separations

MDPI

*Article*

# A Potential Medicinal Food Source: Selenium-Enriched *Osmunda japonica* Spores

**Sheng-Bo Ai, Anna Zheng, Qing-Wu Yang and Jing-Zhou Dong ***

School of Selenium Science and Engineering, Wuhan Polytechnic University, Wuhan 430048, China; 18507345286@163.com (S.-B.A.); zhenganna@zhenganna.work (A.Z.); 18074604493@163.com (Q.-W.Y.)
* Correspondence: djz21cn@aliyun.com

**Abstract**

*Osmunda japonica* sporophyll is a medicinal food source in China and Southeast Asia. However, its functional components are still unknown, and its spores are not utilized. This study aims to analyze the main functional compounds of its sporophylls and spores. Selenium-enriched cultivation of *O. japonica* was conducted, and the main components of sporophylls and spores were comparatively analyzed using ultraviolet–visible spectroscopy, high-performance liquid chromatography and mass spectroscopy. The common phenolics of spores and sporophylls were chlorogenic acid, caffeic acid and kaempferol-3-rutinoside; sporophylls contained one more component, caffeic acid-3-O-(2′-O-butenyl) rahmnoside; caffeic acid was the main phenolic component in spores (2.36 ± 0.1 g/100 g, dried weight); contents of caffeic acid (2.36 ± 0.1 g/100 g, dried weight), kaempferol-3-rutinoside (2.69 ± 0.15 g/100 g, dried weight), polysaccharides (3.53 ± 0.23 g/100 g, dried weight), carotenoids (346 ± 34.9 mg/100 g, fresh weight) and chlorophylls (437 ± 40.2 mg/100 g, fresh weight) of spores were all significantly higher than those of sporophylls ($p < 0.05$). The organic selenium components of spores were Se-Cys (selenocysteine) and Se-Met (selenomethionine); Se-Cys, Se-Met, chlorogenic acid, carotenoids and chlorophylls were all significantly increased by selenium enrichment ($R^2 > 0.9$). In conclusion, selenium-enriched *O. japonica* spores showed high nutritional and medicinal values and should be developed as a new medicinal food source and a selenium supplementation food source.

**Keywords:** *Osmunda japonica*; selenium enrichment; spores; sporophylls; functional components

Check for updates

Academic Editor: Paraskevas D. Tzanavaras

Received: 23 September 2026
Revised: 5 November 2026
Accepted: 14 November 2026
Published: 19 November 2026

**Copyright:** © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

## 1. Introduction

Medicinal foods, as an emerging food category, have attracted the dramatically increased interest of human beings [1]. Many medicinal plants have been developed as functional foods or medicinal foods, and are called "food-medicine homologous medicinal foods". Most food-medicine homologous plants contain rich functional and medicinal components, such as polysaccharides [2,3], flavonoids, phenolic acids, saponins, carotenoids and so on. These functional components are very beneficial for human health protection.

*Osmunda japonica* Thunb is a medicinal plant species of Osmundaceae (Figure 1). In China and Southeast Asia, young sporophylls of *O. japonica* are traditionally processed into dried vegetables as delicious medicinal food. *O. japonica* rhizomes, as a traditional Chinese medicine, called Ziqi Guanzhong [4], were recorded as heat-clearing and detoxifying, hemostatic and antiparasitic in the Chinese medicinal monograph "Ben Cao Gang Mu". There were some reports on *O. japonica* about alleviating skin wrinkles [5], arsenic

accumulation [6], egg development and ovulation [7], chemical constituents of sterile fronds [3], the antibacterial properties of its flavonoids [8], molecular phylogenetics [9] and mycorrhizal formation [10]. However, there are rare reports on the functional component composition of *O. japonica* sporophylls and no reports on *O. japonica* spores.

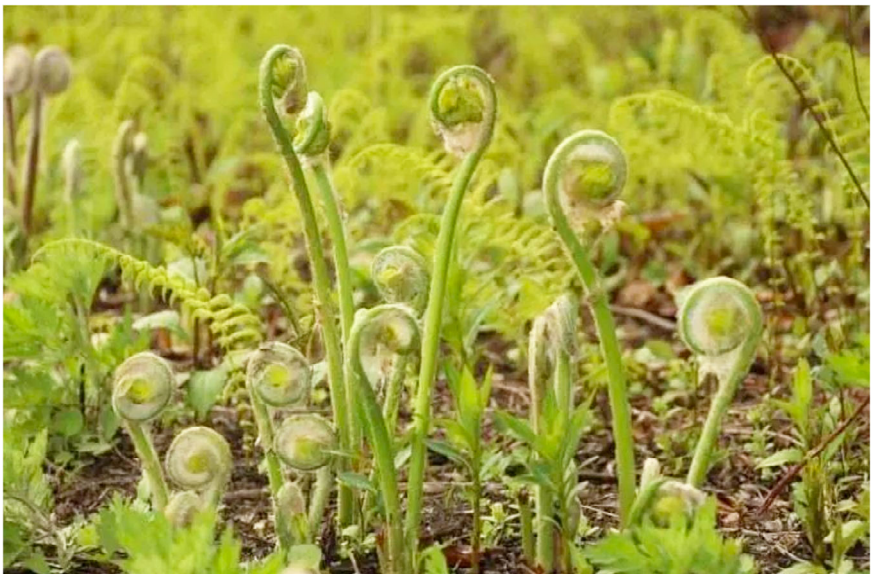


**Figure 1.** *O. japonica* sporophylls.

Spores or pollen, the mature germ cells of fungi or plants, and rich in bioactive components, are becoming important medicinal food sources for human health protection [11]. As typical fungal spores, *Ganoderma lucidum* spores contain the most commonly useful bioactive components, such as polysaccharides and polyunsaturated fatty acids [spore oils] and have been reported to show important bioactivities such as anti-Alzheimer [12], anti-aging [13], antioxidant [14], anti-obesity and anti-inflammation [15] and anti-tumor [16]. As a type of plant spore, pine pollen has also been widely used as medicinal food for their bioactivities, such as antioxidant activity, anti-inflammation [17], and hepatic protection [18]. Fungal spores and plant spores [pollens], as advanced medicinal food sources, are gaining increasing interest among human beings [19].

