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In vitro evaluation of functional properties of extracts of *Fucus vesiculosus* obtained with different conventional solvents

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ABSTRACT

Fucus vesiculosus is a rich source of bioactive substances with many biochemical functions that provide it a variety of biological effects. Over the years, significant research efforts have been made to extract bioactive compounds by applying different methodologies for various applications. There are several solvents used for the extraction of natural products since the choice of solvent must be based primarily on the characteristics of the matrices and the properties of the molecular classes to be obtained. Therefore, the aim of this study was to investigate the efficiency of different conventional solvents to maximize the yield of polyphenol and flavonoid content as well as the antioxidant, antimicrobial and anti-inflammatory capacity. The different extracts of *F. vesiculosus* were analyzed for the Total Polyphenol Content (TPC) and the Total Flavonoid Content (TFC). As well the antioxidant, antimicrobial, and anti-inflammatory capacities were evaluated. The results concerning the content of bioactive molecules disclosed that the extraction carried out with the methanol (50 %) was the one that gave the highest yield in both polyphenol (2.27 ± 0.17 mg GAE/ 50 mg of sample) and flavonoid content (187.12 ± 12.86 mg CE/50 mg of sample) compared to acetone and ethanol extracts. Regarding the functional properties, the results obtained disclose that the extract of *F. vesiculosus* had a high antioxidant capacity (90 % inhibition of radical scavenging activity). Additionally, the growth inhibition assay disclosed that *F. vesiculosus* can reduce significantly ($p < 0.05$) the growth of *E. coli* $F18^+$, in particular when the alga is extracted with methanol and acetone. As well, a concentration of 1 mg/mL of *F. vesiculosus* inhibits the protein denaturation by 60 %, highlighting a potential anti-inflammatory activity. In conclusion, this study discloses the richness of bioactive molecules in *F. vesiculosus* and the resulting functional properties, highlighting also the power of methanol as extraction solvent.

1. Introduction

Marine resources exhibit substantial potential within the nutritional and nutraceutical field due to their abundance of bioactive compounds, such as polysaccharides, peptides, amino acids, and phenolics, including components like phlorotannins, vitamins, and carotenoids. The wide range of applications of algae is due to several factors such as the absence of drug resistance induction, the capability of certain compounds or complexes to act synergistically in reducing toxicity and enhancing drug effectiveness, their environmentally friendly nature due to high availability and easy degradability, their potential to alleviate inflammation and pain and their ability to combat chronic ailments like cancer and diabetes [1,2]. Among all the seaweeds, the genus Fucus is widely distributed and, in recent years, has garnered increased attention. This genus comprises 66 taxonomically accepted species, of which *Fucus vesiculosus* is the best known, mainly findable in the cold-temperate waters of littoral and sublittoral regions along the rocky coasts of the northern hemisphere [3]. Seaweeds belonging to the Fucus genus offer a unique blend of macro- and micronutrients that make them particularly interesting from a nutritional perspective such as the high content of dietary fiber, minerals, and vitamins, associated with low fat levels [4]. From a functional point of view, Fucus *spp.* has gained

Abbreviations: ABTS, (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)); BSA, bovine serum albumin; CE, catechin equivalents; GAE, gallic acid equivalent; $NO^{\bullet}$, nitric oxide; OD, optical density; PI%, percentage of the inhibition of radical scavenging activity; PLE, pressurized liquid extraction; QE, quercetin equivalent; RT, room temperature; TAE, tannic acid equivalent; TFC, total flavonoid content; TPC, total phenolic content.

