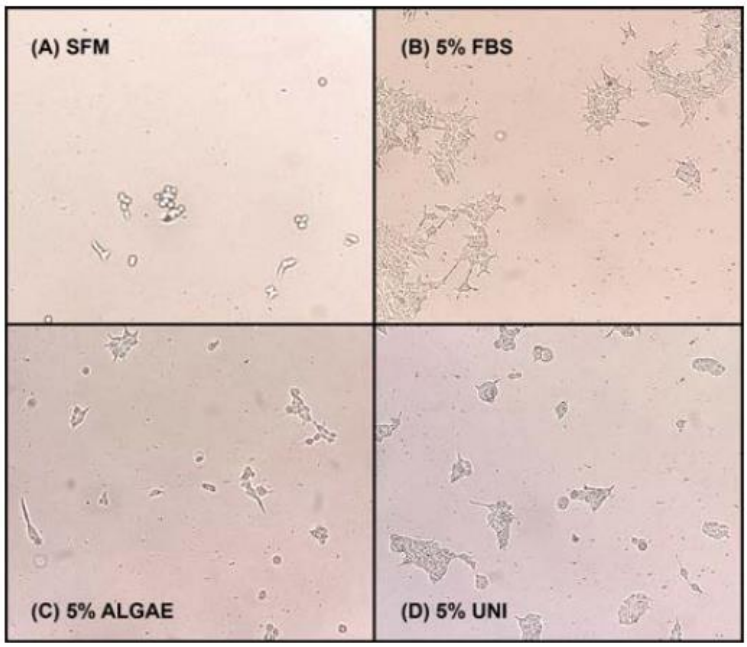

Fig. 3: Light micrographs, captured at 10x magnification, of HEK293 cells cultured in (A) serum-free media (SFM, negative control) or media treated with 5% FBS (B), algae (C), or uni (D) serum concentrations for 72 hours.10%

Sera Treatments

Cells cultured in 10% algae sera exhibited marginal cell growth, demonstrating little to no spreading and sparse clusters. The few clusters that were present appeared stumpy and round, more closely resembling the SFM than the FBS treatment. Cells treated with 10% uni sera displayed a moderate increase in cell density, with cells appearing slightly elongated in shape and in sparse clusters. Confluency was less than cells treated with 10% FBS, and cells appeared more spread out (Fig. 4).

20% Sera Treatments

Cells cultured in both the 20% algae and 20% uni sera exhibited a marked decline in proliferation, demonstrating little to no spreading or clustering, closely resembling the SFM treatment. Dead floating cells could be found in both groups (Fig. 5).

Cell Growth

Cell quantification was conducted in experimental and control groups (Table 1, Fig. 6). The 10% FBS positive control exhibited the highest cell density, with an average of 2.6675x104 cells/mL. The SFM treated cells (negative control) had the lowest cell density, with an average of 7.8575x103 cells/mL. Quantification of cells cultured in the algae sera exhibited an increase in average cell growth with 5% and 10% serum groups but a marked decline observed in the 20% serum group: 5% algae (1.1827x104 cells/mL ±9.10x103), 10% algae (1.415x104 cells/mL ±5.19x103), 20% algae (3.9225x103 cells/mL ±1.51x103). Quantification of cells cultured in uni sera exhibited a marked increase in average cell growth with 5% serum compared to 10% and 20% serum concentrations: 5% uni (2.24875x104 cells/mL ±4.11x103), 10% uni (1.9624x104 cells/mL ±1.01x104), 20% uni (6.563x103 cells/mL ±5.02x103). When compared, each of the treatments (5%, 10%, and 20% of FBS,

uni, and algae) were statistically significantly different from one another ($p = 0.02$). SFM treated cells produced significantly fewer cells than the FBS control, as well as the algae group ($p<0.05$, $p<0.01$). The uni group did not produce significantly different cell counts than the FBS control.

