stresses

MDPI

Article

Efficacy of Green Extracting Solvents on Antioxidant, Xanthine Oxidase, and Plant Inhibitory Potentials of Solid-Based Residues (SBRs) of *Cordyceps militaris*

Truong Ngoc Minh [1], Le Viet Anh [1], Nguyen Quang Trung [1], Bui Quang Minh [1] and Tran Dang Xuan [2,3,*]

[1] Center for Research and Technology Transfer, Vietnam Academy of Science and Technology (VAST), Hanoi 122100, Vietnam
[2] Transdisciplinary Science and Engineering Program, Graduate School of Advanced Science and Engineering, Hiroshima University, Hiroshima 739-8529, Japan
[3] Center for the Planetary Health and Innovation Science (PHIS), the IDEC Institute, Hiroshima University, Hiroshima 739-8529, Japan
* Correspondence: tdxuan@hiroshima-u.ac.jp; Tel./Fax: +81-82-424-6927

Abstract: Solid-based residues (SBRs) of *Cordyceps militaris* are often considered as waste after the cultivation of the fruiting body. To demonstrate the value of this by-product, different ratios of two favorable green solvents (EtOH and water) were employed to optimize the yields of cordycepin (Cor) and adenosine (Ado) and investigate relevant activities of plant growth inhibition (allelopathy), antioxidants, and xanthine oxidase. The SBR extracts of 60% EtOH-40% water (W4) and 40% EtOH-60% water (W6) exhibited the highest antioxidant activity as well as yielded the optimum content of Cor and Ado. The W4 and Wt (hot water) exhibited maximum inhibitory effects on the growth of *Raphanus sativus* (radish), *Lactuca sativa* (lettuce) and two noxious weeds, *Echinochloa crus-galli* (barnyard grass) and *Bidens pilosa* (beggarticks). Furthermore, GC-MS scan analysis revealed the presence of 14 major compounds in the SBRs. W4 is the best solvent to optimize yields of Cor and Ado, as well as having the strongest levels of antioxidant activity, xanthine oxidase, and growth-inhibitory activity. This study reveals that SBRs are a potential source of medicinal and agricultural utilization.

Keywords: *Cordyceps militaris*; solid-based residues; cordycepin; adenosine; antioxidant capacity; growth inhibition

Citation: Minh, T.N.; Anh, L.V.; Trung, N.Q.; Minh, B.Q.; Xuan, T.D. Efficacy of Green Extracting Solvents on Antioxidant, Xanthine Oxidase, and Plant Inhibitory Potentials of Solid-Based Residues (SBRs) of *Cordyceps militaris*. *Stresses* **2023**, *3*, 11–21. https://doi.org/10.3390/stresses3010002

Academic Editors: Marcello Iriti, Georgios Liakopoulos and Eleni Tani

Received: 27 November 2022
Revised: 16 December 2022
Accepted: 19 December 2022
Published: 21 December 2022

1. Introduction

Cordyceps, which are widely recognized as medicinal mushrooms to improve human health and wellbeing, have been emerging as a miracle cure for many common ailments in Eastern and Chinese medicine [1–3]. Among them, the fungus *Cordyceps militaris* (CM), which grows as a parasite in *Lepidopteran pupa* and is yellow or orange in its fruiting body, is one of the members of this genus of *Cordyceps*. Based on historical and research evidence, the mycelium and fruiting body of CM are widely used in the pharmaceutical and health care industries as medical materials, dietary supplements, and food additives. The potential components found in the mycelia and fruiting bodies are cordycepin (Cor), polysaccharides, proteins, and phenolic compounds, which can be extremely effective to enhance oxygen mobilization, improve ATP synthesis, stabilize blood sugar metabolism, and actualize DNA repair [4–8]. Moreover, the health effects of compounds found in this species can positively assist the treatment of cancer and diabetes and enhance the human immune system [4]. Although bioactive compounds extracted from CM are preferably used in healthy foods and dietary supplements, Cor and Adenosine (Ado) (Figure 1) are among the most active compounds that can contribute to tumor cell apoptosis, a decrease in tumor cell proliferation, and several therapeutic potentials (antineoplastic, antioxidant, anti-inflammatory activities) [9].

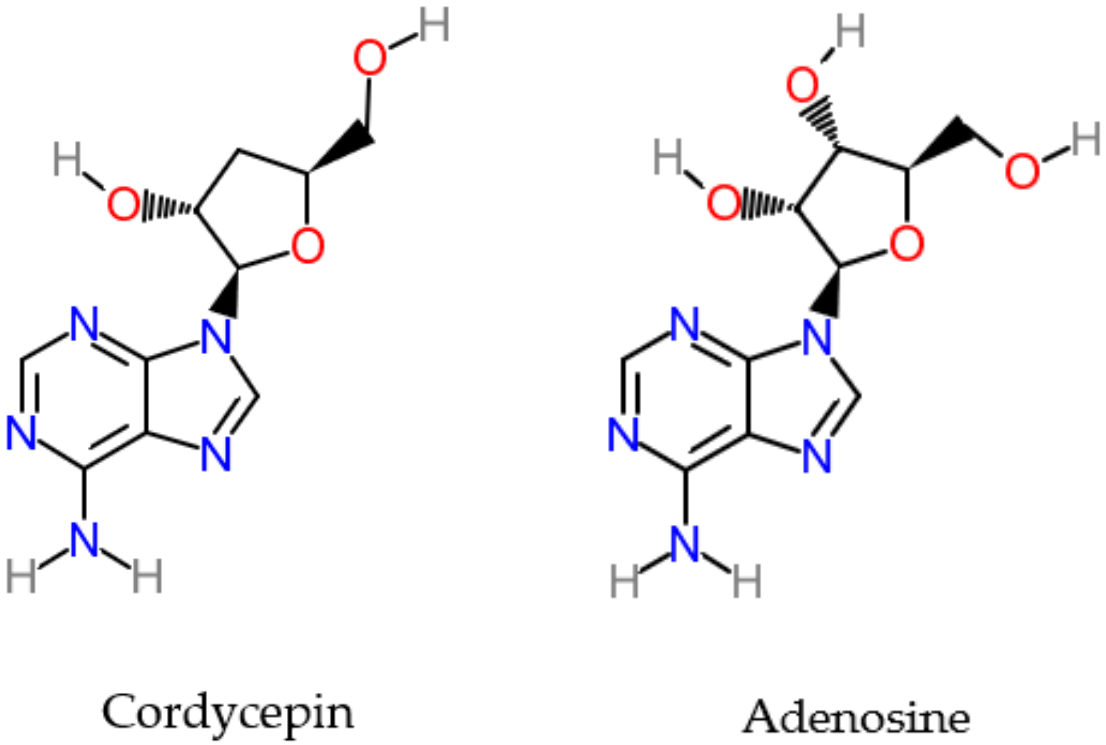

Figure 1. Chemical structures of cordycepin (Cor) and adenosine (Ado).