Nowadays, *O. japonica* is widely cultivated, and the sporophylls are harvested as medicinal vegetables, while *O. japonica* spores, which account for about 7–16% of the dried weight of the sporophylls, are not separated and utilized. In this study, the main functional components, such as phenolics, flavonoids, carotenoids, chlorophylls and polysaccharides of sporophylls and spores, were comparatively analyzed to show the differences between sporophylls and spores.

Selenium has been reported to have physiological functions such as antioxidant [20], anticancer [21], immune stimulation [22], and HIV inhibition [23]. The bioactivity of selenium depends on its chemical form. Organic selenium compounds such as selenomethionine and methylselenocysteine were better tolerated and exhibited anticarcinogenic activity [24]. Many plants showed the ability to transform inorganic selenium into organic selenium [25]. So, selenium-tolerant plants were used for processing organic selenium supplementation. Furthermore, in our previous study, selenium was found to have promotive effects on the accumulation of some secondary metabolites [26,27]. In this study, selenium-enriched cultivation of *O. japonica* was conducted, and an analysis of the composition and quantification of the functional components such as phenolics, flavonoids, chlorophylls,

carotenoids, and organic selenium compounds of selenium-enriched *O. japonica* spores was performed.

## 2. Materials and Methods

### *2.1. Chemicals*

Standards of chlorogenic acid (98% purity, CAS:327-97-9) and caffeic acid (98% purity, CAS:331-39-5) were purchased from Sangon Biotech (Shanghai, China). Se-Cys, Se-Met and proteinase K were obtained from Sigma. Kaempferol-3-rutinoside and caffeic acid-3-O-[2′-O-butenyl] rahmnoside were obtained by an Agilent1260 Infinity II Preparative. Acetonitrile, sodium citrate and hexanesulfonic acid were of HPLC grade. Acetonitrile, ethanol and acetic acid were of HPLC (high-performance liquid chromatography) grade, and the other chemicals were of analytical grade.

### *2.2. Instruments*

An ultrasonic cleaning bath (Shenzhen Jietai Ultrasonic Cleaning Apparatus Co. Ltd., Shenzhen, China, PS-40, 40 kHz, 240 W) was employed for extraction of phenolics, carotenoids, chlorophylls and polysaccharides. A cell wall grinder (SQW-25DI, Yichen Superfine Grinding Tech. Co. Ltd., Jinan, Shandong, China) was used for grinding sporophylls and spores. An ultraviolet–visible spectrophotometer (Shanghai Youke Instrument and Meter Co. Ltd., Shanghai, China) was used for analysis of phenolic components, carotenoids, chlorophylls and polysaccharides. HPLC analysis was performed on an Agilent1100 HPLC system consisting of Agilent1100 ChemStation Rev.A.10.02, G1313A autosampler, G1311A quaternary pump, G1314A variable wavelength ultraviolet (UV) detector and a reverse phase ZORBAX SB-C18 column (5 mm, 4.6 × 250 mm, Agilent Technologies, Santa Clara, CA, USA). LC–MS (liquid chromatography–mass spectrometry) analysis was performed on an Agilent1100 series with an LC–MSD trap SL (Agilent Technologies, Berlin, Germany). Preparation of phenolic components was performed on an Agilent1260 Infinity II Preparative, with a column of Agilent reverse-phase Prep 100Å C18 (50 × 250 mm, 10 μm). Liquid samples were filtered with 0.22 μm microporous membranes (EY-H492) for HPLC and UV–visible analysis.

### *2.3. Sample Preparation*

*O. japonica* samples were collected from the *O. japonica* species conservation base of Hubei Minzu University, China. The *O. japonica* samples were identified by Professor Yi-fa He of Hubei Minzu University, China. Sodium selenite was used as the selenium source in this study. Selenium-enriched cultivation of *O. japonica* was conducted according to our published methods [26,27]. Namely, sodium selenite was added to the MS solution to give final Se-concentrations of 10, 20, 30, 40, and 50 mg/kg dried quartz sands (referred to as the Se-MS solution). Quartz sands were collected using a 30-mesh screen and cleaned with pure water. The dried quartz sands were well mixed with the prepared Se-MS solution in the ratio of 0.25 kg Se-MS solution/1.0 kg dried quartz sands (called Se-soils). *O. japonica* seedlings were planted in the prepared Se-soils. The cultivation was conducted under a glass canopy and irrigated with distilled water to maintain a stable water content (60–70%), which was monitored with a soil moisture detector (FT-TS600). The cultivation was from May to September at Hubei Minzu University, Enshi, China, with an average temperature of 25.5 °C and an altitude of 531.8 m. Sporophylls were harvested before unfolding. The unfolded sporophylls were packaged with filter paper and dried in a silica gel dryer. After the sporophylls were dried to constant weight, 180 g of spores were screened and collected from the sporophylls. Dried spores and sporophylls were ground with a cell wall grinder separately, and the ground powders were used for extraction. The fresh spores were

separated from the fresh sporophylls, and the fresh samples of spores and sporophylls were used for quantification of carotenoids and chlorophylls.

### *2.4. Separation and Identification of the Phenolic Components*

Polyphenols extraction: the ground powders of sporophylls and spores were deoiled with ether in a Soxhlet extractor in an 80 °C water bath for 4 h. Then the deoiled samples were dried at 80 °C for 4 h, and then extracted with 70% ethanol in an ultrasonic cleaning bath for 30 min for three cycles; the ratio of liquid to solid was 30 mL/g, and the extracts were filtered through 0.22 μm microporous membranes for UV (ultraviolet) and HPLC analysis.

The 70% ethanol extracts prepared above were scanned within 190–1100 nm to preliminarily detect the chemical compositions. Based on the UV–visible scanning analysis, HPLC separation of phenolics was conducted: acetonitrile as solvent A (1.0% acetic acid as solvent B) 0–18 min, 15–60%, 18–20 min, 60–100%, and 20–25 min, 100–15%; the column temperature was set at 25 °C, the sample volume injected was 10.0 μL, and the flow rate was 1.0 mL $min^{-1}$; the DAD (diode array detector) detector was set at 190–400 nm. Based on the established HPLC separation method, LC[APCI, Atmospheric Pressure Chemical Ionization]–MS was used for qualitative analysis of the phenolic peaks. The MS parameters were set as follows: ion source, APCI; nebulizer, 50 psi; dry gas temperature, 350 °C; dry gas flow, 10.0 L/min; APCI temperature, 400 °C; HV (high voltage) capillary, 4000 V; and scan range, 50 to 1500 $m/z$. Nitrogen was used as a drying gas (10.0 L/min). The molecular structure of each peak in the HPLC spectra was analyzed using MS data and UV data.

