

Chemical profile of extracts from wild and farmed *Chondrosia reniformis* sponges

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Abstract: Aquaculture is gaining attention as a sustainable method to meet the growing demand for marine-derived products, with the marine sponge *Chondrosia reniformis* emerging as a promising candidate due to its biochemical properties. Rich in collagen, fatty acids and bioactive metabolites, *C. reniformis* holds significant potential for applications in biomedicine, cosmetics, and pharmaceuticals. Cultivating this sponge through aquaculture offers a sustainable alternative to wild harvesting, ensuring a steady supply while alleviating ecological pressure. This study compares the chemical profiles of wild and farmed *C. reniformis* using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Farmed samples exhibited greater chemical consistency and a more diverse fatty acid profile, including unsaturated fatty acids like oleic and vaccenic acid, which are associated with nutritional benefits. In contrast, wild specimens showed higher chemical variability, likely driven by micro-spatial fluctuations in environmental and dietary factors. Bioactive metabolites such as (+)-paupephenone were present in both groups but in low concentrations, limiting their antimicrobial and anticancer activity in assays. Our findings highlight metabolic differences between wild and farmed sponges, possibly shaped by the controlled aquaculture conditions, suggesting that sponge mariculture not only ensures sustainable production but also allows for optimization of metabolite yields. Moreover, the bioremediation capacity of *C. reniformis* enhances aquaculture's environmental sustainability by reducing organic pollutants in surrounding waters. This research underlines the potential of *C. reniformis* as a model organism for integrated aquaculture systems, offering dual benefits of ecological restoration and production of high-value biomolecules.

Keywords: Porifera; Demospongiae; marine sponge farming; chemical fingerprinting; marine natural products; secondary metabolites; primary metabolites; LC-MS/MS dereplication; aquaculture; blue biotechnology; biodiversity sustainability, circular economy

1. Introduction

Aquaculture is increasingly being recognized as a sustainable approach to meet the rising global demand for marine-derived products, with a particular focus on species that offer both economic and ecological benefits. Marine sponges farming has gained considerable interest due to the vast array of natural products these organisms produce. Sponges and their microbial symbionts are a rich source of structurally diverse metabolites with potent bioactive properties, currently accounting for nearly half of all discovered marine-derived drugs [1-5]. Besides their bioproduction potential for maintaining a sustainable supply, the cultivation of marine sponges is also regarded as a bioremediation strategy for alleviating environmental pressures associated with fish aquaculture. Given the steady expansion of the aquaculture sector [6], there is a growing need for sustainable solutions to mitigate the substantial quantities of organic matter and

nutrients released from fish farms [7]. Owing to their natural ability to uptake bacterial [8-13] or algal cells [14-17], dissolved organic [18-20] and inorganic pollutants [21,22], as well as aquaculture effluents [23-25], marine sponges emerge as promising candidates for integration into multitrophic aquaculture systems [26-29].

The marine sponge *Chondrosia reniformis* (Nardo, 1847, Demospongiae, Chondrosiida, Chondrosiidae), stands out for its unique biochemical properties and the broad industrial potential. This collagen-rich sponge with its dense extracellular matrix, is a promising candidate for biomedical, pharmaceutical, and cosmetic applications [30,31]. The cultivation of *C. reniformis* in aquaculture settings offers a sustainable means for harnessing its valuable metabolites. Previous studies have demonstrated the successful growth of this sponge in integrated mariculture systems, particularly near fish farms, where it has shown high survival and growth rates [32,33]. Additionally, our research group has reported its adaptability to eutrophic conditions and aquaculture-related pollutants with notable bioremediation capabilities [17,25]. These findings indicate the potential of *C. reniformis* for commercial-scale cultivation, which could help alleviate fish aquaculture pollution and the pressures on wild populations, while ensuring a consistent supply of collagen. *C. reniformis* remains largely unexplored in terms of secondary metabolite production. Nevertheless, its repertoire of natural products includes the marine sesquiterpene quinone (+)-puupehenone, which is known for its antituberculosis, antibacterial, antifungal, and cytotoxic properties [34-37]. Additionally, its primary metabolites further highlight its potential for bioproduction, as specific fatty acids contribute to sponge's structural integrity and bioactivity [38,39].

This study aimed to showcase the cultivation prospective of *C. reniformis* for the sustainable production of metabolites and to determine whether farmed sponges differ chemically from their wild counterparts. To achieve this, we constructed a comprehensive library of *Chondrosia* metabolites, precursor ions, and MS fragments. The chemical composition of both farmed and wild sponge specimens was then analyzed and compared using high-resolution analytical techniques, specifically liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The biological activity of the sponge-derived extracts was also examined through antimicrobial and anticancer assays.

2. Results and Discussion

Targeted LC-MS/MS analysis of farmed and wild *C. reniformis* revealed a predominance of fatty acids. These presented a diverse range of fatty acids subclasses, including saturated fatty acids such as myristic acid and pentadecanoic acid, branched-chain and methylated acids like 13-methyltetradecanoic acid, and unsaturated fatty acids such as oleic acid (omega-9) and vaccenic acid (omega-7). The farmed specimens displayed a fatty acid profile closely matching that of the wild specimens (Figure 1).

2-Hydroxydocosanoic acid, arachidic acid, 2-hydroxytetracosanoic acid, oleic acid, and 15-methyl-9-hexadecenoic acid were the dominant fatty acids across all samples, contributing significantly (70.86 to 64.93%; 13.85 to 4.46%; 8.02 to 4.30%; 6.12 to 3.68%; and 1.77 to 1.16%, respectively) to the overall fatty acid profile.

Fatty acids are a vital class of metabolites with diverse biotechnological applications across the pharmaceutical, cosmetic, food, feed, and energy industries [40,41]. In particular, 2-hydroxydocosanoic acid, exhibits antioxidant activity, with potential biotechnological applications in dermatological formulations [42]. 2-Hydroxytetracosanoic acid has identified as fatty acid residue in ceramides demonstrating potential antimalarial activity [43,44]. Arachidic acid has been reported to possess antimicrobial properties [45]. Oleic acid is widely used in the food industry for its health benefits, as well as in pharmaceuticals and cosmetics. For instance, it functions as a pancreatic lipase inhibitor, offering prospects in weight management, and as a bone induction agent [46,47]. Additionally, methoxylated lipids have demonstrated antibacterial, antifungal, antitumor, and antiviral [48] Nevertheless, 15-methyl-9-hexadecenoic bioactivity has not been reported.