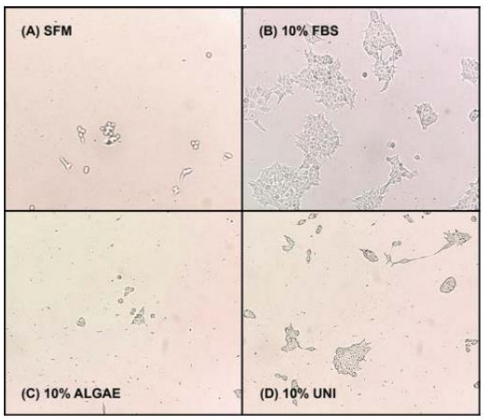

Fig. 4: Light micrographs, captured at 10x magnification, of HEK293 cells cultured in (A) serum-free media (SFM, negative control) or media treated with 10% FBS (B), algae (C), or uni (D) serum concentrations for 72 hours

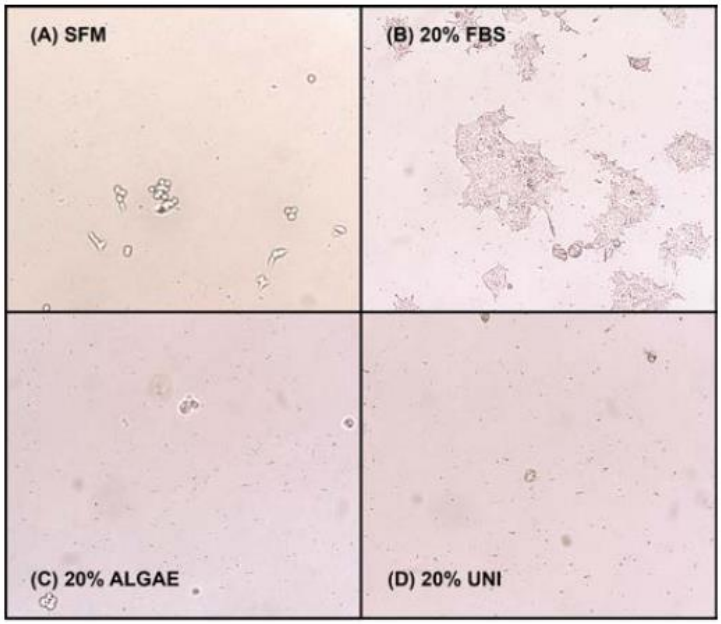

Fig. 5: Light micrographs, captured at 10x magnification, of HEK293 cells cultured in (A) serum-free media (SFM, negative control) or media treated with 20% FBS (B), algae (C), or uni (D) serum concentrations for 72 hours Both the 20% algae treatment and the 20% uni treatment produced significantly fewer cells than the FBS control, exhibiting a decrease in cells from the original seeding ($p<0.01$, $p<0.05$). The 5% and 10% treatments of both extracts did not produce significantly different cell counts than the FBS control

Table 1: Average cell growth in SFM, FBS, Algae, or Uni Treated Groups

	Areroge Cell Viobility (%) ± Standard Deviation			
Extract	0%	5%	10%	20%
Negative Control (SFM)		0.7857		
FBS	2.6675			
Algae		1.1827±0.91	1.415±0.52	0.3923±0.15
Uni		2.2488±0.41	1.9624±1.01	0.65624±0.50