### *2.5. Separation and Identification of the Organic Selenium Components*

Preparation of lipid fraction: dried samples (sporophylls and spores, approximately 30 g, respectively) were ground, and chloroform–methanol (2:1, 60 mL) was added, then shaken vigorously and filtered. The procedure was repeated for three cycles. The extracted samples were vacuum-dried at 80 °C to obtain the lipid-free fraction.

Protein hydrolysis: sodium hydroxide solution (0.1 mol $L^{-1}$, 10 mL) was added to 1.0 g of the lipid-free sample prepared above. After agitation (Vortex), the mixture was centrifuged (10 min,5000 rpm), and the solid residue was again treated with sodium hydroxide solution for three cycles. The combined solutions (20 mL) were neutralized to pH 7.0 with 4 mol $L^{-1}$ phosphoric acid, and acetone was added to make the final concentration 80%. The sample was kept at −20 °C for 30 min, and the precipitate (proteins) obtained by centrifugation (10 min, 5000 rpm) was washed with acetone and vacuum-dried at 60 °C. The precipitate was solubilized in acetic acid–ammonium acetate buffer (50 mmol $L^{-1}$, pH 4.5) containing 5% sodium dodecyl sulfate (water bath 60 °C, 5 min) and filtered through a hydrophilic low-protein-binding polysulfone filter (0.45 μm). The filtrates were used as protein samples. The proteins obtained above were solubilized in 5 mL phosphate buffer (pH 7.5) and incubated with 1.0 mL of proteinase K (20 mg $mL^{-1}$, 37 °C, overnight), then acetone was added to a final concentration of 80%, and the mixture was centrifuged at 5000 rpm for 10 min. The supernatant was vacuum-dried at 60 °C, and the residue containing selenoamino acids was solubilized in the mobile phase for HPLC–ICP–MS analysis. The mobile phase for analysis of Se-Cys and Se-Met using HPLC–ICP–MS was 5 mmol/L sodium citrate: 5 mmol/L hexanesulfonic acid = 95:5, with a flow rate of 1.0 mL/min and an injection volume of 20 μL.

### *2.6. Quantitation of the Phenolic Components, Polysaccharides, Carotenoids, Chlorophylls, Se-Cys and Se-Met*

Phenolic components: Based on the established HPLC separation method above, the 70% ethanol extracts of phenolics were separated using preparative HPLC. The two

separated peaks in the HPLC spectra (unknown components, Peak 3, Peak 4) were collected, respectively, and vacuum-dried at 80 °C to constant weight. Calibration curves of the components, such as caffeic acid, chlorogenic acid, Peak 3 and Peak 4, were established, respectively.

Chlorogenic acid: Y = 59762X + 18057, r = 0.9998, linearity range = 4.87–9.16 μg/mL (1)

Caffeic acid: Y = 32586X + 17668, r = 0.9998, linearity range = 6.034–10.11 μg/mL (2)

Peak 3: Y = 32316X + 13741, r = 0.9999, linearity range = 3.62–13.72 μg/mL (3)

Peak 4: Y = 43336X + 19637, r = 0.9999, linearity range = 5.56–17.15 μg/mL (4)

Selenium components: The standards of Se-Cys and Se-Met were used to establish calibration curves, respectively.

Se-Cys: Y = 46773X + 15682, r = 0.9998, linearity range = 3.55–8.32 μg/mL (5)

Se-Met: Y = 52537X + 17448, r = 0.9998, linearity range = 3.67–9.15 μg/mL (6)

where Y is the peak area, and X is the mass concentration.

Polysaccharides: The ground powders of the sporophylls and spores were deoiled as described above, and then refluxed with 80% (*v*/*v*) ethanol at 95 °C in a water bath in a Soxhlet extractor for 4 h to remove sugars, polyphenols and flavonoids. Finally, the samples were dried at 80 °C for 4 h and then extracted with 80 °C pure water and ultrasonicated for 30 min, for three cycles; the ratio of liquid to solid was 30 mL/g; the extracts were filtered through 0.22 μm microporous membranes as polysaccharide samples. The phenol–sulfuric acid method was used for determination of *O. japonica* polysaccharides. Polysaccharide concentrations were calculated according to the calibration curve, with glucose as a standard:

y = 0.0075x + 0.0056, r = 0.9998, linearity range = 7.5–28.1 μg/mL (7)

where y is the absorbance at 490 nm, x is the mass concentration of glucose (μg/mL), and r is the correlation coefficient.

Carotenoids: Estimation of carotenoids was conducted based on our published methods [26] with some modifications. Namely, samples were extracted with absolute ethanol, and the extracts were measured at 450 nm, using $E_{1cm}^{1\%}$ for quantification of carotenoids.

Chlorophylls: Concentrations of chlorophyll a, chlorophyll b and chlorophyll a + b were determined using the reported methods [28] with some modifications. Namely, 0.2 g samples were ground with 10 mL absolute ethanol, then placed in a 50 mL centrifuge tube and filled to 30 mL with the same solvent, then kept in a dark place until the sample color changed to white. One millimeter of filtered extract was diluted with 3 mL absolute ethanol, and the absorbance was measured at 645 nm, 652 nm and 663 nm. The concentrations (mg/mL) of chlorophyll a ($C_a$), chlorophyll b ($C_b$), and chlorophyll a + b ($C_{a+b}$) were calculated according to the formulae:

$$C_a = 0.0127D_{663} - 0.00269D_{645} \quad (8)$$

$$C_b = 0.0229D_{645} - 0.00468D_{663} \quad (9)$$

$$C_{a+b} = ((0.0202D_{645} + 0.00802D_{663} + D_{652})/34.5)/2 \quad (10)$$

where D is absorbance, and C is mass concentration (mg/mL).