The sesquiterpene quinone (+)-puupehenone, an antituberculosis, antibacterial, antifungal, and cytotoxic agent originally isolated from *Chondrosia chucalla* marine sponges, was also identified in the *C. reniformis* samples [34,49]. The LC-MS/MS peak intensity of the major fatty acids, along with puupehenone, was intently compared between the wild

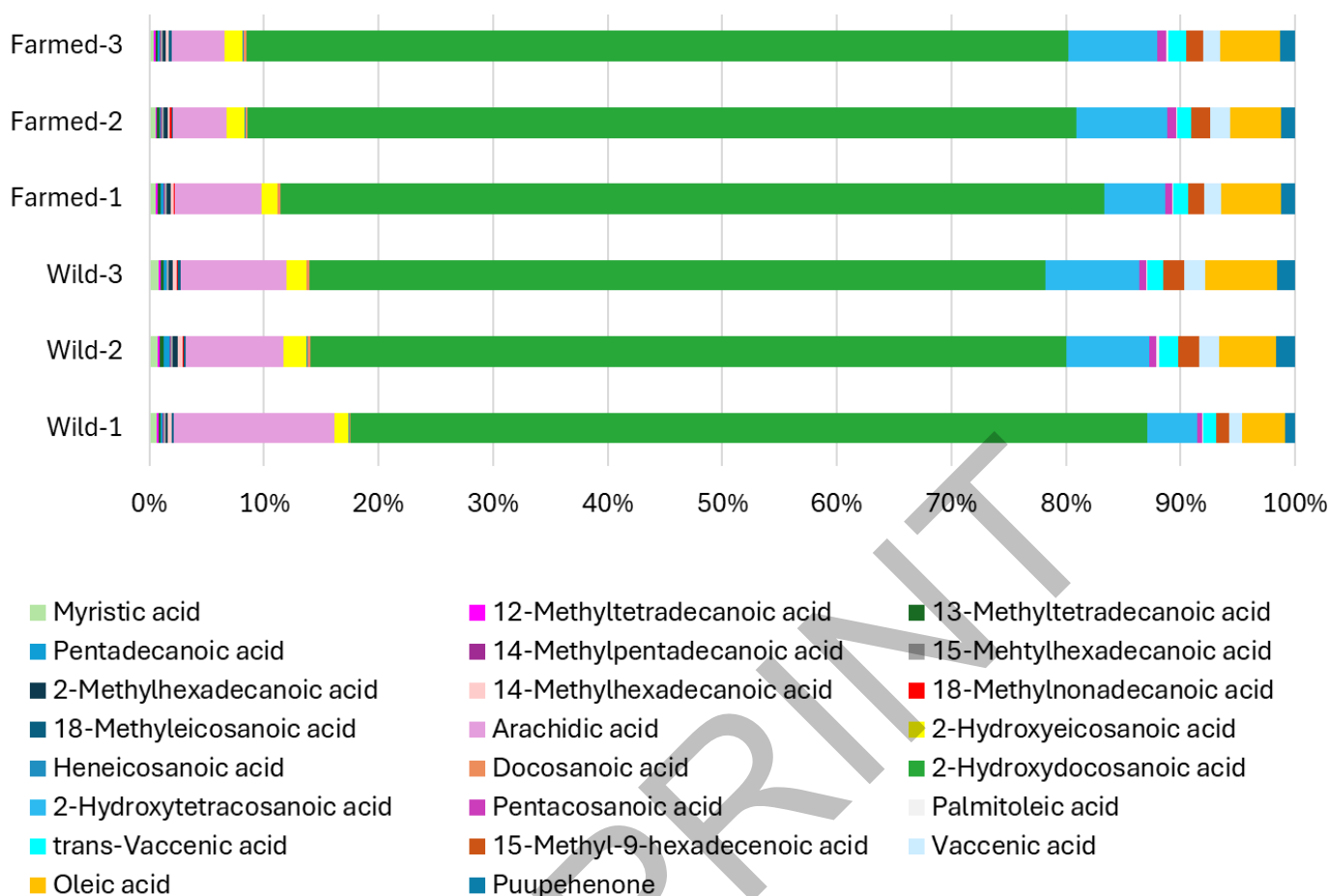


Figure 1. Fatty acids and sesquiterpene composition of farmed and wild *Chondrosia reniformis* sponges.

and farmed groups (Figure 2). In the farmed group, 2-hydroxydocosanoic acid and 2-hydroxytetraacosanoic acid exhibited a higher median intensity, whereas arachidic acid, 15-methyl-9-hexadecenoic acid, and puupehenone showed a higher median intensity in the wild group. Oleic acid median intensity was similar in both groups.

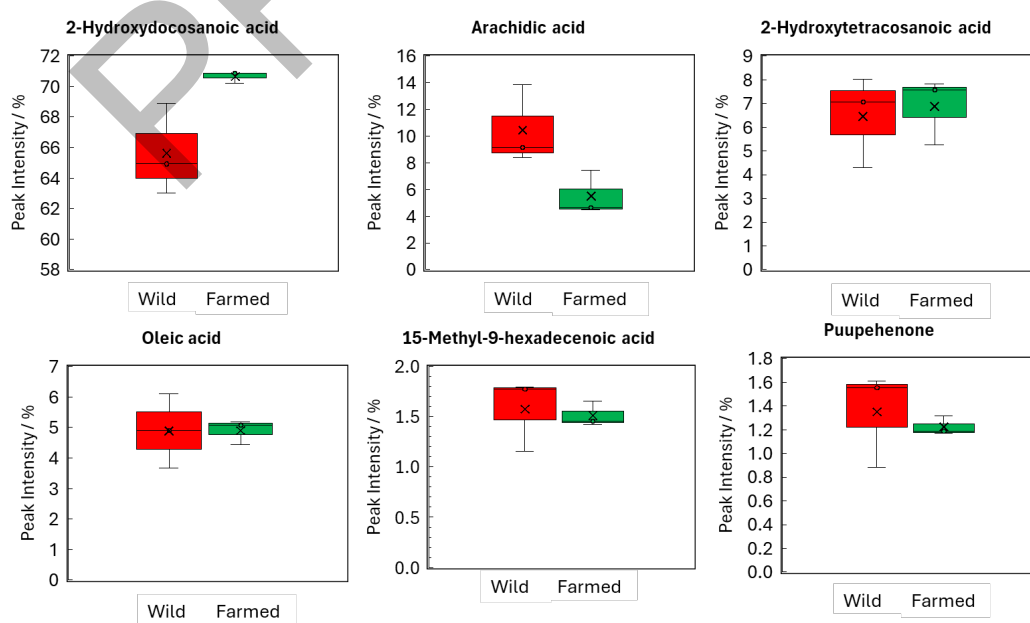


Figure 2. Boxplot of relative abundances of dominant metabolites across wild and farmed *C. reniformis* sponges.

Overall, farmed specimens demonstrated lower variability across all metabolites compared to the wild group, suggesting more consistent metabolites concentrations. Wild specimens displayed greater variability and higher peak intensities across most fatty acids, exceptions include 14-methylpentadecanoic acid, 15-methylhexadecanoic acid, 18-methylnonadecanoic acid, docosanoic acid, pentacosanoic acid, and vaccenic acid, as well as previously mentioned 2-hydroxydocosanoic acid and 2-hydroxytetracosanoic acid, which exhibited higher median intensities in the farmed group, Figure S1. Additionally, 18-methyleicosanoic acid, 18-methylhexadecanoic acid, and 12-methyltetradecanoic acid are the only primary metabolites that exhibited higher variability in the farmed group.

The observed differences in fatty acids composition may result from variations in the dietary sources and environmental conditions influencing wild populations, while the controlled conditions of farmed groups contribute to more uniform fatty acid profiles. It is worth stressing that farmed sponges should primarily feed on organic matter derived from fish excretions and uneaten fish feed, which likely differs significantly from the natural diet of wild sponges.

