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Extraction methods: Effects on the contents of bioactive compounds and anti-oxidant activity of *Coriolosis aspera* mycelia

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Abstract:
Coriolosis aspera has been known as a medicinal mushroom commonly used in Vietnam, China, and certain regions in South Asia. It has many health-beneficial effects, namely anti-inflammatory, anti-cancerous, and anti-antioxidant. Despite these advantages, the rigid and durable cell walls of *C. aspera* pose challenges during chemical or mechanical extraction processes.
We aimed to identify the optimal method for extracting bioactive compounds from *C. aspera* among hot-water extraction, ultrasound-assisted extraction, microwave-assisted extraction, ultrasound-assisted alkali extraction, and ultrasound-assisted liquid nitrogen extraction.
Among these methods, a combination of liquid nitrogen treatment (with a material-to-nitrogen ratio of 1:6) and ultrasound-assisted extraction (15 min) proved to be the most effective. This method yielded the highest concentrations of polyphenols (4.69 ± 0.02 mg GAE/g dry weight), flavonoids (0.88 ± 0.01 mg QE/g dry weight), and triterpenoids (1.28 ± 0.01 mg OAE/g dry weight). Additionally, it exhibited a notable antioxidant activity of 3.48 ± 0.01 μg ascorbic acid/g dry weight. The scanning electron microscope images indicated that ultrasound-assisted liquid nitrogen extraction was the only method able to effectively disrupt the cell walls of *C. aspera*.
Our study contributes to the potential application of *C. aspera* in developing functional foods. It emphasizes the importance of effective extraction techniques in discovering medicinal properties of the mushroom.

Keywords: Anti-oxidant activity, *Coriolopsis aspera*, flavonoids, mycelia, triterpenoids, ultrasound-assisted extraction

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INTRODUCTION

Fungal species on earth have been estimated to number around 140 000 to 160 000. However, only 10% of them have been identified, and only about 2000 species are considered safe for humans as edible or medicinal mushrooms [1, 2]. For example, *Coriolopsis aspera*, a member of the *Polyporaceae* family, is a medicinal mushroom commonly used in Vietnam, China, and some regions in South Asia. It has been reported to exhibit health-promoting effects, including anti-inflammatory, anti-oxidant, and anti-cancerous activities [3]. Several other strains belonging to the *Polyporaceae* family are also used to treat diabetes, oxidative stress-related diseases, or bacterial infections. They include *Coriolopsis rigida*, *Coriolopsis gallica*, and *Coriolopsis polyzona* [4–6]. These fungi are a promising source of valuable compounds, such as anti-oxidant (polyphenols, flavonoids), immuno-chemotherapy (polysaccharide Krestin, lentinan, and schizophyllan), as well as anti-inflammatory and anti-cancerous (triterpene) compounds [7, 8].

Fungal cell walls possess complex components and textures. They mainly contain chitins, glucans, and some proteins, including hydrophobins and mannoproteins [9]. For instance, the cell wall of *Agaricus bisporus*, a common edible mushroom, is composed of 12.0–14.3% of protein, 33.0–37.0% polysaccharides, and 15.8–17.4% chitin [10]. Furthermore, the cell walls of *Ganoderma resinaceum*, a medicinal mushroom, possess mannogalactan, *β*-D-glucans, (1→3)-*α*-D-glucan, (1→3)-*β*-D-glucan, chitin, and *β*-D-glucan complex, O-2-*β*-D-mannosyl-(1→6)-*α*-D-galactan and (1→3)(1→4)(1→6)-*β*-D-glucan [11]. These components and their arrangement in fugal cell walls provide the cell shape and rigidity. In addition, they protect fungal cells from chemical or mechanical destruction, which in turn lowers the

extraction yield of bioactive compounds in medicinal mushrooms. Aside from the traditional hot-water extraction, some new extraction techniques have been developed recently to break down fugal cell walls and enhance the efficiency of extracting active ingredients from plant and fugal materials. They include chemical treatments (enzymatic hydrolysis, acid hydrolysis, ethanol-acid treatment, and alkaline extraction) and mechanical methods (high-speed centrifugal shearing pulverization, ultrasound-assisted extraction, microwave-assisted extraction, and cavitation-based extraction) [12–14]. The ultrasound- and microwave-assisted extractions have been reported to increase the extraction yield of bioactive compounds. They were also considered as green technologies with many advantages, such as a shorter extraction time, as well as solvent and energy saving [15]. Sun *et al.* used a method of pulverizing plant materials with liquid nitrogen that punctured the fibers of *Astragalus mongholicus*, thus improving the extraction yield of bioactive compounds [16]. In another study, Liang *et al.* revealed that pulverization with liquid nitrogen significantly changed the physical properties of the raw material, benefiting the extraction bioactive substances in *Astragalus mongholicus* [17].

Although there has been extensive research into the bioactivities of *C. aspera*, an optimal method of extracting bioactive components from this species has not been found so far. Therefore, we aimed to study a range of methods (hot-water extraction, ultrasound- and microwave-assisted extraction, NaOH treatment coupled with ultrasound-assisted extraction, and liquid nitrogen treatment coupled with ultrasound-assisted extraction) to select an optimal method for extracting bioactive compounds (polyphenol, flavonoids, and triterpenoids) from *C. aspera* to ensure its antioxidant activity.

STUDY OBJECTS AND METHODS

Chemicals and reagents. Most chemicals and reagents, such as the Folin-Ciocalteu reagent, gallic acid, oleanolic acid, ascorbic acid, quercetin, and 2,2-Diphenylpicrylhydrazyl (DPPH) were purchased from Sigma Aldrich (Sigma Chemicals Co., St. Louis, MO, USA), while others were obtained commercially and were of analytical grade.

Fugal materials. Fresh mycelia of *Coriolopsis aspera* were obtained from the Experimental Garden, Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City (Fig. 1). The specimens were dried at 45°C in the oven to less than 6% moisture prior to being ground into a fine powder using a hammer mill. The powder was then sifted through a 20-mesh sieve and distributed into polyethylene bags, with 2 g of powder per bag. The samples were stored at 4°C in a refrigerator for further analysis.