Due to the precious properties it can bring to human health and the high demand of the medicine industry, the marketing requirements for this species are continuously increasing. However, hosts for the growth of CM are specialized and rare in nature; the natural development of this fungus happens slowly only in specific places and ends with a small fruiting body [10]. Several solid media such as rice, green beans, corn, sunflower seeds, and insects are reported as possible alternative protein sources to cultivate CM at both laboratory and industrial levels [11]. Chemical components in CM cultivated via these artificial methods, such as Cor, Ado, proteins, carotenoids, and trace minerals, are in line with those of the fungus grown naturally [12]. Bioactive compounds are mostly found in the fruiting body of this fungus. Thus, the fruiting body of CM is considered the most valuable part, which is usually collected and utilized as a food product for human consumption. The remaining part of CM, so-called "solid media" or "solid-based residues" (SBRs), is usually discarded as waste [13,14]. The unused SBRs of CM cause problems for the environment [15]. However, SBRs may contain bioactive substances [13]. A considerable amount of Cor was extracted, separated, and purified successfully from this waste medium of CM [13].

The idea of "green solvents" has been raised since the 1990s from the concept of green chemistry [16,17]. The environmental and health hazards of conventional solvents, such as benzene, carbon disulfide, carbon tetrachloride, diethyl ether, hexane, nitromethane, and ethylene glycol dimethyl ether, has required the use of less hazardous solvents [16,18]. Thus, several solvent selection guides have emerged from many organizations including GSK [19–21], ACS [22], Pfizer [23], and Sanofi [24]. Ethanol and water are among the most recommended extractants [19–24]. Although ethanol shares the same attributes as other alcohols (methanol, isopropanol), e.g., solvent strength, dielectric constant, critical point, and hydrogen-donation ability, it has become the favorable selection because it is safe [25], easily available in high purity, low in price, and completely biodegradable [26]. Ethanol is well known as an efficient solvent to extract polyphenols [27], flavonoids, and other polar compounds [28]. Meanwhile, water also plays an important role in green chemistry and is categorized as "green" because it is non-flammable, affordable, readily available, and, more

importantly, non-toxic. Moreover, water is also a high polar solvent, having advantages regarding reactivity and selectivity, and thus it is favorable for organometallic catalysis [29]. A mixture of EtOH and water was found to be environmentally preferable [17].

In this study, two solvents, ethanol (EtOH) and water, and their combinations were used to evaluate optimum yields of Cor and Ado and relevant biological activities in relation to allelopathy, antioxidants, and xanthine oxidase (XOD). Allelopathy was determined according to the inhibitory levels of SBRs against the growth of *R. sativus*, *L. sativa*, and the two harmful weeds *E. crus-galli* and *B. pilosa*.

2. Results

2.1. Adenosine and Cordycepin Content in Cordyceps Militaris SBRs

A reversed-phase column was employed to quantify the content of the two bioactive compounds in the SBRs of CM. A good separation of Ado and Cor was conducted by the HPLC system integrated with a UV-Vis detector at a wavelength of 260 nm. Two sharp peaks of Ado and Cor were detected at the retention times of 9.110 min and 12.617 min in the standard chromatogram (Figure 2), respectively. Table 1 shows that Ado and Cor were detected and quantified in most of the extracts, except for W8–W10 and Wt where the contents of Ado were lower than LOD (0.274 μg/mL). With modifications of the solvent ratio of 10% (*v*/*v*), the levels of Ado and Cor varied unproportionately. The optimum yields of both Ado and Cor were recorded in fraction W4 (E:W = 6:4) and W6 (E:W = 4:6) when the content of these two compounds reached levels of 0.381 ± 0.002 mg/g and 0.561 ± 0.011 mg/g (W4), followed by 0.255 ± 0.001 mg/g and 0.511 ± 0.014 mg/g (W6), respectively. The highest content of Cor (16.515 ± 0.283 mg/g) was determined by hot water (Wt); however, Ado was not detected in this fraction.

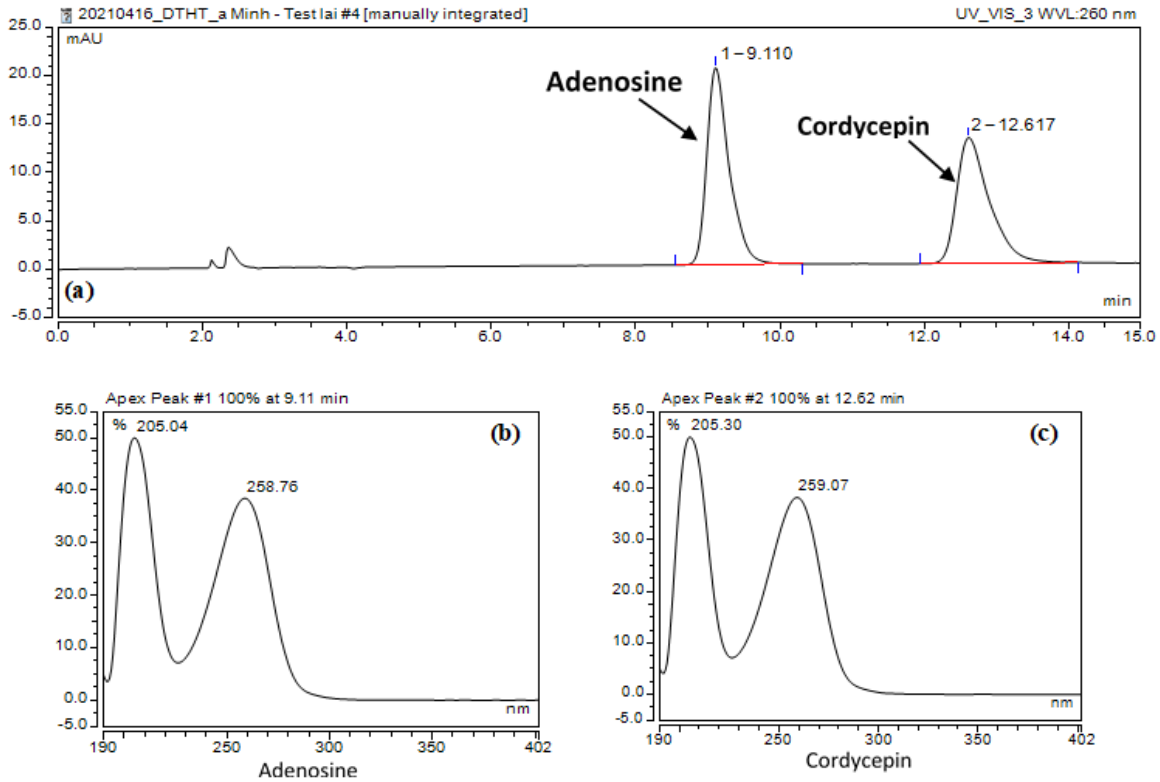

Figure 2. Chromatogram of Cor, Ado (**a**) and their ultraviolet-visible spectrophotometry (**b**,**c**).

Table 1. Adenosine and cordycepin content in *Cordyceps militaris* SBRs extracted by different solvent systems.