Statistical analysis using t-tests was conducted to identify significant features in the dataset and determine statistically relevant compounds. Several fatty acids were detected, including myristic acid, arachidic acid, 2-hydroxydocosanoic acid, 14-methylhexadecanoic acid, and 12-methyltetradecanoic acid. Among these, myristic acid demonstrated the highest t-statistic (3.3106) and the lowest p-value (0.029637), making it the most differentially abundant feature in the dataset. While 2-hydroxydocosanoic acid and arachidic acid also showed potential importance, their statistical significance is comparatively weaker. For the remaining compounds, the p-value threshold of 0.1 suggests moderate significance, but none meet the conventional $p < 0.05$ level. This suggests that the design of future experiments should include larger sample sizes.

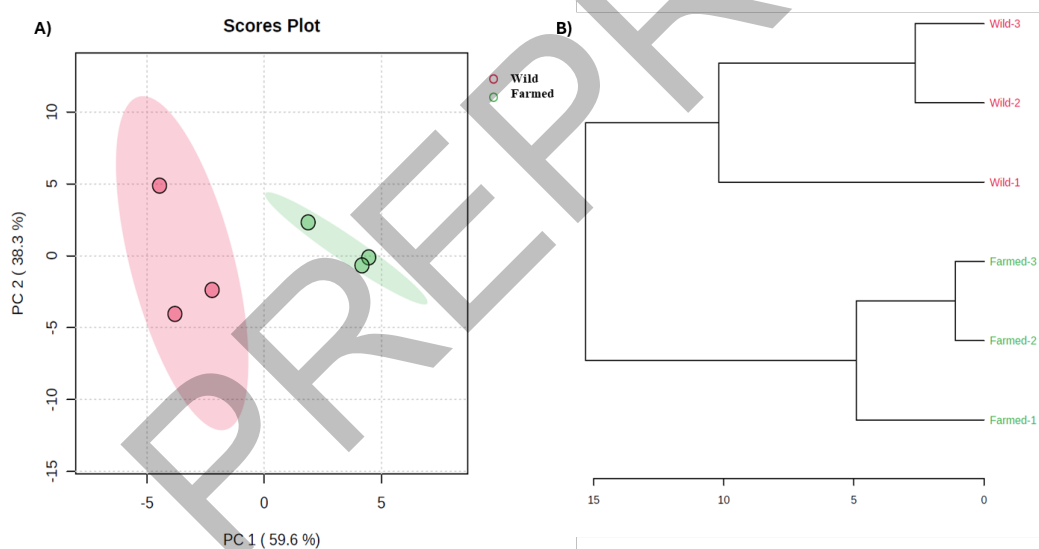


Figure 3. Comparison of wild and farmed sponges. A) Principal Component Analysis (PCA) and B) Hierarchical clustering analysis.

Principal Component Analysis (PCA) clustering pattern showed that wild and farmed samples are distinguishable based on their metabolic profiles, Figure 3 A. PCA explains 97.9% of the variability in the dataset, reducing the data to two principal components, PC1 and PC2, which describe 59.6% and 38.3% of the variance, respectively. The resulting plot reveals two distinct clusters corresponding to wild and farmed sponges, with confidence ellipses indicating variance within each group. The cluster of wild sponges shows greater spread along both axes, suggesting higher compositional variability, while the cluster of farmed ones appears more compact, indicating lower variation, corroborating the previous box plot analysis. The hierarchical clustering analysis (Figure 3 B) further distinguishes wild and farmed groups, revealing a strong group-specific structure in the dataset. The hierarchical clustering dendrogram classifies samples into two distinct clusters: Cluster 1 (Wild-1, Wild-2, Wild-3) and Cluster 2 (Farmed-1, Farmed-2, Farmed-3), highlighting differences between them.

PCA and hierarchical clustering suggest that the primary sources of variance in the data clearly separate wild and farmed into only two groups. However, K-means clustering divides the samples into three groups: Cluster 1 (Wild-1), Cluster 2 (Wild-2 and Wild-3), and Cluster 3 (Farmed-1, Farmed-2, and Farmed-3), indicating that Wild-1 is more distinct from Wild-2 and Wild-3, while all farmed samples cluster together. In contrast, Self-Organizing Map (SOM) clustering groups the samples differently, with Cluster 1 comprising of Wild-1, Cluster 2 including Wild-2, Wild-3 and Farmed-1, and Cluster 3 consisting of Farmed-2 and Farmed-3. This suggests that Farmed-1 shares some features with wild samples, making it less distinguishable from them, whereas Farmed-2 and Farmed-3 form a separate cluster. Altogether, our results demonstrate the successful metabolites production of farmed *C. reniformis* marine sponges and similarities of the identified metabolites with wild specimens, advocating for their inclusion in integrated aquaculture systems.

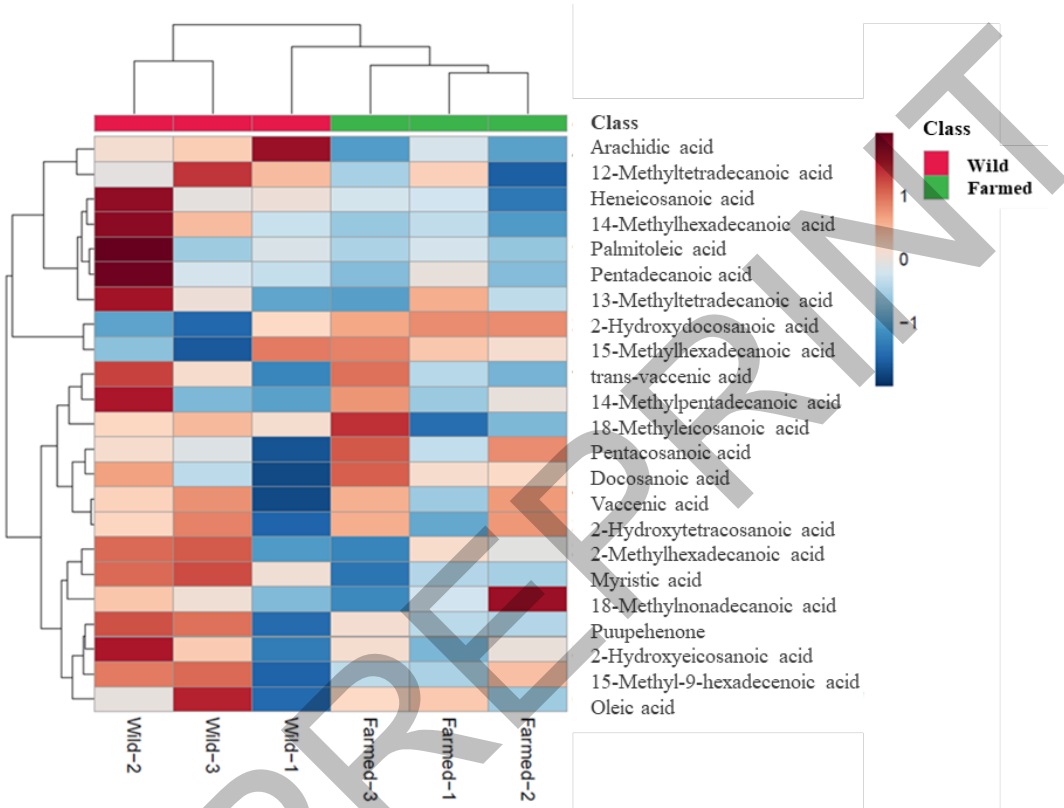


Figure 4. Heatmap representation of wild and farmed *C. reniformis* sponges. The color scale, from blue (low) to red (high), represents standardized expression levels.