Ultrasound equipment (Fig. 2). Product type: ultrasonic processor. Model: GE 750. Power: 750 W. Freq: 20 kHz. Min Sample Size (mL): 0.25. Max Sample Size (mL): 1900. Amplitude: 0–100%.

Ultrasound-assisted extraction (USE). Single factor experimental designs were employed to investigate the effects of ultrasonic power and extraction time on

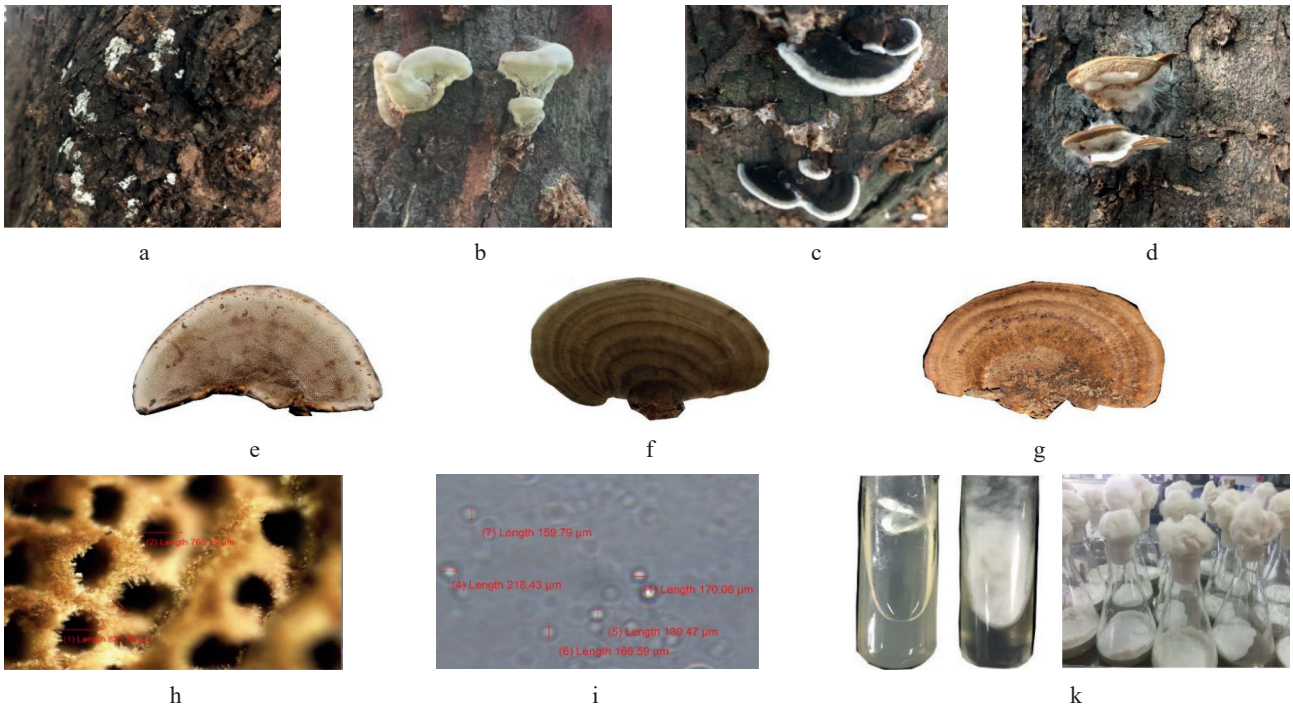

Figure 1 Natural samples of *Coriolopsis aspera* on wood and its laboratory-cultivated samples. Mycelia (a) and immature fruiting bodies after 3 days (b), 7 days (c), and 10 days (d). Lower (e) and upper (f) surface of a fruiting body after 15–18 days. The mature fruiting body (g) and microscopic images of pores in lower surface (h) and spores (i). Laboratory-cultivated *Coriolopsis aspera* strains and mycelia (k)

Figure 2 Ultrasonic processor equipment GE 750

the extraction yield of bioactive compounds. In particular, we determined the effect of different ultrasonic power levels on the contents of polyphenols (TPC), flavonoids (TFC), triterpenoids (TTC), and antioxidant activity (radical scavenging activity, RSA) of the extracts. For this, a *C. aspera* sample, with a material-to-water ratio of 1:60 (w/v), was treated with a range of ultrasonic power levels (150–525 W, equivalent to an amplitude range of 20–70% at 20 kHz) for 25 min at 30°C to break down the cell walls. Following this, an extract was collected through centrifugation at 6000 rpm. The solid residue of the sample was then dried until 6% moisture was reached. Upon drying out, the sample was extracted twice with 96% ethanol for 30 min each time. All fractioned extracts were combined to obtain the final extract. The effect of extraction time on the contents of polyphenols, flavonoids, triterpenoids, and antioxidant activity of the extracts was determined using the same procedure with minor modifications. In particular, the extraction time ranged from 5 to 35 min, while the constant ultrasonic power was obtained from the above experiment. The control sample was treated with the same procedure without ultrasound-assisted extraction. The M1 (USE-treated) sample was considered an optimal sample since it possessed the highest values of TPC, TFC, TTC, and RSA.

Microwave-assisted extraction (MAE). Single factor experimental designs were employed to clarify the effect of microwave energy and extraction time on the extraction yield of bioactive compounds. In particular, we determined the effect of different microwave power levels on the contents of polyphenols (TPC), flavonoids (TFC), triterpenoids (TTC), and antioxidant activity (radical scavenging activity, RSA) of the extracts. For this, a *C. aspera* sample, with a material-to-water ratio of 1:60 (w/v) set as constant, was treated with a range of microwave power levels (110–140 W) for 10 min. Following this, an extract was collected through centrifugation at 6000 rpm. The solid residue of the samples was then dried until 6% moisture was reached. Upon drying out, the sample was extracted twice with 96% ethanol for 30 min each time. All fractioned extracts were combined to obtain the final extract. The effect of extraction time on the TPC, TFC, TTC, and RSA of the extracts was determined using the same procedure with minor modifications. In particular, the extraction time ranged from 5 to 35 min, while the constant microwave power was obtained from the above experiment. The control sample was treated with the same procedure without microwave-assisted extraction. The M2 (MAE-treated) sample was considered an optimal sample since it possessed the highest values of TPC, TFC, TTC, and RSA.