## 3. Results

### *3.1. The Main Phenolic Compositions: Chlorogenic Acid, Caffeic Acid, Kaempferol-3-rutinoside and Caffeic Acid-3-O-[2′-O-butenyl] Rahmnoside*

The UV–visible spectra of the absolute ethanol extracts of spores and sporophylls indicated the possible compositions, such as phenolics (326 nm), carotenoids (440 nm) and chlorophylls (660 nm) (Figure 2). Based on the MS and UV–visible spectra data of the separated peaks, the three separated peaks of spores were identified as kaempferol-3-rutinoside, chlorogenic acid and caffeic acid (Table 1, Figure 3). The four peaks of sporophylls were identified as chlorogenic acid, caffeic acid, kaempferol-3-rutinoside and caffeic acid-3-O-(2′-O-butenyl) rahmnoside (Table 1, Figure 4). Chlorogenic acid, caffeic acid and kaempferol-3-rutinoside were the common phenolics of sporophylls and spores, while sporophylls contained one more phenolic component: caffeic acid-3-O-(2′-O-butenyl) rahmnoside (Table 1, Figure 4).

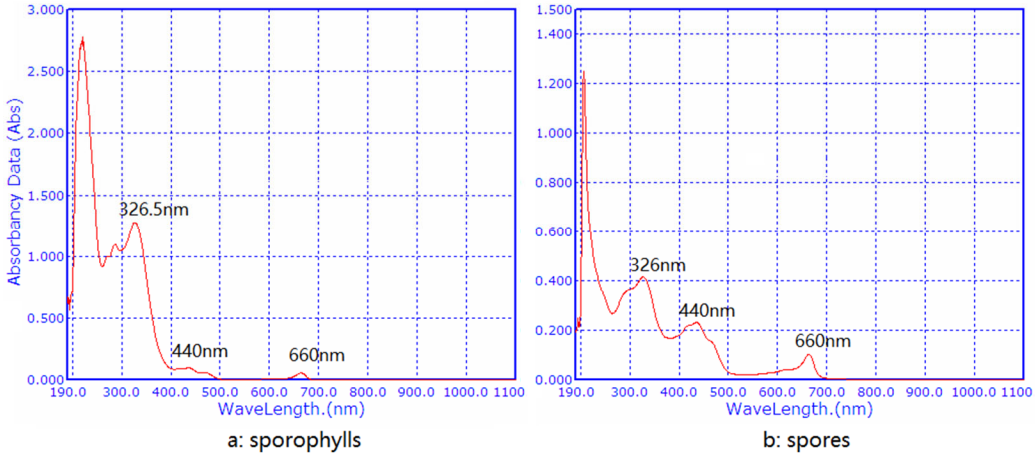



**Figure 2.** UV–visible spectra of the absolute ethanol extracts of (**a**) sporophylls and (**b**) spores (660 nm = chlorophylls; 440 = carotenoids; 326/326.5 = phenolics).

**Table 1.** UV–MS data of the main phenolic components.

| Peaks | Components | MW | MS Data | | UV Spectra (nm) | Retention Time (min) |
|---|---|---|---|---|---|---|
| | | | $[M + H]^+$ | $[M - H]^-$ | | |
| 1 | Chlorogenic acid | 354 | 355.1/163.1/145.2/117.3 | 353.0/190.6/172.6/110.6 | 297 (sh), 326.5 | 8.565 |
| 2 | Caffeic acid | 180 | 181.3/163.1 | 178.9/134.7 | 295 (sh), 326.5 | 15.873 |
| 3 | Kaempferol-3-rutinoside | 594 | 595.0/449.2/287.0/243.1/157.1 | 593.7/447.2/284.8/255.1 | 265, 301(sh), 351.5 | 22.240 |
| 4 | Caffeic acid-3-O-(2′-O-butenyl) rahmnoside | 382 | 383.0/327.5/181.1 | 381.3/178.6/134.8 | 251, 341.5 (sh) | 26.113 |

UV = ultraviolet spectrum; MS = mass spectrum; MW = molecular weight.

In the spores, caffeic acid was the main phenolic compound, with an average content of 2.36 ± 0.1 g/100 g dried weight, while in sporophylls, caffeic acid showed the least content, 1.35 ± 0.1 g/100 g dried weight (Table 2). There was no significant differences in chlorogenic acid contents between spores and sporophylls ($p = 0.765$), while the contents of caffeic acid and Kaempferol-3-rutinoside in spores were both significantly higher than those in sporophylls ($p = 0.0003, 0.046$) (Table 2); caffeic acid-3-O-(2′-O-butenyl) rahmnoside was only detected in sporophylls, with an average content of 2.07 ± 0.15 g/100 g dried weight (Table 2, Figure 3).

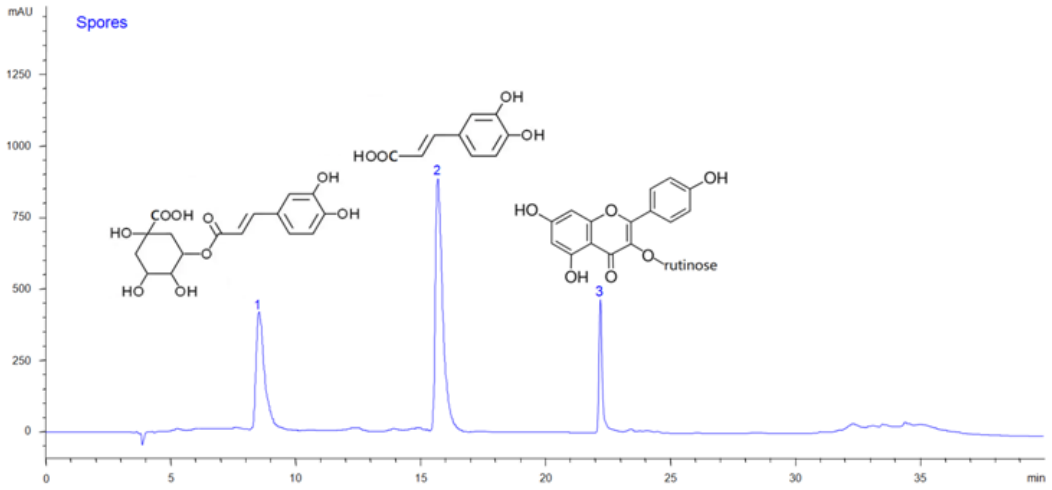



**Figure 3.** HPLC separation of the phenolic components of the spores. (1 = chlorogenic acid; 2 = caffeic acid; 3 = Kaempferol-3-rutinoside).