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popularity due to its high iodine content [5]. Additionally, this genus serves as a rich source of bioactive compounds like fucoidans, phlorotannins, and fucoxanthin, which have demonstrated significant therapeutic potential in treating blood clot formation, rheumatoid arthritis, asthma, atherosclerosis, diabetes, psoriasis, skin ailments, cancer, and various oxidative and inflammatory conditions [6]. These characteristics make Fucus *spp.* a promising functional and/or active ingredient with substantial potential not only in the cosmetic and pharmaceutical sectors but also in the food and nutraceutical industries. Additionally, to the better-known applications, *Fucus vesiculosus* could also find its use in animal nutrition, where it could be employed to promote animal health while counteracting the issue of antibiotic resistance. Since its well-known bioactive properties, *Fucus vesiculosus* has been previously studied due to the wealth and diversity of natural products that can be extracted from it and their promising applications [7]. Therefore, the extraction of bioactive compounds, which may be the cause of the discordant results reported in the literature, becomes a key process to profit from both their uses and the residue. Various extraction methods have been developed. The more traditional method was conventional solvent extraction, which involves using organic solvents to dissolve and extract bioactive compounds from algae. It is widely used due to its simplicity and effectiveness in extracting a broad range of compounds. However, it often involves toxic solvents, which pose environmental and health risks [8,9]. To address the problem of toxicity in recent years, organic solvents have been replaced with water. However, this type of extraction has proven to be less effective by requiring longer extraction times and temperatures [10]. Other techniques widely used were the Microwave-Assisted Extraction (MAE), which exploit microwave energy to heat the solvent and matrix, enhancing the extraction efficiency [11,12] and the Ultrasound-Assisted Extraction (UAE) which through ultrasonic waves, disrupts cell walls, facilitating the release of intracellular compounds proving effective in the extraction of both polar and nonpolar compounds [12,13]. Recent advancements have introduced novel extraction techniques like Supercritical Fluid Extraction (SFE). This method utilizes supercritical fluids, primarily carbon dioxide (CO_2), to extract compounds from various matrices, offering numerous advantages such as environmental friendliness, absence of solvent residues, and enhanced extraction efficiency. This technology was particularly advantageous in the food industry for extracting edible oils and, in recent years, gained importance also for the extraction of secondary metabolites, offering new avenues for bioactivity-guided isolation and the discovery of novel compounds [14–16]. Regardless of the technology used in the extraction process the extraction conditions, including the solvent and its concentration, solvent/sample ratio, temperature, and time, are one of the major factors that can influence the type, amount, and activity of the extracted compounds. For example, in fact, an excessive extraction time and elevated temperatures might cause the degradation of the bioactive molecules or allow interaction with the solvent itself that can lead to a change in the chemical structure of the molecule [17–20]. Additionally, another important thing that must be considered is the choice of extraction solvent is it polarity. Indeed, it is known that different bioactive compounds move within extraction solvents according to the polarity of the latter. As concerns plant matrices, according to the literature reported, from 18 to 94 % of phenolic compounds, from 3 to 55 % of total flavonoids, 11 % of total phenols, from 20 to 30 % of tannins, 60 % of carotenoids, and from 40 to 88 % of chlorophyll are recovered by hydrophilic solvents, such as water, acetone, methanol, ethanol [21–24]. Although solvents such as acetone, ethanol, and methanol are able to ensure excellent yield of the bioactive component of the extracted matrix their potential toxicity must also be taken into account. For this reason, they are they are preferably used within non-toxic concentration levels. However, the effectiveness of solvents and extraction methodologies is not solely dictated by solvent polarity but also by the solvent class (mixing polar and non-polar solvents), which can directly impact compound solubility and the desorption capacity of seaweed cellular walls. Given, therefore, the high variability in terms of yield that different extraction solvents can have towards the same matrix this study aims to evaluate the extraction efficacy of three different solvents, acetone, ethanol, and methanol, on the determination of the main bioactive compounds, polyphenols and flavonoids, and related functional activities, such as antioxidant, antimicrobial and anti-inflammatory, peculiar to *Fucus vesiculosus*. Given the recent interest in *Fucus vesiculosus*, the results of this study may provide a useful reference for the evaluation of bioactive components found in *Fucus vesiculosus* extracts depending on the extraction solvent used, as well as provide an overview of the functional activities of this alga.

2. Materials and methods

2.1. Seaweed biomass

Fucus vesiculosus was purchased from Sevecom S.p.a (Milan, Italy) as dried whole seaweed with 90 % dry matter content. The whole seaweed was coarsely pounded by a mortar. Then ten subsamples of 5 g each were taken and ground at 0.05 mm through a mill (Retsch, Bergamo, Italy), then mixed to obtain a representative sample of the whole seaweed to be used in subsequent analyses (Fig. 1).

2.2. Extraction procedure

For the extraction, 1 g of algae powder was mixed with either 20 ml of 70 % aqueous acetone (VWR Chemicals, Milan, Italy), 80 % ethanol (VWR Chemicals, Milan, Italy) or 50 % methanol (VWR Chemicals, Milan, Italy) (*v*/v) for 1 h at 200 rpm at room temperature (RT). After that, the solutions were centrifuged at 5000 rpm for 10 min at 4 °C, and the extracts were filtered with a 0.22 μm RC filter [25]. The extract's filtration guarantees its microbiological purity, as reported by the Food and Drug Administration (FDA, USA) and the United States Pharmacopoeia (USP) [26,27]. The samples were stored at −20 °C for future analysis.

2.3. Evaluation of total polyphenol content (TPC) and total flavonoid content (TFC)

Starting from a concentration of 50 mg/ml and proceeding with 1:2 scalar dilutions until the concentration of 1.56 mg/ml the phenolic content of *Fucus vesiculosus* extracts was evaluated by the Folin-Ciocalteu method, according to Shakya et al. [28]. Briefly, the assay was performed by reacting 10 μL of extracted sample/standard with 100 μL of Folin-Ciocalteu reagent (Sigma-Aldrich, Darmstadt, Germany), and 90 μL sodium carbonate (1 M) (Sigma-Aldrich, Darmstadt, Germany). The reaction mixture was incubated for 1 h at RT in the dark. Total phenolic content was determined spectrophotometrically at 765 nm (BioTek Epoch, Agilent, Santa Clara, CA, United States). The same concentrations were also tested for the flavonoid content according to the method described by Herald et al., [29]. Briefly, 25 of extract, 100 μl of deionized water, and 10 μL of $NaNO_2$ (50 g/L) (Sigma-Aldrich, Darmstadt, Germany) were combined and left for 5 min of incubation at RT. Subsequently, 15 μL of $AlCl_3$ (100 g/L) (Sigma-Aldrich, Darmstadt, Germany) was added to the solution. The solution was left to stand for 6 min, after which 50 μL of NaOH (1 mol/L) and 50 μL of deionized water were added. Finally, the absorbance at 510 nm was measured against the reagent blank.

