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Research article

Valorization of coastal seagrass biomass: Enzyme-assisted extraction of bioactive compounds from *Posidonia oceanica*

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Abstract: In this study, we focus on the enzyme-assisted extraction (EAE) of bioactive compounds from *Posidonia oceanica*, a Mediterranean seagrass of ecological importance that is often treated as waste. Increasing evidence highlights its beneficial properties, making it a promising source of compounds for the production of value-added compounds. A Taguchi experimental design was employed to optimize the EAE, using the cellulolytic commercial enzyme preparation Cellic CTec3® HS. The effects of key parameters, namely extraction time, solid-to-liquid ratio, and enzyme loading, were evaluated for total phenolic content (TPC) and total flavonoid content (TFC). Optimization was achieved using "the larger-the-better" approach, and under optimal conditions (solid-to-liquid ratio 1% w/v, enzyme loading 200 U/g, and extraction time 6 h), TPC and TFC reached 27.54 ± 0.84 mg GAE/g DW and 9.22 ± 0.84 mg CAE/g DW, respectively, representing approximately a ten-fold and more than five-fold increase compared to conventional ethanol:water extraction. An additional hemicellulolytic enzyme preparation, Viscoferm® was evaluated under the optimized conditions, and EAE with Cellic CTec3® HS yielded higher TPC and TFC. The optimized extracts obtained with Cellic CTec3® HS and Viscoferm® were further assessed for their antioxidant activity, anti-aging activity (inhibition of tyrosinase activity), anti-diabetic activity (inhibition of amylase and α-glucosidase activity), and antibacterial activity (inhibition of *E. coli* growth). The Cellic CTec3® HS extract yielded highest antioxidant activity (**IC_{50}** =203.72 ± 23.99 μL extract/mL) and antibacterial activity (21.37 ± 2.00 %), whereas the Viscoferm® extracts demonstrated stronger antidiabetic activity

with IC_{50} values of IC_{50} = 38.42 ± 2.45 mg/mL (α-amylase) and 43.84 ± 1.29 mg/mL (a-glucosidase). Both extracts showed relatively low tyrosinase inhibition; at 200 mg/mL, Cellic CTec3® HS and Viscoferm® exhibited 41.62 ± 0.10% and 18.69 ± 0.57% inhibition, respectively. These findings confirm the potential of *Posidonia oceanica* as a sustainable source of bioactive compounds with promising applications.

Keywords: *Posidonia oceanica*; enzyme-assisted extraction (EAE); Taguchi design; Cellic CTec3® HS; Viscoferm®; phenolic compounds; flavonoids; biological activities

1. Introduction

Posidonia oceanica, a seagrass species endemic to the Mediterranean Sea, is ecologically indispensable due to its role in carbon sequestration, sediment stabilization, and habitat provision [1–4], and is widely protected under European and regional conservation frameworks [5–7]. Its meadows are classified as habitat type 1120 ("Posidonia beds") within the Natura 2000 network and are subject to strict protection and sustainable management measures, ensuring the preservation of *P. oceanica* as a marine ecosystem engineer [8,9]. During its life cycle, *P. oceanica* leaves detach and accumulate on coastlines, and the resulting deposits are periodically removed and typically landfilled, despite representing an underutilized biomass with clear potential for valorization and recovery of bioactive compounds. Reports from Mediterranean destinations indicate that *P. oceanica* management can involve hundreds to several thousand tons per year, creating a recurring logistical and economic burden for municipalities. In parallel, published estimates for beach-wrack collection and transport in coastal municipalities are commonly in the ~€100-per-ton range, reinforcing the value of valorization routes that can reduce disposal needs [10]. Concurrently, studies have demonstrated the potential of *P. oceanica* waste biomass in environmental and agricultural engineering applications, including its conversion into biochar for soil amendment and carbon sequestration [11], its use as a biomass source for energy generation [12], incorporation into polymer composites [13], and valorization through green bioprocessing for bioeconomy frameworks [14]. These approaches align with circular economy principles and offer sustainable pathways for resource recovery and coastal waste mitigation. *P. oceanica* has garnered increasing attention due to its biochemical composition. Its leaves and banquette-derived biomass contain a wide array of phenolic compounds, notably gallic acid, ferulic acid, and caffeic acid, alongside flavonoids such as quercetin and kaempferol. These constituents have been associated with antioxidant and antimicrobial activities in vitro. For example, Benito-González et al. [15] characterized extracts from waste biomass and reported radical-scavenging capacity and antimicrobial efficacy against foodborne pathogens in vitro. Similarly, Astudillo-Pascual et al. [16] expanded the phenolic profile using high-resolution mass spectrometry, identifying additional compounds of potential interest for nutraceutical and packaging applications. In parallel, Rubio-Portillo et al. [17] conducted metagenomic analyses of *P. oceanica* banquette samples, revealing the presence of carbohydrate-active enzymes (CAZymes) and genes linked to the synthesis of bioactive metabolites, suggesting their utility in biomass degradation and bioenergy production.

Beyond polyphenols, *P. oceanica* contains phytosterols such as β-sitosterol, which exhibit anti-inflammatory and cholesterol-lowering effects, and unsaturated fatty acids like oleic and linoleic acid, which have been reported in other systems to exhibit anti-inflammatory and cholesterol-modulating

properties [13]. These compounds are particularly relevant for cosmetic and pharmaceutical formulations, where natural bioactive compounds are increasingly favored. Karima et al. [18] demonstrated that Eastern Algerian *P. oceanica* extracts inhibit cholinesterase and urease enzymes, indicating potential neuroprotective and anti-ulcer applications, with minimal cytotoxicity observed *in vitro*. Although these findings support the safe incorporation of P. *oceanica* derivatives into functional foods and therapeutic agents, further investigation, including in vivo validation and safety assessment, is required before therapeutic or clinical relevance can be established.

Given that *P. oceanica* biomass is structurally rich in cellulose, hemicelluloses (including xylans and xyloglucans), and pectins [19], conventional solvent extraction may be limited by restricted mass transfer and incomplete release of intracellular and matrix-bound phenolic compounds. Enzyme-assisted extraction (EAE) provides a targeted approach to partially depolymerize these structural polysaccharides, enhance cell wall permeability, and improve the recovery of associated bioactive compounds under mild and environmentally friendly conditions [20]. Although EAE has been increasingly investigated for the recovery of bioactive compounds from marine biomasses (e.g., macroalgae), studies on washed-up *P. oceanica* waste biomass (WPO) remain limited, particularly those integrating systematic process optimization with multifunctional bioactivity assessment of the resulting extracts [21].

The Taguchi experimental design was selected as an efficient screening and optimization tool, enabling the evaluation of multiple process parameters with a limited number of experimental runs. Compared to full factorial designs, the Taguchi approach reduces experimental burden while enabling identification of the most influential factors through signal-to-noise (S/N) analysis [22]. Given the exploratory and optimization-focused scope of this study, this method pprovides a practical balance between statistical evaluation and experimental feasibility.

