WORKING GROUP 4

BIOACTIVE CHEMICALS AND *ULVA*-ASSOCIATED MICROORGANISMS

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CO-LEADER

RATIONALE AND OBJECTIVES

Describe rational and objectives of the WG. Similar to Memorandum of Understanding but including changes and adjustments. This will be used in Final Report)

The green seaweed *Ulva* (commonly known as sea lettuce) is a widely distributed green macroalga with high ecological and industrial importance. *Ulva* is recognized as a valuable source of bioactive compounds, including polysaccharides (ulvans), proteins, fatty acids, sterols, pigments, and vitamins. These metabolites are of interest for applications in food, feed, nutraceuticals, cosmetics, pharmaceuticals, and as biostimulants in agriculture (Ozogul et al. 2024). The COST Action CA20106 (SeaWheat) and its Working Group 4 (WG4: Bioactive Products) have aimed to explore, characterize, and valorise bioactive metabolites from *Ulva*. The main objectives include: (**i**) Identifying and characterizing extractable metabolites from *Ulva* relevant for industrial uses by the participating research groups. (**ii**) Developing analytical and biorefinery approaches to optimize extraction and applications within workshop and training schools. (**iii**) Evaluating the antioxidant, antibacterial, antiviral, and other bioactivities of *Ulva* metabolites and their associated microbiome. (**iv**) Promoting collaboration among European and international laboratories to build a comprehensive understanding of *Ulva*'s bioactive potential through STSMs, newly established collaboration for the preparation of a review article, and presenting our results at conferences.

References

Ozogul F, Trif M, Rusu A (Editors) (2024). Seaweeds and Seaweed-derived compounds. Meeting the growing need for healthy biologically active compounds

ACTIVITIES AND KEY FINDINGS

Main activities and key findings. Please mind that reports of workshops and Training Schools will be included separately in the final report document.

Working Group 4 (WG4) has made substantial progress in exploring the bioactive potential of ***Ulva*** species, focusing on both the diversity of extractable compounds and their functional applications. Research under this WG has revealed a broad array of substances with relevance to the food, cosmetic, pharmaceutical, and other industrial sectors. The deliverables D 4.1 report on the following topics:

a. Extractable substances from Ulva, utilised as food additives, in cosmetics and for various other purposes (D4.1a).

The bioactive potential of *Ulva* species was thoroughly investigated by working group 4, with an emphasis on chemicals that can be extracted sustainably and used in a variety of industries. The discovery and characterisation of multiple classes of extractable compounds with great potential for use in food, cosmetics, medicines, and biotechnological applications was one of the group's major accomplishments. Specifically, a WG4 core team has written a review article outlining the potential of bioactives obtained from *Ulva* (**Wichard et al. 2025**, to be submitted).

(1) *Ulva* was found to contain a diverse profile of lipids and fatty acids, including omega-3 polyunsaturated fatty acids (PUFAs), glycolipids, and phospholipids (**Hoffman et al. 2024; Wichard et al. 2025**). These compounds are of particular interest to the nutraceutical and cosmetic industries, where they are associated with anti-inflammatory, skin barrier-supporting, and cardiovascular health-promoting properties (**Table 1**). A dedicated lipidomics training, hosted in Aveiro in 2024, enabled WG4 participants to deepen their expertise in advanced

analytical techniques and to harmonise lipid profiling approaches across laboratories. The workshop also facilitated collaboration with external experts and supported early-career researchers in developing specialised skills (**Monteiro et al. 2022**).

(2) Further investigation led to the identification of various sterols with potential implications for cholesterol metabolism (**Mulder et al**. **2025**, presentation on COST Action conference, Bremerhaven, Germany), as well as photosynthetic pigments such as chlorophylls and carotenoids. These pigments were confirmed to have antioxidant activity, reinforcing their value as natural colourants and functional additives in food and cosmetic products.

(3) Another promising category of bioactives found in *Ulva* includes proteins and bioactive peptides. Preliminary screening has shown that *Ulva*-derived peptides may support nutritional enhancement, making them attractive candidates for use in protein-enriched or functional food products (**Steinhagen et al. 2025**). Ongoing studies are investigating their digestibility, bioavailability, and potential health claims.

(4) The significance of these findings was further emphasized during the 2024 WG4 Workshop and Training School in Aveiro (Portugal, **see reports**), where researchers evaluated and practised extraction technologies and discussed economic and environmental considerations for market entry. Discussions also touched on the potential application and integration of *Ulva*-based compounds into existing product pipelines and the potential for co-products in circular bioeconomy models and scalable biorefinery pipelines for the production of algae-based bioactive (presented by **H. Pereira of the GreenCoLab,** Portugal).

(5) In a collaborative effort with WG1, *Ulva* samples were collected across Europe by WG1 as part of the **eUlva** initiative (**O. deCleck**) (https://sites.google.com/view/eulva) for microbiome analysis, while WG4 focused on the quantification of biochemical constituents. This newly established and ongoing network aims to correlate *Ulva* biodiversity with its associated microbiome and biochemical composition, offering an integrated perspective on ecological and functional diversity.