All assays were performed in technical triplicate and with three biological replicates meant to verify the replicability of the experiment using the same procedures, which included repeating the investigation starting from the sample extraction and repeating the test on different days. The calibration curves were constructed using the average absorbance of the two-calibration series, and the coefficient of determination (R^2) of the calibration curve was used to evaluate the linearity of the curve. Specifically, gallic acid (Sigma-Aldrich, Darmstadt, Germany)

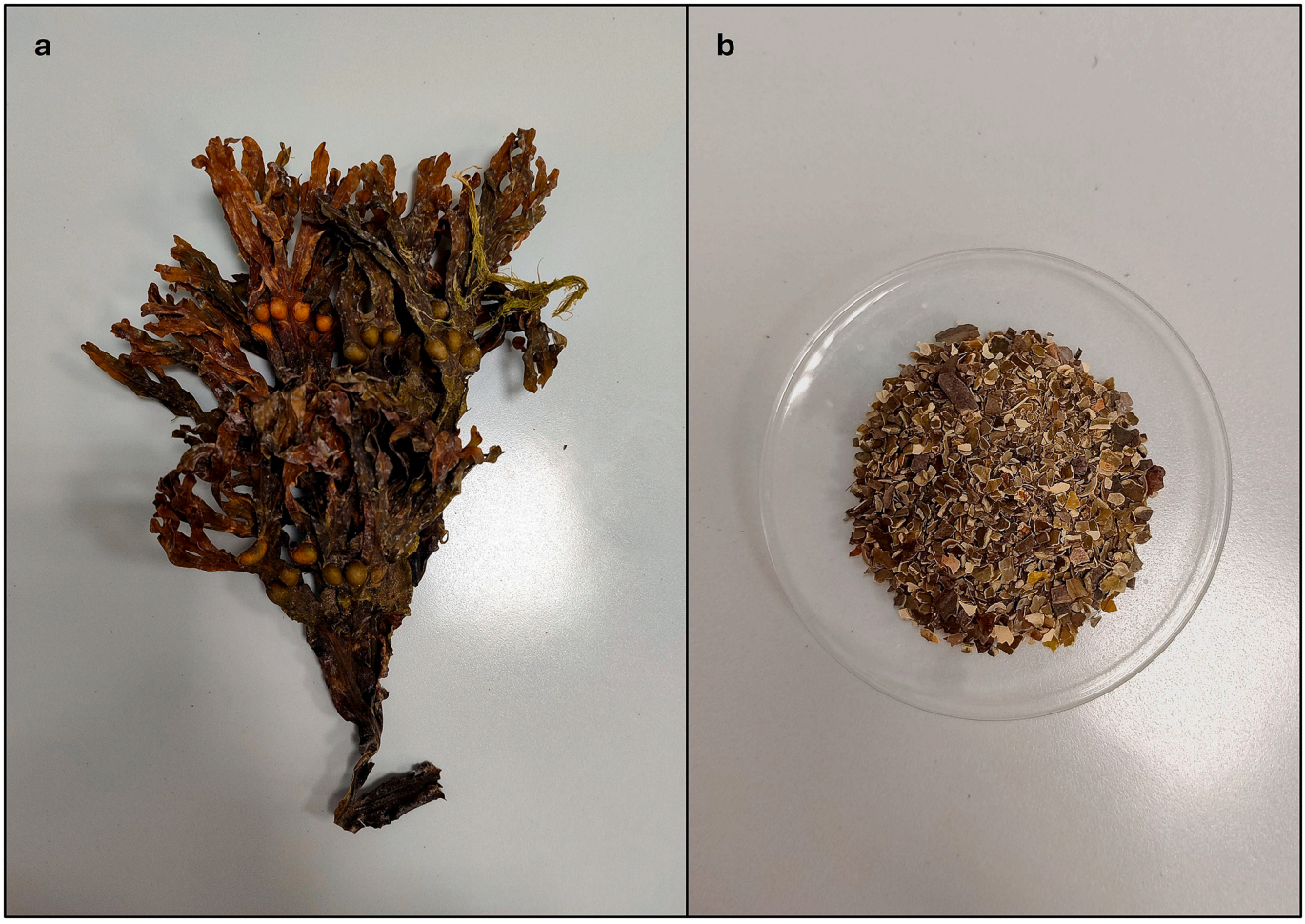

Fig. 1. (a) *Fucus vesiculosus* as dried whole seaweed. (b) subsample of *Fucus vesiculosus.*

was used as a standard for TPC in the 0.010–0.50 μg/mL range. While catechin (Sigma-Aldrich, Darmstadt, Germany) was used as a standard for TFC in the 7–250 μg/mL range. The limit of detection (LOD) was calculated as 3.3σ/s, where σ is the standard deviation of the response, and S is the slope of the calibration curve [30]. The LOD was 0.0039 mg GAE/mL and 4.386 mg CE/mL for polyphenols and flavonoid detection, respectively. The gallic acid and catechin concentration in each extract was calculated from the regression equation using their absorbance. The results were then converted to the TPC as milligrams of Gallic acid equivalent per gram of dry extract (mg GAE/g) and to TFC as milligrams of catechin equivalent per gram of dry extract (mg CE/g) using the following equation [31]:

$$\mathbf{T = C^{*}\frac{V}{M}}$$

where T is the TPC or the TFC, expressed as mg GAE/g or mg CE/g respectively; C is the concentration of the standard (gallic acid or catechin) established from the calibration curve in mg/mL, V is the volume of the extract in mL, and m is the weight of the dry plant extract in g.

2.4. Evaluation functional properties

2.4.1. ABTS radical scavenging activity

The scavenging activity of *Fucus vesiculosus* was evaluated through ABTS assay as previously done in our works [1]. Briefly, 10 μL of the sample was added to 1 mL of $ABTS^{\bullet+}$ working solution. The absorbance was recorded after 6 min of incubation in the dark, and all determinations were performed in technical triplicate and with three biological replicates. The results were expressed as the percentage of the inhibition of radical scavenging activity (PI%), where 100 % inhibition was considered as Trolox's capacity at a concentration of 2000 μM to inhibit the radical scavenging activity of the $ABTS^{\bullet+}$ working solution.