Hot-water extraction (HWE). Single factor experimental designs were employed to clarify the effect of extraction temperature and time on the extraction yield of bioactive compounds. In particular, we determined the effect of different extraction temperatures on the contents of polyphenols (TPC), flavonoids (TFC), triterpenoids (TTC), and antioxidant activity (radical scavenging activity, RSA) of the extracts. For this, a *C. aspera* sample, with a material-to-water ratio of 1:60 (w/v) set as constant, was treated with a range of temperatures (70–100°C) for 10 min. Following this, an extract was collected through centrifugation at 6000 rpm. The solid residue of the samples was then dried until 6% moisture was reached. Upon drying out, the sample was extracted twice with 96% ethanol for 30 min each time. All fractioned extracts were combined to obtain the final extract. The effect of extraction time on the TPC, TFC, TTC, and RSA of the extracts was determined using the same procedure with minor modifications. In particular, the extraction time ranged from 5 to 35 min while the constant temperature was obtained from the above experiment. The control sample (M0) was treated with the same procedure without hot-water extraction. The M3 (HWE-treated) sample was considered an optimal sample since it possessed the highest values of TPC, TFC, TTC, and RSA.

Alkali treatment coupled with ultrasound-assisted extraction (AKE). The effect of different alkali treatments on the contents of polyphenols (TPC), flavonoids (TFC), triterpenoids (TTC), and antioxidant activity (radical scavenging activity, RSA) of the extracts was determined by pre-treating the materials with NaOH solutions at concentrations ranging from 3 to 9% for 20 min. For this, a *C. aspera* sample, with a material-to-water ratio of 1:60 (w/v) set as constant, was treated with ultrasonic power at 375 W and 30°C for 15 min. Following this, an extract was collected through centrifugation at 6000 rpm. The solid residue of the samples was dried until 6% moisture was reached. Upon drying out, the sample was extracted twice with 96% ethanol for 30 min

each time. All the fractions were combined and adjusted to the neutral pH to obtain the final extract. The effect of extraction time on the TPC, TFC, TTC, and RSA of the extracts was performed using the same procedure with minor modifications. In particular, the extraction time ranged from 5 to 35 min, while the NaOH solution at the constant concentration was obtained from the above experiment. The control sample was treated with the same procedure without liquid nitrogen pre-treatment and ultrasound-assisted extraction. The M4 (AKE-treated) sample was considered an optimal sample since it possessed the highest values of TPC, TFC, TTC, and RSA.

Liquid nitrogen treatment coupled with ultrasound-assisted extraction (LNE). The effect of different liquid nitrogen pre-treatments on the contents of polyphenols (TPC), flavonoids (TFC), triterpenoids (TTC), and antioxidant activity (radical scavenging activity, RSA) was determined by pulverizing the materials using liquid nitrogen with a material-to-liquid nitrogen ratio of 1:2–1:8. For this, a *C. aspera* sample, with a material-to-water ratio of 1:60 (w/v) set as constant, was treated with ultrasonic power at 375 W and 30°C for 15 min. Following this, an extract was collected through centrifugation at 6000 rpm. The solid residue of the samples was dried until 6% moisture was reached. Upon drying out, the sample was extracted twice with 96% ethanol for 30 min each time. All the fractions were then combined and adjusted to the neutral pH to obtain the final extract. The effect of extraction time on the TPC, TFC, TTC, and RSA of the extracts was determined using the same procedure with minor modifications. In particular, the extraction time ranged from 5 to 35 min, while the constant material-to-liquid nitrogen ratio was obtained from the above experiment. The control sample was treated with the same procedure without liquid nitrogen pre-treatment and ultrasound-assisted extraction. The M5 (LNE-treated) sample was considered an optimal sample since it possessed the highest values of TPC, TFC, TTC, and RSA.

Determining the total polyphenol content (TPC) of the extract. The total polyphenol content of the extracts was evaluated by colorimetric assay with a Folin-Ciocalteu reagent and a UV-Vis spectrophotometer. A 1 mL aliquot of a diluted extract was added into a test tube containing 0.5 mL of the Folin-Ciocalteu reagent. The mixture was vortexed and maintained at room temperature for 5 min. The mixture was neutralized with 2.5 mL of a saturated Na_2CO_3 solution before it was brought to 10 mL with distilled water and vigorously shaken. After 30 min of incubation in the darkness, the absorbance of the solution at 765 nm was recorded and the polyphenol content was calculated from the standard curve of gallic acid with a concentration range of 0–100 ppm. The results were expressed as mean ± standard deviation of triplicate experiments and the unit was set as mg gallic acid equivalent per one gram of dry weight of the sample (mg GAE/g dry weight) [18].

Determining the total flavonoids content (TFC) of the extract. The total flavonoid content of the extracts was evaluated by aluminum chloride assay [19]. For this, a 1 mL aliquot of a diluted extract was mixed with 0.3 mL of a 5% $NaNO_2$ solution in a test tube and maintained at room temperature for 5 min. The mixture was then mixed with 0.3 mL of a 10% $AlCl_3$ solution and maintained at room temperature for 5 min. It was then mixed with 2 mL of a 1M NaOH solution, brought to 10 mL with distilled water, and vigorously shaken. The absorbance of the mixture at 510 nm was recorded and the flavonoid content was calculated from the standard curve of quercetin with a concentration range of 0–100 ppm. The results were expressed as mean ± standard deviation of triplicate experiments and the unit was set as mg quercetin equivalent per one gram of dry weight of the sample (mg QE/g dry weight) [18].

Determining the triterpenoid content (TTC) of the extract. The triterpenoid content of the extracts was evaluated by colorimetric assay using a UV-Vis spectrophotometer, as described in previous studies [20]. For this, a 0.2 mL aliquot of a sample was mixed with 0.2 mL of a 5% vanillin-glacial acetic acid solution and 1.2 mL of perchloric acid (70–72%) in a test tube. Subsequently, the mixture was briefly vortexed and incubated in a thermostatic water bath at 70°C for 15 min. The reaction mixture was rapidly cooled down for 2 min and brought to 5 mL with ethyl acetate. The absorbance of the solution at 550 nm was recorded and the triterpenoid content was calculated from the standard curve of oleanolic acid with a concentration range of 0–10 ppm. The results were expressed as mean ± standard deviation of triplicate experiments and the unit was set as mg oleanolic acid equivalent per one gram of dry weight of the sample (mg OAE/g dry weight) [18].