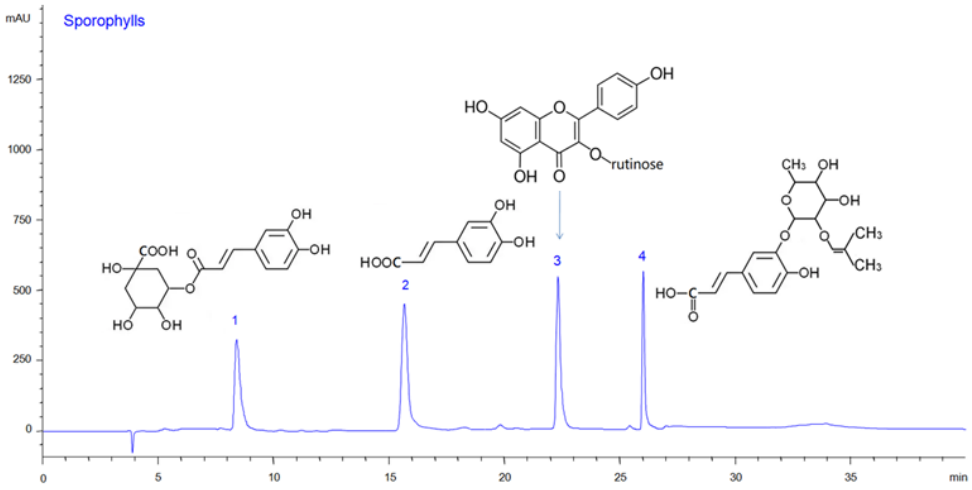



**Figure 4.** HPLC separation of the phenolic components of sporophylls. (1 = chlorogenic acid; 2 = caffeic acid; 3 = Kaempferol-3-rutinoside; 4 = Caffeic acid-3-O-(2′-O-butenyl) rahmnoside).

**Table 2.** Analysis of the components between spores and sporophylls ($N = 5$).

| Components | Spores | Sporophylls | *p*-Value |
|---|---|---|---|
| Chlorogenic acid (g/100 g DW) | 2.15 ± 0.09 | 2.17 ± 0.07 | 0.765 |
| Caffeic acid (g/100 g DW) | 2.36 ± 0.1 | 1.35 ± 0.1 | 0.0003 |
| Kaempferol-3-rutinoside (g/100 g DW) | 2.69 ± 0.15 | 2.13 ± 0.22 | 0.046 |
| Caffeic acid-3-O-(2′-O-butenyl) rahmnoside(g/100 g DW) | ND * | 2.07 ± 0.15 | |
| Polysaccharides (g/100 g DW) | 3.53 ± 0.23 | 1.67 ± 0.16 | 0.004 |
| Carotenoids (mg/100 g FW) | 346 ± 34.9 | 209 ± 41 | 0.012 |
| Chlorophyll a (mg/100 g FW) | 217 ± 21.1 | 139 ± 15.5 | 0.008 |
| Chlorophyll b (mg/100 g FW) | 195 ± 22.6 | 126 ± 19.5 | 0.008 |
| Chlorophyll a + b (mg/100 g FW) | 437 ± 40.2 | 261 ± 16.6 | 0.002 |

* not detected, LOD = 0.05 mg/100 g; DW = dried weight; FW = fresh weight.

### *3.2. Other Functional Components: Polysaccharides, Carotenoids and Chlorophylls*

Apart from phenolic compounds, there were other functional components, such as polysaccharides, carotenoids and chlorophylls (Figure 2; Table 2). Spores contained an average polysaccharide content of 3.53 ± 0.23 g/100 g dried weight, significantly higher than that of sporophylls (1.67 ± 0.16 g/100 g dried weight) (Table 2). The average contents of

carotenoids and chlorophylls in spores were 346 ± 34.9 mg/100 g and 437 ± 40.2 mg/100 g, respectively, both significantly higher than those of sporophylls (209 ± 41 mg/100 g, $p = 0.012$; 261 ± 16.6 mg/100 g, $p = 0.002$) (Table 2). Except for caffeic acid-3-O-(2′-O-butenyl) rahmnoside and chlorogenic acid, the contents of the other components, such as caffeic acid, kaempfero-3-rutinoside, polysaccharides, carotenoids and chlorophylls in spores were all significantly higher than those of sporophylls (Table 2).

### *3.3. Selenium Components in Sporophylls and Spores: Selenomethionine and Selenocysteine*

The main organic selenium components in selenium-enriched *O. japonica* plants were Se-Met (selenomethionine) and Se-Cys (selenocysteine) (Figure 5). The contents of Se-Cys and Se-Met in spores and sporophylls were increased in a selenium-dependent manner ($R^2 > 0.97$). Furthermore, the contents of Se-Cys and Se-Met in spores were both significantly higher than those of sporophylls (Table 3), which indicated that selenium is crucial for plant development, and so *O. japonica* spores, as reproductive organs, could absorb and store more organic selenium for propagation. In this sense, selenium-enriched *O. japonica* spores could be developed for selenium-supplemented food.

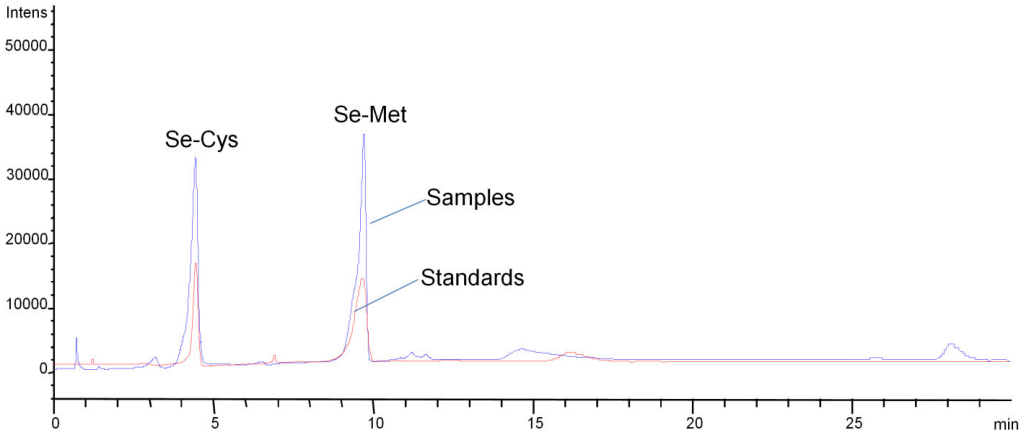



**Figure 5.** Composition of organic selenium components from *O. japonica* spores (Se-Cys = Selenocysteine; Se-Met = Selenomethionine).