In this context, our aim of this study was to develop and optimize an EAE process for the efficient recovery of bioactive compounds from WPO using a Taguchi experimental design to systematically evaluate the effects of extraction time, solid-to-liquid ratio, and enzyme loading on total phenolic content (TPC) and total flavonoid content (TFC). Specifically, the study sought to identify optimal extraction conditions using the cellulolytic enzyme preparation Cellic CTec3® HS to compare its performance with the hemicellulolytic preparation Viscoferm® and with conventional ethanol:water extraction, and to evaluate the antioxidant, anti-aging, antidiabetic, and antibacterial potential of the resulting extracts. The novelty of this work lies in the valorization of an underutilized marine residue (WPO), the comparative evaluation of two commercial enzyme preparations for EAE, and the assessment of the recovered extracts in view of their potential application in cosmetic and nutraceutical formulations within a circular bioeconomy framework.

2. Materials and methods

2.1. Material

Waste (dead) biomass of *Posidonia oceanica* (WPO) was used as the raw material for the experiments. WPO was collected from Lake Moustos, Arcadia, Greece (coordinates: 37°23'43.8"N, 22°45'09.3"E), a protected Natura 2000 site, in March 2024. The material was ground and sieved to a final particle size of <1.981 mm and stored at room temperature in a dark, dry place throughout the duration of the experiments.

2.2. Chemical and reagents

The following reagents were used: 2,2-Diphenyl-1-picrylhydrazyl (DPPH·); absolute methanol (CH3OH); and Folin–Ciocalteu from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of the highest purity commercially available and were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Enzyme preparations

The commercial enzyme preparations used in this study (Viscoferm® and Cellic® CTec3 HS) were multi-enzyme mixtures and were kindly provided by Novozymes A/S (Bagsværd, Denmark). The enzymatic activities were experimentally determined using established assay methods. Xylanase activity (Viscoferm®) was determined via the DNS method using xylan as substrate and expressed as Units/mL; and total cellulolytic activity (Cellic® CTec3 HS) was quantified according to the standard filter paper assay (FPU method) and expressed as FPU/mL. The measured activities were 4500 U/mL for Viscoferm® (xylanase activity) and 120 FPU/mL for Cellic® CTec3 HS (total cellulolytic activity). Based on these values, the enzyme dosages applied in the extraction experiments were calculated and expressed as units per gram of dry material (U/g or FPU/g). Tyrosinase (EC 1.14.1.8.1) was derived from mushroom, α-amylase (EC 3.2.1.1) from porcine pancreas, and α-glucosidase (EC 3.2.1.20) from *Saccharomyces cerevisiae*. The latter three enzymes were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.4. Experimental design

In this study, the Taguchi experimental design was employed to investigate and optimize the conditions for the enzyme-assisted extraction of bioactive compounds from WPO. This statistical approach was chosen for its efficiency in minimizing the number of required experimental trials while maintaining analytical robustness and reproducibility. Three critical factors were examined: (i) The enzyme loading of the cellulolytic preparation Cellic CTec 3® HS, (ii) the solid-to-liquid ratio of WPO biomass to the extraction medium, and (iii) the extraction time of the process. These three factors and their ranges were chosen based on preliminary experiments. An L9 orthogonal array (3^3 design) was adopted to evaluate the effects of these factors at three levels each, providing a balanced and independent assessment of their individual and combined influences. The design matrix used in the study is presented in Table 1.

Table 1. L9 orthogonal array for EAE of bioactive compounds from WPO.

A/A	Solid to liquid ratio (% w/v)	Enzyme loading (Units/g)	Extraction time (h)
1	1	50	2
2	1	100	4
3	1	200	6
4	5	50	4
5	5	100	6
6	5	200	2
7	7	50	6
8	7	100	2
9	7	200	4

The responses obtained from each run were transformed into signal-to-noise (S/N) ratios to evaluate the robustness and performance of the extraction process. Since we are concerned with the maximization of the responses, the S/N ratio for the "the larger-the-better" approach was evaluated as follows [22] (Eq (1)):

$$\frac{S}{N} = -10 \log\left(\frac{1}{n}\sum_{i=1}^{n} 1/Y_i^2\right) \quad (1)$$

where, n is the total number of replications of each test run; Y_i is the number of responses realized in the replication experiment; and I was carried out under the same experimental conditions of each test run.

The data obtained from the Taguchi method for each response were then subjected to regression analysis and fitted to the following quadratic polynomial model (Eq (2)).

$$y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j + \sum_{i=1}^{n} \beta_{ii} X_{ii}^{\ 2} \quad (2)$$

where Y is the predicted response; β_0 is an offset term; β_i, β_{ii}, and β_{ij} are the linear, quadratic, and interactive coefficients, respectively; n is the number of independent variables; and X_i and X_j are the levels of the independent variables.

2.5. Enzyme-assisted extraction

The milled material was mixed with 50 mM phosphate buffer pH = 5.5 and treated with the appropriate enzyme at the solid-to-liquid ratio and extraction time depicted by Taguchi design. The enzyme was first dissolved in the buffer before the addition of plant material. Extractions were performed in a thermoshaker (Thermomixer®, Eppendorf) at 50 °C under constant agitation (1300 rpm), with a final working volume of 1.0 mL per run. The pH was adjusted at the beginning of the extraction. Following extraction, the samples were centrifuged at 10,000 rpm for 10 min at 4 °C. The resulting supernatants were collected and stored at −18 °C until further analysis. The samples were centrifuged at 10000 rpm for 10 min at 4 °C. The supernatants were collected and heated at 85 °C for 10 min to inactivate residual enzymes, and stored at −18 °C until further analysis. All extraction experiments were carried out in triplicate.

2.6. Conventional extraction

The milled material was mixed with ethanol:water mixtures of varying ethanol concentrations at a solid-to-liquid ratio of 4% w/v for 24 h. Six solvent systems with increasing ethanol content (0, 20, 40, 60, 80, and 100% v/v) were investigated. Extractions were performed in a thermoshaker (Thermomixer®, Eppendorf) at 25 °C under constant agitation (1300 rpm), with a final working volume of 1.0 mL per run. The samples were centrifuged at 10000 rpm for 10 min at 4 °C, and the supernatants were collected and stored at −18 °C until further analysis.

2.7. Determination of total phenolic content

The total phenolic content (TPC) was assessed using the Folin–Ciocalteu method, following the procedure described previously [23]. The extract was combined with 250 µL of Folin–Ciocalteu reagent and 3.95 mL of distilled water, and the mixture was allowed to react at room temperature for 1 hour. Absorbance was then recorded at 755 nm. Results were expressed as milligrams of gallic acid equivalents per gram of dry material (mg GAE/g DW). All measurements were conducted in triplicate.

2.8. Determination of total flavonoid content

The total flavonoid content (TFC) was determined using the aluminum chloride colorimetric assay, as described in another study [23]. In summary, the extract was sequentially combined with sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium hydroxide (NaOH), and distilled water. The resulting mixture was allowed to rest at ambient temperature for 15 minutes. Absorbance was recorded at 510 nm and TFC values were expressed as milligrams of catechin equivalents per gram of dry material (mg CAE/g DW). All measurements were conducted in triplicate.

2.9. Antioxidant activity

The antioxidant capacity of the extracts was evaluated using the DPPH radical scavenging assay, following the protocol previously described [23]. In brief, varying concentrations of the extract were combined with a 2.5% (w/v) methanolic DPPH solution and incubated in the dark at ambient temperature for 30 minutes. Absorbance was recorded at 515 nm, and radical scavenging activity was expressed as the IC_{50} value, defined as the extract concentration required to inhibit 50% of the initial DPPH absorbance. All assays were performed in triplicate. IC_{50} values were derived from linear regression plots correlating extract concentration with percentage inhibition, and results were reported as microliters of extract per milliliter of sample (µL extract/mL).

