EFFECT OF SOLVENT IN COMBINATION WITH ULTRASONIC WAVE ASSISTANCE ON TRITERPENOID EXTRACTION FROM RED LINGZHI MUSHROOM (Ganoderma lucidum)

Nguyen Duc Tien* and Nguyen Thi Huyen

Vietnam Institute of Agricultural Engineering and Postharvest Technology, 60 TrungKinhRoat, CauGiay District, Hanoi, Vietnam. *Corresponding author email: nguyenductienviaep@gmail.com

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ABSTRACT

Triterpenoid is one of the compounds that contribute to red lingzhi mushroom's major significant therapeutic activities (G. lucidum). Triterpenoids have anti-allergic, anti-cancer, anti-tumor, and anti-aging effects, enhance oxygen utilization, free radical scavenging, lower blood pressure, inhibit cholesterol biosynthesis, enhance liver function and blood pressure, inhibit cholesterol biosynthesis, and improve liver function. The beneficial effects of triterpenoids in Ganoderma lucidum on human health have generated interest in developing methods for extracting them from the fruit bodies of G. lucidum. This article presents the effect of solvent in combination with ultrasonic wave assistance in triterpenoid extraction ability from red lingzhi mushroom. Before carrying out triterpenoid extraction; Red lingzhi mushroom was ground 2mm in size. Four parameters of the extraction process, including the ethanol concentration, ratio (v:w) between ethanol solvent and mushroom, extraction time, extraction temperature, ultrasonic time and ultrasonic intensity were determined. The extraction without ultrasonic waves for 180 minutes was used as a control. The total triterpenoid content was collected during the extraction process. The results showed that red lingzhi mushroom was extracted by ethanol solvent at a concentration of 85%, the ratio between ethanol solvent and mushroom was 3:1 (v:w), ultrasound intensity was 58 W/cm², frequency was 20 kHz, extracting temperature was 55°C, removing time was 5 min had total triterpenoid content 3.06 times higher than the control.

Keywords: Redlingzhi mushroom, triterpenoid, ultrasound, extraction.

INTRODUCTION

Recently, the red lingzhi mushroom (Ganoderma lucidum) has been successfully cultivated in Vietnam, with a total yield of about 10 tons annually, bringing high economic efficiency (Duong et al., 2020). Several studies have shown that the red lingzhi mushroom (RLM) provides many biologically active substances. RLM contains more than 400 compounds including triterpenoids, polysaccharides, nucleotides, sterols, steroids, fatty acids, proteins, peptides, and trace elements, with biological activities such as anti-atherosclerotic, anti-inflammatory, immune-boosting, anti-cancer, anti-bacteria and viruses, protect the liver, anti-aging, etc. (Sanodiya et al., 2009, Russell et al., 2006). More than 150 triterpenoid compounds have been extracted from RLM. In which, there are 50 triterpenoids found only on RLM. Therefore, RLM is identified and confirmed as one of the plants that has rich triterpenoid compounds (Chang and Miles, 2004). Triterpenoids from RLM are considered important substances because they can fight cancer cells, enhance oxygen use, redox free radicals in the body, anti-aging, lower blood pressure, inhibit cholesterol biosynthesis, and to improve liver function (Sakai and Chihara, 1995). Extracting triterpenoids from dried RLM is not easy even though we also use traditional extraction methods such as decoction in water, extraction with methanol, alcohol or concentrated form according to the standard ratio. However, traditional methods usually only obtain triterpenoids in mycelium and fruit bodies, but it is difficult to separate the shell of fungal spores (Chen et al., 2011). The extraction of biologically active substances in RLM depends on many factors such as solvent type, extraction temperature, extraction time, etc. There are many methods to assist the extraction of triterpenoids in plant materials such as enzymes, microwaves, supercritical CO₂, etc. The basis of using ultrasound energy in the extraction process increases the mass transfer rate of the extract, making it easier to diffuse the section from the inside of the material to the outside (Liu et al., 2010). Therefore, in this study, we evaluated the effect of some parameters (concentration of ethanol solvent, material/solvent ratio, extracting temperature, ultrasound intensity, ultrasound time) on the total content of triterpenoids from RLM during processing with the assistance of ultrasonic
The effect of the ethanol solvent's concentration on triterpenoid extraction. The impact of the concentration of ethanol solvent on the ability to extract triterpenoid in the red lingzhi mushrooms by ultrasonic wave assistance is shown in Figure 1. In general, different ethanol concentration gave different triterpenoid ability extraction.