Determining the antioxidant activity (RSA) of the extract. The antioxidant activity of the extracts was evaluated by DPPH radicals scavenging assay according to the procedure described by Chu *et al.* with minor modifications [21]. For this, 0.1 mL aliquot of an extract was mixed with 4 mL of a 0.1 mM DPPH solution and 0.9 mL of ethanol in a test tube. The reaction mixture was kept in the darkness at room temperature for 30 min before the absorbance was measured at 517 nm. Ascorbic acid was used as a reference substance with a concentration range of 0–10 ppm and the antioxidant activity of the extract (DDPH radical scavenging activity, RSA) was estimated from the standard curve between various concentrations of ascorbic acid and changes in the absorbance of the samples. The results were expressed as mean ± standard deviation of triplicate experiments and the unit was set as μg ascorbic acid equivalent per one gram of dry weight of the sample (μg AAE/g dry weight) [18].

Statistical analysis. The statistical analysis of all analytic experiments was implemented using Statgraphics Centurion XV. The differences among the treatments were determined by ANOVA One-way analysis, followed by Multiple Sample Comparison and Fisher's Least Significant Difference procedures. The statistical significance threshold was set as $p < 0.05$.

RESULTS AND DISCUSSION

The effects of ultrasonic power levels and extraction time on bioactive compounds and antioxidant activity of the extracts. Table 1A presents the effects of ultrasonic power levels on the contents of polyphenols (TPC), flavonoids (TFC), triterpenoids (TTC), and antioxidant activity (radical scavenging activity, RSA) of the samples treated with ultrasound-assisted extraction (UAE). The results indicate that the samples treated with ultrasound at 150, 225, 300, 375, 450, and 525 W had higher values of TPC, TFC, TTC, and RSA than the untreated samples. The increase in ultrasonic power levels was associated with higher contents of TPC, TFC, and TTC, as well as greater antioxidant activity (RSA) of the extracts. For example, the TPC, TFC, TTC, and RSA of the samples treated with 525 W were 1.2, 1.5, 1.5, and 1.1 fold higher, respectively, than those of the samples treated with 150 W. Notably, there was no significant difference among the ultrasound-treated samples at higher power levels (375, 450, and 525 W). Among those levels, 375 W was considered an optimal UAE power level in terms of cost and energysaving issues.

Data were expressed as Mean ± SD of three experiments, and different lowercase letters (a, b, c, d, e) indicated statistically significant differences among the treatments ($p < 0.05$). TPC is total polyphenol content, TFC is total flavonoid content, TTC is total triterpenoid content, and RSA is antioxidant activity

Table 1B presents the effects of different UAE times on the TPC, TFC, TTC, and RSA of the samples. There was an increase in the TPC, TFC, TTC, and RSA values with longer extraction times. For example, the TPC, TFC, TTC, and RSA of the samples treated for 35 min were respectively 1.6, 2.5, 2.1, and 1.5 fold higher than those of the samples treated for 5 min. Notably, we found no significant difference between the samples treated for 30 and 35 min ($p > 0.05$). Taken together, the optimal UAE conditions were 375 W power and 30 min extraction time (UAE-treated M1 sample).

Medicinal mushrooms are a rich source of bioactive ingredients and precious natural products but they possess hard, rigid, and durable cell walls which protect them from mechanical or chemical treatment. Therefore, rupturing fugal cell walls to improve bioactive compound extraction is a challenge for food and pharmaceutical industries. UAE is a modern and green technology for natural product extraction. In addition to saving the extraction time and energy, it is eco-friendlier compared to other conventional extraction methods, such as organic solvent extraction. Our data are in line with several previous studies, which indicated the effectiveness of UAE, especially for bioactive compound extraction [22, 23]. For example, Machado-Carvalho *et al.* suggested that UAE could be a better choice for bioactive compound isolation [22]. According to the authors, the optimal conditions for extracting polyphenols and antioxidants from the medicinal mushroom *Inonotus hispidus* were a 1:75 (w/v) ratio of material and 40% ethanol solution and an extraction time of 20 min.

In a study by Zheng *et al.*, the optimal extraction procedure involved using UAE with ultrasonic power of 210 W, a 1:50 (w/v) ratio of material to 50% ethanol solution, and extraction at 80°C for 100 min [24, 25]. Notably, these findings implied that prolonging the extraction time was a more efficient strategy for bioactive compound extraction than increasing ultrasonic power levels. Although the thermal effect of ultrasound was minimized by using a thermostatic water bath (30°C), the higher power of ultrasound can also degrade bioactive compounds (antioxidants, polyphenols, flavonoids, and triterpenoids) or generate too many bubbles, leading to a decline in cavitation effect, which in turn may decrease the extraction yield [26].

Table 1 Changes in bioactive compounds and antioxidant activity of the extracts under different ultrasonic power levels and ultrasound-assisted extraction times

(A)	Ultrasonic power, W						
	0	150	225	300	375	450	525
TPC, mg GAE/g DW	1.12 ± 0.01^a	2.60 ± 0.01^b	2.70 ± 0.01^c	2.81 ± 0.01^d	3.13 ± 0.01^e	3.14 ± 0.03^e	3.15 ± 0.01^e
TFC, mg QE/g DW	0.15 ± 0.01^a	0.18 ± 0.01^b	0.21 ± 0.02^c	0.23 ± 0.01^d	0.26 ± 0.02^e	0.27 ± 0.01^e	0.27 ± 0.01^e
TTC, mg OAE/g DW	0.11 ± 0.02^a	0.36 ± 0.01^b	0.48 ± 0.01^c	0.49 ± 0.01^c	0.54 ± 0.01^d	0.54 ± 0.02^d	0.55 ± 0.01^d
RSA, µg ascorbic acid/g DW	0.37 ± 0.02^a	1.52 ± 0.05^b	1.61 ± 0.01^c	1.68 ± 0.01^c	1.71 ± 0.03^d	1.73 ± 0.02^d	1.74 ± 0.01^d
(B)	Extraction times, min							
	0	5	10	15	20	25	30	35
TPC, mg GAE/g DW	1.12 ± 0.01^a	2.12 ± 0.01^b	2.13 ± 0.01^b	2.26 ± 0.01^c	2.31 ± 0.04^d	3.13 ± 0.01^e	3.41 ± 0.03^f	3.42 ± 0.02^f
TFC, mg QE/g DW	0.15 ± 0.01^a	0.12 ± 0.02^b	0.26 ± 0.02^c	0.26 ± 0.01^c	0.26 ± 0.01^c	0.26 ± 0.02^c	0.30 ± 0.01^d	0.30 ± 0.02^d
TTC, mg OAE/g DW	0.11 ± 0.02^a	0.31 ± 0.01^b	0.54 ± 0.01^c	0.54 ± 0.01^c	0.55 ± 0.02^c	0.54 ± 0.01^c	0.66 ± 0.02^d	0.66 ± 0.01^d
RSA, µg ascorbic acid/g DW	0.37 ± 0.02^a	1.58 ± 0.01^b	1.71 ± 0.03^c	1.70 ± 0.02^c	1.72 ± 0.02^c	1.71 ± 0.03^c	2.43 ± 0.02^d	2.44 ± 0.02^d