Extracts	Code	Adenosine (mg/g)	Cordycepin (mg/g)
EtOH	W0	0.002 ± 0.0003 d	0.018 ± 0.0004 d
E:W = 9:1	W1	0.029 ± 0.001 d	0.25 ± 0.008 c
E:W = 8:2	W2	0.096 ± 0.001 c	0.298 ± 0.012 c
E:W = 7:3	W3	0.092 ± 0.001 c	0.278 ± 0.007 c
E:W = 6:4	W4	0.381 ± 0.002 a	0.561 ± 0.011 b
E:W = 5:5	W5	0.089 ± 0.0004 c	0.251 ± 0.008 c
E:W = 4:6	W6	0.255 ± 0.001 b	0.511 ± 0.014 bc
E:W = 3:7	W7	0.074 ± 0.0004 c	0.316 ± 0.014 c
E:W = 2:8	W8	nd	0.26 ± 0.008 c
E:W = 1:9	W9	nd	0.212 ± 0.001 c
Water	W10	nd	0.423 ± 0.007 c
Hot water	Wt	nd	0.67 ± 0.012 a
Glue	Wg	nd	nd
HPLC		Standards	
Retention time (min)	-	9.117 ± 0.108	12.617 ± 0.122
LOD (μg/mL)	-	0.274	0.366
LOQ (μg/mL)	-	0.831	1.110

Values represent means ± SD (standard deviation). Alphabetic letters indicate statistical symbols generated by one-way ANOVA using Duncan's test (α = 5%). Values with similar letters in each column are not significantly different ($p < 0.05$) ($n = 3$). LOD: limit of detection; LOQ: limit of quantification; nd: not detectable; '-': not available.

2.2. Antioxidant Activities and In Vitro Inhibition of Xanthine Oxidase (XOD)

The free radical inhibitory activity of 13 extracts is presented in Table 2. It is obvious that the antioxidant activity of the SBR extracts was not proportional to the alteration of the solvent system ratio and varied unpredictably. The highest inhibitory activity of the extract was identified in two fractions, W4 and W6, where the IC_{50} of all three assays (DPPH, ABTS, and XOD) ranged from 118.5 ± 2.9 to 194.2 ± 4.9 μg/mL and from 118.2 ± 5.4 to 198.0 ± 7.7 μg/mL, respectively. These fractions confirmed a certain inhibition effect on oxidative agents due to their comparable values to the controls, including BHT (18.0 ± 0.3 and 40.0 ± 0.6 μg/mL) and allopurinol (20.8 ± 0.7 μg/mL). W10 was found to exert the lowest antioxidant activity, of which the IC_{50} ranged from 1103.5 ± 6.4 to 2007.0 ± 6.8 μg/mL. However, in the DPPH and ABTS assays, the inhibitory capability against oxidants of Wt was much higher than W10, revealing IC_{50} values of 413.5 ± 7.7 and 413.4 ± 6.7 μg/mL, respectively.

Table 2. Antioxidant activity of *Cordyceps militaris* SBRs extracted by different solvent systems.

Extract	Code	IC_{50} (μg/mL)		IC_{50} (μg/mL)
		DPPH	ABTS	XOD
EtOH	W0	1199.9 ± 6.0 b	1227.8 ± 8.2 b	782.2 ± 4.6 b
E:W = 9:1	W1	1182.2 ± 7.8 b	1246.0 ± 7.5 b	731.6 ± 7.2 b
E:W = 8:2	W2	411.5 ± 7.4 c	457.4 ± 4.9 c	451.7 ± 10.0 c
E:W = 7:3	W3	424.1 ± 7.3 c	459.9 ± 5.8 c	448.5 ± 9.9 c
E:W = 6:4	W4	118.5 ± 2.9 d	194.2 ± 4.9 d	186.0 ± 1.9 d
E:W = 5:5	W5	225.2 ± 5.3 cd	234.1 ± 3.1 cd	450.8 ± 3.7 cd
E:W = 4:6	W6	118.2 ± 5.4 d	198.0 ± 7.7 d	185.2 ± 6.0 d
E:W = 3:7	W7	851.2 ± 7.8 bc	846.6 ± 7.2 bc	466.8 ± 10.0 bc
E:W = 2:8	W8	1234.8 ± 8.7 b	1230.3 ± 6.7 b	778.2 ± 13.6 b
E:W = 1:9	W9	1232.5 ± 9.5 b	1246.2 ± 8.7 b	750.5 ± 5.0 b
Water	W10	1964.3 ± 8.0 a	2007.0 ± 6.8 a	1103.5 ± 6.4 a
Hot water	Wt	413.5 ± 7.7 c	413.4 ± 6.7 c	1190.7 ± 7.7 c
Glue	Wg	nd	nd	nd
BHT	-	18.0 ± 0.3 e	40.0 ± 0.6 e	-
Allopurinol	-	-	-	20.8 ± 0.7 e

Values represent means ± SD (standard deviation). Alphabetic letters indicate statistical symbols generated by one-way ANOVA using Duncan's test (α = 5%). Values with similar letters in each column are not significantly different ($p < 0.05$) ($n = 3$). BHT: Butylated hydroxytoluene; nd: not detectable; '-': not available.

2.3. Screening Analysis of GC-MS

By GC-MS, major compounds in the SBRs relating to the different extracts were identified, and these are shown in Table 3. They included 14 compounds: 1H-pyrazole,1,3,5-trimethyl-; 1-tetradecanol; acetic acid; aqua cera; bis(2-ethylhexyl) phthalate; glycerin; hexadecanoic acid; methyl oleate; myristic acid; oxacyclododecan-2-one; phloroglucinol; ricinoleic acid; stearic acid; and trehalose. These chemicals belonged to disaccharides, fatty acid, and phenolic compounds. The presence of the chemicals varied among the extracting protocols, of which trehalose accounted for greater peak areas as compared to other putative constituents. Ricinoleic acid was found in W0–W6 and W7, whilst stearic acid was detected in W0, W2, W3, W4, and W5.

Table 3. Principal compounds identified in *Cordyceps militaris* SBRs by GC-MS screening analysis.

Extract	Codes	Major Constituents	Retention Times (min)	Peak Area (%)
EtOH	W0	Ste, Tre, Met, Ric, Oxa, Myr	20.30, 21.45, 22.94, 23.70, 26.56, 27.40	24.79, 7.33, 36.12, 8.69, 8.8, 4.22
E:W = 9:1	W1	Gly, Tre, Met, Hex, Ric	20.80, 22.65, 22.98, 23.22, 23.72,	4.94, 5.76, 28.9, 34.52, 7.79, 0
E:W = 8:2	W2	Aqu, Tre, Gly, Hex, Ric, Ste, Met	20.31, 20.79, 21.45, 23.28, 23.73, 25.60, 29.04	11.58, 1.81, 1.27, 18.49, 7.88, 7.98, 45.95
E:W = 7:3	W3	Aqu, Tre, Gly, Ric, Hex, Ste, Met	20.32, 20.81, 21.48, 22.89, 23.29, 23.74, 29.15	14.07, 6.76, 1.25, 16.44, 13.14, 4.57, 38.56
E:W = 6:4	W4	Tre, Gly, Ric, Hex, Ste, Met	20.82, 21.49, 22.82, 23.27, 25.65, 29.16	19.22, 10.23, 16.68, 17.26, 5.4, 18.93
E:W = 5:5	W5	Ace, Tre, Gly, Ric, Hex, Ste, Met	11.93, 20.85, 21.53, 22.95, 23.25, 25.62, 29.12	2.83, 3.86, 3.25, 12.85, 23.61, 5.18, 35.78
E:W = 4:6	W6	Ace, Tre, Gly, Ric, Met	11.91, 20.83, 21.50, 22.56, 28.35	3.67, 7.75, 9.24, 23.34, 24.19
E:W = 3:7	W7	Ace, Tre, Gly, Ric, Hex, 1-T	11.91, 20.83, 21.51, 22.19, 22.86, 23.85	4.57, 14.37, 9.76, 32.22, 7.06, 6.28
E:W = 2:8	W8	Ace, Tre, Gly, 1-T	11.88, 20.80, 21.50, 23.83	6.09, 15.61, 10.21, 9.41
E:W = 1:9	W9	Ace, 1H-, Tre, Gly, Hex, Phl	11.92, 13.44, 20.83, 21.52, 22.74, 23.88	8.18, 3.85, 18.19, 25.46, 5.13, 32.83
Water	W10	Ace, Tre, Gly, 1-T	11.95, 20.86, 21.56, 23.90	3.28, 8.06, 12.26, 7.69
Hot water	Wt	Ace, Tre, Gly	11.89, 20.79, 21.48	15.43, 8.49, 18.34
Glue	Wg	Tre	20.77	30.16