The metabolic signatures of wild and farmed groups were further illustrated using heatmap analysis, Figure 4. Metabolites contribute to two classes differentiation and may serve as potential biomarkers for distinguishing wild and farmed populations. Clustering of metabolites reveals distinct chemical patterns, with compounds like 2-hydroxydocosanoic acid, 15-methylhexadecanoic acid and docosanoic acid showing higher expression in the farmed group, while arachidic acid, 12-methyltetradecanoic acid, 18-methylpentadecanoic acid, and myristic acid were more associated with the wild group (Figure 4).

Additionally, secondary metabolite jaspisamide, a cytotoxic diterpene lactone previously reported in *Jaspis* sp. and *Chondrosia corticata* sponges, was detected in one of the wild samples [50,51]. Our analysis also identified a family of compounds with an m/z range of 800-900 Da present in all samples, consistent with the molecular weights of macrolide jaspisamide and halidondramide class. This family includes triazole-containing macrolides compounds, previously reported from *C. corticata*, known for their cytotoxicity and antifungal properties[51-53]. Further isolation and

identification of these compounds are warranted to fully explore the biotechnological potential of *C. reniformis* sponges for drug discovery and aquaculture applications.

2.2 Antimicrobial and anticancer activities evaluation

We examined the antimicrobial and anticancer activities of extracts from wild and farmed *C. reniformis* sponges. Antimicrobial tests were performed against two human pathogens: methicillin-resistant *Staphylococcus aureus* (MRSA, COL) as a representative Gram-positive bacterium, and *Escherichia coli* (ATCC 25922) as a representative Gram-negative bacterium. Additionally, the anticancer effects and general toxicities of the extracts were evaluated using the human colorectal carcinoma cell line HCT-116 (ECACC 91091005), Figure S2. None of the extracts demonstrated antimicrobial or anticancer activity, likely due to the predominance of fatty acids in the extracts, which have no reported activity against the tested bacteria or cell line [38,39]. Furthermore, metabolites with known biological activity, such as puerpehnone and jaspisamide, were detected in lower concentrations when compared to fatty acids [34,54].

3. Materials and Methods

3.1 Sponge samples

In June 2020, individuals of *C. reniformis* collected from natural habitats were introduced into an integrated aquaculture system. They were transferred live and affixed to PVC discs attached to vertical hanging ropes. Those rearing arrays were positioned in close proximity (5-10 m) to the fish cages of an active fish farm in Souda Bay, northwest Crete, Aegean Sea (35.4801/24.1117), at a depth of 7 to 10 meters. Reared sponges' individuals were derived from biomass cuttings (explants) taken from a nearby wild population in Souda Bay (35.4783/24.1091). Over a continuous period of 19 months, the sponges remained in open-sea cultivation, exhibiting minimal mortality while demonstrating regeneration and growth. In February 2022, tissue samples were collected from three replicate individuals of both wild and farmed populations, totalling 6 samples, following the methodology of Varamogianni-Mamatsi et al. (2022) [17]. The wild specimens were sampled from their respective original collection sites used for the initial experimental seeding. Sampling in all cases was performed selectively by divers, ensuring that only excess biomass was taken, thereby allowing the donor sponges to regenerate. In both farmed and wild populations, samples were excised underwater from the parent sponge using a razor blade and placed in individually labelled sterile bags. They were subsequently preserved in cooler boxes with ice packs and transported to the Hellenic Centre for Marine Research within three hours, where they were stored at -20 °C until further analysis.

3.2 Sponge extracts

Freeze-dried samples from both wild and farmed sponges were ground into a fine powder (1 to 4 g per sample) using a standard mixer, according to a previously described method [55]. The resulting powder underwent three rounds of extraction in an ice-cold sonication bath for 15 minutes per round, utilizing a solvent mixture of methanol and dichloromethane (20 mL of a 1:1 v/v ratio per gram of sponge). Following each extraction, the sponge suspensions were centrifuged at 8000× g for 7 minutes at 20 °C. The supernatants collected were filtered through paper and dried using a centrifugal vacuum evaporator (EZ-2 Plus; Genevac, United Kingdom). The dried extracts were subsequently dissolved in 4 mL of the same solvent mixture (MeOH:DCM 1:1), transferred to 50 mL Falcon tubes, and mixed with 16 mL of acetonitrile. To precipitate proteins, the samples were kept at -20 °C overnight. Afterwards, they were centrifuged at 10,000× g for 10 minutes at 4 °C, and the resulting supernatants were evaporated to dryness. To remove neutral lipids, each sample was loaded onto a glass column (8 mm inner diameter) packed with 1.5 g of silica gel (silica gel 60, particle size: 0.060–0.200 mm, Merck), which had been activated at 300 °C for 3 hours. Elution was carried out sequentially with

20 mL of 2% ethyl acetate in hexane, followed by 15 mL of ethyl acetate and 15 mL of methanol. The latter two fractions were pooled, evaporated to dryness, and stored at +4 °C until further analysis.

3.3. Antibacterial Assays

The antibacterial potential of the wild and farmed *C. reniformis* crude sponge extracts was assessed through growth inhibition assays targeting two bacterial strains representing Gram-positive and Gram-negative human opportunistic pathogens. The strains tested included the methicillin-resistant *Staphylococcus aureus* COL (MRSA) and the *Escherichia coli* ATCC 25922 strain. The methodology employed was adapted from Pinto-Almeida et al. (2022) [56]. Specifically, *S. aureus* cultures were maintained in tryptic soy broth (TSB; Becton Dickinson, Germany), while *E. coli* was grown in Lysogeny broth (NZYtech), both incubated at 37 °C. The assays were conducted using 96-well polystyrene flat-bottom microplates. Bacterial overnight cultures were diluted to an optical density (OD_{600nm}) of 0.005 and exposed to various concentrations of the crude extracts, which were dissolved in DMSO (1% w/v). The cultures underwent two-fold serial dilution, yielding extract concentrations ranging from 250 to 0.4935 µg/mL. Following a 24-hour incubation at 37 °C, the minimal inhibitory concentration (MIC) was determined through visual examination. MIC is defined as the lowest concentration of an antimicrobial agent required to prevent visible bacterial growth after overnight incubation [57]. The MIC values obtained were compared against a positive control (vancomycin for MRSA and tetracycline for *E. coli*), a DMSO solvent control, and a negative control consisting of inoculated medium without any extract added, all within the same experimental plate.