2.10. Antibacterial activity

The antibacterial activity of the extracts against *Escherichia coli* was evaluated using the broth microdilution technique, as previously described [23]. Prior to testing, extracts were filter-sterilized (0.22 µm) to ensure sterility and prevent microbial contamination. Sterile extracts were first diluted in sterile culture medium. In a 96-well microplate, 50 µL of culture medium was dispensed into each well. Subsequently, 50 µL of each extract was added to the wells in the first column and thoroughly mixed.

A serial two-fold dilution was then performed by transferring 50 µL from one column to the next, continuing through column 10. Each well then received 50 µL of a bacterial suspension (10^8 CFU/mL). Column 11 served as the growth control (containing only bacterial suspension and culture medium), while column 12 functioned as the sterility control (containing only culture medium). Matrix controls were included where applicable (culture medium supplemented with the corresponding extraction medium, i.e., buffer or ethanol–water (ethanol concentration under 1% v/v), without extract) to account for any potential interference from the extraction solvent or buffer. The plate was incubated at 37 ℃ for 24 hours, after which the optical density was measured at 600 nm. All experiments were conducted in triplicate. The percentage of bacterial growth inhibition was calculated using the following equation:

$$\text{Inhibition of growth (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (3)$$

where $A_{control}$ is the absorbance of the growth control and A_{sample} represents the absorbance of cells containing the extracts.

2.11. Anti-aging activity (Tyrosinase inhibition)

Tyrosinase inhibition (EC 1.14.1.8.1) was assessed using a modified dopachrome assay with L-DOPA as the substrate, following the procedure described elsewhere [24]. All samples were diluted in phosphate buffer (50 mM, pH 6.8). In each well of a 96-well microplate, 25 µL of sample, 40 µL of tyrosinase solution (200 U/mL in the same buffer), 100 µL of buffer, and 40 µL of L-DOPA (10 mM in buffer) were added. The reaction mixtures were incubated at 25 °C for 10 minutes, after which absorbance was measured at 492 nm. All assays were performed in triplicate. Kojic acid, a well-established tyrosinase inhibitor, served as the positive control, representing complete enzymatic inhibition.

2.12. Anti-diabetic activity

2.12.1. Inhibition of a-amylase activity

α-Amylase inhibition was evaluated using a modified Caraway–Somogyi iodine/potassium iodide (IKI) assay [25]. In a 96-well microplate, 25 µL of sample was mixed with 50 µL of α-amylase solution (0.5 U/mL in phosphate buffer, pH 6.9, containing 6 mM NaCl) and pre-incubated at 37 ℃ for 10 minutes. The reaction was initiated by adding 50 µL of starch solution (0.025% w/v in the same buffer), followed by a second 10-minute incubation at 37 ℃. The reaction was terminated with 25 µL of 1 M HCl, and 100 µL of iodine–potassium iodide reagent was added. Absorbance was measured at 630 nm. Acarbose, an α-amylase inhibitor, served as the positive control, representing complete enzymatic inhibition. All measurements were performed in triplicate.

2.12.2. Inhibition of α-glucosidase activity

α-Glucosidase inhibition was assessed following an established protocol [23], utilizing p-nitrophenyl-α-D-glucopyranoside as the enzymatic substrate. Absorbance was recorded at 405 nm. Acarbose, a well-characterized α-glucosidase inhibitor, served as the positive control, representing

complete enzymatic inhibition. All measurements were conducted in triplicate.

2.13. Statistical analysis

Statistical analyses were conducted using Minitab® 18 software (Minitab Inc., State College, PA, USA). All experiments were performed in a randomized order to minimize systematic error and were conducted in triplicate. Results are presented as mean values ± standard deviation (SD) of three independent measurements (n = 3). Statistical analysis was carried out using analysis of variance (ANOVA), and differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Conventional extraction

Conventional extraction was performed in six different ratios of ethanol:water with increasing ethanol content (0, 20, 40, 60, 80, 100% v/v). Figure 1 shows the results of the total phenolic content (TPC) and total flavonoid content (TFC) measurements in all experiments performed.

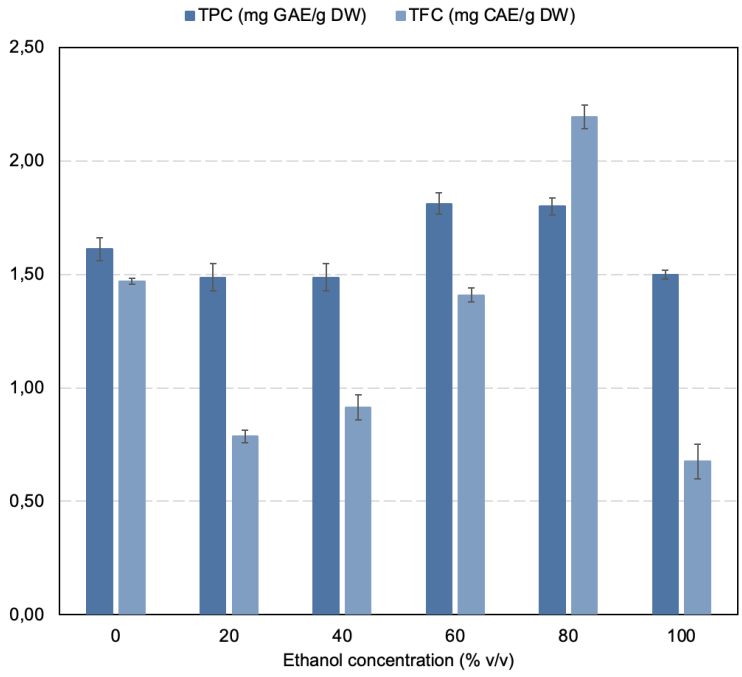


Figure 1. Total phenolic content (TPC) and total flavonoid content (TFC) results from the conventional extraction of WPO. Data are shown as mean values ± SD.

In general, as the ethanol content increased, both measured variables initially rose, reaching their respective maxima. Notably, both TPC and TFC yields were high at ethanol:water ratio 0:100 though lower than the maxima of both. Water efficiently extracts low-molecular-weight phenolic acids and polar flavonoid glycosides, which are often abundant in seagrass and other marine plants [26,27]. The highest TPC was observed at a 60:40 ethanol:water ratio, reaching 2.19 ± 0.05 mg GAE/g, whereas

the maximum TFC at an 80:20 ethanol:water ratio was 1.81 ± 0.04 mg CAE/g, showing a noticeable difference. Beyond this point, further increases in ethanol content led to a gradual decline, likely due to the differing polarities of the two solvents. As ethanol concentration increases, the overall polarity of the solvent mixture changes, which can become less effective at dissolving substances that are either too polar or too non-polar for the new solvent environment. This phenomenon is common in chemical processes, such as the extraction of compounds from plant materials, where the specific ratio of solvent polarities is crucial for achieving the desired outcome [28]. Moreover, the performance of ethanol:water systems can vary markedly across plant matrices and experimental designs. In general, intermediate ethanol–water mixtures often provide an effective balance for recovering phenolic compounds, although the optimal solvent composition is strongly matrix-dependent. For example, Lohvina et al. examined the effect of ethanol concentration on extraction yield and phenolic recovery from fenugreek seeds, showing that 70% ethanol provided the highest phenolic extraction efficiency [29]. Similarly, Kim and Chin (2017) found that 0–75% ethanol produced comparable yields, whereas 100% ethanol gave both the lowest yield and TPC [30]. In contrast, Mani and Thomas (2014) reported higher TPC in aqueous extracts of *Pittosporum dasycaulon* stem bark compared with methanolic extracts, highlighting the variability in solvent performance across plant matrices [31].