2.4.2. Growth inhibition assay

A liquid culture-based growth inhibition assay with *Escherichia coli* O138, belongs to our strain collection [32], was performed to evaluate their ability to inhibit bacterial growth. An overnight culture of *E. coli* O138 in Luria–Bertani (LB) broth was used as inoculum for the experiments. The growth inhibition assay was performed according to Frazzini et al. on different concentrations (1:2; 1:4; 1:8; 1:16; 1:32). Briefly, 100 μL of diluted extract was added in a microtiter 96-well plate to which 30 μL *E. coli* inoculum was also added. All samples were incubated at 37 °C in a shaking incubator for 6 h. The growth rate of *E. coli* was estimated every hour for 6 h by measuring the absorbance with a microplate reader spectrophotometer (BioTek Epoch, Agilent, Santa Clara, CA, United States) at an optical density (OD) of 620 nm. The measured OD was converted into $\log_{10}$ of the number of cells/mL, considering 1 OD = $1^{*}10^{9}$ cells/mL. The assays were performed in technical quadruplicate and with three biological replicates meant to verify the replicability of the experiment using the same procedures, which included repeating the investigation starting from the sample extraction and repeating the test on different days.

2.4.3. Minimal inhibitory concentration (MIC)

Minimum inhibitory concentrations (MICs) were determined through the broth microdilution method. Briefly, a total of 100 μL of the

different concentrations of *Fucus vesiculosus* extracts were plated in a 96-well microplate, and 10 µL of an overnight culture of *E. coli* O138 in Luria–Bertani (LB) broth (approximately 10^6 CFU/mL) was inoculated according to plate design and incubated at 37 °C for 20 h. The change in absorbance determined bacterial growth after reading the microplates at 620 nm in a microplate reader spectrophotometer (Scan-Ready P-800, Life Real, Hangzhou, China). The following formula estimated the inhibition rate:

$$\textit{Inhibition Rate}\ (\%) = 100^{*}\left(\frac{ODCTRL^{-} - ODsample}{ODCTRL^{-} - ODBlank}\right)$$

The MIC was defined as the lowest extract concentration that did not produce turbidity compared to a positive control (0 mg of extract/mL). The experiment was performed in technical triplicate and with three biological replicates.

2.4.4. Inhibition of protein denaturation

Inhibition of protein denaturation was evaluated as a possible marker of the inflammatory process according to Osman et al., [33]. Briefly, 1 mL of *Fucus vesiculosus* extract at different concentrations (1000–500–250–125 µg/mL) was mixed with 450 µl of 5 % (*w/v*) bovine serum albumin solution and 1.4 ml of phosphate-buffered saline. Afterward, the mixtures were incubated at 37 °C for 15 min and then heated at 70 °C for 5 min. After cooling, their absorbance was measured spectrophotometrically at 660 nm (BioTek Epoch, Agilent, Santa Clara, CA, United States). Acetone 70 %, ethanol 80 %, and methanol 50 % were used as negative controls for the different extraction methods. Ibuprofen was taken as a positive control. The experiment was carried out in technical triplicate with three biological replicates and the percent inhibition for protein denaturation is calculated as follows:

$$\%\textit{Inhibition of denaturation} = (1 - D/C)^{*}100$$

where D is the absorbance of the sample and C is the absorbance of the negative control.

2.5. Statistical analysis

All the data were analyzed using GraphPad Prism (version 9.0.0). The normality of the distribution of the data and residuals was evaluated by D'Agostino–Pearson tests. Data were analyzed using two-way analysis of variance (two-way-ANOVA), which included the effects of treatment, time, and their interaction. *Post hoc* pairwise comparisons were performed using Bonferroni Sidak's test. The data are reported as the mean ± standard deviation, and differences were considered to be statistically significant at $p \leq 0.05$.

3. Results

3.1. Bioactive compounds

The evaluation of the bioactive compounds disclosed that regardless of the extraction solvent, the content of polyphenols and flavonoids is positively correlated with sample concentration (Fig. 2). Considering the highest concentration tested (50 mg/mL), our results demonstrate that methanol is the solvent that ensures the major yield in both polyphenols and flavonoid content. The methanolic extract of *Fucus vesiculosus* revealed a TPC equal to 2.27 ± 0.17 mg GAE/ 50 mg of sample, corresponding to 45.57 ± 3.43 mg GAE/g of dry sample. In comparison, the acetone and ethanol extract disclosed a value of TPC of 2.13 ± 0.15 and 1.90 ± 0.21 mg GAE/ 50 mg of the sample, respectively, which correspond to 42.70 ± 3.11 and 38.12 ± 4.33 mg GAE/g of dry sample. Likewise, for the flavonoids, we observed that the *F. vesiculosus* extracted with methanol had the highest TFC (182.16 ± 3.37 mg CE/g of sample) compared to the acetone and methanol extract (158.12 ± 1.71 and 127.09 ± 1.46 mg CE/g of sample, respectively).