3.4. Anticancer Assays

Crude extracts from wild and farmed populations of *C. reniformis* were evaluated in vitro for their anticancer activity against the human colorectal carcinoma cell line HCT-116 (ECACC 91091005, Porton Down, UK), following protocols described by Florindo et al. (2016) [58] and Prieto-Davó et al. (2016) [59]. The HCT-116 cells were maintained in McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic/antimycotic solution (Gibco, Thermo Fisher Scientific, Paisley, UK), under a humidified atmosphere of 5% CO₂ at 37 °C. For the cell viability assays, HCT-116 cells were seeded in 96-well plates at a density of 0.5×10^4 cells per well. Following a 24-hour incubation period, the cells were treated with different concentrations of sponge extracts (ranging from 0.30 to 125 µg/mL), DMSO (vehicle control), or 10 µM 5-fluorouracil (5-Fu, positive control) for 72 hours. Cell viability was determined by MTS metabolism using the CellTiter 96® Aqueous Non-Radioactive Cell Proliferation Assay (Promega, Fitchburg, WI, USA), in accordance with the manufacturer's guidelines. Absorbance was measured at 490 nm using a Glomax®-MultiDetection System (Promega). Results were presented as mean ± standard error of the mean (SEM) from at least three independent experiments. Data analysis was conducted with GraphPad Prism 8.4.2 software (La Jolla, CA, USA), and dose-response curves were plotted to determine IC₅₀ values using the log-(inhibitor) vs. response-variable slope (four parameters) model.

3.5 LC-MS/MS Analysis

3.5.1. Chemicals and Reagents

Ultra-High-Performance Liquid Chromatography-Mass Spectrometry (UHPLC-MS) grade analytical solvents, including methanol and acetonitrile, as well as LC-MS grade formic acid, were sourced from Carlo Erba® Reagents S.A.S (Le Vaudreuil, France). Ultrapure water was obtained from a Milli-Q® ultrapure water system, which features a Milli-Q® Reference and a Q-POD® element.

3.5.2. LC-MS/MS equipment

LC–MS/MS analysis was conducted using a Dionex® Ultimate 3000 System (UHPLC, Thermo Scientific, Germany) coupled with a TSQ Quantis™ triple-stage quadrupole mass spectrometer (Thermo Scientific, Waltham, MA, USA). The UHPLC system included four key modules: an SR–3000 Solvent Rack with an LPG–3400RS pump, a WPS–3000TRS auto sampler with temperature control, and a TCC–3000RS column compartment. The tandem mass spectrometer utilized an electrospray ionization (ESI) source. Operation and data acquisition were managed through the XCalibur™ 4.2SP1 Thermo Scientific SP1 (0388–00CD–7B33, USA) software.

3.5.3. Sample Preparation for LC–MS/MS Analysis

To prevent contamination, all plastic materials and glassware were thoroughly cleaned. Prior to use, organic solvents (LC–MS grade) and distilled water were analyzed to reduce background interference and avoid contaminations. Before injection into the LC–MS/MS system, the extracts were dissolved in 1 mL of methanol, filtered using a 13-mm, 0.22- μ m nylon syringe filter (Filter-Lab®, Sant Pere de Riudebitlles, Spain) with a 500 μ L syringe (Gastight 1750 Hamilton®, Vernon Hills, IL, USA), and diluted tenfold with methanol. The prepared liquid extract was transferred through a syringe filter into a conical insert within a sterile 2-mL vial (9–425 C0000752) equipped with a screw cap and red PTFE/white silicone septa (Alwsci® Technologies, Shaoxing, China) [55].

3.5.4. Chromatographic and Mass Spectrometry Conditions

Compound separation was performed using an Accurore™ RP–MS Column (2.6 μ m, 150 $\times$ 2.1 mm, Thermo Fisher Scientific) with a sample injection volume of 10 μ L. The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile (B). The gradient elution was set as follows: re-equilibration at 5% B for 1 minute, 0.0–15.0 minutes at 5–50% B, 15.0–20.0 minutes at 50–99% B, 20.0–29.0 minutes at 99% B, and 29.0–30.0 minutes at 99–5% B. A flow rate of 0.200 mL/min was maintained throughout the process. Mass spectrometry (MS) analysis was performed using the triple-stage quadrupole mass spectrometer with the following electrospray ionization (ESI) parameters: a spray voltage of +3500/–3000 V, sheath gas flow of 50 L/min, auxiliary gas flow of 10 L/min, sweep gas flow of 0 L/min, ion transfer tube temperature of 320 °C, and vaporizer temperature of 30 °C. The cycle time was established at 0.5 seconds, with a calibrated radio frequency (RF) lens and collision-induced dissociation (CID) gas pressure of 1.5 mTorr. Collision energy values of 10, 20, and 40 V were tested. The samples were injected in selective reaction monitoring (SRM) mode using multiple reaction monitoring (MRM), with targeted analyte transitions detailed in Tables S1, provided in the supplementary materials along with fragmentation parameter settings. A comprehensive literature review was conducted for each studied sponge species to identify previously reported compounds and their respective mass ions, adducts, and fragments. Detected metabolites were identified and classified by cross-referencing with literature sources and public databases such as DrugBank, FoodB, GNPS, HMDB, MoNA, Metabolomics Workbench, and PubChem.

3.6. Statistical Analysis

The peak intensity data generated from the LC–MS/MS analysis were utilized to assess qualitatively the content of each identified metabolite in the extracts. Statistical data analysis was performed with MetabolAnalyst 6.0 software [60] to evaluate similarities and differences in the chemical composition of the extracts derived from wild and farmed sponges populations. Performed tests included t-tests, hierarchical clustering, k-means clustering and self-organizing map (SOM). Principal component analysis (PCA) and heatmap generation were also performed using the same software.

4. Conclusion

This study highlights the novelty, biotechnological importance, and sustainability of cultivating *Chondrosia reniformis* sponges as components of integrated aquaculture systems. By comparing the chemical profiles and biological

activities of wild and farmed sponge extracts, we have demonstrated that aquaculture can provide a consistent and sustainable source of *C. reniformis* biomass while reducing the environmental pressure on wild populations. Our findings reveal significant differences in the metabolic profiles of wild and farmed sponges, with farmed samples exhibiting a broader diversity of fatty acids and more consistent concentrations of key metabolites. These characteristics underscore the adaptability of *C. reniformis* to aquaculture conditions and its potential to produce high-value compounds in controlled settings.

From a biotechnological perspective, *C. reniformis* offers substantial potential for applications in the pharmaceutical, biomedical, and cosmetic industries. While the extracts tested in this study did not exhibit antimicrobial or anticancer activities, the presence of bioactive compounds such as puerpene, jaspisamide and potential halicondramide derivatives, even in low concentrations, underscores the species' potential for drug discovery. Future efforts should focus on optimizing cultivation practices to enhance the production of these valuable secondary metabolites, as well as exploring novel extraction and isolation techniques to maximize yield.

The integration of sponge aquaculture with fish farming presents a compelling model of circular bioeconomy. By leveraging the natural filter-feeding properties of *C. reniformis*, aquaculture operations can simultaneously reduce organic pollution and generate economically valuable biomass. This dual benefit exemplifies a sustainable approach to marine resource utilization, aligning with global efforts to promote ecological balance and economic resilience.