3.2. Taguchi method

The data of TPC and TFC obtained from the Taguchi method are presented in Table 2. The results indicated that phenolic and flavonoid extracted responded differently to the tested factors, emphasizing the importance of optimizing extraction conditions according to the target compound class.

Table 2. Results of total phenolic content (TPC) and total flavonoid content (TFC) of Taguchi design.

A/A	Solid to liquid ratio (% w/v)	Enzyme loading (Units/g)	Extraction time (h)	TPC (mg GAE/g)	TFC (mg CAE/g)	S/N (TPC)	S/N (TFC)
1	1	50	2	15.18 ± 0.15	4.99 ± 0.15	23.63	13.04
2	1	100	4	12.53 ± 0.95	6.06 ± 0.95	21.96	14.35
3	1	200	6	27.54 ± 0.84	9.22 ± 0.84	28.80	19.30
4	5	50	4	7.59 ± 0.19	3.80 ± 0.19	17.40	11.40
5	5	100	6	12.82 ± 0.39	4.41 ± 0.39	22.15	12.89
6	5	200	2	18.7 ± 1.03	3.24 ± 1.03	25.43	10.21
7	7	50	6	6.62 ± 0.28	4.05 ± 0.28	16.42	12.15
8	7	100	2	12.15 ± 0.64	2.85 ± 0.64	21.69	9.09
9	7	200	4	13.58 ± 0.36	3.57 ± 0.36	22.66	11.05

The mean response analysis showed clear effects of the tested factors on the extraction of phenolics and flavonoids from WPO (Figure 2). The solid-to-liquid ratio exhibited a strong influence, with the lowest ratio (1% w/v) yielding the highest TPC (27.54 ± 0.84mg GAE/g) and TFC (9.22 ± 0.84mg CAE/g). Increasing the solid-to-liquid ratio to 5% and 7% led to substantial decreases in both responses, indicating that higher solvent availability enhances mass transfer and promotes the release of soluble phenolic compounds [32]. Enzyme loading had the most pronounced effect on TPC, while

increasing the loading from 50 to 200 U/g resulted in a nearly twofold rise in mean TPC, across all solid-to-liquid ratios, confirming the importance of enzymatic hydrolysis in disrupting the cell wall matrix of WPO. TFC also increased with enzyme loading, particularly at the solid-to-liquid ratio of 1% w/v, where an approximately twofold enhancement was observed. In the higher ratios, the increase was more modest, suggesting that flavonoids require less extensive hydrolysis for their release compared to other phenolic subclasses. Extraction time influenced TPC and TFC differently. TPC showed a non-linear pattern, with high values at 2 h, a decline at 4 h, and recovery at 6 h. In contrast, TFC increased steadily with time, reaching its maximum at 6 h, indicating continuous liberation of flavonoids during prolonged hydrolysis. This trend possibly reflects a competing extraction and degradation processes that act differently on simple phenolic acids and on flavonoids. At the beginning of the extraction, easily accessible, low-molecular-weight phenolic acids and other simple phenolics diffuse rapidly into the solvent, giving the initial rise in TPC. However, in the literature, phenolic acids are reported to be readily hydrolyzed and oxidized in the presence of oxygen, light, and moderate heating, and longer extraction times increase the probability of such reactions [33,34]. Extended contact with the plant matrix also enables endogenous oxidative enzymes (polyphenol oxidase, peroxidase, laccase) to act, converting o- and p-diphenols into quinones that can further polymerize or bind to proteins and cell-wall polysaccharides, thereby becoming less soluble and less reactive in the Folin–Ciocalteu assay [35,36]. Kinetic studies of polyphenol extraction frequently describe this behavior as an interplay between a diffusion-controlled extraction term and a simultaneous first-order degradation term; when the degradation rate temporarily exceeds the net release rate, a local minimum in TPC is observed at intermediate times, as reported for various plant matrices under prolonged extractions. At longer times, further disruption of the plant structure and continued enzymatic hydrolysis of esterified or cell-wall-bound phenolics can release additional, more strongly bound phenolic fractions, causing the TPC to increase again despite the ongoing degradation of the most sensitive compounds [23,37,38]. Nevertheless, elucidating the exact cause would require targeted measurements (e.g., oxidation markers and polyphenol oxidase activity) and control of oxygen/light exposure in future studies.

In contrast, the TFC does not show a comparable decrease because flavonoids are, on average, structurally more stable and are extracted more slowly than simple phenolic acids. Several processing studies have demonstrated that TPC declines markedly with heating or extended treatment, whereas TFC either decreases less, remains unchanged, or increases, and this behavior has been explicitly attributed to the greater thermal and oxidative stability of flavonoid aglycones and glycosides compared with free phenolic acids [39,40]. Reports on polyphenol stability further emphasize that stability is strongly structure-dependent, with flavonoids (particularly glycosylated flavonols and flavanones) generally more resistant to oxidation than hydroxybenzoic and hydroxycinnamic acids [41].