3.2. Functional properties

3.2.1. Antioxidant activity

To evaluate the antioxidant capacity of *Fucus vesiculosus* extract the radical scavenger by ABTS assay was used. The assay revealed that the antioxidant capacity was positively correlated with the concentration of the sample, and for the bioactive compounds, the methanolic extract was the one that highlighted better the antioxidant property of *F. vesiculosus* (Fig. 3). At a concentration equal to 50 mg/ml, the percentage of inhibition was 90.39 ± 0.34 % for the methanolic extract, while for acetone and ethanol extract, values of 86.46 ± 1.06 % and 81.01 ± 1.46 % were determined. Considering as standard the Trolox solution is observable that the antioxidant capacity of the extract obtained with the most performant solvent, methanol, is equal to 36,717.77 ± 527.66 µM Trolox equivalent/g of sample.

3.2.2. Antimicrobial activity

The growth of *E. coli* F18^{+} strains was tested in the absence or presence of different *F. vesiculosus* extracts. For all the extracts, the logarithmic growth phase started after 1 h, and the inhibitory activity started after 2 h for the extract obtained with acetone and ethanol. For the methanolic extract, the inhibitory activity started after 3 h of incubation and remained so until the last hour of incubation. Although the methanolic extract sees inhibitory activity begin an hour after the other two extracts it results, at the point of maximum inhibition, as the extract

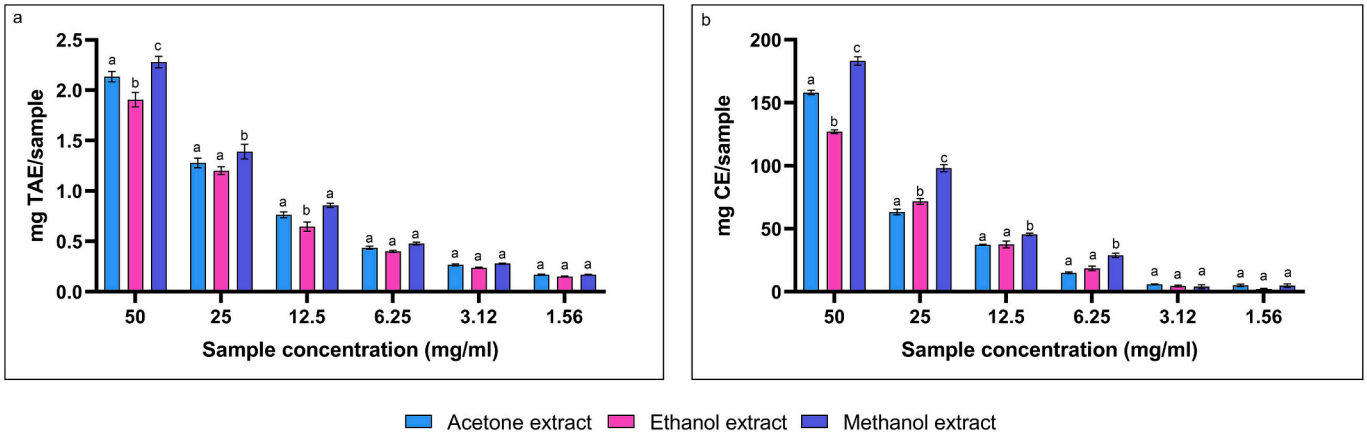

Fig. 2. Principal bioactive compounds in acetone, ethanol and methanol extract of *Fucus vesiculosus*. (a) Total phenolic content (TPC). (b) Total Flavonoid Content (TFC). TAE: tannic acid equivalent; CE: catechin equivalent. The data are shown as the means ± standard deviations (SDs). $^{a–c}$ Means ($n = 3$) with different superscripts are significantly different ($p < 0.05$).

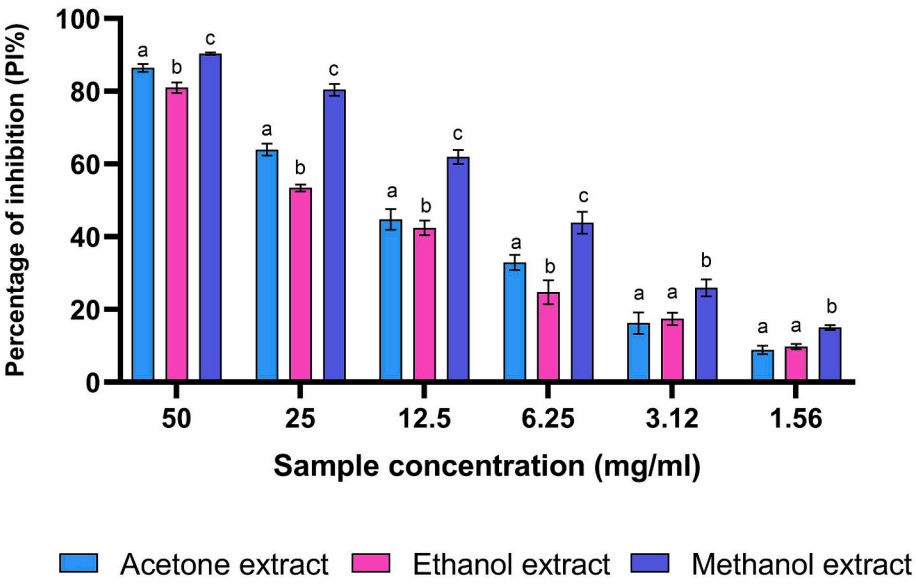

Fig. 3. Percentage inhibition of radical scavenging activity (PI%) at six different concentrations (50; 25; 12.5; 6.25; 3.12; 1.56 mg/ml) of *Fucus vesiculosus* extract. The data are shown as the means ± standard deviations (SDs). $^{a-c}$ Means (n = 3) with different superscripts are significantly different ($p < 0.05$).

with the greatest inhibitory capacity. In fact, at the peak of inhibition, found after 4 h of incubation, the extract in methanol can reduce the growth of *E. coli* F18^{+} by 12.22 % while for the extracts in acetone and ethanol this reduction of the pathogen strain results in 10.59 % and 9.99 %, respectively. Additionally, to evaluate the growth inhibition and prove the antimicrobial activity of *F. vesiculosus*, the minimal inhibitory concentration (MIC) was also evaluated as shown in Table 1.