**Table 3.** Analysis of the components in Se spores from different Se-concentration cultivation (N = 5).

| Items | Concentrations of Sodium Selenite(mg/kg) | | | | | $R^2$ |
|---|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 | |
| Chlorogenic acid (g/100 g DW) | 2.23 ± 0.11 | 2.51 ± 0.05 | 2.65 ± 0.21 | 2.77 ± 0.24 | 2.83 ± 0.11 | 0.957 |
| Caffeic acid (g/100 g DW) | 2.42 ± 0.18 | 2.57 ± 0.09 | 2.68 ± 0.15 | 2.72 ± 0.18 | 2.82 ± 0.18 | 0.963 |
| Kaempferol-3-rutinoside (g/100 g DW) | 2.8 ± 0.15 | 2.97 ± 0.12 | 3.18 ± 0.22 | 3.08 ± 0.25 | 3.58 ± 0.15 | 0.82 |
| Polysaccharides (g/100 g DW) | 3.65 ± 0.51 | 3.57 ± 0.63 | 2.95 ± 0.31 | 3.05 ± 0.26 | 3.75 ± 0.51 | 0.02 |
| Carotenoids (mg/100 g FW) | 363 ± 27.5 | 375 ± 21.3 | 383 ± 25.7 | 391 ± 22.2 | 413 ± 25.5 | 0.934 |
| Chlorophyll a (mg/100 g FW) | 238 ± 23.5 | 244 ± 15.5 | 258 ± 21.5 | 268 ± 23.5 | 271 ± 33.5 | 0.955 |
| Chlorophyll b (mg/100 g FW) | 218 ± 19.9 | 235 ± 15.1 | 247 ± 16.5 | 279 ± 19.5 | 284 ± 29.5 | 0.967 |
| Chlorophyll a + b (mg/100 g FW) | 455 ± 35.5 | 471 ± 25.3 | 494 ± 15.9 | 525 ± 37.3 | 539 ± 25.1 | 0.912 |
| Se-Cys in spores (μg/100 g DW) | 15.68 ± 2.5 | 31.7 ± 5.6 | 52.91 ± 8 | 72.55 ± 7.5 | 75.3 ± 9.1 | 0.975 |
| Se-Met in spores (μg/100 g DW) | 21.3 ± 3.5 | 40.22 ± 4.8 | 66.35 ± 7.7 | 89.13 ± 8.2 | 95.11 ± 9 | 0.975 |
| Se-Cys in sporophylls (μg/100 g DW) | 6.33 ± 1.5 | 8.1 ± 1.6 | 13.45 ± 2.7 | 15.05 ± 3.5 | 18.6 ± 3.1 | 0.985 |
| Se-Met in sporophylls (μg/100 g DW) | 7.25 ± 1.1 | 9.32 ± 1.8 | 12.31 ± 2.7 | 15.51 ± 3.2 | 21.5 ± 3.6 | 0.975 |
| Spore production (%) | 15 ± 3.3 | 16.5 ± 3.8 | 15.5 ± 4.1 | 14 ± 4.4 | 12 ± 6.5 | 0.753 |

Se-Cys = Selenocysteine; Se-Met = Selenomethionine; DW = dried weight; FW = fresh weight; Spore production (%) = spores weight/(spores weight + sporophyll weight).

### *3.4. Selenium Significantly Increased the Main Functional Components of Spores: Chlorogenic Acid, Caffeic Acid, Kaempferol-3-rutinoside, Carotenoids and Chlorophylls*

The other functional components, such as chlorogenic acid, caffeic acid, kaempferol-3-rutinoside, carotenoids, and chlorophylls, were all increased in a very significant, selenium-dependent manner ($R^2$ = 0.957/0.963/0.82/0.934/0.912), which was mostly in agreement with our previous studies [26,27]. But the contents of polysaccharides were not concentration-dependent on selenium, although they increased with selenium at 50 mg/kg, which might mean that spores synthesized more polysaccharides to protect cells under the stress of high selenium concentrations. There have been many published studies on the structures and functionalities of selenium polysaccharides, but there are few reports on the correlation between polysaccharide accumulation and selenium. The reason might be that selenium promotes the biosynthetic pathways of chlorogenic acid, caffeic acid, kaempferol-3-rutinoside, carotenoids, and chlorophylls, but is not involved in the synthesis of polysaccharides. The molecular mechanisms deserve further research.

The production of spores was also increased by selenium at relatively low concentrations, but decreased at higher concentrations (≥40 mg/kg, Table 3). Selenium promotes the development of reproductive organs at relatively low concentrations, but inhibits reproductive organ growth at high selenium concentrations, which was partially in agreement with our previous study [27]. The components, such as polysaccharides, carotenoids and chlorophylls, in spores were all significantly higher than those of sporophylls (Table 2), indicating that the spores showed higher functional values than sporophylls.

Unlike the traditional fungal spores of *Ganoderma lucidum*, whose main functional components were polysaccharides [28], *O. japonica* spores contained more functional components, such as phenolics, carotenoids and chlorophylls (Figure 2), indicating higher functional values than *G. lucidum* spores.

## 4. Discussion

As described above, *O. japonica* spores contained significantly higher bioactive components than those of sporophylls. The reason might be that, as a reproductive organ, spores were the most active metabolic center of *O. japonica* plants and produced more bioactive components for germination and self-protection against environmental stress. *Ganoderma lucidum* spores were widely used as functional food and a traditional medicine in China and Southeast Asia. The *G. lucidum* polysaccharides were considered the main functional components in *G. lucidum* spores with a reported content of 2.98 g/100 g [29], lower than that of *O. japonica* spores (3.53 g/100 g; Table 2). In this sense, *O. japonica* spores could be a better medicinal food. Except for the polysaccharides, the *O. japonica* spores contained other bioactive components, such as phenolics, carotenoids and chlorophylls, compared to *G. lucidum* spores (Table 2). Phenolics, a large group of natural products in functional foods, are known for many important medicinal values. Polysaccharides were considered important active components in foods and traditional medicines and showed many physiological functions, such as antioxidant, antifungal, blood sugar level reduction, immunological, anti-radiation, anti-blood coagulation, anticancer, anti-HIV and hypoglycemic activities [30]. Carotenoids showed antioxidant, anticancer and eyesight-enhancing activities [31]. Chlorophylls, as a traditional natural medicine, have been reported to have many important physiological functions such as anti-inflammatory, antioxidant, metal-chelating and anticancer activities [32]. So, in this sense, *O. japonica* spores might be a more valuable functional food source than *G. lucidum* spores. Nowadays, fungal spores from *G. lucidum* are widely used as functional food and traditional medicine, while spores from *O. japonica* plants are still not separated and utilized. In our cultivation of *O. japonica* plants, the dried weight of spores proved to be 7–16% of the dried sporophylls. In this study, *O. japonica* spores proved

to contain many functional components as described above. Hence, *O. japonica* spores should be developed as a new source for functional food and natural medicine. In order to provide a scientific basis for industrial utilization of *O. japonica* spores, comparison of the health-protective functions and medicinal values between *O. japonica* spores and *G. lucidum* spores, cultivation techniques to increase these functional components and related intensive processing techniques deserve further research.