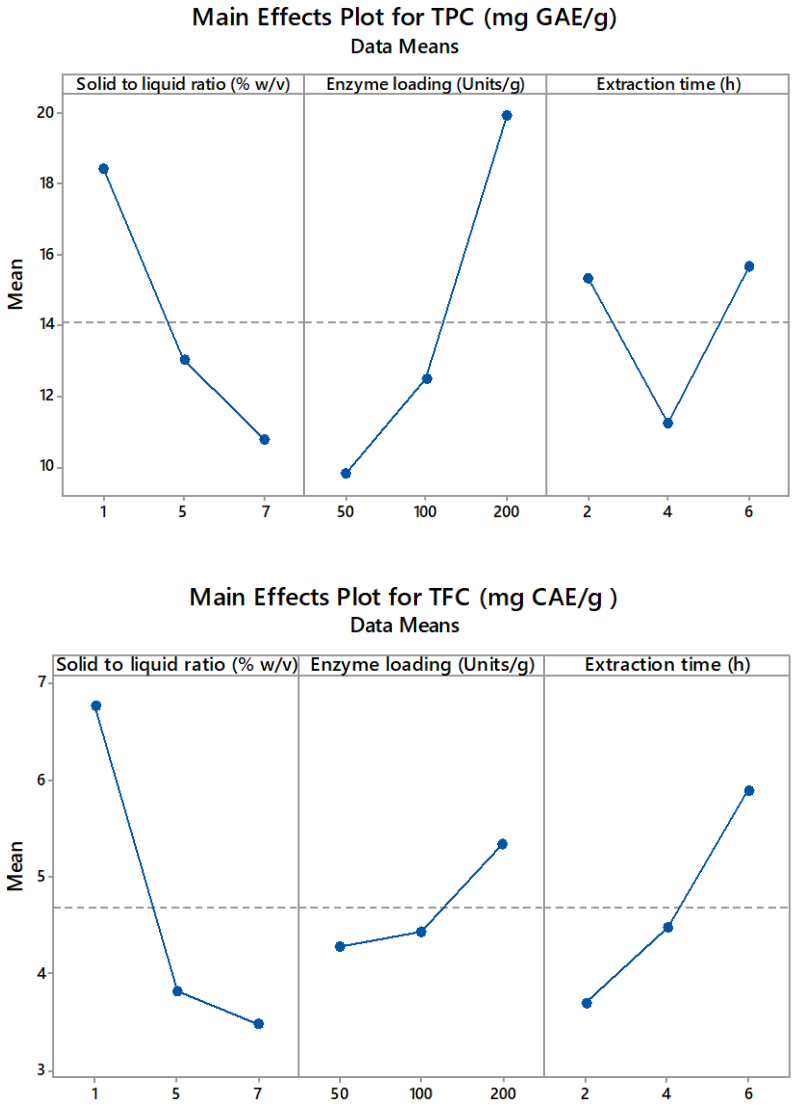


Figure 2. Major effects of factors on the mean values of the TPC and TFC from EAE of WPO.

Signal-to-noise (S/N) ratios were calculated using the "larger-the-better" criterion to maximize both responses independently based on Eq (1) (Table 2). Among the tested conditions, Run 3 stands out as the best-performing experiment for TPC and TFC. More importantly, its corresponding S/N ratios were also the highest in the dataset, indicating not only superior extraction yields but also high robustness and low susceptibility to random variability. In the context of Taguchi methodology, higher S/N ratios reflect more stable and reliable performance [22], meaning that Run 3 delivers consistently high extraction efficiency even under potential uncontrolled fluctuations.

The TPC and TFC data were fitted to the following quadratic models (Eqs (4) and (5)).

$$\mathrm{TPC} = 32.81 + 0.20\mathrm{SLR} - 0.20\mathrm{E} - 7.24\mathrm{t} + 0.04SLR^2 + 0.00067E^2 + 1.48t^2 + 0.01SLR * E - 0.86SLR * t \quad (4)$$

$$\mathrm{TFC} = 6.02 - 0.70\mathrm{SLR} - 0.04\mathrm{E} + 0.53\mathrm{t} + 0.09SLR^2 + 0.00014E^2 + 0.10t^2 + 0.001SLR * E - 0.17SLR * t \quad (5)$$

where SLR is the solid to liquid ratio (% w/v); E is enzyme loading (Units/g); and t is extraction time (h).

Both models showed a good fit, with coefficients of determination for TPC R^2 being 0.983 and R^2(adjusted) 0.933 and for TFC R^2 being 0.947 and R^2(adjusted) 0.787. Based on the ANOVA results, both models were statistically significant ($p < 0.05$). The choice of a second-order model was based on the expectation that extraction responses may exhibit nonlinear behavior. Unlike a first-order (linear) model, a second-order model includes quadratic terms, enabling it to capture response curvature, estimate local maxima/minima, and better represent non-linear trends and interactions among process variables within the studied range. Percentage contribution analysis (based on ANOVA) showed that TPC variability was driven primarily by enzyme loading ($p < 0.05$), which accounted for 53.50% of the total variation, followed by solid-to-liquid ratio with 29.82% and extraction time with 11.82%; the residual contribution was 4.86%. In contrast, for TFC, the dominant factor was solid-to-liquid ratio ($p < 0.05$), contributing 63.66% of the total variation, followed by extraction time with 24.45% and enzyme loading with 6.46%, while the residual contribution was 5.43%. Based on the response yields and the corresponding S/N ratios, the optimal extraction conditions were identified as a solid-to-liquid ratio of 1% w/v, an enzyme loading of 200 U/g, and an extraction time of 6 h. A confirmation experiment showed good agreement between predicted and experimental values, supporting the use of this model for preliminary optimization within the studied range, as under optimized conditions the predicted TPC and TFC values (30.26 mg GAE/g DW and 9.57 mg CAE/g DW, respectively) closely matched those obtained in the validation experiment (27.54 ± 0.84 mg GAE/g DW and 9.22 ± 0.84 mg CAE/g DW, respectively). Compared to the results of conventional extraction, EAE of bioactive compounds from WPO resulted in approximately 90% higher yields of TPC, while 75% higher TFC, while in 4-times lower extraction time. It should be noted that the conventional extraction was not independently optimized and it was included as a commonly used reference system. As operating conditions (e.g., temperature, pH, and agitation) can influence extraction performance, the comparison is intended to provide an indicative assessment of the added value of EAE. This method balances yield and efficiency, achieving high bioactive compound extraction within a significantly short extraction time. This not only enhances the process economic and environmental sustainability, but also minimizes the potential for oxidative degradation during extraction.

3.3. Effect of Viscoferm® on the extraction of WPO bioactive compounds

Following the experimental design and analysis of the results, an additional EAE was conducted using hemicellulolytic enzyme preparation Viscoferm® to evaluate its performance under the optimal extraction conditions determined by the Taguchi design. Among these, Viscoferm® achieved comparatively lower extraction efficiency, yielding only 10.54 mg GAE/g DW TPC and 6.93 mg CAE/g DW TFC, which represents approximately a 40% reduction relative to Cellic CTec3®. This reduced performance can be attributed to the differences in enzymatic selectivity between the two preparations.

A wide range of techniques has been applied in the literature to extract bioactive compounds, from mainly the WPO, each yielding markedly different levels of TPC, TFC, and antioxidant activity

(Table 3). These variations largely reflect differences in solvent polarity, extraction intensity, and the degree of structural disruption achieved prior to chemical extraction. However, direct comparison with the literature is inherently limited by differences in biomass origin, preprocessing, and reporting units; therefore, the cited values are discussed as contextual benchmarks rather than strict one-to-one comparisons.

Conventional solvent extractions generally produce the highest phenolic yields when medium- to high-polarity organic solvents are employed. For instance, sequential solvent extraction using ethyl acetate produced the highest TPC (85.71 mg GAE/g DW) and TFC (74.43 mg CAE/g DW) among all reviewed methods [42], indicating that semi-polar solvents such as ethanol, methanol, and ethyl acetate efficiently concentrate phenolic metabolites such as phenolic acids and flavonoids. This observation aligns with recent evidence showing that solvent polarity is a critical determinant of phenolic recovery and antioxidant activity, with semi-polar solvents consistently outperforming purely aqueous or highly non-polar systems [43,44].

Ethanol- and methanol-based extractions produced phenolic yields, with efficiency strongly dependent on solvent concentration and sample preprocessing. For example, extraction with 100% ethanol yielded 20.02 mg GAE/g DW TPC [42], while a 4-hour extraction with 60% ethanol resulted in very low TPC yields (0.126 mg GAE/g DW) [45]. Hydroalcoholic extraction at 70% ethanol yielded divergent results. Barletta et al. (2015) reported low phenolic recovery (7.4 ± 0.4 mg GAE/g DW) in a 3-hour extraction [46], while Messina et al. (2021) achieved higher yields (19.71 ± 0.50 mg GAE/g DW) when combining the solvent extraction with repeated grinding [47]. Moreover, methanol extractions were generally less efficient; solvent extraction with 50% methanol for 16h yielded <9 mg GAE/g DW phenolics, while sequential methanol/ether/ethyl acetate extraction increased yields to ~27 mg GAE/g DW and ~18 mg CAE/g DW [48]. Taken together, the literature shows that prolonged solvent extraction does not necessarily guarantee high phenolic recovery, as very long extraction times (e.g., 16 h) can result in relatively low yields, depending on the extraction system and biomass pretreatment.