In summary, WG4's work has created the framework for *Ulva*'s holistic valorisation plan, which will use its biochemical variety to support innovation across different sectors. The enhanced extraction techniques (for example, in lipidomics and thallusin quantification), knowledge transfer efforts during training schools and STMS, and scientific publications produced during the COST Action all contribute to the widespread dissemination of this knowledge.

Table 1: Overview of extractable compounds in *Ulva* and their bioactive potential (**Wichard et al. 2025**; review of WG4 to be submitted)

Compound Group	Examples	Bioactives
Polysaccharides	Ulvan	Antioxidant, antiviral, anticoagulant
Phenolics	Flavonoids, phenolic acids	Antioxidant, anti-inflammatory
Proteins/peptides	ACE-inhibitory peptides	Antihypertensive, antimicrobial
Fatty acids	αLEA	Anti-inflammatory, cardioprotective
Pigments	carotenoids	Antioxidant, anticancer
Vitamins	vitamins A, C, E	General health, metabolism support
Sterols	stigmasterol	anticancer
Minerals	Ca, Fe	General health

b. The potential of *Ulva* strain extracts as natural antioxidants, antibacterial and antiviral activities and isolation of bioactive molecules (D4.1b).

In parallel with the compositional analysis of *Ulva*, Working Group 4 (WG4) placed significant emphasis on investigating the biological activity of its extracts and individual compounds in multiple research groups (**Ulrich et al. 2022; Palou et al. 2023; Gnayem et al. 2024; Schultze et al. 2024**).
(1) New identification techniques were tested, such as Raman spectroscopy or near infrared spectroscopy **(Palou et al. 2023 and Schultze et al. 2024**). These bioactivity studies formed a crucial part of the group's contribution to the COST Action, supporting the identification of functional properties that could translate into the identification of new substances, and which might be applied across pharmaceuticals, nutraceuticals, cosmetics, and sustainable health products.

(2) One of the most consistently observed properties was antioxidant activity. Extracts from multiple *Ulva* strains exhibited high levels of radical-scavenging capacity, suggesting strong potential for preventing oxidative stress and associated cellular damage. Importantly, WG4's research demonstrated that antioxidant potency was not uniform across strains or cultivation conditions. Instead, it was influenced by a range of environmental and physiological variables, including light intensity, seasonal changes, nutrient availability, and even cultivation method (e.g., wild-harvested vs. aquaculture-grown). These findings, supported by work such as **Cardoso et al. (2025)**, point to the need for strain-specific selection and optimized cultivation strategies when targeting antioxidant functionality for commercial applications.

(3) Another promising avenue of investigation involved the antibacterial and antiviral properties of *Ulva*-derived compounds. In vitro studies carried out across partner laboratories showed that ulvan-rich extracts and specific lipid fractions demonstrated inhibitory activity against a range of bacterial and viral pathogens. These bioactivities were especially relevant in the context of developing eco-friendly alternatives to synthetic preservatives in cosmetics and bioactive ingredients for dermaceutical or topical pharmaceutical formulations. For instance, ulvans can be versatile bioactive building blocks for the development of hybrid biomaterials for biomedical applications (presented by V. **Roussis** of the University of Athen**, GreeceS)**
The antimicrobial properties of *Ulva* also hold promise for incorporation into food packaging and coatings, aligning with EU goals for reducing chemical preservatives and promoting biobased materials.

(4) A third significant development emerged from WG4's work on the isolation and characterization of specific bioactive molecules also derived from bacteria associated with the seaweed host. Notably, progress was made in identifying and isolating rare compounds such as morphogenetic substances (including thallusin), which play roles in algal development and interspecies signaling (**Ulrich et al. 2022**; and ongoing collaborations within the COST Action network). These compounds have sparked interest for their potential in plant health, aquaculture, and microbial community management. In addition, the discovery of novel sterols opens new directions for research into cholesterol-lowering nutraceuticals and cell membrane-targeting therapies. These achievements were supported by methodological advances shared through WG4 training activities, which introduced improved chromatographic and spectrometric techniques tailored to marine matrices.

(5) The cumulative outcomes of these efforts highlight the multifunctional potential of *Ulva* as a bioactive resource. WG4 demonstrated antioxidant, antimicrobial, and biochemical properties make it uniquely positioned to address multiple market needs with a single, sustainable raw material. WG4 has ensured that this knowledge is not only documented through scientific publications but also accessible through open-access and was taught in the training school. The group's targeted capacity-building activities, including short-term scientific missions and

hands-on training schools (**see report**), have helped train the next generation of researchers and enhanced research capabilities across the COST network.