Data were expressed as Mean ± SD of three experiments, and different lowercase letters (a, b, c, d, e) indicated statistically significant differences among the treatments ($p < 0.05$). TPC is total polyphenol content, TFC is total flavonoid content, TTC is total triterpenoid content, and RSA is antioxidant activity. DW is dry weight.

Table 2 Changes in bioactive compounds and antioxidant activity of the extracts under different microwave power and microwave-assisted extraction times

(A)	Microwave power, W				
	0	110	120	130	140
TPC, mg GAE/g DW	1.12 ± 0.01[a]	1.73 ± 0.03[b]	1.96 ± 0.02[c]	2.08 ± 0.01[d]	2.10 ± 0.01[d]
TFC, mg QE/g DW	0.05 ± 0.01[a]	0.19 ± 0.02[b]	0.24 ± 0.01[c]	0.27 ± 0.02[d]	0.28 ± 0.01[d]
TTC, mg OAE/g DW	0.11 ± 0.02[a]	0.39 ± 0.02[b]	0.47 ± 0.03[c]	0.57 ± 0.03[d]	0.59 ± 0.01[d]
RSA, µg ascorbic acid/g DW	0.37 ± 0.02[a]	1.89 ± 0.04[b]	2.02 ± 0.01[c]	2.10 ± 0.02[d]	2.18 ± 0.02[d]
(B)	Extraction times, min				
	0	5	10	15	20	25
TPC, mg GAE/g DW	1.12 ± 0.01[a]	2.00 ± 0.01[b]	2.08 ± 0.01[c]	3.17 ± 0.01[d]	3.17 ± 0.03[d]	3.19 ± 0.02[d]
TFC, mg QE/g DW	0.05 ± 0.01[a]	0.19 ± 0.01[b]	0.27 ± 0.02[c]	0.29 ± 0.01[c]	0.29 ± 0.01[c]	0.30 ± 0.02[c]
TTC, mg OAE/g DW	0.11 ± 0.02[a]	0.46 ± 0.01[b]	0.57 ± 0.03[c]	0.66 ± 0.02[d]	0.66 ± 0.01[d]	0.67 ± 0.01[d]
RSA, µg ascorbic acid/g DW	0.37 ± 0.02[a]	1.65 ± 0.01[b]	2.10 ± 0.02[c]	2.21 ± 0.01[d]	2.21 ± 0.02[d]	2.22 ± 0.01[d]

Data were expressed as Mean ± SD of three experiments, and different lowercase letters (a, b, c, d, e) indicated statistically significant differences among the treatments ($p < 0.05$). TPC is total polyphenol content, TFC is total flavonoid content, TTC is total triterpenoid content, and RSA is antioxidant activity. DW is dry weight.

The effects of microwave power levels and extraction time on bioactive compounds and antioxidant activity of the extracts. As shown in Table 2A, microwave could effectively extract bioactive compounds from the medical mushroom. The contents of polyphenols (TPC), flavonoids (TFC), triterpenoids (TTC), and antioxidant activity (radical scavenging activity, RSA) of the samples treated with microwave at 110 W were remarkably higher those of the untreated samples ($p < 0.05$). Additionally, there was an increase in TPC, TFC, TTC, and RSA depending on the microwave power level. For example, the samples treated with microwave-assisted extraction (MAE) at 130 W yielded the highest TPC, TFC, TTC, and RSA, followed by the samples treated with MAE at 120 W, 110 W, and the untreated samples ($p < 0.05$). We observed that the MAE level of 140 W did not improve the TPC, TFC, TTC, and RSA as compared to 130 W ($p > 0.05$). The microwave power of 130 W was therefore identified as the optimal power for extraction.

Aside from the microwave power, the extraction time was also found to enhance the extraction efficacy (Table 2B). There was a positive correlation between the MAE time and bioactive compound extraction efficacy (TPC, TFC, TTC, and RSA). The MAE-treated samples obtained their maximal values of TPC, TTC, and RSA after 15 min ($p < 0.05$), with no noticeable differences observed under the longer extraction time. Notably, the TFC of the samples reached a plateau at 10 min and remained constant until 25 min. These results indicated that the duration of 15 min was the sufficient time to extract bioactive compounds (MAE-treated M2 sample).

MAE has been one of the green extraction techniques widely used in bioactive compound extraction, especially from medicinal mushrooms. As microwave heats and dries fugal cells, increasing temperature and aqueous vapor inside the cells cause their walls to stretch and break down, which in turn facilitates the extraction process [27]. According to Maeng *et al*., MAE is a better practice to obtain the maximal values of polyphenols and antioxidants from turkey tail or Yun Zhi mushroom in contrast to the aqueous-based reflux method [28]. Smiderle *et al.* also used MAE to extract *β*-D-glucan, a bioactive constituent of cell walls acting as an immuno-stimulant, from two medicinal mushrooms, *Ganoderma lucidum* and *Pleurotus ostreatus* [29]. They found some slight differences in the optimal conditions between a previous study (125 W, 3.8 min, 40% ethanol) and the present study (130 W, 15 min, water), which could be mainly due to the different solvents used for extraction in these experiments. The selection of optimal conditions for MAE is a critical requirement for bioactive constituent extraction, since high microwave power and long extraction time are often accompanied by a higher risk of loss of bioactive compounds, especially in the substances susceptible to thermal degradation [27].