Aqueous UAE produced relatively low TPC (7.6 mg GAE/g DW) compared to alcohol-based solvent techniques, yet the hybrid method combining ultrasound pretreatment with Soxhlet extraction achieved much higher yields (90.2 mg GAE/g DW), indicating that pretreatment increasing tissue permeability can significantly improve solvent penetration and phenolic recovery [15]. However, this high recovery was obtained after a 24 h extraction, which represents a substantial processing time.

In comparison, the EAE performed in this study, using an aqueous buffer (pH 5.5, 50 °C, 4 h), produced 27.54 ± 0.84 mg GAE/g DW TPC and 9.22 ± 0.84 mg CAE/g DW, TFC. While the TPC of EAE are lower than that achieved by strong organic solvent systems such as ethyl acetate extraction or ultrasound–Soxhlet hybrid extraction, its performance is comparable to or higher than several aqueous or mild solvent extractions (Table 3). Extraction duration is another critical factor influencing yield and sustainability. Conventional solvent extractions often require extended times to achieve appreciable recovery (Table 3). In contrast, the EAE conducted in this study was completed in 6 h under mild aqueous conditions (pH 5.5, 50 °C). The duration of EAE compares favorably with many conventional methods, particularly when considering that no organic solvents or prolonged heating are required. The relatively short extraction time, combined with mild operating parameters, reduces energy consumption and minimizes degradation of thermolabile phenolics, offering a practical balance between efficiency and sustainability. By contrast, solvent-intensive methods often achieve higher yields but at the cost of long extraction times (16–24 h), elevated temperatures, and greater environmental burden. Importantly, EAE represents a green and sustainable alternative, as it avoids

the use of hazardous solvents, reduces chemical waste, and operates under conditions that preserve sensitive metabolites [49,50]. Enzymatic hydrolysis facilitates the release of bound phenolic compounds from the cell wall matrix, explaining why yields approach those of solvent extractions despite using only buffer and biocatalysts. Compared to conventional methods, EAE thus offers a favorable balance between extraction efficiency, environmental safety, and product purity, making it particularly suitable for applications where solvent-free extracts are required, including food, nutraceutical, and cosmetic formulations. Although further optimization may be needed to enhance antioxidant potency, our findings confirm that EAE can deliver competitive yields while aligning with the principles of green chemistry and sustainable bioprocessing [20,51–53].

3.4. Biological activities

The obtained extracts from Cellic CTec 3® HS and Viscoferm® were subsequently analyzed for their biological activities, including, antioxidant, anti-aging, anti-diabetic, and antimicrobial.

3.4.1. Antioxidant activity

Antioxidant in vitro properties are associated with phenolic compounds as they contribute to the protection against chronic diseases, heart and cancer related, by neutralizing reactive oxygen species (ROS) such as hydroxyl radicals and peroxy radicals. In this study, the antioxidant activity of the extracts was assessed with the DPPH method and expressed as IC_{50} values. The extract derived from Cellic CTec3® HS and Viscoferm® exhibited 203.72 ± 23.99 µL extract/mL and 316.11 ± 29.37 µL extract/mL, respectively.

Numerous extraction methods have been employed for *P. oceanica* biomass, and the resulting antioxidant yields vary considerably depending on solvent polarity, extraction conditions, and the degree of prior structural disruption (Table 3). However, direct comparison with the literature is inherently limited by differences in in vitro assays and reporting units; therefore, the cited values are discussed as contextual benchmarks. For example, sequential solvent extraction with ethyl acetate produced strong antioxidant activity (DPPH IC_{50} = 1.19 mg/mL) [42], whereas hydroalcoholic extraction at 70% ethanol yielded inconsistent outcomes, ranging from moderate activity reported by Barletta et al. [46] (IC_{50} = 6.01 ± 0.55 mg/mL) to markedly higher potency observed by Messina et al. [47] (IC_{50} = 0.090 µg/µL). In contrast, methanolic extracts generally exhibited antioxidant capacity (~7 µmol TE/mg DW), and a sequential methanol/ether/ethyl acetate protocol produced similar results (~8 µmol TE/mg DW) [48].

Table 3. Total phenolic content, total flavonoid content, and antioxidant activity of *Posidonia oceanica* extracts obtained by different extraction methods across the literature.

Extraction method	Conditions	TPC (mg GAE/g DW)	TFC (mg CAE/g DW)	Assay	Antioxidant activity	Ref
Aqueous extraction	Water, 90 ℃, 2h	37.9 ± 8.0	n.d.	ATBS	236.5 ± 16.5 μmol Trolox equivalents (TE)/g extract	[15]
Solvent extraction	Solvent: 50% (v/v) aqueous ethanol, 40 ℃, 3 h	27.7 ± 2.4	15.33 ± 1.4 mg QE/g DW	DPPH	IC_{50} = 4.0 ± 0.03 mg/mL	[54]
Solvent extraction (x2)	Solvent: 60% (v/v) aqueous ethanol, room temperature, 4 h	0.13	n.d.	DPPH,	IC_{50} = 32.0 ± 2.0 μg/mL	[45]
Solvent extraction	Solvent: 100% Ethanol	20.0 ± 1.57	16.89 ± 2.58	DPPH	IC_{50} = 1.78 ± 0.018 mg/mL	[42]
Solvent extraction	Solvent: 70% (v/v) aqueous ethanol, room temperature, overnight, 3h, at 65 ℃	7.4 ± 0.4	n.d.	DPPH	IC_{50} =6.01 ± 0.55 mg/mL	[46]
Solvent extraction	four grinding cycles and extraction with 70% (v/v) aqueous ethanol	19.7 ± 0.496	n.d.	DPPH	IC_{50} = 0.090 μg/μL	[47]
Solvent extraction	Solvent: 50% (v/v) aqueous methanol, 5 ℃, 16h	< 9	~4.0	ATBS	~7 μmol TE/mg DW	[48]
Solvent extraction	Solvent: ethyl acetate	85.7 ± 0.59	74.43 ± 4.25	DPPH	IC_{50} = 1.19 ± 0.055 mg/mL	[42]
Ultrasound-assisted extraction (UAE)	Solvent: water power 400 W, frequency 24 kHz, 30 min	7.6 ± 2.2	n.d.	ATBS	275.9 ± 10.2 μmol Trolox equivalents (TE)/g extract	[15]
Combined ultrasound pretreatment and Soxhlet extraction	Ultrasound (Solvent: water power 400 W, frequency 24 kHz, 30 min) Soxhlet (solvent: toluene/ethanol (2/1), 24 h)	90.2 ± 17.6	n.d.	ATBS	730.0 ± 12.2 μmol Trolox equivalents (TE)/g extract	[15]

Continued on next page

Extraction method	Conditions	TPC (mg GAE/g DW)	TFC (mg CAE/g DW)	Assay	Antioxidant activity	Ref
Sequential solvent extraction	Extraction with 80% (v/v) aqueous methanol, 5 °C, 18h, acidification, extraction with diethyl ether/ethyl acetate	27.0	~18.0	ATBS	~8 µmol TE/mg DW	[48]
EAE	Solvent: buffer pH 5.5 50 °C, 6 h	27.5 ± 0.8	9.2±0.8	DPPH	IC_{50} = 203.72 ± 23.99 µL extract/mL	Present study

3.4.2. Antibacterial activity

The antibacterial activity of the extracts was measured based on monitoring *E. coli* growth (OD_{600} nm) in the presence and absence of the extracts (Figure 3) and was determined to be 21.37 ± 2.00 % and 17.36 ± 1.67 % for the extract derived using Cellic Ctec 3® HS and Viscoferm®, respectively.