The successful cultivation of *C. reniformis* not only opens new avenues for biotechnological innovation but also reinforces the role of aquaculture as a key driver of sustainability and circular bioeconomy in the marine sector. Future research should aim to scale up sponge farming systems, improve metabolite production, and explore further applications of this remarkable species in diverse industries.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, **Table S1.** Selected compounds for *Chondrosia reniformis* targeted SRM detection by tandem mass spectrometry, **Figure S1.** Minor fatty acids composition of farmed and wild *C. reniformis* sponges, and **Figure S2.** HCT-116 antiproliferation results for wild and farmed sponges *C. reniformis*.

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Data Availability Statement: The original data presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Jimenez, P.C.; Wilke, D.V.; Branco, P.C.; Bauermeister, A.; Rezende-Teixeira, P.; Gaudencio, S.P.; Costa-Lotufo, L.V. Enriching cancer pharmacology with drugs of marine origin. *British journal of pharmacology* **2019**, doi:10.1111/bph.14876.
2. Barreca, M.; Spane, V.; Montalbano, A.; Cueto, M.; Marrero, A.R.D.; Deniz, I.; Erdogan, A.; Bilela, L.L.; Moulin, C.; Taffin-de-Givenchy, E.; et al. Marine Anticancer Agents: An Overview with a Particular Focus on Their Chemical Classes. *Marine Drugs* **2020**, *18*, doi:10.3390/md18120619.
3. Rotter, A.; Varamogianni-Mamatsi, D.; Pobirk, A.; Matjaz, M.; Cueto, M.; Díaz-Marrero, A.; Jónsdóttir, R.; Sveinsdóttir, K.; Catalá, T.; Romano, G.; et al. Marine cosmetics and the blue bioeconomy: From sourcing to success stories. *iScience* **2024**, *27*, doi:10.1016/j.isci.2024.111339.
4. Li, P.; Lu, H.; Zhang, Y.; Zhang, X.; Liu, L.; Wang, M.; Liu, L. The natural products discovered in marine sponge-associated microorganisms: structures, activities, and mining strategy. *Frontiers in Marine Science* **2023**, *10*, doi:10.3389/fmars.2023.1191858.
5. Mehbub, M.; Yang, Q.; Cheng, Y.; Franco, C.; Zhang, W. Marine sponge-derived natural products: trends and opportunities for the decade of 2011-2020. *Frontiers in Marine Science* **2024**, *11*, doi:10.3389/fmars.2024.1462825.
6. Garlock, T.; Asche, F.; Anderson, J.; Ceballos-Concha, A.; Love, D.; Osmundsen, T.; Pincinato, R. Aquaculture: The missing contributor in the food security agenda. *Global food security-agriculture policy economics and environment* **2022**, *32*, doi:10.1016/j.gfs.2022.100620.
7. Ackefors, H.; Enell, M. The release of nutrients and organic-matter from aquaculture systems in nordic countries. *Journal of Applied Ichthyology-Zeitschrift fur Angewandte Ichthyologie* **1994**, *10*, 225-241.
8. Claus, G.; Madri, P.; Kunen, S. Removal of microbial pollutants from waste effluents by redbear sponge. *Nature* **1967**, *216*, 712-+.
9. Longo, C.; Corriero, G.; Licciano, M.; Stabili, L. Bacterial accumulation by the Demospongiae Hymeniacidon perlevis: A tool for the bioremediation of polluted seawater. *Marine Pollution Bulletin* **2010**, *60*, 1182-1187, doi:10.1016/j.marpolbul.2010.03.035.
10. Maldonado, M.; Zhang, X.; Cao, X.; Xue, L.; Cao, H.; Zhang, W. Selective feeding by sponges on pathogenic microbes: a reassessment of potential for abatement of microbial pollution. *Marine Ecology Progress Series* **2010**, *403*, 75-89, doi:10.3354/meps08411.
11. Stabili, L.; Licciano, M.; Giangrande, A.; Longo, C.; Mercurio, M.; Marzano, C.; Corriero, G. Filtering activity of *Spongia officinalis* var. adriatica (Schmidt) (Porifera, Demospongiae) on bacterioplankton: Implications for bioremediation of polluted seawater. *WATER RESEARCH* **2006**, *40*, 3083-3090, doi:10.1016/j.watres.2006.06.012.
12. Wehrl, M.; Steinert, M.; Hentschel, U. Bacterial uptake by the marine sponge *Aplysina aerophoba*. *Microbial Ecology* **2007**, *53*, 355-365, doi:10.1007/s00248-006-9090-4.
13. Zhang, X.; Zhang, W.; Xue, L.; Zhang, B.; Jin, M.; Fu, W. Bioremediation of bacteria pollution using the marine sponge *Hymeniacidon perlevis* in the intensive mariculture water system of turbot *Scophthalmus maximus*. *Biotechnology and Bioengineering* **2010**, *105*, 59-68, doi:10.1002/bit.22522.
14. Frost, T. Insitu measurements of clearance rates for freshwater sponge *Spongilla-lacustris*. *Limnology and Oceanography* **1978**, *23*, 1034-1039.
15. Osinga, R.; Kleijn, R.; Groenendijk, E.; Niesink, P.; Tramper, J.; Wijffels, R. Development of in vivo sponge cultures: Particle feeding by the tropical sponge *Pseudosuberites aff. andrewsi*. *Marine Biotechnology* **2001**, *3*, 544-554.