The effects of hot-water extraction temperature and time on bioactive compounds and antioxidant activity of the extracts. The effects of hot-water extraction (HWE) temperature on the extraction efficacy are presented in Table 3A. Briefly, we found a positive correlation between the extraction temperature and the contents of polyphenols (TPC), flavonoids (TFC), triterpenoids (TTC), and antioxidant activity (radical scavenging activity, RSA) of the extracts. For example, the samples treated at 90°C exhibited a noticeable increase in the TPC, TFC, TTC, and RSA (1.6, 3.2, 2.8, and 3.6 fold, respectively, $p < 0.05$) in comparison with the control treated at room temperature (25°C). The TPC, TFC, TTC, and RSA continuously increased until reaching the maximal values at 100°C. Our findings were consistent with a previous study by Sharma and Tulsawani, where higher extraction temperatures were associated with a greater amount of TPC and TFC. Noteworthily, the temperature higher than 100°C could lead to a degradation of some antioxidants [30]. Therefore, we chose the extraction temperature of 100°C for further experiments. We also observed that longer extraction

Table 3 Changes in bioactive compounds and antioxidant activity of the extracts under different hot-water extraction temperatures and times

(A)	Extraction temperature, °C				
	25	70	80	90	100
TPC, mg GAE/g DW	1.12 ± 0.01[a]	1.55 ± 0.01[b]	1.66 ± 0.01[c]	1.81 ± 0.02[d]	2.08 ± 0.01[e]
TFC, mg QE/g DW	0.05 ± 0.01[a]	0.08 ± 0.01[b]	0.12 ± 0.01[c]	0.16 ± 0.01[d]	0.19 ± 0.02[e]
TTC, mg OAE/g DW	0.11 ± 0.02[a]	0.23 ± 0.01[b]	0.31 ± 0.02[c]	0.31 ± 0.01[c]	0.36 ± 0.01[d]
RSA, µg ascorbic acid/g DW	0.37 ± 0.02[a]	1.04 ± 0.02[b]	1.14 ± 0.01[c]	1.28 ± 0.02[d]	1.34 ± 0.02[e]
(B)	Extraction time, min				
	0	5	10	15	20
TPC, mg GAE/g DW	1.12 ± 0.01[a]	1.43 ± 0.01[b]	2.08 ± 0.02[c]	2.35 ± 0.01[d]	2.37 ± 0.01[d]
TFC, mg QE/g DW	0.05 ± 0.01[a]	0.09 ± 0.02[b]	0.19 ± 0.01[c]	0.24 ± 0.02[d]	0.25 ± 0.01[d]
TTC, mg OAE/g DW	0.11 ± 0.02[a]	0.24 ± 0.02[b]	0.34 ± 0.01[c]	0.38 ± 0.01[d]	0.39 ± 0.01[d]
RSA, µg ascorbic acid/g DW	0.37 ± 0.02[a]	1.20 ± 0.01[b]	1.34 ± 0.01[c]	1.51 ± 0.01[d]	1.52 ± 0.02[d]

Data were expressed as Mean ± SD of three experiments, and different lowercase letters (a, b, c, d, e) indicated statistically significant differences among the treatments ($p < 0.05$). TPC is total polyphenol content, TFC is total flavonoid content, TTC is total triterpenoid content, and RSA is antioxidant activity. DW is dry weight.

Table 4 Changes in bioactive compounds and antioxidant activity of the extracts pretreated with different NaOH concentrations and at different ultrasound extraction times

(A)	NaOH concentration, %				
	0	3	5	7	9
TPC, mg GAE/g DW	1.19 ± 0.02[a]	2.19 ± 0.01[b]	2.48 ± 0.01[c]	2.49 ± 0.01[c]	2.49 ± 0.02[c]
TFC, mg QE/g DW	0.08 ± 0.01[a]	0.16 ± 0.01[b]	0.21 ± 0.02[c]	0.22 ± 0.02[c]	0.22 ± 0.01[c]
TTC, mg OAE/g DW	0.17 ± 0.02[a]	0.53 ± 0.01[b]	0.88 ± 0.02[c]	0.89 ± 0.02[c]	0.90 ± 0.02[c]
RSA, µg ascorbic acid/g DW	0.40 ± 0.02[a]	1.23 ± 0.01[b]	2.15 ± 0.01[c]	2.15 ± 0.02[c]	2.16 ± 0.02[c]
(B)	Ultrasound extraction time, min				
	0	5	10	15	20
TPC, mg GAE/g DW	1.19 ± 0.02[a]	2.12 ± 0.03[b]	2.22 ± 0.02[c]	2.48 ± 0.01[d]	2.49 ± 0.01[d]
TFC, mg QE/g DW	0.08 ± 0.01[a]	0.11 ± 0.01[b]	0.17 ± 0.02[c]	0.21 ± 0.01[d]	0.22 ± 0.01[d]
TTC, mg OAE/g DW	0.17 ± 0.02[a]	0.22 ± 0.02[b]	0.61 ± 0.01[c]	0.88 ± 0.02[d]	0.89 ± 0.01[d]
RSA, µg ascorbic acid/g DW	0.40 ± 0.02[a]	1.29 ± 0.01[b]	1.46 ± 0.02[c]	2.15 ± 0.02[d]	2.15 ± 0.01[d]

Data were expressed as Mean ± SD of three experiments, and different lowercase letters (a, b, c, d, e) indicated statistically significant differences among the treatments ($p < 0.05$). TPC is total polyphenol content, TFC is total flavonoid content, TTC is total triterpenoid content, and RSA is antioxidant activity. DW is dry weight.

times improved the TPC, TFC, TTC, and RSA of the extracts (Table 3B). Among five times of extraction (0–20 min), all of bioactive compound contents obtained a plateau at 15 min ($p < 0.05$) and remained unchanged until 20 min. Thus, the HWE temperature of 100°C and HWE time of 15 min (HWE-treated M3 sample) were found to be optimal conditions to extract polyphenols, flavonoids, triterpenoids, and antioxidants.