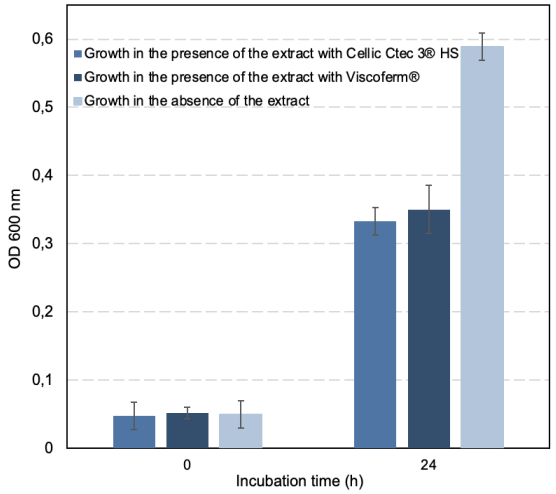


Figure 3. Effect of WPO extract derived from EAE with different enzyme preparations on *E. coli* growth. Bacterial growth was monitored by measuring optical density at 600 nm (OD_{600}) in the presence and absence of the extracts at 0 and 24 h of incubation. Data are expressed as mean values ± SD.

Several studies have reported the antibacterial properties of WPO extracts obtained through different extraction techniques. Berfad et al. [55] reported that the antibacterial effects of WPO depended on the bacterial strain and the extracting solvent. The largest inhibition zones were recorded against *Pseudomonas aerug*inosa and *Staphylococcus aureus*, while *Proteus mirabilis* was highly resistant. Among the solvents tested, the cyclohexane extract displayed a high concentration of phytochemicals (notably strong phlobatannins and sterols) and produced one of the strongest inhibition zones, while acetone also extracted the most compounds and showed pronounced activity. Ethanol and ethyl-acetate extracts yielded sizeable zones against *P. aeruginosa* and, to a lesser extent*, S. aureus*; ethyl acetate was noted as having weaker activity overall. The study linked the observed antibacterial activity to the presence of specific secondary metabolites identified in each extract (e.g., sterols, phlobatannins, reducing sugars, and phenols), suggesting that solvents that solubilized higher amounts of these compounds corresponded to greater antibacterial effects. This perspective is further supported by Felemban et al. [56], who studied the aqueous extract of *P. oceanica* leaves prepared by Soxhlet extraction for 30 min and showed strong antibacterial activity against several pathogenic strains. The inhibition zones measured were 21.33 mm for *E. coli* and 13.33 mm for *Listeria monocytogenes*; comparable activity was observed against *Salmonella typhi* (20.33 mm) and *S. aureus* (20.67 mm). The authors attribute the antibacterial activity mainly to the rich polyphenolic profile of the extract revealed from GC-MS, noting that hydroxyl-rich phenolics enhance membrane disruption and intracellular inhibition in bacteria and linked the observed activity to their synergistic effects within the crude extract.

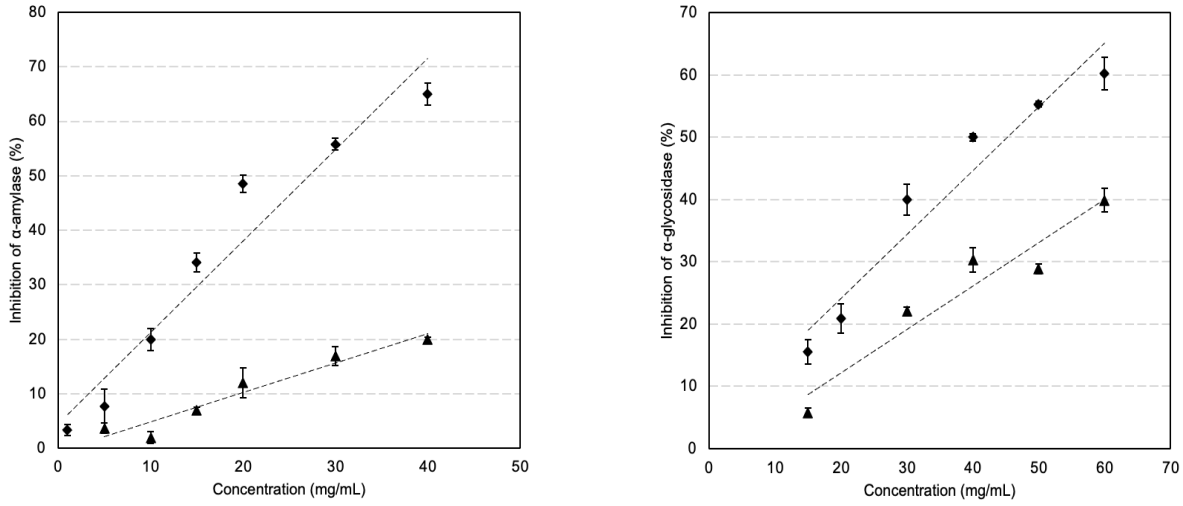
3.4.3. Antidiabetic activity

Inhibitors of α-amylase and α-glucosidase have attracted considerable interest due to their therapeutic relevance in managing post-prandial hyperglycemia and type 2 diabetes. They play key roles in carbohydrate digestion, as α-amylase initiates the hydrolysis of complex starches and α-glucosidase catalyzes the final step that releases absorbable glucose. Their inhibition slows carbohydrate breakdown and glucose absorption, resulting in a moderated glycemic response after meals and reduced metabolic stress. This mechanism underlies the clinical use of synthetic inhibitors such as acarbose; however, their gastrointestinal side effects have prompted growing interest in natural, plant-derived alternatives with improved tolerability. Extracts capable of inhibiting these enzymes may therefore offer dual benefits by supporting glycemic control and contributing to the broader prevention of metabolic disorders [57–60].

The extracts obtained with Cellic CTec3® HS and Viscoferm® exhibited relatively weak α-glucosidase and α-amylase inhibitory activity compared to the positive control acarbose (1 mg/mL, 100% inhibition). Their α-glucosidase IC_{50} values were 74.42 ± 3.61 mg/mL and 43.84 ± 1.29 mg/mL, respectively, while the corresponding α-amylase IC_{50} values were 93.53 ± 7.01 mg/mL and 38.42 ± 2.45 mg/mL (Figure 4). Although both extracts were less potent than acarbose, the Viscoferm® extract consistently demonstrated stronger inhibitory capacity, particularly against α-amylase. The stronger α-amylase and α-glucosidase inhibition observed for the Viscoferm® extract likely reflects the nature of the compounds released during hemicellulolytic degradation. Hemicellulose-rich regions of the *P. oceanica* cell wall may contain flavonoid glycosides, complex phenolics, and low-molecular-weight oligosaccharides, which are molecules known to inhibit carbohydrate-digesting enzymes [61,62]. Although Cellic CTec3® yielded higher overall TPC and TFC, these compounds may be less effective

inhibitors, as glycosidase inhibition depends more on specific structural features than on total phenolic quantity. The breakdown of hemicellulose by Viscoferm® likely liberated a more active mixture of inhibitory metabolites, explaining its superior antidiabetic activity. However, targeted compositional profiling (e.g., HPLC-DAD and/or LC–MS) is needed to identify the compounds responsible for the observed differences and verify the hypothesis.