3.2.3. Inhibition of protein denaturalization

The addition of *Fucus vesiculosus* extracts to the BSA-containing solution allowed, at a concentration of 1000 μg/ml, to inhibit protein denaturation by about 60 %. Specifically, the extracts obtained with the acetone and methanol were found to perform equally effectively, while the extract in ethanol showed lower efficacy (64.56 ± 0.20 %; 64.50 ± 0.35 %; 63.02 ± 0.10 %, respectively) (Fig. 4). Protein denaturation, which occurs at the moment in which protein loses its secondary and tertiary structure, is often associated with the inflammation process. In fact, when the protein denaturation occurs, the denatured protein ends up forming the antigens that can establish the inflammation process. In this assay, the denaturation of the protein is generated by using BSA, and the anti-inflammatory property can be evaluated, considering the ability of the tested substance to inhibit the denaturation of protein [34]. Therefore, the obtained results disclosed that *F. vesiculosus* being able to prevent most of the protein from being degraded could be considered as a substance with good anti-inflammatory power.

4. Discussion

4.1. Polyphenols and flavonoids content

The extraction of bioactive compounds from various natural sources is a critical area of research, with numerous studies exploring different solvents and methods to optimize yield and activity. Among the various bioactive molecules, the polyphenol class is extremely well studied as it is closely related to various functional properties, such as antioxidant, antimicrobial, and anti-inflammatory [35,36]. Our study disclosed that *Fucus vesiculosus* was rich in polyphenols since their amount was between 1.90 and 2.27 mg GAE/ 50 mg of sample, depending on the extraction solvent used. These values were in line with those found in literature where the TPC content of *F. vesiculosus* was estimated to between 7.8 g GAE 100 g-1 dw and 311.30 mg GAE/g of dry seaweed extract [37,38]. As well as for polyphenols, *F. vesiculosus* was rich also in flavonoids. In fact, our analysis highlighted a flavonoid content that varied between 187 and 127 mg CE/g of the sample, according to the solvent used. These results are in line with those found by Soares and colleagues, which disclose a content of flavonoid equal to 3.5 ± 0.1 g CE/100 g of dry sample [39]. Although the literature agreed with the high flavonoid content of *F. vesiculosus* pointed out also that the achievable results could differ between different studies. The difference could be due to the origin geographic region and reproductive phase, as was highlighted in the studies of Obluchinskaya et al. (2022) and Cox and colleagues (2010) that disclosed a huge range of TFC in *Fucus vesiculosus* extract [40,41]. The variability in polyphenolic and flavonoid content evidenced both within our study and in the literature can be explained by the fact that the evaluation of its bioactive components is affected by the choice of solvent, which affects the yield, composition, and bioactivity of the extracts [42]. The successful extraction of polyphenolic compounds from plant material hinges on two critical parameters: the chosen extractant's polarity and the target compounds' solubility within that extractant [43]. Generally, polyphenols exhibit greater solubility in less polar extractants, and a recommended approach was to involves a mixture of water and either methanol, ethanol, or acetone [44]. When comparing acetone, ethanol, and methanol for the extraction of polyphenols, it is evident that each solvent has distinct advantages and limitations depending on the plant material and the specific polyphenols targeted. In the context of our study specifically, it was seen that methanol had a superior extraction efficiency compared to acetone and ethanol. This superior performance could be attributed to methanol's polarity, which enhances its ability to dissolve polar compounds, making it an effective choice for polyphenol extraction [45]. This finding was in line with those reported in literature. In fact, different studies on several matrices reported the high potential of methanol as an extraction solvent. For instance, methanol extracts from olive oil mill wastewater showed the highest total phenolic content (950

Table 1
Minimal inhibitory concentration (MIC) of different extracts of *Fucus vesiculosus* on *E. coli* F18 + .

Extraction solvent	Extract concentration (mg/mL)	Inhibition rate (%)
Acetone	10	87.06
Ethanol	12	85.54
Methanol	7.5	90.15