HWE is a conventional method for extracting plant bioactive components. It shortens the extraction time and improves the yield, compared to cold-water extraction [31]. For some particular phenolic substances, such as gallic acid and *p*-hydroxybenzoic acid, hot-water extraction had a greater efficacy than methanol extraction [32]. However, its efficacy for natural product extraction was less than that of some modern techniques, such as ultrasound-assisted extraction [24, 25]. In this study, the maximal extraction yields of HWE in terms of TPC, TFC, TPC, and RSA were lower than those of ultrasound- or microwave-assisted extraction. For example, the TPC of the UAE-treated sample (M1) was the highest, followed by that of the MAE-treated (M2) and HWE-treated (M3) samples ($p < 0.05$). Therefore, ultrasound-assisted extraction was considered the most appropriate extraction method for *C. aspera*.

The effects of NaOH pretreatment and ultrasound extraction time on bioactive compounds and antioxidant activity of the extracts. The effects of NaOH concentration (alkali pretreatment) and ultrasound extraction time on the extraction efficacy are presented in Table 4. As can be seen, alkali treatment (AKE) remarkably increased the contents of bioactive compounds in the extracts (Table 4A). For example, the samples treated with a 3% NaOH solution improved the extraction efficacy of polyphenols (TPC), flavonoids (TFC), triterpenoids (TTC), and antioxidant activity (radical scavenging activity, RSA) (1.84, 2.00, 3.11, and 3.07 fold, respectively), as compared to the untreated samples. Since the maxi-

mal values of TPC, TFC, TTC, and RSA were obtained in 5% NaOH pretreatment, this concentration was used in the further optimization experiment. We also observed that longer extraction times (0–20) increased the TPC, TFC, TTC, and RSA, which subsequently reached a plateau at the time point of 15 min. These results indicated that the optimal AKE conditions were 5% NaOH, 375 W, and 15 min extraction (AKE-treated M4 sample). The samples treated under the optimal AKE conditions possessed higher TPC and TTC, compared to those treated with ultrasound only (375 W, 25 min) (Table 1). This suggested that the alkali pretreatment facilitated the extraction of bioactive compounds.

Alkali treatment is one of the most popular methods for extracting polysaccharides, a group of bioactive compounds found in mushrooms [33]. Medicinal mushroom cell walls are rich in polysaccharides, most of which are easily dissolved under alkaline conditions [33]. The alkaline solution not only punctures the fugal cell walls but also breaks down cellular fibers and links within peptides, as well as glucan [33, 34]. Therefore, alkali pretreatment effectively enhances the extraction yield of bioactive compounds from medicinal mushrooms, which was also demonstrated in this study. However, the maximal values of TPC, TTC, and RSA of the ultrasound-treated samples under the optimal conditions (375 W, 25 min) were higher than those of the AKE-treated samples. This indicated that ultrasound-assisted extraction was a better method to extract these bioactive compounds.

The effects of different ratios of material to liquid nitrogen and ultrasound extraction times on bioactive compounds and antioxidant activity of the extracts. The effects of liquid nitrogen pre-treatment and ultrasound extraction times on the extraction efficacy are presented in Table 5. As shown in Table 5A, pulverizing the samples with liquid nitrogen significantly increased their contents of polyphenols (TPC), flavonoids (TFC), triterpenoids (TTC), and antioxidant activity (radical scavenging activity, RSA) compared to the control sample ($p < 0.05$). We also found a positive correlation between the material-to-liquid nitrogen ratio (w/v) and the TPC, TFC, TTC, and RSA. For example, the samples pre-treated with liquid nitrogen in the ratio of 1:8 had their TPC, TFC, TTC, and RSA increased by 94.2, 128.2, 112.5, and 17.9%, respectively, compared to the samples pre-treated with liquid nitrogen in the ratio of 1:2. Among four ratios under study, the maximal values of TPC, TFC, TTC, and RSA were obtained with the ratios of 1:6 and 1:8 (w/v). In addition, there was no significant difference between the optimal ratios of 1:6 and 1:8 ($p > 0.05$). We also found that longer ultrasound extraction times increased the efficacy of bioactive compound extraction in terms of TPC, TFC, TTC, and RSA, which reached the optimal values at 15 min (Table 5B). Therefore, the material-to-liquid nitrogen ratio of 1:6 for pulverizing materials and the ultrasound extraction time of 15 min were identified as the optimal conditions for extracting polyphenols, flavonoids, triterpenoids, and antioxidants.

Our results were consistent with some previous studies. For example, in a study by Razumov *et al.*, pulverizing the Chaga mushroom, *Inonotus obliquus*, with liquid nitrogen enhanced the extraction of such bioactive compounds as methionine, leucine, melanin, asparagine, glutamine, and flavonoids [35]. Furthermore, cryogenic grinding with liquid nitrogen is a common method used in the food industry due to its effectiveness in producing super-fine materials. Besides, the low temperature during the grinding process accelerates the extraction of natural products, especially in phytosterols, soluble dietary fiber, and glucosinolate [36, 37]. Our findings proved the efficacy of pulverization in liquid nitrogen coupled with ultrasound-assisted extraction. In particular, the samples pretreated with liquid nitrogen (1:4) and treated with ultrasound extraction (375 W, 15 min) possessed higher TPC, TFC, TTC, and RSA, compared to the samples treated with ultrasound only (375 W, 15 min,

Table 5 Changes in bioactive compounds and antioxidant activity of the extracts under different ratios of material to liquid nitrogen and ultrasound extraction times