Our selenium-enriched cultivation of *O. japonica* plants showed that selenium enrichment significantly increased production of spores and the main functional components of spores. The organic selenium components, such as Se-Cys and Se-Met, were both significantly higher than those of sporophylls. So, selenium-enriched *O. japonica* spores could be developed as a functional food source and a selenium-supplementation food source.

## 5. Conclusions

Although chlorogenic acid, caffeic acid and kaempferol-3-rutinoside are the common phenolic components of spores and sporophylls, and there were no significant differences in chlorogenic acid contents between spores and sporophylls, the contents of caffeic acid, Kaempferol-3-rutinoside, polysaccharides, carotenoids and chlorophylls in spores were all significantly higher than those of sporophylls. Caffeic acid, polysaccharides, carotenoids and chlorophylls were the main active components in spores. So, spores should be separated and utilized as a new food source and natural medicine, and related techniques, including cultivation, processing and extraction techniques, deserve further research.

Furthermore, selenium could increase the production of *O. japonica* spores, and selenium-enriched *O. japonica* spores contained rich organic selenium components, such as Se-Cys and Se-Met, and significantly higher levels of chlorogenic acid, caffeic acid, kaempferol-3-rutinoside, carotenoids, and chlorophylls than those of sporophylls. So, selenium enrichment could be used in the development of *O. japonica* to increase the production and quality of *O. japonica* spores and provide a selenium-supplemented food source.

**Author Contributions:** S.-B.A.: conceptualization; methodology; formal analysis; data curation; visualization; resource; writing—review and editing. A.Z.: methodology; writing—review and editing; formal analysis; data curation; writing—original draft; visualization. Q.-W.Y.: formal analysis; data curation; writing—original draft; visualization. J.-Z.D.: conceptualization; methodology; resources; project administration; supervision; writing—original draft; writing—review and editing. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was partially funded by the Doctor Research Project (53210052493) from Wuhan Polytechnic University, Wuhan, China.

**Data Availability Statement:** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Acknowledgments:** We are grateful to the anonymous reviewers and scientific editors for critical review and valuable suggestions, to Yi-fa He (Hubei Minzu University, China) for identification of *O. japonica*, and to Ji-Hua Yang (Hubei Minzu University, China) for sampling of *O. japonica* spores.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. Marilisa, A.; Monica, A. Re-thinking functional food development through a holistic approach. *J. Funct. Foods.* **2021**, *81*, 104466. [CrossRef]
2. Liu, X.C.; Xu, R.; Miao, Y.T.; Zang, Y.N.; Huang, Y.X.; Zheng, Y.Y.; Li, F.W.; Wei, M.; Xiao, R.; Yu, X.H. Exploring the immunomodulatory mechanisms of *Osmunda japonica* Thunb polysaccharides: Activation of MAPK and NF-κB signaling pathways. *Food Biosci.* **2025**, *63*, 105695. [CrossRef]