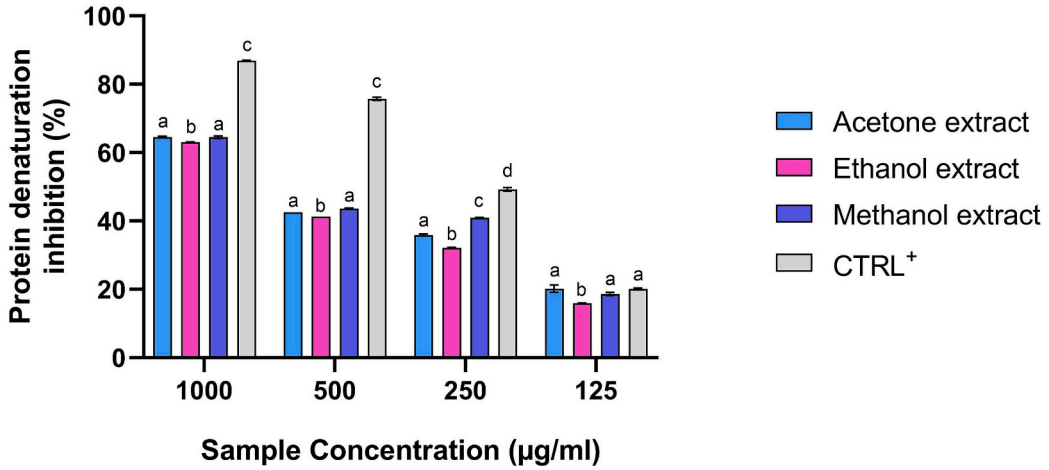

Fig. 4. Percentage of protein denaturation inhibition at four different concentrations (1000, 500, 250, 125 µg/ml) of *Fucus vesiculosus* extract. $CTRL^{+}$ is represented by the ibuprofen. The data are shown as the means ± standard deviations (SDs). $^{a–c}$ Means (n = 3) with different superscripts are significantly different ($p < 0.05$).

± 14.2 µg GAE/mg of extract) and flavonoid content (80.6 ± 17.27 µg QE/mg of extract) compared to other solvents [46]. Similarly, methanol was found to be the most effective solvent for extracting polyphenols from Bassia muricata, yielding the highest total phenolic content (122.15–144.82 mg GAE/g) and antioxidant activity [47]. Furthermore, methanol was also the most effective solvent for extracting phenolic compounds from *Pluchea indica* leaves, with the highest total phenol levels (147.91 mg GAE/g) and flavonoid content (69.72 mg QE/g) observed at 75 % methanol concentration [48]. Although methanol is known for its highest extractive power, it is often not used because of its toxicity. Therefore, other solvents, such as ethanol and acetone, could be used since they yield lower amounts of polyphenols than methanol, they were still a viable option, especially considering their safety profile for food applications [45]. Our study was in line with this statement. In fact, the content of polyphenols quantified through Folin-Ciocalteu assay was found higher in the methanol extract, followed by acetone and ethanol ones. The lower extraction efficiency of ethanol compared to acetone can be explained by the fact that the length of polymers extracted with ethanol is typically shorter than that of polymers extracted with acetone, indicating a difference in the structural characteristics of the polyphenols obtained. Acetone, particularly at a concentration of 70 %, has been noted to have superior extraction capabilities, as it produces more condensed polyphenols and longer polymer lengths [49]. Despite this our study focused on *Fucus vesiculosus* disclosed that all the solvents used were able to highlight the high content of bioactive molecules present in this brown seaweed. However, methanol was the solvent that allowed for the most remarkable recovery.

4.2. Evaluation of functional properties

The high content of bioactive molecules makes *Fucus vesiculosus* an alga with great functional properties, such as antioxidant, antimicrobial, and anti-inflammatory.

4.2.1. Antioxidant activity

Our study disclosed that *Fucus vesiculosus* could inhibit the ABTS cation radical almost completely (around 90 %) at a concentration of 50 mg/mL, demonstrating high antioxidant capacity. Commonly, the antioxidant potential of seaweed extracts is associated with phenolic compounds [38,50]. Particularly was reported that phlorotannins, which are phenolic compounds especially present in brown algae, have shown strong radical scavenging activity, particularly towards nitric oxide (NO•), and have been effective in inhibiting lipopolysaccharide-induced NO• production in macrophages, thus exhibiting anti-inflammatory properties as well [51]. Although the available data in the literature are highly variable, depending on the type of extract used, our study confirmed the widely recognized antioxidant property of *Fucus vesiculosus*. Obluchinskaya et al. (2023) reported that ethanolic extract of *Fucus vesiculosus* had a scavenging activity around 70 % at a concentration of 0.12 mg/mL [52]. Hermund and colleagues (2022) evaluated the concentration of an *F. vesiculosus* extract that provides half of the maximal response (50 %) of the antioxidant pathway (EC50), disclosing that the ethanol extract had a higher radical scavenging capacity in comparison to the water one [38]. As well Farvin & Jacobsen reported EC50 values of the radical scavenging capacity of 8.3 and 9.9 µg dw/mL for water and ethanolic extracts of *F. vesiculosus*, respectively [53]. These studies indicate that a lower concentration of *F. vesiculosus* extract could exhibit a higher radical scavenging activity compared to the results found in the present study. This may be due to the type of bioactive compounds present in the extracts. In fact, the positive correlation between polyphenol content and antioxidant capacity has been well documented [53]. In turn, this difference may be due to the extraction method used. For example, the employment of pressurized liquid extraction (PLE) has been demonstrated to yield high phenolic content with excellent radical scavenging and metal chelating abilities, proving the efficiency of PLE in obtaining potent antioxidant extracts from *F. vesiculosus* [54]. Moreover, the antioxidant activity of *F. vesiculosus* extracts has been evaluated in food systems, such as fish-oil-enriched milk and mayonnaise, where the ethyl acetate fraction demonstrated high radical scavenging and metal chelating abilities, contributing to the stability of these food products during storage [55].