(A)	Ratios of material to liquid nitrogen, w/v				
	0	1:2	1:4	1:6	1:8
TPC, mg GAE/g DW	1.13 ± 0.04^a	2.43 ± 0.02^b	3.59 ± 0.01^c	4.69 ± 0.02^d	4.72 ± 0.01^d
TFC, mg QE/g DW	0.06 ± 0.01^a	0.39 ± 0.01^b	0.61 ± 0.03^c	0.88 ± 0.01^d	0.89 ± 0.01^d
TTC, mg OAE/g DW	0.12 ± 0.02^a	0.56 ± 0.01^b	0.82 ± 0.02^c	1.18 ± 0.01^d	1.19 ± 0.01^d
RSA, µg ascorbic acid/g DW	0.38 ± 0.03^a	2.96 ± 0.02^b	3.18 ± 0.02^c	3.48 ± 0.01^d	3.49 ± 0.01^d
(B)	Ultrasound extraction time, min				
	0	5	10	15	20
TPC, mg GAE/g DW	1.13 ± 0.04^a	3.10 ± 0.02^b	4.01 ± 0.01^c	4.69 ± 0.02^d	4.71 ± 0.01^d
TFC, mg QE/g DW	0.06 ± 0.01^a	0.38 ± 0.01^b	0.64 ± 0.02^c	0.88 ± 0.01^d	0.87 ± 0.01^d
TTC, mg OAE/g DW	0.12 ± 0.02^a	0.60 ± 0.02^b	0.96 ± 0.01^c	1.28 ± 0.01^d	1.29 ± 0.01^d
RSA, µg ascorbic acid/g DW	0.38 ± 0.03^a	2.91 ± 0.02^b	3.24 ± 0.02^c	3.48 ± 0.01^d	3.48 ± 0.02^d

Data were expressed as Mean ± SD of three experiments, and different lowercase letters (a, b, c, d, e) indicated statistically significant differences among the treatments ($p < 0.05$). TPC is total polyphenol content, TFC is total flavonoid content, TTC is total triterpenoid content, and RSA is antioxidant activity. DW is dry weight.

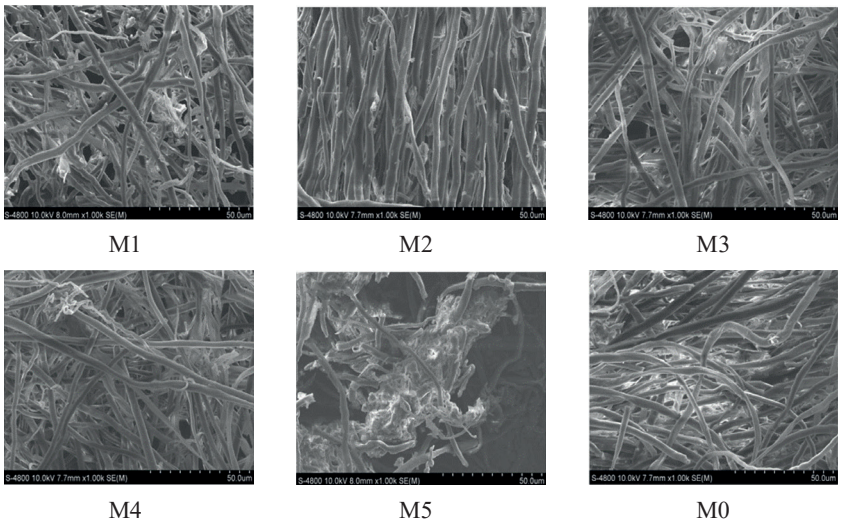

Figure 3 SEM images of *Coriolosis aspera* mycelia after treatments. The mycelia of the control samples (M0) are intact, complex, and compactly organized, while those of the samples treated with ultrasound-assisted extraction (M1), microwave-assisted extraction (M2), hot-water extraction (M3), and alkali extraction (M4) are partly destroyed. Only the mycelia of the samples treated with liquid nitrogen (M5) have visible damages

Table 1). This implied the superior effect of liquid nitrogen pretreatment coupled with UAE (LNE) against purely ultrasound-assisted extraction (UAE).

Among the five treatments under study, LNE was the best extraction method for TPC, TFC, TTC, and RSA. In the optimal LNE conditions with the material-to-liquid nitrogen ratio of 1:6 (w/v) and UAE (15 min), the extract contained the highest amounts of polyphenols (4.69 ± 0.02 mg GAE/g dry weight), flavonoids (0.88 ± 0.01 mg QE/g dry weight), triterpenoids (1.28 ± 0.01 mg OAE/g dry weight), and antioxidant activity (3.48 ± 0.01 μg acid ascorbic/g dry weight). These results were consistent with the SEM analysis of the mycelia of the LNE-treated samples (M5, Fig. 3), in which LNE was the only method able to rupture fugal mycelia, unlike UAE, MAE, AKE, and HWE. Noteworthily, the efficacy of the extraction methods varied depending on the bioactive compounds. The order of maximal values was LNE > UAE > MAE > AKE > HWE for polyphenols and antioxidants, LNE > AKE > MAE > UAE > HWE for triterpenoids, as well as LNE > MAE > UAE > HWE > AKE for flavonoids. These findings suggested that different methods and optimal conditions should be selected to optimize the extraction process for particular groups of natural products derived from medicinal mushrooms.

CONCLUSION

We studied the efficiency of extracting polyphenols, triterpenoids, and flavonoids from *Coriolosis aspera* by ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), hot-water extraction (HWE), ultrasound-assisted alkali extraction (AKE), and ultrasound-assisted liquid nitrogen extraction (LNE). The orders of maximal values were LNE > UAE > MAE > AKE > HWE for polyphenols, LNE > AKE > MAE > UAE > HWE for triterpenoids, and LNE > MAE > UAE > HWE > AKE for flavonoids. Thus, LNE was the most optimal method in all the cases.

Furthermore, the material-to-liquid nitrogen ratio of 1:6 (w/v), ultrasound extraction time of 15 min, and ultrasonic power of 375 W (frequency of 20 kHz; amplitude of 50%) were shown to be the optimal conditions for the LNE method, with the highest concentrations of polyphenols (4.69 ± 0.02 mg GAE/g dry weight), flavonoids (0.88 ± 0.01 mg QE/g dry weight), and triterpenoids (1.28 ± 0.01 mg OAE/g dry weight), as well as high antioxidant activity (3.48 ± 0.01 μg acid ascorbic/g dry weight). These findings could be used as supportive evidence for the potential application of the extracts from *C. aspera* in functional food production or in the pharmaceutical industry.

CONTRIBUTION

L.M. Thu developed the research concept and methodology, as well as performed the experiments. D.S. Mai designed the study, as well as reviewed and proofread the manuscript. N.N. Thuan collected the data and wrote the draft of the manuscript. L.T. Nguyen reviewed, edited, and proofread the manuscript. D.V. Phuong wrote the abstract, conducted formal analysis, finalized the manuscript and is the corresponding author who submitted the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this article.

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