3. Liu, X.; Yu, X.; Lyu, J.; Li, F.; Shang, Y.; Lyu, Y.; Liu, J. Chemical structure characterization of polysaccharide from *Osmunda japonica* Thunb and its inhibitory activity on uterine fibroids. *Arab. J. Chem.* **2022**, *15*, 103724. [CrossRef]
4. The State Pharmacopoeia Commission of China. *Pharmacopoeia of China*; Chemical Industry Press: Beijing, China, 2020; Volume I, p. 321.
5. Kim, T.W.; Hur, N.M.; Jo, K.Y.; Kim, Y.J.; Cho, S.I.; Yun, E.S. Cosmetic Composition for Skin Anti-Wrinkle Comprising *Osmunda japonica* Extract as Effective Component. KR20180107616A, 21 August 2022.
6. Srivastava, M.; Santos, J.; Srivastava, P.; Ma, L.Q. Comparison of arsenic accumulation in 18 fern species and four *Pteris vittata* accessions. *Bioresour. Technol.* **2010**, *101*, 2691–2699. [CrossRef]
7. Cao, J.G.; Dai, X.F.; Wang, Q.X. Cytological features of oogenesis and their evolutionary significance in the fern *Osmunda japonica*. *Sex. Plant Reprod.* **2012**, *25*, 61–69. [CrossRef]
8. Li, F.; Hu, Y. Antibacterial and Antifungal Properties of Flavonoid Compounds from *Osmunda japonica* thumb. *Asian J. Chem.* **2012**, *24*, 4964–4966. Available online: https://www.researchgate.net/publication/295626264 (accessed on 15 November 2025).
9. Tsutsumi, C.; Hirayama, Y.; Kato, M.; Yatabe-Kakugawa, Y.; Zhang, S.Z. Molecular Evidence on the Origin of *Osmunda* ×*mildei* (Osmundaceae). *Am. Fern J.* **2012**, *102*, 55–68. [CrossRef]
10. Ogura-Tsujita, Y.; Sakoda, A.; Ebihara, A.; Yukawa, T.; Imaichi, R. Arbuscular mycorrhiza formation in cordate gametophytes of two ferns, *Angiopteris lygodiifolia* and *Osmunda japonica*. *J. Plant Res.* **2013**, *126*, 41–50. [CrossRef]
11. Xu, J.H.; Li, P. Researches and application of ganoderma spores powder. In *Ganoderma and Health*; Advances in Experimental Medicine and Biology; Springer: Singapore, 2019; pp. 157–186. [CrossRef]
12. Qin, L.H.; Wang, C.; Qin, L.W.; Liang, Y.F.; Wang, G.H. Spore powder of *Ganoderma lucidum* for alzheimer's disease: A protocol for systematic review. *Medicine* **2019**, *98*, e14382. [CrossRef]
13. Song, H.; Kim, M.S. Anti-aging & Skin Hydration Effects of Spore oil Extracted from *Ganoderma lucidum*. *J. Converg. Inf. Technol.* **2020**, *10*, 232–238. [CrossRef]
14. Senphan, T.; Takeungwongtrakul, S.; Kaewthong, P. Extraction and antioxidant activities of broken *Ganoderma lucidum* spore. *Int. J. Agric. Technol.* **2021**, *17*, 2303–2316. Available online: https://li04.tci-thaijo.org/index.php/IJAT/article/view/6392 (accessed on 15 November 2025). [CrossRef]
15. Sang, T.; Guo, C.; Guo, D.; Wu, J.; Wang, Y.; Wang, X. Suppression of obesity and inflammation by polysaccharide from sporoderm-broken spore of *Ganoderma lucidum* via gut microbiota regulation. *Carbohydr. Polym.* **2021**, *256*, 117594. [CrossRef]
16. Sun, M.; Ni, L.; Huang, Y.; Wang, B.; Zhang, M.; Ma, C.; Wu, M. Volatile Components and Antitumor Activity of *Ganoderma lucidum* Spore Oil and Its Molecular Distillation Components. *Asian Agric. Res.* **2023**, *15*, 30–35. [CrossRef]
17. Zhou, C.; Yang, Q.; Chen, L.; Fang, K.; Lu, X.; Song, X.; Sun, L. Antioxidant and anti-inflammatory properties of pine pollen extract promote wound healing. *Mater. Express.* **2023**, *13*, 327–336. [CrossRef]
18. Cong, T.; Jin, X.; Zhao, L.; Ma, L.; Guo, C. The protective effects of mass on pine pollen aqueous extract on ccl4-induced oxidative damage of human hepatic cells. *Int. J. Clin. Exp.* **2015**, *8*, 17773–17780. Available online: https://pubmed.ncbi.nlm.nih.gov/26770368 (accessed on 15 November 2025).
19. Rupanar, S.V.; Satpute, R.; Gadhave, P.; Kakade, N. Bee pollen beyond nutrition: An integrated review of its chemical, pharmacological, and biological properties. *J. Drug Res. Ayurvedic Sci.* **2024**, *9*, 211–221. [CrossRef]
20. Wen, S.; Hui, Y.; Chuang, W. Biosynthesis and antioxidation of nano-selenium using lemon juice as a reducing agent. *Green Process. Synth.* **2021**, *10*, 178–188. [CrossRef]
21. Subha, V.; Shanmugam, K.; Sahadevan, R. *InVivo* Studies and Flow Cytometric Investigation on Anticancer Potential of Selenium Nanoparticles Synthesized via Aqueous Extract of *Clerodendron phlomidis*. *Proc.Anticancer.Res.* **2024**, *8*, 71–81. Available online: https://openalex.org/works/W4392791067 (accessed on 15 November 2025). [CrossRef]
22. Debata, N.R.; Sethy, K.; Swain, R.K.; Mishra, S.K.; Panda, N.; Maity, S. Supplementation of nano-selenium [SeNPs] improved growth, immunity, antioxidant enzyme activity, and selenium retention in broiler chicken during summer season. *Trop. Anim. Health Prod.* **2023**, *55*, 260. [CrossRef]
23. Pourmoradian, S.; Rezazadeh, L.; Tutunchi, H.; Ostadrahimi, A. Selenium and zinc supplementation in HIV-infected patients. *Int. J. Vitam. Nutr. Res.* **2023**, *94*, 153–159. [CrossRef]
24. Hadrup, N.; Ravn-Haren, G. Toxicity of repeated oral intake of organic selenium, inorganic selenium, and selenium nanoparticles: A review. *J. Trace Elem. Med. Biol.* **2023**, *79*, 127235. [CrossRef]
25. Collins, J.F. Chapter 39: Could Selenium Be a Double-Edged Sword? In *Molecular, Genetic, and Nutritional Aspects of Major and Trace Minerals*; Elsevier: Amsterdam, The Netherlands, 2016. [CrossRef]
26. Dong, J.Z.; Wang, Y.; Wang, S.H.; Yin, L.P.; Xu, G.J.; Zheng, C.; Lei, C. Selenium increases chlorogenic acid, chlorophyll and carotenoids of *Lycium chinense* leaves. *J. Sci. Food Agric.* **2013**, *93*, 310–315. [CrossRef]
27. Lei, C.; Ma, Q.; Tang, Q.; Ai, X.; Zhou, Z.; Yao, L.; Wang, Y.; Wang, Q.; Dong, J. Sodium selenite regulates phenolica and tuber development of purple potatoes. *Sci. Hortic.* **2014**, *165*, 142–147. [CrossRef]

28. Sanchini, A.; Grosjean, M. Quantification of chlorophyll a, chlorophyll b and pheopigments a in lake sediments through deconvolution of bulk UV–VIS absorption spectra. *J. Paleolimnol.* **2020**, *64*, 243–256. [CrossRef]
29. Fu, Y.J.; Liu, W.; Zu, Y.G.; Shi, X.G.; Liu, Z.G.; Schwarz, G.; Efferth, T. Breaking the spores of the fungus *Ganoderma lucidum* by supercritical $CO_2$. *Food Chem.* **2009**, *112*, 71–76. [CrossRef]
30. Yang, J.; Sun, H.; Guo, H.; Zhang, X.L.; Zhang, W.T. Research progress on the preparation, structure-activity relationship, and functional mechanism of plant polysaccharides with anti-obesity activity: A review. *Int. J. Biol. Macromol.* **2025**, *331*, 148438. [CrossRef]
31. Tanumihardjo, S.A. Carotenoids: Health effects. In *Encyclopedia of Human Nutrition*, 4th ed.; Elsevier: Amsterdam, The Netherlands, 2023; pp. 154–161. Available online: https://www.sciencedirect.com/science/article/abs/pii/B9780128218488001852 (accessed on 15 November 2025).
32. Sun, D.; Wu, S.; Li, X.; Ge, B.; Zhou, C.; Yan, X.; Cheng, P. The structure, functions and potential medicinal effects of chlorophylls derived from microalgae. *Mar. Drugs.* **2024**, *22*, 65. [CrossRef]

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.