1H-Pyrazole,1,3,5-trimethyl-: 1H-; 1-Tetradecanol: 1-T; acetic acid: Ace; aqua cera: Aqu; bis(2-ethylhexyl) phthalate: Bis; glycerin: Gly; hexadecanoic acid: Hex; methyl oleate: Met; myristic acid: Myr; oxacyclododecan-2-one: Oxa; phloroglucinol: Phl; ricinoleic acid: Ric; stearic acid: Ste; trehalose: Tre.

2.4. Growth-Inhibitory Activity on Plants of Cordyceps Militaris SBRs Extract

The inhibitory effect of the SBR extracts on the development of two indicator plants and two noxious weeds, expressed in IC_{50} values, is illustrated in Table 4. As can be seen, water played an important role in the solvent system to prove the weed-resistance activity of the SBR extracts. The MeOH extract fraction (W0) yielded the highest IC_{50} value for all four weed species, ranging from 438.0 ± 13.0 to 529.8 ± 15.4 μg/mL. In contrast, the inhibitory concentration of the solvent systems from W1 to W10 ranged below 178.3 ± 8.7 μg/mL, which was at least three times lower than W0. Among the 14 extracts, Wt exhibited the highest inhibitory activity against the growth of the two indicator plants and the two noxious weeds (IC_{50} value ranged from 54.9 ± 3.8 to 64.7 ± 1.0 μg/mL), followed by the extract W4 (IC_{50} value ranged from 64.7 ± 1.4 to 78.1 ± 3.9 μg/mL).

Table 4. Inhibitory effects of different *Cordyceps militaris* SBR extracts on the growth of *R. sativus*, *E. crus-galli*, *L. sativa*, and *B. pilosa* L.

Extract (Code)	*R. sativus* IC_{50} (μg/mL)		*E. crus-galli* IC_{50} (μg/mL)		*L. sativa* IC_{50} (μg/mL)		*B. pilosa L.* IC_{50} (μg/mL)	
	Shoot	**Root**	**Shoot**	**Root**	**Shoot**	**Root**	**Shoot**	**Root**
W0	512.2 ± 10.8 b	529.8 ± 15.4 b	459.6 ± 16.3 b	438.0 ± 13.0 b	449.7 ± 13.5 b	439.7 ± 3.7 b	522.5 ± 10.5 b	523.3 ± 12.2 b
W1	163.0 ± 5.6 c	172.8 ± 2.6 c	151.0 ± 3.5 c	147.5 ± 3.7 c	150.7 ± 6.7 c	140.9 ± 7.8 c	178.3 ± 8.7 c	172.1 ± 8.8 c
W2	125.4 ± 2.0 c	126.4 ± 4.5 c	114.7 ± 4.3 c	108.4 ± 3.7 c	110.4 ± 3.8 c	108.0 ± 3.5 c	128.5 ± 2.6 c	129.9 ± 5.9 c
W3	122.4 ± 1.5 c	119.8 ± 5.8 c	113.3 ± 1.5 c	108.8 ± 2.5 c	113.2 ± 1.8 c	106.7 ± 5.7 c	127.9 ± 7.5 c	123.4 ± 4.3 c
W4	77.5 ± 4.4 d	78.1 ± 3.9 d	73.1 ± 3.4 d	64.7 ± 1.4 d	71.9 ± 1.5 d	65.6 ± 0.8 d	77.3 ± 1.2 d	76.4 ± 3.2 d
W5	119.8 ± 3.5 c	128.9 ± 5.1 c	115.6 ± 5.3 c	106.8 ± 2.4 c	109.8 ± 4.9 c	107.5 ± 2.3 c	121.7 ± 5.1 c	127.9 ± 2.2 c
W6	79.6 ± 1.0 d	82.0 ± 1.3 d	76.0 ± 3.9 d	70.7 ± 0.8 d	74.4 ± 0.6 d	67.7 ± 2.4 d	86.3 ± 4.1 d	85.5 ± 3.7 d
W7	116.8 ± 2.6 c	121.5 ± 5.6 c	111.4 ± 1.3 c	105.5 ± 4.2 c	107.6 ± 2.6 c	101.4 ± 6.4 c	119.6 ± 3.4 c	122.6 ± 4.1 c
W8	148.7 ± 6.1 c	152.0 ± 6.6 c	136.1 ± 4.9 c	129.9 ± 5.1 c	130.5 ± 6.6 c	126.5 ± 8.8 c	154.7 ± 1.5 c	154.7 ± 1.7 c
W9	164.0 ± 5.0 c	175.4 ± 9.0 c	157.2 ± 9.8 c	145.3 ± 0.7 c	148.8 ± 6.0 c	147.2 ± 10.4 c	176.9 ± 11.7 c	174.7 ± 1.7 c
W10	99.1 ± 1.6 cd	106.2 ± 5.0 cd	94.6 ± 3.7 cd	84.5 ± 1.6 cd	87.4 ± 3.8 cd	84.6 ± 5.8 cd	101.6 ± 2.8 cd	100.4 ± 6.3 cd
Wt	62.0 ± 1.8 d	63.4 ± 1.0 d	57.4 ± 0.7 d	54.5 ± 2.8 d	56.9 ± 1.9 d	54.9 ± 3.8 d	64.7 ± 1.0 d	63.6 ± 3.5 d
Wg	1025.1 ± 28.6 a	1074.7 ± 24.2 a	950.3 ± 27.7 a	834.6 ± 22.5 a	915.2 ± 25.3 a	844.2 ± 12.9 a	1070.0 ± 28.5 a	1054.9 ± 21.7 a

Values represent means ± SD (standard deviation). Alphabetic letters indicate statistical symbols generated by one-way ANOVA using Duncan's test (α = 5%). Values with similar letters in each column are not significantly different ($p < 0.05$) ($n = 3$).

3. Discussion

Bioactive compounds, including Cor, Ado, polysaccharides, ergosterol, mannitol, and phenolic compounds, are reported to play important roles in pharmacological and medicinal activities of *C. militaris* [30]. Ado is regarded as the principal active compound in CM fruiting body cultured in China [31,32]. Meanwhile, Cor has shown capability in cancer cell inhibition [32]. In this study, these two compounds were both observed in the SBRs, although their doses varied among the applied solvents of EtOH-water and hot water. Pintathong et al. [15] employed barley, white rice, riceberry rice, and wheat to culture CM and obtained contents of Cor and Ado of between 0.78 and 1.09 mg/g dried weight [15]. Elsewhere, the concentration of Ado in CM waste medium was 0.47 mg/g DW [33]. The contents of Cor and Ado in the corpus of CM were 3.6 and 0.6 mg/g of CM, respectively [34]. Compared to the mentioned papers, the contents of Ado and Cor in this research (0.002–0.381 mg/g and 0.018–0.67 mg/g, respectively) were lower. The medium used to culture CM, which has a major effect on the yield of Ado and Cor from the fruiting body, may regulate the contents of Ado and Cor of this study [35,36].