In conclusion, the bioactivity profile of *Ulva* confirmed during the Action reaffirms its value as a renewable marine resource. These findings serve as a springboard for continued exploration, product development, and cross-sectoral innovation in the blue bioeconomy.

500 words per task, including key findings + link to deliverable document (paper, report, dataset)

FUTURE DIRECTIONS & RECOMMENDATIONS

500 words Future research recommendations, identified knowledge gaps

Prospective Directions and Suggestions: The results and accomplishments of WG4 in the COST Action on marine bioactives highlight the significant potential of *Ulva* species as a sustainable supply of valuable bioactive chemicals. Based on the advancements achieved throughout the Action, many prospective directions and proposals arise to inform ongoing research, implementation, and policy formulation (WG5).

Enhancing Bioprospecting and Functional Characterization: Future research should broaden the scope of bioprospecting to encompass a wider variety and a defined range of *Ulva* strains and growth conditions. Environmental factors, including salinity, light intensity, and nutrient availability, substantially impact the metabolite profiles of Ulva, hence altering the yield and efficacy of bioactive chemicals (e.g., Gnayem et al. 2024). Targeted omics methodologies, such as transcriptomics, metabolomics, and lipidomics, as well as mechanistic studies, will be crucial for elucidating the molecular mechanisms underlying these variances to overcome the descriptive approaches in phycology. However, the data-driven approach will facilitate the discovery of new chemicals with unexploited commercial or medicinal potential.

Standardization and Scalability of Extraction Protocols: Although WG4 achieved notable advancements in optimizing extraction procedures in collaboration with WG1 and due to training schools, further efforts are needed to standardize these protocols across laboratories and guarantee consistency. Special emphasis must be placed on the environmental and economic sustainability of extraction methods, prioritizing green chemistry principles and scalable procedures as reviewed in our WG achievement (Wichard et al. (2025) submitted). Joint pilot initiatives with industry collaborators can assess the techno-economic viability of scaling up *Ulva*-derived product lines, including nutraceuticals, cosmeceuticals, and bioplastics.

Enhancing Intersectoral Cooperation: The communication established between academia and industry (SME) during workshops in Lisbon and Aveiro (Portugal) in 2023 and 2024 setup new networks and collaborations. These networks will now enable technology transfer, regulatory compliance, and market access. Promoting interdisciplinary collaboration among marine ecologists, chemical engineers, and regulatory specialists would expedite the creation of commercially feasible solutions.

Regulatory Alignment and Policy Support: The rapid commercialization of *Ulva*-derived goods necessitates strict adherence to food safety, pharmaceutical, and cosmetic standards. It is advisable to engage with regulatory authorities at both national and EU levels to ensure that innovative *Ulva*-based products comply with safety and efficacy standards. Additionally, policy frameworks must facilitate the sustainable cultivation of macroalgae, encompassing provisions for integrated multi-trophic aquaculture (IMTA). The uptake and sequestration of metals and micropollutants should be particularly monitored (Hardegen et al. 2025, Vargas-Murga et al. 2025)

Instruction and teaching the next generation of (applied) researchers: The efficacy of COST Action initiatives, especially short-term scientific missions and training schools,

underscores the importance of investing in early career researchers and fostering multidisciplinary skill enhancement. Future projects ought to enhance accessibility to practical training in sophisticated analytical techniques, bioinformatics, and process engineering. Although the overall importance of seaweed research is acknowledged, the involved and funded scientists are a small group in the scientific landscape.

Conclusion: The encouraging outcomes produced by WG4 establish a robust basis for ongoing study, innovation, and valorization of *Ulva* bioactives and particular new networks of scientists who are now seeking Horizon Europe-based funding. Here, future initiatives must emphasize ecological sustainability, technical scalability, and regulatory integration, while enhancing cross-sector collaboration and education. By utilizing these guidelines, *Ulva* can transform from a still underexploited marine resource into a fundamental component of the blue bioeconomy in Europe.

PUBLICATIONS OF WG4 (CHRONOLOGICAL ORDER DURING COST ACTION)

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SUMMARY OF WG4 OBJECTIVES AND DELIVERABLES

Table 2

Objective	Activities	Deliverables	Timeline
Identification of *Ulva* bioactives	Compositional analysis of polysaccharides, lipids, proteins, pigments by various groups	Catalog of Ulva metabolites (review article to be submitted in Sept 2025); individual peer-reviewed research articles	2022–2025
Development of analytical workflows	Training schools in lipidomics, metabolomics, glycomics; protocol harmonization	Protocol Book (Aveiro 2024); Training the next generation of scientists	2023–2024
Evaluation of bioactivities	Antioxidant, antibacterial, antiviral assays; cross-lab collaborations	Reports and publications on *Ulva* bioactivity	2023–2025
Capacity building and training	Workshops, Training Schools, STSMs, and industry collaboration (e.g., ALGAPlus Ltd, Aveiro, Portugal),	Trained researchers; knowledge exchange; dissemination events	2022–2025