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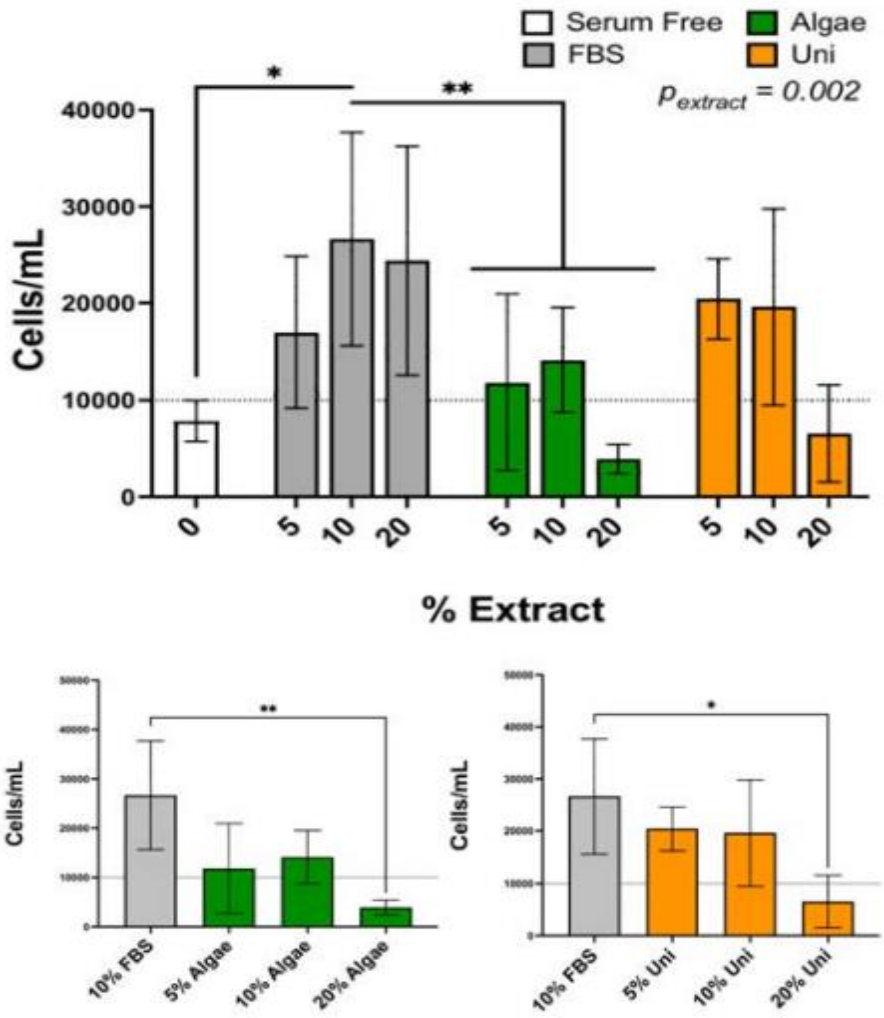

Fig. 6: Comparative Cell Growth of HEK295 Cells Cultured in Algae or Uni Sera. Cell counts (cells/mL) measured after 72 hours of culture in media supplemented with varying concentrations of algae (green) and uni (orange) extracts, compared to 10% FBS (gray) and SFM (white). Dotted line represents initial seeding density (10,000 cells/mL). Graphs depicting an overall comparison between treatment groups(top), comparison of algae groups to 10% FBS (left bottom), and comparison of uni groups to 10% FBS (right bottom) are shown. Significance determined by one-way ANOVA with post-hoc testing; * p≤0.005, ** p≤0.01

These findings suggest that marine extracts may support cell survival at lower sera concentrations as cell growth was observed when compared to cells grown in serum-free media. However, may be toxic at higher sera concentrations, as cell growth declined, parallel to the morphological abnormalities observed.

Cell Viability

Percent viability results for cells after 72 hours are shown in Table 2 and Fig. 7.

Table 2: Average Cell Viability in SFM, FBS, Algae, or Uni Treated Groups

Areroge Cell Viobility (%)± Standard Deviation				
Extract	0%	5%	10%	20%
Negative Control (SFM)	70.6±5.02			
FBS		99.02±2.71	100	86.88±4.39
Algae		60.92±250	47.87±2.04	41.63±4.75
Uni		68.76±3.64	58.39±2.98	50.06±6.46

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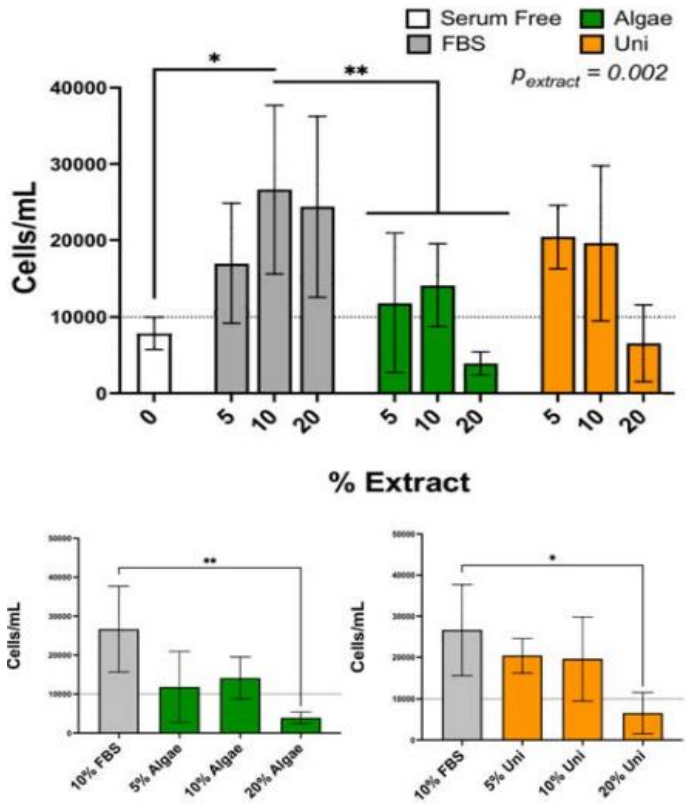