Reactive oxygen species (ROS) is the main factor for oxidative stress to cause various diseases, such as hepatopathies, atherosclerosis, and aging [37,38]. The mechanism of antioxidants, which comprises their direct reaction with free radicals including hydrogen peroxide (H_2O_2), superoxide anion ($O_2^{\bullet -}$) radicals, and hydroxyl (OH^-) radicals, etc., to prevent the formation of peroxide, is the factor that mitigates the impact of those radicals on cells [33]. CM might be a good source to scavenge the activities of certain types of ROS, including xanthine oxidase (XOD), DPPH$^\bullet$, and ABTS$^\bullet$ radicals [39–41]. In this study, hot water was the best medium to optimize the yields of the major constituents of CM, and it also exhibited the strongest antioxidant capacity. In addition, the combination of EtOH and water at the ratio of 4:6 (W6) was the ideal fraction to scavenge the activities of XOD, DPPH$^\bullet$, and ABTS$^\bullet$. The concentrations of SBRs to inhibit the three ROS were 185.2 ± 6.0 μg/mL, 118.2 ± 5.4 μg/mL, and 198.0 ± 7.7 μg/mL (IC_{50}), respectively (Table 2). Wang et al. (2015) indicated that, at a concentration of 15 mg/mL, CM waste medium extract can inhibit 50% of TBARS formation, while the highest values of scavenging activity against DPPH$^\bullet$ (within the range of 40.12 ± 0.85 to 66.62 ± 2.10 mg TEAC/g extract) were found in hot water extracts of SBRs [15,33].

Bioactive compounds such as polyphenols, flavonoids, polysaccharides, sterols, and peptides are associated with antioxidant capacity in CM extracts [42]. Polysaccharides are the most

potential compounds in mushrooms to be responsible for their antioxidant properties [43,44]. Polysaccharides separated from CM decreased the level of malondialdehyde, which is considered an indicator for oxidant damage in cells, in mice blood [45,46]. The GC-MS analysis of this study revealed the presence of 14 compounds, of which trehalose, stearic acid, and methyl oleate have been described as increasing antioxidant activities [47]. The antioxidant activity, xanthine oxidase, and inhibitory activity of W4 and W6 may be largely attributed to these compounds. Trehalose can modify the structural conformation of the enzyme, therefore enhancing the activity of urate oxidase and improving its catalytic efficiency [48–51].

Allelochemicals from fungi have been described as having potential for weed control in agriculture [52–54]. The toxic effect of fungi on plant growth is driven by the variety and concentration of allelochemicals [55]. This study revealed that Wt and W4 exerted the maximum inhibitory effects on the germination, shoot height, and root length of two indicator plants and two noxious weeds, as compared to other fractions (Table 2). This suggests that Wt and W4 might contain certain allelochemical content relevant to inhibitory activity. Cor isolated from CM strongly inhibited the growth of radish seedlings [39]. Particularly, at a concentration of 0.04 mg/mL, Cor inhibited the germination and growth of *R. sativus* 3.8–5.9 and 3.3–3.7 times more than benzoic acid and glyphosate, respectively [56]. Meanwhile, the analysis of HPLC also confirmed the noticeable presence of Cor in Wt and W4. The immersion of EtOH provided an amount of Cor, but the highest yield of Cor was found in Wt (hot water treatment), followed by W4 and W6 (treatments of EtOH 60% and EtOH 40%, respectively) (Table 1). The amounts of Cor varied among the extracts and are proportional to the observed inhibitory activities against the tested plants (Tables 1 and 3). Cor has been described as a potential compound to alter glyphosate, which has recently been debated and removed from the market in some countries [56].

4. Materials and Methods

4.1. Materials

SBRs of *C. militaris* were obtained from Center for Research and Development of Mushroom, Agricultural Genetics Institute (AGI), Vietnam in January 2021. The drying process of SBRs was conducted at 40 ± 1 °C for 3 days until SBR weight was constant. The dried SBRs were then ground into powders. An alteration of 10% of the solvent system (EtOH:water; E:W) was applied to each extraction procedure to identify the most active fraction. A 50 g sample was mixed with a solvent (500 mL) at a solid–liquid ratio of 1:10 w/v, then the obtained product was processed with an orbital shaker, conditioning at 125 rpm for 6 h. Exceptionally, hot water extraction was conducted with an incubator shaker, conditioned at 95 ± 1 °C with a rate of 125 rpm for 4 h. All experiments were replicated thrice. Then, the filtrate of the mixture was collected via a Whatman filter (no.1) in a vacuum filtration system. Solvent vaporization was conducted using a rotary evaporator to obtain the concentrated crude extract, which was then kept in MeOH and stored at −20 °C for further analysis.

4.2. Reagents

1,1-dipheynyl 2-picrylhydrazyl (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), dimethyl sulfoxide (DMSO), monopotassium phosphate, dipotassium phosphate, sodium hydroxide, hydrochloric acid, xanthine, microbial xanthine oxidase, and allopurinol were purchased from Sigma-Aldrich Pte Ltd., Singapore. Analytical grade reagents were utilized for all experimentation.

4.3. Adenosine and Cordycepin Content in Cordyceps militaris SBRs

Quantification of Cor and Ado was performed by HPLC system, adapting the method of Li et al. [57]. In brief, 5 mL of SBR extract (in MeOH) was filtered through a hydrophilic filter (0.2 µm) before being injected into the HPLC system (Thermo UltiMate 3000, column Hypersil Gold 250 × 4.6 mm–5 µm). Two solvent systems, (A)—H_2O, ammonium acetate 10 mM, acetic acid 0.1% and (B)—MeOH 90%, ammonium acetate 10 mM, acetic acid 0.1%,

were employed for the gradient program. This included 3 phases: The first 20 min involved a gradient concentration of B (5% to 95%), which inclined to 100% in the next 5 min and decreased to 5% in the last 5 min. The HPLC system was conditioned with a wavelength of 260 nm, flow rate of 1.6 mL/min, column temperature of 40 °C, and sample temperature of 15 °C.

4.4. Antioxidant and Xanthine Oxidase Inhibition (XOD) Activities

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) radical cation decolorization assays were used to determine the antioxidant activity of all fractions [58,59]. Slight modifications from the reported spectrophotometric technique were used to measure the inhibitory effect on xanthine oxidase (XO) of all fractions [60].

4.5. GC-MS Screening Analysis

A GC-MS system (GC Trace 1310, Thermo Fisher Scientific, Waltham, USA) equipped with TriPlus RSH liquid autosampler and coupled with electron impact ionization-tandem mass spectrometry (TSQ 9000, Thermo Fisher Scientific, Waltham, USA) was employed to determine the chemical constituents of fractions from W0 to Wg [61]. A Thermo Scientific TG-5MS column (30 m × 0.25 mm ID× 0.25 µm in thickness) was utilized for chemical separation. Splitless mode was selected for the liquid autosampler, of which the injection volume and temperature were set at 1 µL and 280 °C, respectively. The carrier gas was helium (99.999% purity), and the flow rate was maintained at 1.0 mL min^{-1} throughout separation time. The temperature program of the GC oven was as follows: A hold time of 3 min and an initial temperature of 80 °C were set for the first stage. Then, the temperature was increased to 200 °C with a rate of 15 °C min^{-1} before continuously rising to 300 °C with a lower rate of 8 °C min^{-1}. Finally, a temperature of 300 °C was held for 5 min to end the program. Constant temperatures of 280 °C and 150 °C were used for the electron impact ionization source and quadrupole, respectively. Volatile compound detection was performed by positive EI-MS/MS under MRM mode and at an energy ionization level of 70 eV.