16. Riisgard, H.; Thomassen, S.; Jakobsen, H.; Weeks, J.; Larsen, P. Suspension-feeding in marine sponges *Halichondria panicea* and *Haliclona urceolus* - effects of temperature on filtration-rate and energy-cost of pumping. *Marine Ecology Progress Series* **1993**, *96*, 177-188. 357-359
17. Varamogianni-Mamatsi, D.; Anastasiou, T.; Vernadou, E.; Papandroulakis, N.; Kalogerakis, N.; Dailianis, T.; Mandalakis, M. A Multi-Species Investigation of Sponges' Filtering Activity towards Marine Microalgae. *Marine Drugs* **2022**, *20*, doi:10.3390/md20010024. 360-362
18. Camacho, F.; Chileh, T.; García, M.; Mirón, A.; Belarbi, E.; Gómez, A.; Grima, E. Sustained growth of explants from Mediterranean sponge *Crambe crambe* cultured in vitro with enriched RPMI 1640. *Biotechnology Progress* **2006**, *22*, 781-790, doi:10.1021/bp050341m. 363-365
19. Aresta, A.; Marzano, C.; Lopane, C.; Corriero, G.; Longo, C.; Zambonin, C.; Stabili, L. Analytical investigations on the lindane bioremediation capability of the demosponge *Hymeniacidon perlevis*. *Marine Pollution Bulletin* **2015**, *90*, 143-149, doi:10.1016/j.marpolbul.2014.11.003. 366-368
20. Ribes, M.; Yahel, G.; Romera-Castillo, C.; Mallenco, R.; Morganti, T.; Coma, R. The removal of dissolved organic matter by marine sponges is a function of its composition and concentration: An in situ seasonal study of four Mediterranean species. *Science of The Total Environment* **2023**, 871, doi:10.1016/j.scitotenv.2023.161991. 369-371
21. Ferrante, M.; Vassallo, M.; Mazzola, A.; Brundo, M.; Pecoraro, R.; Grasso, A.; Copat, C. In vivo exposure of the marine sponge *Chondrilla nucula* Schmidt, 1862 to cadmium (Cd), copper (Cu) and lead (Pb) and its potential use for bioremediation purposes. *Chemosphere* **2018**, *193*, 1049-1057, doi:10.1016/j.chemosphere.2017.11.144. 372-374
22. Gravina, M.; Longo, C.; Puthod, P.; Rosati, M.; Colozza, N.; Scarselli, M. Heavy metal accumulation capacity of *Axinella damicornis* (Esper, 1794) (Porifera, Demospongiae): a tool for bioremediation of polluted seawaters. *Mediterranean Marine Science* **2022**, *23*, 125-133, doi:10.12681/mms.27792. 375-377
23. Fu, W.; Wu, Y.; Sun, L.; Zhang, W. Efficient bioremediation of total organic carbon (TOC) in integrated aquaculture system by marine sponge *Hymeniacidon perleve*. *Biotechnology and Bioengineering* **2007**, *97*, 1387-1397, doi:10.1002/bit.21352. 378-380
24. Pronzato, R. Sponge-fishing, disease and farming in the Mediterranean Sea. *Aquatic Conservation-Marine and Freshwater Ecosystems* **1999**, *9*, 485-493. 381-382
25. Varamogianni-Mamatsi, D.; Anastasiou, T.; Vernadou, E.; Kouvarakis, N.; Kagiampaki, E.; Kalogerakis, N.; Dailianis, T.; Mandalakis, M. Uptake of aquaculture-related dissolved organic pollutants by marine sponges: Kinetics and mechanistic insights from a laboratory study. *Science of The Total Environment* **2023**, 899, doi:10.1016/j.scitotenv.2023.165601. 383-386
26. Fu, W.; Sun, L.; Zhang, X.; Zhang, W. Potential of the marine sponge *Hymeniacidon perleve* as a bioremediator of pathogenic bacteria in integrated aquaculture ecosystems. *Biotechnology and Bioengineering* **2006**, *93*, 1112-1122, doi:10.1002/bit.20823. 387-389
27. Gökalp, M.; Mes, D.; Nederloff, M.; Zhao, H.; de Goeij, J.; Osinga, R. The potential roles of sponges in integrated mariculture. *Reviews in Aquaculture* **2021**, *13*, 1159-1171, doi:10.1111/raq.12516. 390-391
28. Osinga, R. Bioreactor system useful for cultivation of aquatic organisms such as sponges to produce natural compounds useful in, e.g. pharmaceutical industry comprises separate enclosures for enrichment of water and cultivation of the organisms. EP1508272-A1. 392-394
29. Milanese, M.; Chelossi, E.; Manconi, R.; Sarà, A.; Sidri, M.; Pronzato, R. The marine sponge *Chondrilla nucula* Schmidt, 1862 as an elective candidate for bioremediation in integrated aquaculture. *Biomolecular Engineering* **2003**, *20*, 363-368, doi:10.1016/S1389-0344(03)00052-2. 395-397
30. Rocha, M.; Fassini, D.; Martins, E.; Alves, A.; Reis, R.; Silva, T. Contributing of "omics" for the understanding of *Chondrosia reniformis* collagen aggregation phenomenon. *ISJ-Invertebrate Survival Journal* **2020**, *17*, 30-30. 398-399

31. Imhoff, J.; Garrone, R. Solubilization and characterization of *Chondrosia reniformis* sponge collagen. *Connective Tissue Research* **1983**, *11*, 193-197. 400
401
32. Orel, B.; Giovine, M.; Ilan, M. On the Path to Thermo-Stable Collagen: Culturing the Versatile Sponge *Chondrosia reniformis*. *Marine Drugs* **2021**, *19*, doi:10.3390/md19120669. 402
403
33. Gökalp, M.; Wijgerde, T.; Sarà, A.; de Goeij, J.; Osinga, R. Development of an Integrated Mariculture for the Collagen-Rich Sponge *Chondrosia reniformis*. *Marine Drugs* **2019**, *17*, doi:10.3390/md17010029. 404
405
34. Quideau, S.; Lebon, M.; Lamidey, A. Enantiospecific synthesis of the antituberculosis marine sponge metabolite (+)-puupehenone. The arenol oxidative activation route. *Organic Letters* **2002**, *4*, 3975-3978, doi:10.1021/ol026855t. 406
407
35. Pina, I.C.; Sanders, M.L.; Crews, P. Puupehenone congeners from an indo-pacific *Hyrtios* sponge. *Journal of Natural Products* **2003**, *66*, 2-6, doi:10.1021/np020279s. 408
409
36. Zjawiony, J.K.; Bartyzel, P.; Hamann, M.T. Chemistry of puupehenone: 1,6-conjugate addition to its quinone-methide system. *Journal of Natural Products* **1998**, *61*, 1502-1508, doi:10.1021/np9802062. 410
411
37. Tripathi, S.; Feng, Q.; Liu, L.; Levin, D.; Roy, K.; Doerksen, R.; Baerson, S.; Shi, X.; Pan, X.; Xu, W.; et al. Puupehenone, a Marine-Sponge-Derived Sesquiterpene Quinone, Potentiates the Antifungal Drug Caspofungin by Disrupting Hsp90 Activity and the Cell Wall Integrity Pathway. *MSphere* **2020**, *5*, doi:10.1128/mSphere.00818-19. 412
413
414
38. Carballeira, N.; Reyes, E.; Shalabi, F. Identification of novel iso anteiso nonacosadienoic acids from the phospholipids of the sponges *Chondrosia reniformis* and *Myrmekioderma styx*. *Journal of Natural Products* **1993**, *56*, 1850-1855. 415
416
39. Nechev, J.; Christie, W.; Robaina, R.; Ivanova, A.; Popov, S.; Stefanov, K. Chemical composition of the sponge *Chondrosia reniformis* from the Canary Islands. *Hydrobiologia* **2002**, *489*, 91-98. 417
418
40. Cunha, M.; Jorge, A.; Nunes, M.; Sousa, J.; Lança, M.; da Silva, M.; Gaudêncio, S. GC/MS Fatty Acid Profile of Marine-Derived Actinomycetes from Extreme Environments: Chemotaxonomic Insights and Biotechnological Potential. *Marine Drugs* **2025**, *23*, doi:10.3390/md23010001. 419
420
421
41. Eroldogan, O.T.; Glencross, B.; Novoveska, L.; Gaudencio, S.P.; Rinkevich, B.; Varese, G.C.; de Fatima Carvalho, M.; Tasdemir, D.; Safarik, I.; Nielsen, S.L.; et al. From the sea to aquafeed: A perspective overview. *Reviews in Aquaculture* **2022**, doi:10.1111/raq.12740. 422
423
424
42. Al-Hamoud, G.; Al-Musayeib, N.; Amina, M.; Ibrahim, S. Abubidentin A, New Oleanane-type Triterpene Ester from *Abutilon bidentatum* and its antioxidant, cholinesterase and antimicrobial activities. *PEERJ* **2022**, *10*, doi:10.7717/peerj.13040. 425
426
427
43. Liu, L.; Hu, L.; Dong, Z. A glucosylceramide with a novel ceramide and three novel ceramides from the basidiomycete *Cortinarius umidicola*. *Lipids* **2003**, *38*, 669-675. 428
429
44. Farokhi, F.; Grellier, P.; Clement, M.; Roussakis, C.; Loiseau, P.; Genin-Seward, E.; Kornprobst, J.; Barnathan, G.; Wielgosz-Collin, G. Antimalarial Activity of Axidjiferosides, New β -Galactosylceramides from the African Sponge *Axinyssa djiferi*. *Marine Drugs* **2013**, *11*, 1304-1315, doi:10.3390/md11041304. 430
431
432
45. Lykholat, Y.; Khromykh, N.; Liashenko, O.; Sklyar, T.; Anishchenko, A.; Balalaiev, O.; Holubieva, T.; Lykholat, Y. Phytochemical profiles and antimicrobial activity of the inflorescences of *Sorbus domestica*, *S. aucuparia*, and *S. torminalis*. *Biosystems Diversity* **2023**, *31*, 290-296, doi:10.15421/012333. 433
434
435
46. Li, X.; Morita, S.; Yamada, H.; Koga, K.; Ota, W.; Furuta, T.; Yamatsu, A.; Kim, M. Free Linoleic Acid and Oleic Acid Reduce Fat Digestion and Absorption In Vivo as Potent Pancreatic Lipase Inhibitors Derived from Sesame Meal. *Molecules* **2022**, *27*, doi:10.3390/molecules27154910. 436
437
438
47. Cardoso, G.; Chacon, E.; Chacon, P.; Bordeaux-Rego, P.; Duarte, A.; Saad, S.; Zavaglia, C.; Cunha, M. Fatty acid is a potential agent for bone tissue induction: In vitro and in vivo approach. *Experimental Biology and Medicine* **2017**, *242*, 1765-1771, doi:10.1177/1535370217731104. 439
440
441
48. Carballeira, N. New advances in the chemistry of methoxylated lipids. *Progress In Lipid Research* **2002**, *41*, 437-456. 442