Fig. 7: Comparative Cell Viability of HEK295 Cells Cultured in Algae or Uni Sera for 72 hours. Concentrations of algae (green) and uni (orange) extracts, compared to 10% FBS (gray) and SFM (white) are shown. Graphs depicting an overall comparison between treatment groups (top), comparison of algae groups to 10% FBS (left bottom), and comparison of uni groups to 10% FBS (right bottom) are shown. Significance determined by oneway ANOVA with post-hoc testing; ***p≤0.001

The 10% FBS treatment (positive control) exhibited the highest viability and served as the 100% benchmark. The SFM treatment (negative control) compared to the 10% FBS positive control had a cell viability of 70.6%. Percent viability of the FBS groups exhibited: 5% FBS (99.02%), 10% FBS (100%), 20% FBS (86.88%). The percent viability of the algae groups exhibited: 5% algae (60.92%), 10% algae (47.87%), 20% algae (41.63%). The percent viability of the uni groups exhibited: 5% uni (68.76%), 10% uni (58.39%), 20% uni (50.06%). In general, SFM and experimental groups exhibited a significant decrease in cell viability ($p<0.001$).

These initial MTT results suggest there may be compounds within marine extracts leading to metabolic dysfunction, as cell viability was significantly reduced compared to controls and further shown to decline as sera concentrations of marine extracts increase.

Discussion

The search for ethical and sustainable alternatives to Fetal Bovine Serum (FBS) in cell culture has intensified due to concerns over cost, variability, and animal welfare. In this study, two marine-derived extracts were evaluated for their potential to support mammalian cell proliferation in vitro. The invasive algae Gracilaria salicornia and sea urchin roe were selected from Hawai‘i sources, and the findings demonstrate that both extracts exhibited dosedependent effects on cell morphology, proliferation, and viability, with lower concentrations supporting limited growth and higher concentrations leading to cytotoxic outcomes.

Cell morphology and proliferation analyses using imaging and counting assays revealed that both extracts, particularly at 5% concentration, were capable of partially supporting cell growth. The sea urchin roe extract at 5% concentration (averaged 22,487.5 cell/mL) showed the most promising results, achieving 84.3% of the cell density observed in the 10% FBS control

(averaged 26,675 cells/mL) after 72 hours. These findings suggest that certain components in the marine extracts may provide nutrients or signaling molecules essential for cell survival and division. However, at 10% and 20% concentrations, cell viability declined sharply, accompanied by visible morphological abnormalities, indicating the presence of cytotoxic compounds or metabolic imbalances introduced by the crude extracts.

These results partially support the initial hypothesis that marine-based supplements could serve as viable alternatives to FBS under optimized conditions. Although neither extract matched the proliferative effect of 10% FBS, the observed growth at lower concentrations underscores their potential utility as partial or supplemental serum replacements. Further optimization through dilution, purification, or fractionation could enhance their bioactivity while minimizing toxic effects. Follow-up studies should focus on lower concentration gradients, e.g., 0.5–4%, to identify minimal effective doses with acceptable cytocompatibility. Extract concentrations of 5%, 10%, and 20% were evaluated to reflect commonly used serum ranges in mammalian cell culture. Lower concentration ranges below 5% were not assessed in this initial investigation but may improve cytocompatibility and metabolic performance.

Comparison with previous literature reinforces the plausibility of these findings. Studies utilizing marine algae such as Chlorella vulgaris and Ulva lactuca have demonstrated similar dose-dependent trends, where low concentrations promote proliferation and high concentrations exhibit toxicity [17, 21]. This study's findings for G. salicornia are consistent with these trends. Moreover, the inclusion of sea urchin roe extract is novel. To the best of the researchers' knowledge, this is the first documented attempt to evaluate its use in mammalian cell culture. Its relatively strong performance at 5% concentration highlights the potential of marine invertebrate biomaterials as untapped sources of bioactive compounds for regenerative and in vitro applications [16, 18].