4.6. Growth-Inhibitory Activity Bioassays

Echinochloa crus-galli, Bidens pilosa L., Raphanus sativus L., and *Lactuca sativa L.* seeds were selected as experimental subjects to evaluate the growth-constraint potential of the SBR extracts. The seeds were germinated in a growth chamber, which was conditioned with a photoperiodic setting of 12/12 h (day/night) and corresponding temperatures of 25/23 °C. The preparation of the SRB-derived test solution was conducted by diluting the extract in Tween water (0.2%) to attain different concentrations (10, 100, 500, 1000, and 2000 µg/mL). An aliquot of the SRB solution (100 µL) was dropped through filter papers laid on top of a 12-well plate (22 mm diameter × 18 mm height of each well). The selected seeds were put in corresponding wells filled with 100 µL of distilled water. Changes in length of the root and shoot were monitored and recorded daily for 7 days. Six replications were applied in this bioassay. The half-maximal concentration (IC_{50}) of the SBRs inhibiting shoot and root growth was also determined following the previous method [62].

4.7. Statistical Analysis

Results are presented as mean ± standard deviation (SD). Duncan's test (α = 5%) was employed to present significant differences in the results. Moreover, significant differences of the solvent system were determined by one-way analysis of variance (ANOVA) using the statistical software Minitab®19.2020.1 (Philadelphia, PA, USA).

5. Conclusions

This study optimized the quantification of cordycepin (Cor) and adenosine (Ado) in solid-based residues of *C. militaris* using different combinations of EtOH-water and hot water and examined the relevant potential of antioxidant activities, antihyperuricemia, and plant growth-inhibitory activities. The combination of 60% EtOH-40% water (W4)

obtained the highest amounts of Cor and Ado and the strongest levels of the examined biological activities. Hot water (Wt) displayed the maximum inhibition on the growth of lettuce, radish, and the noxious weeds *E. crus-galli* and *B. pilosa*. From this study, SBRs of *C. militaris* appear to be a potential source to exploit its potential in antioxidant and xanthine oxidase activity as well as in weed control.

Author Contributions: Conceptualization and methodology, T.N.M.; formal analysis, L.V.A. and T.N.M.; investigation, N.Q.T. and B.Q.M.; resources, T.N.M. and L.V.A.; writing—original draft preparation, L.V.A. and T.N.M.; writing—review and editing, T.D.X.; supervision, T.D.X. and N.Q.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Vietnam Academy of Science and Technology under grant number THTEXS.02/21-24.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Center for Research and Technology Transfer, Vietnam is appreciated for partly supporting this research. We thank Do Hoang Giang and Nguyen Tien Dat for helpful advice on this research.

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

References

1. Pan, S.-Y.; Litscher, G.; Gao, S.-H.; Zhou, S.-F.; Yu, Z.-L.; Chen, H.-Q.; Zhang, S.-F.; Tang, M.-K.; Sun, J.-N.; Ko, K.-M. Historical Perspective of Traditional Indigenous Medical Practices: The Current Renaissance and Conservation of Herbal Resources. *Evid. Based Complement. Altern. Med.* **2014**, *2014*, 525340. [CrossRef] [PubMed]
2. Chang, S.T.; Wasser, S.P. The Role of Culinary-Medicinal Mushrooms on Human Welfare with a Pyramid Model for Human Health. *Int. J. Med. Mushrooms* **2012**, *14*, 95–134. [CrossRef] [PubMed]
3. Valverde, M.E.; Hernández-Pérez, T.; Paredes-López, O. Edible Mushrooms: Improving Human Health and Promoting Quality Life. *Int. J. Microbiol.* **2015**, *2015*, 376387. [CrossRef] [PubMed]
4. Nguyen, L.T.; Van Le, V.; Nguyen, B.T.T.; Ngo, N.X.; Nguyen, H.T.T.; Nguyen, Q.D.; Mulla, S. Cultural Characteristics and Cordycepin Production of Some *Cordyceps militaris* Strains under Artificial Cultivation Conditions. *BioTechnologia* **2020**, *101*, 135–145. [CrossRef]
5. Kim, H.O.; Yun, J.W. A Comparative Study on the Production of Exopolysaccharides between Two Entomopathogenic Fungi *Cordyceps militaris* and *Cordyceps sinensis* in Submerged Mycelial Cultures. *J. Appl. Microbiol.* **2005**, *99*, 728–738. [CrossRef]
6. Yu, X.-Y.; Zou, Y.; Zheng, Q.-W.; Lu, F.-X.; Li, D.-H.; Guo, L.-Q.; Lin, J.-F. Physicochemical, Functional and Structural Properties of the Major Protein Fractions Extracted from *Cordyceps militaris* Fruit Body. *Food Res. Int.* **2021**, *142*, 110211. [CrossRef]
7. Dong, C.-H.; Yang, T.; Lian, T. A Comparative Study of the Antimicrobial, Antioxidant, and Cytotoxic Activities of Methanol Extracts from Fruit Bodies and Fermented Mycelia of Caterpillar Medicinal Mushroom *Cordyceps militaris* (Ascomycetes). *Int. J. Med. Mushrooms* **2014**, *16*, 485–495. [CrossRef]
8. Prieto-Bermejo, R.; Romo-González, M.; Pérez-Fernández, A.; Ijurko, C.; Hernández-Hernández, Á. Reactive Oxygen Species in Haematopoiesis: Leukaemic Cells Take a Walk on the Wild Side. *J. Exp. Clin. Cancer Res.* **2018**, *37*, 125. [CrossRef]
9. Tuli, H.S.; Sharma, A.K.; Sandhu, S.S.; Kashyap, D. Cordycepin: A Bioactive Metabolite with Therapeutic Potential. *Life Sci.* **2013**, *93*, 863–869. [CrossRef]
10. Holliday, J.; Matt, C. On the Trail of the Yak Ancient Cordyceps in the Modern World. Available online: https://www.academia.edu/81461467/On%20the%20Trail%20of%20The%20Yak%20Ancient%20Cordyceps%20in%20the%20Modern%20World (accessed on 24 June 2022).
11. Silva, D.; Ximenes, J.M. *Using Different Cereals to Cultivate Cordyceps militaris*; Assumption University: Bangkok, Thailand, 2019.
12. Wang, Y.; Yang, Z.; Bao, D.; Li, B.; Yin, X.; Wu, Y.; Chen, H.; Tang, G.; Li, N.; Zou, G. Improving Hypoxia Adaption Causes Distinct Effects on Growth and Bioactive Compounds Synthesis in an Entomopathogenic Fungus *Cordyceps militaris*. *Front. Microbiol.* **2021**, *12*, 698436. [CrossRef]
13. Ni, H.; Zhou, X.-H.; Li, H.-H.; Huang, W.-F. Column Chromatographic Extraction and Preparation of Cordycepin from *Cordyceps militaris* Waster Medium. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2009**, *877*, 2135–2141. [CrossRef]
14. Roda, A.; Lucini, L.; Torchio, F.; Dordoni, R.; De Faveri, D.M.; Lambri, M. Metabolite Profiling and Volatiles of Pineapple Wine and Vinegar Obtained from Pineapple Waste. *Food Chem.* **2017**, *229*, 734–742. [CrossRef]
15. Pintathong, P.; Chomnunti, P.; Sangthong, S.; Jirarat, A.; Chaiwut, P. The Feasibility of Utilizing Cultured *Cordyceps militaris* Residues in Cosmetics: Biological Activity Assessment of Their Crude Extracts. *J. Fungi* **2021**, *7*, 973. [CrossRef]
16. Winterton, N. The Green Solvent: A Critical Perspective. *Clean Technol. Environ. Policy* **2021**, *23*, 2499–2522. [CrossRef]