For comparison, extracts from the green seaweed *Halimeda tuna* have also been evaluated for antidiabetic activity, with the ethyl acetate fraction showing α-amylase inhibition comparable to acarbose (IC_{50} = 0.88 ± 0.20 mg/mL vs. 0.76 ± 0.04 mg/mL), whereas the methanol extract and ethyl acetate fraction exhibiting α-glucosidase inhibitions of 0.05 ± 0.01 mg/mL and 0.01 ± 0.00 mg/mL, respectively, relative to acarbose (0.27 ± 0.13 mg/mL) [63]. Similarly, a large screening study of 45 crude seaweed extracts from 31 species of Ochrophyta, Rhodophyta, and Chlorophyta collected along the Mexican coastline reported a wide range of α-amylase and α-glucosidase inhibitory activities [64]. Notably, species such as *Cladophora dalmatica*, *Ectocarpus siliculosus*, *Padina boergesenii*, and *P. gymnospora* exhibited varying inhibitory strengths across the two enzymes. These activities in both studies were attributed to the distinct metabolite profiles of each species. Importantly, the variability across species highlights how differences in matrix composition, phytochemical diversity, and dominant metabolite classes can strongly influence α-amylase and α-glucosidase inhibition, underscoring that inhibitory potency is highly species- and chemistry-dependent rather than directly comparable across marine matrices.



(a) (b)

Figure 4. Inhibitory activity of the extracts against (a) α-amylase and (b) tyrosinase at various concentrations. Results are expressed as percentage inhibition (%). The dashed line represents the data fitted to linear trendline. (▲) Extract derived from Cellic CTec3® HS and (◆) Extract derived from Viscoferm®. The data are expressed as mean values ± SD.

3.4.4. Antiaging activity

Melanin is a biological pigment found in many organisms, and functions as a protective barrier against ultraviolet (UV) radiation and reactive oxygen species (ROS), contributing to skin

photoprotection and phenotype. Excessive melanin production, however, can lead to skin discoloration driven by heightened melanocyte activity and overactivation of tyrosinase. Tyrosinase, a copper-containing metalloenzyme also known as polyphenol oxidase, catalyzes the two initial, rate-limiting steps of melanin biosynthesis: The monophenolase reaction, in which L-tyrosine is hydroxylated to L-DOPA, and the diphenolase reaction, which oxidizes L-DOPA to DOPAquinone. Such skin hyperpigmentation disorders include lentigo, melanoma, melasma, and melanosis. Treatment strategies typically rely on skin-lightening agents that reduce melanin levels either by directly inhibiting tyrosinase activity or by suppressing its expression at the transcriptional or translational level [65–67].

In this study, the extract derived from Cellic CTec3® HS and Viscoferm® at a concentration of 200 mg/mL exhibited 41.62 ± 0.10% and 18.69 ± 0.57%, respectively, showing the distinct difference in enzyme's selectivity, as mentioned above. Compared to the positive control, kojic acid (1 mg/mL, 100% inhibition), the extract demonstrated weak activity; nevertheless, it was promising after optimization. *P. oceanica* has not been extensively investigated for its anti-melanogenic properties; however, an ethanolic extract has been reported to inhibit mushroom tyrosinase with an IC_{50} of approximately 50 μg/mL, while exhibiting low cytotoxicity (IC_{50} > 100 μg/mL in MeWo melanoma cells) [45]. The extract demonstrated dose-dependent skin-whitening activity, characterized by a weak and non-logistic tyrosinase inhibition profile indicative of multiple inhibitory constituents; yet, it elicited a comparatively stronger demelanizing effect in melanoma cells. This suggests that its whitening activity likely involves partial tyrosinase inhibition and additional modulation of melanogenesis-related pathways, supporting its potential relevance in anti-hyperpigmentation formulations. Researchers identified dozens of marine-derived metabolites capable of inhibiting tyrosinase, the rate-limiting enzyme in melanogenesis, thereby highlighting their therapeutic potential for hyperpigmentation disorders [68]. Among the most potent inhibitors reported are 7-phloroeckol from *Ecklonia cava* (IC_{50} = 0.85 μM), arenarol from *Dysidea arenaria* (IC_{50} < 3 μM in B16 melanoma cells), luteolin-7-sulfate, fucoidan, and fucoxanthin. Additional phlorotannins, including eckol, dieckol, and phloroglucinol, also exhibit notable tyrosinase-inhibitory activity. These marine compounds are considered promising leads to replace conventional agents such as hydroquinone, which carry safety concerns.

4. Conclusions

This study demonstrates that EAE, particularly with the cellulolytic preparation Cellic CTec3®, markedly enhances the recovery of phenolics and flavonoids from WPO, yielding extracts with measurable antioxidant, anti-aging, and antibacterial activities in vitro. The hemicellulolytic preparation Viscoferm® produced comparatively lower phenolic yields but showed greater α-amylase and α-amylase inhibition, highlighting the impact of enzyme specificity on bioactive compound profiles. The biological activities reported provide a useful screening-level evaluation of crude WPO extracts and support their potential as multifunctional bioactive ingredients. As expected for complex natural extracts, the observed IC_{50} values were higher than those of pure reference compounds; however, their practical relevance will depend on achievable concentrations in real formulations and on factors such as safety, stability, and formulation compatibility. These results therefore offer a strong basis for future formulation-oriented studies. Overall, the results confirm WPO as a sustainable and valuable source of multifunctional bioactive compounds with promising applications in health, nutraceutical, and cosmetic formulations. Future studies may expand our dataset by evaluating biomass

from different collection sites and seasons to capture potential variability in composition and bioactivity. The encouraging in vitro bioactivities reported here also motivate the next application-oriented steps, including toxicity/safety assessment of the extracts, as well as stability and formulation compatibility studies. In parallel, researchers should focus on compound-level identification (e.g., HPLC and LC-MS/MS) of the most active constituents released through EAE to better understand the compounds responsible for the observed biological activities. Exploring additional enzyme cocktails, including tailored combinations of cellulolytic and hemicellulolytic activities, may further enhance extraction efficiency and diversify the recovered bioactive profiles. Moreover, scaling up the optimized extraction process and evaluating the stability and efficacy of WPO extracts in real cosmetic and nutraceutical formulations will be essential to assess their commercial potential. Finally, environmental and economic assessments could support the development of sustainable valorization strategies for WPO, contributing to circular bioeconomy approaches that convert an underutilized marine resource into high-value products.

Author contributions

Conceptualization: DM, TL; Data curation: SK; Formal Analysis: ZL, SK; Investigation: DA; Methodology: DM, TL; Resources: DM; Supervision: DM, TL; Validation: DA, ZL, SK; Visualization: DA, ZL, SK; Writing – original draft: ZL; Writing – review & editing: DM.

Use of Generative-AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare they have no conflict interest in this paper

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