While cell proliferation and morphology appeared promising at low concentrations, mitochondrial activity, measured via MTT assay, was significantly reduced across all treatment groups. This disconnect suggests that, although the extracts can sustain some degree of proliferative capacity for the HEK293 cells, they do not fully support their metabolic functionality or mitochondrial health. Potential explanations include the absence of essential growth factors, presence of metabolic inhibitors, or oxidative stress induced by extract constituents. Comprehensive biochemical analyses, including compositional profiling of proteins, lipids, and carbohydrates, as well as targeted metabolomic studies, are necessary to identify both beneficial and deleterious components within the extracts. The reduced metabolic activity found in the treatment groups represents incomplete metabolic support rather than the cytotoxicity of the extracts. These findings reinforce that the tested marine-derived formulations are preliminary or partial supplements rather than direct replacements for fetal bovine serum based on the current experimental conditions.

Experimental variables in this study, such as seedingdensity, cell passage number, and prior acclimation to fetal bovine serum, may have influenced observed viability outcomes. Although these parameters were standardized across treatment groups using established laboratory protocols, increased optimization of seeding density and conditions will be critical to improve the performance in the next stages.

Future studies should employ more stringent decontamination protocols and refined extraction techniques, such as extended centrifugation or chromatographic separation, to improve reproducibility and purity. It may be possible the algae collection process had introduced biological contaminants, such as sponges, tunicates, and polychaetes, which in turn may have altered the chemical profile of the final extract. Additionally, the low initial cell seeding density, chosen to prevent confluency, may have affected growth dynamics due to reduced intercellular signaling. Increasing seeding density in future experiments could help to clarify extract performance under more physiologically relevant conditions.

Heat sterilization by autoclaving was implemented to ensure sterility of base biological extracts during the initial feasibility testing. However, exposure to elevated temperatures may reduce the activity of thermolabile proteins or growth factors present in the extracts. Future studies will evaluate alternative sterilization approaches, such as sterile filtration without heat treatment. The purpose is to preserve bioactive components more effectively and optimize functional performance.

This study was conducted as an initial investigation to evaluate whether marine-derived extracts can support mammalian cell growth under controlled laboratory conditions. All treatment conditions were performed in quadruplicates using a single standardized extract batch to maintain consistency across experimental groups. While this design enabled controlled comparison among treatments, additional biological replicates, repeated extraction batches, and independent experimental runs are necessary to establish reproducibility and thoroughness of reported findings. Experimental validation and statistical reliability will be strengthened by incorporating these works.

Although cell morphology and counts were collected at multiple time points during the experiment, full growth curve visualization and expanded temporal sampling were not included in this initial study design. Future studies will incorporate more time points and additional experimental cycles.

Conclusion

The sea urchin roe extract, in particular, offered a viable promise as a supplement. Its local availability in Hawai'i, ethical sourcing, and low cost make it a compelling candidate for further development. Though the G. salicornia extract showed modest results, its partial efficacy at low concentrations suggests value in pursuing improved formulations. Given that this species is an invasive threat to Hawaiian ecosystems, the development of a commercially viable extract could support both research and environmental management goals. Its rampant growth makes it suitable for mass aquaculture and, if chosen as an industry standard, would not affect local ecosystems.

This study presents preliminary evidence that marinederived extracts, particularly sea urchin roe, may serve as supplemental alternatives to FBS in mammalian cell culture. While neither extract achieved full equivalence to FBS, their capacity to support proliferation at optimized concentrations justifies further investigation. Continued refinement, compositional analysis, and bioactivity screening of these marine resources could contribute to the development of more ethical, sustainable, and costeffective cell culture supplements.

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Author's Contributions

Kian Sanchez: Conducted the experimental testing and data collection and prepared the first draft of the manuscript.
Jennifer Seki-Wong, Kayla Hallam, and Lucia Seale: Provided laboratory support and contributed to manuscript review.
Mark Oleksak and Courtney Chang: Contributed to manuscript preparation and editing. All authors read and approved the final manuscript.

Ethics

The authors hereby declare that there is no conflict of interest regarding the publication of this research paper. All authors have approved the final manuscript and have no financial, personal, or professional affiliations that could be perceived to influence the investigation presented in this paper.

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