17. Capello, C.; Fischer, U.; Hungerbühler, K. What Is a Green Solvent? A Comprehensive Framework for the Environmental Assessment of Solvents. *Green Chem.* **2007**, *9*, 927–934. [CrossRef]
18. Welton, T. Solvents and Sustainable Chemistry. *Proc. Math. Phys. Eng. Sci.* **2015**, *471*, 20150502. [CrossRef]
19. Curzons, A.D.; Constable, D.C.; Cunningham, V.L. Solvent Selection Guide: A Guide to the Integration of Environmental, Health and Safety Criteria into the Selection of Solvents. *Clean Technol. Environ. Policy* **1999**, *1*, 82–90. [CrossRef]
20. Jiménez-González, C.; Curzons, A.D.; Constable, D.J.C.; Cunningham, V.L. Expanding GSK's Solvent Selection Guide—Application of life cycle assessment to enhance solvent selections. *Clean Technol. Environ. Policy* **2004**, *7*, 42–50. [CrossRef]
21. Henderson, R.K.; Jiménez-González, C.; Constable, D.J.C.; Alston, S.R.; Inglis, G.G.A.; Fisher, G.; Sherwood, J.; Binks, S.P.; Curzons, A.D. Expanding GSK's Solvent Selection Guide—Embedding Sustainability into Solvent Selection Starting at Medicinal Chemistry. *Green Chem.* **2011**, *13*, 854. [CrossRef]
22. ACS GCI Pharmaceutical Roundtable Solvent Selection Guide Version 2.0 Issued March 21, 2011. Available online: https://www.acs.org/content/dam/acsorg/greenchemistry/industriainnovation/roundtable/acs-gci-pr-solvent-selection-guide.pdf (accessed on 11 November 2022).
23. Alfonsi, K.; Colberg, J.; Dunn, P.J.; Fevig, T.; Jennings, S.; Johnson, T.A.; Peter Kleine, H.; Knight, C.; Nagy, M.A.; Perry, D.A.; et al. Green Chemistry Tools to Influence a Medicinal Chemistry and Research Chemistry Based Organisation. *Green Chem.* **2008**, *10*, 31–36. [CrossRef]
24. Prat, D.; Pardigon, O.; Flemming, H.-W.; Letestu, S.; Ducandas, V.; Isnard, P.; Guntrum, E.; Senac, T.; Ruisseau, S.; Cruciani, P.; et al. Sanofi's Solvent Selection Guide: A Step toward More Sustainable Processes. *Org. Process Res. Dev.* **2013**, *17*, 1517–1525. [CrossRef]
25. Tekin, K.; Hao, N.; Karagoz, S.; Ragauskas, A.J. Ethanol: A Promising Green Solvent for the Deconstruction of Lignocellulose. *ChemSusChem* **2018**, *11*, 3559–3575. [CrossRef]
26. Chemat, F.; Vian, M.A.; Cravotto, G. Green Extraction of Natural Products: Concept and Principles. *Int. J. Mol. Sci.* **2012**, *13*, 8615–8627. [CrossRef]
27. Do, Q.D.; Angkawijaya, A.E.; Tran-Nguyen, P.L.; Huynh, L.H.; Soetaredjo, F.E.; Ismadji, S.; Ju, Y.-H. Effect of Extraction Solvent on Total Phenol Content, Total Flavonoid Content, and Antioxidant Activity of *Limnophila aromatica*. *J. Food Drug Anal.* **2014**, *22*, 296–302. [CrossRef]
28. Chaves, J.O.; De Souza, M.C.; Da Silva, L.C.; Lachos-Perez, D.; Torres-Mayanga, P.C.; Machado, A.P.D.F.; Forster-Carneiro, T.; Vázquez-Espinosa, M.; González-de-Peredo, A.V.; Barbero, G.F.; et al. Extraction of Flavonoids from Natural Sources Using Modern Techniques. *Front. Chem.* **2020**, *8*, 507887. [CrossRef]
29. Shanab, K.; Neudorfer, C.; Schirmer, E.; Spreitzer, H. Green Solvents in Organic Synthesis: An Overview. *Curr. Org. Chem.* **2013**, *17*, 1179–1187. [CrossRef]
30. Elkhateeb, W.A.; Daba, G.M.; Thomas, P.W.; Wen, T.-C. Medicinal Mushrooms as a New Source of Natural Therapeutic Bioactive Compounds. *Egypt. Pharm. J.* **2019**, *18*, 88.
31. Dong, J.Z.; Liu, M.R.; Lei, C.; Zheng, X.J.; Wang, Y. Effects of Selenium and Light Wavelengths on Liquid Culture of *Cordyceps militaris* Link. *Appl. Biochem. Biotechnol.* **2012**, *166*, 2030–2036. [CrossRef]
32. Guo, M.; Guo, S.; Huaijun, Y.; Bu, N.; Dong, C.-H. Comparison of Major Bioactive Compounds of the Caterpillar Medicinal Mushroom, *Cordyceps militaris* (Ascomycetes), Fruiting Bodies Cultured on Wheat Substrate and Pupae. *Int. J. Med. Mushrooms* **2016**, *18*, 327–336. [CrossRef]
33. Wang, C.-L.; Chiang, C.-J.; Chao, Y.-P.; Yu, B.; Lee, T.-T. Effect of *Cordyceps militaris* Waster Medium on Production Performance, Egg Traits and Egg Yolk Cholesterol of Laying Hens. *J. Poult. Sci.* **2015**, *52*, 188–196. [CrossRef]
34. Hur, H. Chemical Ingredients of *Cordyceps militaris*. *Mycobiology* **2008**, *36*, 233–235. [CrossRef]
35. Shih, I.-L.; Tsai, K.-L.; Hsieh, C. Effects of Culture Conditions on the Mycelial Growth and Bioactive Metabolite Production in Submerged Culture of *Cordyceps militaris*. *Biochem. Eng. J.* **2007**, *33*, 193–201. [CrossRef]
36. Iamtham, S.; Kaewkam, A.; Chanprame, S.; Pan-utai, W. Effect of Spirulina Biomass Residue on Yield and Cordycepin and Adenosine Production of *Cordyceps militaris* Culture. *Bioresour. Technol. Rep.* **2022**, *17*, 100893. [CrossRef]
37. Sies, H.; Jones, D.P. Reactive Oxygen Species (ROS) as Pleiotropic Physiological Signalling Agents. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 363–383. [CrossRef]
38. Yu, H.M.; Wang, B.-S.; Huang, S.C.; Duh, P.-D. Comparison of Protective Effects between Cultured *Cordyceps militaris* and Natural *Cordyceps sinensis* against Oxidative Damage. *J. Agric. Food Chem.* **2006**, *54*, 3132–3138. [CrossRef]
39. Quy, T.; Xuan, T. Xanthine Oxidase Inhibitory Potential, Antioxidant and Antibacterial Activities of *Cordyceps militaris* (L.) Link Fruiting Body. *Medicines* **2019**, *6*, 20. [CrossRef]
40. Eiamthaworn, K.; Kaewkod, T.; Bovonsombut, S.; Tragoolpua, Y. Efficacy of *Cordyceps militaris* Extracts against Some Skin Pathogenic Bacteria and Antioxidant Activity. *J. Fungi* **2022**, *8*, 327. [CrossRef]
41. Liu, W.; Dun, M.; Liu, X.; Zhang, G.; Ling, J. Effects on Total Phenolic and Flavonoid Content, Antioxidant Properties, and Angiotensin I-Converting Enzyme Inhibitory Activity of Beans by Solid-State Fermentation with *Cordyceps militaris*. *Int. J. Food Prop.* **2022**, *25*, 477–491. [CrossRef]
42. Shahidi, F.; Zhong, Y. Novel Antioxidants in Food Quality Preservation and Health Promotion. *Eur. J. Lipid Sci. Technol.* **2010**, *112*, 930–940. [CrossRef]