4.2.2. Antimicrobial properties

Additionally, to the antioxidant properties, our analysis revealed the antimicrobial properties of *Fucus vesiculosus* that had the capacity to inhibit the growth of *Escherichia coli* with an inhibition rate between 85 and 90 % in base of the extraction solvent. This feature was widely reported in literature where different studies confirmed it also through a metabolomic approach that has identified galactolipids and phlorotannins as key antimicrobial components, with reference to specific monogalactosyldiacylglycerol derivatives and phlorethol-type phlorotannins [56]. Such as for the antioxidant activity, the methanolic extract is the one that highlights the highest antimicrobial capacity. This was reported also in literature where is showed that the antimicrobial activity of *F. vesiculosus* is also linked to its methanol-extractable contaminants, which contribute to its cytotoxicity and antibacterial properties [57]. Additionally, fucoidans, sulfated polysaccharides found in the cell walls of brown algae, have shown bacteriostatic effects against both Gram-positive and Gram-negative bacteria, with *Escherichia coli*

being particularly sensitive [58]. Also, solvent extracts from *F. vesiculosus*, particularly water extracts, have demonstrated potent antimicrobial activity against multiple strains of Methicillin-resistant *Staphylococcus aureus* (MRSA), including the ability to prevent and disrupt biofilms [59]. Furthermore, fucoidan derived from *F. vesiculosus* has shown bacteriostatic and bactericidal effects against *Listeria monocytogenes* and *Salmonella enterica serovar Typhimurium*, with its efficacy being influenced by concentration, temperature, and exposure time [60].

4.2.3. Anti-inflammatory properties

Among the different bioactive properties, our studies disclosed that *F. vesiculosus* could exhibit anti-inflammatory properties. In fact, the extracts tested in this study highlight their ability to inhibit protein precipitation. This capacity could be correlated with anti-inflammatory properties since protein denaturation, that is the process in which the proteins lose their quaternary, tertiary, and secondary structures due to exposure to external stress or compounds, generally leads to a loss of their biological functions that leads to onset of inflammatory processes [61]. The results obtained are supported by the TPC, which, as reported, was strictly correlated with the different bioactive properties and effectively inhibited protein denaturation and stabilized cell membrane integrity, suggesting their utility in treating inflammatory-related diseases [57]. Additionally, methanolic extracts of *F. vesiculosus* have shown central and peripheral analgesic potential in mice, further supporting its anti-inflammatory properties [62]. Moreover, the cold-water extract of *F. vesiculosus* showed a high inhibition of IL-8 production in TNF-α challenged Caco-2 cells and significantly reduced the expression of multiple inflammatory mediators, cytokines, chemokines, cell adhesion molecules, and components of NF-κB, MAPK, and AP-1 pathways in *ex-vivo* porcine colonic tissue, indicating its potent immunomodulatory effects [63]. Furthermore, fucoidan, extracted from *F. vesiculosus*, demonstrated to upregulate anti-inflammatory markers like CD206 and IL-10 in mouse bone-marrow-derived macrophages (BMDMs), while reversing LPS-induced inflammation *in vivo* by reducing macrophage activation [59].

With its substantial polyphenols and flavonoid content, *Fucus vesiculosus* stands out as a valuable resource for diverse bioactivities. This uniqueness makes it a promising candidate for various applications in different fields. The bioactivity found in *Fucus vesiculosus* opens up a new avenue in animal nutrition. It could potentially serve as a viable alternative to antibiotics, addressing a long-standing need in the field. In this context, the topic of discussion is how the algae itself is administered. In fact, on the one hand, administration of the extracts may improve the nutritional profile of the feed by providing concentrated bioactive compounds, which have been seen to have strong antimicrobial properties [64]. This would make the algae effective substitutes for antibiotics in animal feed. This can reduce antibiotic dependency and improve animal health by mitigating issues like medicament tolerance and secondary infections [65]. On the other hand, whole algal matter is rich in proteins, essential amino acids, vitamins, minerals, and other beneficial compounds like polyunsaturated fatty acids and carotenoids, which contribute to the nutritional quality of the feed [66,67]. The use of whole algae can also contribute to sustainable livestock production by utilizing non-competitive feed resources and improving feed security [68]. While both forms of algae offer significant benefits, the choice between extracts and whole matter should be guided by specific nutritional needs, desired health outcomes, and the type of livestock being fed. For instance, whole algae might be more suitable for comprehensive nutritional support and improving meat quality, while extracts could be more effective for targeted health interventions and reducing antibiotic use. Ultimately, integrating both forms could provide a balanced approach, leveraging the concentrated benefits of extracts and the broad nutritional advantages of whole algal matter to optimize animal health and productivity [69–71].

5. Conclusion

Given the known content of bioactive components of *Fucus vesiculosus* and its possible use in various fields including animal and human nutrition, pharmaceutical, nutraceutical, and cosmetic this study aimed to evaluate which of the conventional and most widely used solvents (acetone, ethanol, and methanol) provided the highest yield for the determination of bioactive components and consequently for the evaluation of bioactive properties such as antioxidant, antimicrobial, and anti-inflammatory. Collectively this study according to those reported in literature highlight that overall, all the solvent employed in the diverse extraction process guarantee a high yield of bioactive molecules, such as polyphenols and flavonoid, despite this the methanol is the ones that better highlight the functional potentiality of *Fucus vesiculosus*. The results obtained not only highlight the unquestionable bioactivity of brown algae such as *Fucus vesiculosus* but also open a reflection on the fact that it is difficult to compare data in the literature because the methods used for extraction can significantly influence the results.

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CRediT authorship contribution statement

Luciana Rossi: Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Conceptualization. **Benedetta Canala:** Writing – review & editing, Investigation. **Anna Paola Fifi:** Writing – review & editing, Supervision. **Sara Frazzini:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data presented in this study are not deposited in an official repository. Data are available within the article and from the corresponding author upon reasonable request.

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