49. Ravi, B.; Perzanowski, H.; Ross, R.; Erdman, T.; Scheuer, P.; Finer, J.; Clardy, J. Recent research in marine natural-products - puerphenones. *Pure And Applied Chemistry* **1979**, *51*, 1893-1900. 443
444
50. Kobayashi, J.; Murata, O.; Shigemori, H.; Sasaki, T. Jaspisamides A-C, new cytotoxic macrolides from the Okinawan sponge *Jaspis* sp. *Journal Of Natural Products* **1993**, *56*, 787-791. 445
446
51. Shin, Y.; Kim, G.; Jeon, J.; Shin, J.; Lee, S. Antimetastatic Effect of Halichondramide, a Trisoxazole Macrolide from the Marine Sponge *Chondrosia corticata*, on Human Prostate Cancer Cells via Modulation of Epithelial-to-Mesenchymal Transition. *Marine Drugs* **2013**, *11*, 2472-2485, doi:10.3390/md11072472. 447
448
449
52. Bae, S.; Kim, G.; Jeon, J.; Shin, J.; Lee, S. Anti-proliferative effect of (19Z)-halichondramide, a novel marine macrolide isolated from the sponge *Chondrosia corticata*, is associated with G2/M cell cycle arrest and suppression of mTOR signaling in human lung cancer cells. *Toxicology in Vitro* **2013**, *27*, 694-699, doi:10.1016/j.tiv.2012.11.001. 450
451
452
53. Bae, S.; Song, J.; Shin, Y.; Kim, W.; Oh, J.; Choi, T.; Jeong, E.; Park, S.; Jang, E.; Kang, J.; et al. Anti-proliferative effect of (19Z)-halichondramide from the sponge *Chondrosia corticata* via G2/M cell cycle arrest and suppression of mTOR signaling in human lung cancer cells. *Cancer Research* **2014**, *74*, doi:10.1158/1538-7445.AM2014-4235. 453
454
455
54. Shin, J.; Lee, H.; Kim, J.; Shin, H.; Ahn, J.; Paul, V. New macrolides from the sponge *Chondrosia corticata*. *Journal of Natural Products* **2004**, *67*, 1889-1892, doi:10.1021/np040124f. 456
457
55. Varamogianni-Mamatsi, D.; Nunes, M.J.; Marques, V.; Anastasiou, T.I.; Kagiampaki, E.; Vernadou, E.; Dailianis, T.; Kalogerakis, N.; Branco, L.C.; Rodrigues, C.M.P.; et al. Comparative Chemical Profiling and Antimicrobial/Anticancer Evaluation of Extracts from Farmed versus Wild *Agelas oroides* and *Sarcotragus foetidus* Sponges. *Marine Drugs* **2023**, *21*, doi:10.3390/md21120612. 458
459
460
461
56. Pinto-Almeida, A.; Bauermeister, A.; Luppino, L.; Grilo, I.R.; Oliveira, J.; Sousa, J.R.; Petras, D.; Rodrigues, C.F.; Prieto-Davo, A.; Tasdemir, D.; et al. The Diversity, Metabolomics Profiling, and the Pharmacological Potential of Actinomycetes Isolated from the Estremadura Spur Pockmarks (Portugal). *Marine Drugs* **2022**, *20*, doi:10.3390/md20010021. 462
463
464
465
57. Andrews, J. Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy* **2001**, *48*, 5-16. 466
58. Florindo, P.; Pereira, D.; Borralho, P.; Costa, P.; Piedade, M.; Rodrigues, C.; Fernandes, A. New [(η⁵-C₅H₅)Ru(N-N)(PPh₃)] [PF₆] compounds: colon anticancer activity and GLUT-mediated cellular uptake of carbohydrate-appended complexes. *Dalton Transactions* **2016**, *45*, 11926-11930, doi:10.1039/c6dt01571a. 467
468
469
59. Prieto-Davo, A.; Dias, T.; Gomes, S.E.; Rodrigues, S.; Parera-Valadezl, Y.; Borralho, P.M.; Pereira, F.; Rodrigues, C.M.P.; Santos-Sanches, I.; Gaudencio, S.P. The Madeira Archipelago As a Significant Source of Marine-Derived Actinomycete Diversity with Anticancer and Antimicrobial Potential. *Frontiers in Microbiology* **2016**, *7*, doi:10.3389/fmicb.2016.01594. 470
471
472
473
60. Pang, Z.; Lu, Y.; Zhou, G.; Hui, F.; Xu, L.; Viau, C.; Spigelman, A.; Macdonald, P.; Wishart, D.; Li, S.; et al. MetaboAnalyst 6.0: towards a unified platform for metabolomics data processing, analysis and interpretation. *Nucleic Acids Research* **2024**, *52*, W398-W406, doi:10.1093/nar/gkae253. 474
475
476
477