43. Zeng, Y.; Zhang, Y.; Zhang, L.; Cui, S.; Sun, Y. Structural Characterization and Antioxidant and Immunomodulation Activities of Polysaccharides from the Spent Rice Substrate of *Cordyceps militaris*. *Food Sci. Biotechnol.* **2015**, *24*, 1591–1596. [CrossRef]
44. Yu, R.; Yang, W.; Song, L.; Yan, C.; Zhang, Z.; Zhao, Y. Structural Characterization and Antioxidant Activity of a Polysaccharide from the Fruiting Bodies of Cultured *Cordyceps militaris*. *Carbohydr. Polym.* **2007**, *70*, 430–436. [CrossRef]
45. Wang, M.; Meng, X.Y.; Yang, R.L.; Qin, T.; Wang, X.Y.; Zhang, K.Y.; Fei, C.Z.; Li, Y.; Hu, Y.L.; Xue, F.Q. *Cordyceps militaris* Polysaccharides Can Enhance the Immunity and Antioxidation Activity in Immunosuppressed Mice. *Carbohydr. Polym.* **2012**, *89*, 461–466. [CrossRef] [PubMed]
46. Yilmaz, N.; Dulger, H.; Kiymaz, N.; Yilmaz, C.; Gudu, B.O.; Demir, I. Activity of Mannitol and Hypertonic Saline Therapy on the Oxidant and Antioxidant System during the Acute Term after Traumatic Brain Injury in the Rats. *Brain Res.* **2007**, *1164*, 132–135. [CrossRef] [PubMed]
47. Xu, L.; Wang, F.; Zhang, Z.; Terry, N. Optimization of Polysaccharide Production from *Cordyceps militaris* by Solid-State Fermentation on Rice and Its Antioxidant Activities. *Foods* **2019**, *8*, 590. [CrossRef]
48. Sokołowska, E.; Sadowska, A.; Sawicka, D.; Kotulska-Bąblińska, I.; Car, H. A Head-to-head Comparison Review of Biological and Toxicological Studies of Isomaltulose, D-tagatose, and Trehalose on Glycemic Control. *Crit. Rev. Food Sci. Nutr.* **2022**, *62*, 5679–5704. [CrossRef]
49. Taherimehr, Z.; Zaboli, M.; Torkzadeh-Mahani, M. New Insight into the Molecular Mechanism of the Trehalose Effect on Urate Oxidase Stability. *J. Biomol. Struct. Dyn.* **2022**, *40*, 1461–1471. [CrossRef]
50. Wang, Z.-J.; Liang, C.-L.; Li, G.-M.; Yu, C.-Y.; Yin, M. Stearic acid protects primary cultured cortical neurons against oxidative stress. *Acta Pharmacol. Sin.* **2007**, *28*, 315–326. [CrossRef]
51. Boumerfeg, S.; Baghiani, A.; Messaoudi, D.; Khennouf, S.; Arrar, L. Antioxidant Properties and Xanthine Oxidase Inhibitory Effects of *Tamus communis* L. Root Extracts. *Phytother. Res.* **2009**, *23*, 283–288. [CrossRef]
52. Holighaus, G.; Rohlfs, M. Fungal Allelochemicals in Insect Pest Management. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 5681–5689. [CrossRef]
53. Quy, T.N.; Xuan, T.D.; Andriana, Y.; Tran, H.-D.; Khanh, T.D.; Teschke, R. Cordycepin Isolated from *Cordyceps militaris*: Its Newly Discovered Herbicidal Property and Potential Plant-Based Novel Alternative to Glyphosate. *Molecules* **2019**, *24*, 2901. [CrossRef]
54. Cimmino, A.; Masi, M.; Evidente, M.; Superchi, S.; Evidente, A. Fungal Phytotoxins with Potential Herbicidal Activity: Chemical and Biological Characterization. *Nat. Prod. Rep.* **2015**, *32*, 1629–1653. [CrossRef] [PubMed]
55. Uddin, M.N.; Robinson, R.W.; Buultjens, A.; Al Harun, M.A.Y.; Shampa, S.H. Role of Allelopathy of Phragmites Australis in Its Invasion Processes. *J. Exp. Mar. Bio. Ecol.* **2017**, *486*, 237–244. [CrossRef]
56. Henderson, A.M.; Gervais, J.A.; Luukinen, B.; Buhl, K.; Stone, D.; Strid, A.; Cross, A.; Jenkins, J. Glyphosate Technical Fact Sheet. Available online: http://npic.orst.edu/factsheets/archive/glyphotech.html (accessed on 24 June 2022).
57. Li, S.P.; Li, P.; Dong, T.T.; Tsim, K.W. Determination of Nucleosides in Natural *Cordyceps sinensis* and Cultured *Cordyceps mycelia* by Capillary Electrophoresis. *Electrophoresis* **2001**, *22*, 144–150. [CrossRef] [PubMed]
58. Molyneux, P. The Use of the Stable Free Radical Diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant. *Warasan Songkhla Nakharin* **2004**, *26*, 211–219.
59. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
60. Unno, T.; Sugimoto, A.; Kakuda, T. Xanthine Oxidase Inhibitors from the Leaves of *Lagerstroemia speciosa* (L.) Pers. *J. Ethnopharmacol.* **2004**, *93*, 391–395. [CrossRef]
61. Zhao, X.; Wang, B.; Xie, K.; Liu, Y.; Zhang, Y.; Wang, Y.; Guo, Y.; Bu, X.; Liu, C.; Zhang, G.; et al. Determination of Dinitolmide and Its Metabolite 3-ANOT in Chicken Tissues via ASE-SPE-GC–MS/MS. *J. Food Compost. Anal.* **2018**, *71*, 94–103. [CrossRef]
62. Minh, T.N.; Xuan, T.D.; Van, T.M.; Andriana, Y.; Viet, T.D.; Khanh, T.D.; Tran, H.-D. Phytochemical Analysis and Potential Biological Activities of Essential Oil from Rice Leaf. *Molecules* **2019**, *24*, 546. [CrossRef]