Figure 1: Effect of the ethanol solvent's concentration on triterpenoid content of the G. lucidum extract

Ethanol at concentration of 70, 75, 80, 85, 90 and 96% obtaining the triterpenoid content in the extract respectively were 0.761; 0.832; 1.065; 1.570; 1.321 and 1.23%. These results demonstrated that the concentration of ethanol solvent affects the ability to extract triterpenoids from red lingzhi mushrooms. This can be explained that at each given solvent concentration in addition to the ability to dissolve triterpenoid, ethanol also can dissolve impurities, reducing the ability to dissolve triterpenoid into the solvent. The highest extract yield was found in the ethanol concentration of 85% (1.570% triterpenoid). Thus, the ethanol concentration of 85% was selected as the solvent.
for extracting triterpenoidin theredlingzi mush-
rooms by ultrasonic wave. Cai et al., (2019) found
that the total triterpenoid extraction from the
medicinal fungus Sanghuang russanghuang was
optimal at an ethanol concentration of 80% under
the conditions of a 1:15 solidliquid ratio, 15 min
extraction time 100 W of extraction power and a
60°C extraction temperature. Chew et al., (2011)
found that 40% ethanol had a significant (p<0.05)
effect on phenolic contents (total phenolic content,
total flavonoid content and 2,2'–azino-bis(3-ethyl-
benzo-thiazoline-6-sul-phonic acid) and antioxi-
dant capacities (2,2’-azi-no-bis(3-ethyl–benzothia-
zoline-6-sulphonicacid and 2,2-diphenyl-1-picryl-
hydrazyl) of crude extract from Cente-llasiatica.

The effect of the ratio of ethanol solvent and
mushroom on the extraction of triterpenoids:
Using more solvents for extraction, the greater the
ability to diffuse triterpenoids from mushroom to
the solvent, but if the amount of solvent is too
much, the ability to obtain triterpenoid active
ingredients is insignificant, it will not be parac-tical
due to costing solvent, time and energy to recover
solvent. Therefore, determining the ratio between
ethanol solvent and mushroom for triterpenoids
extraction to achieve high efficiency is necessary.

Figure 2 showed that using a solvent with
different ratios, the extraction capacity of triter-
penoids was different, when increasing the amount
of the solvent, the triterpenoids concentration in
the extract also grew.

Figure 2: Effect of the proportion between
ethanol solvent and G. lucidum (V:w) on the
triterpenoid content

Tritrpenoid content increased sharply at the ratio
of solvent/mushroom was 1:1, 2:1 and 3:1 (l/kg)
(0.835, 1.086 and 1.547%, respectively). The
results showed that when at a ratio of 1:1, the
amount of solvent used is small, not enough to
penetrate the entire extraction material, the
diffusion of solute into the solvent, and a certain
amount of solvent will be kept in the raw material
is the cause of the reduction in extraction effi-
ciency. Increasing the solvent ratio will promote
the dissolution of triterpenoids into the solvent at
an early stage. The solutes have good solubility
conditions in the solvent because a large amount
of solvent will increase the contacting ability of the
cell with the solvent, leading to an increase in the
concentration difference between the environment
inside and outside of the cell. It increases the
osmotic pressure difference, and the solutes will
diffuse into the solvent, leading to an increase in
the triterpenoid content of the extract. Initially, the
concentration of Triterpenoids in the material is
high, so their diffusions out of the cell is also fast.
At high solvent ratios of 4, 5 and 6, the triterpenoid
extraction capacity did not increase significantly,
respectively 1.562, 1.586 and 1.591 % when com-
pared to a ratio of 3:1 (1.547%). From these results,
to save powerful solvent and energy, the ratio
between solvent and mushroom of 3:1 (v:w) was
the most ideal for extracting triterpenoid in the
redlingzhi mushrooms. Cai et al., (2019) found
that the total triterpenoid yield was removed from the
medicinal fungus Sanghuang-porussanghuang
markedly increased when the solid-liquid ratio
ranged from 1:10 to 1:20. In this condition,
dissolved medicinal powder increased as the solid
liquid ratio increased. When the dissolution of
triterpenoid reached an equivalence point, an
increase in the solvent volume did not dissolve
more triterpenoid; therefore, the extraction yield no
longer increased when the solid-liquid ratio
reached 1:20.

The effect of ultrasonic temperature on tri-
terpenoid yield: The higher the extracting tempe-
rate will increase the porosity of the material (due
to the swelling of the material), the viscosity
decreases and the active ingredient to dissolve
easily into the solvent. Extracting by temperature
with ultrasonic waves assistance increased the
number of air bubbles formed. However, when the
temperature increases, air bubble’ intensity break-
ing will be reduced due to the effect of vapour
pressure increasing and playing a role as a buffer
layer, preventing the collision of surrounding mol-
ecules when the air bubble is broken. Extracting
temperature too highly increase, can increase the
solubility of some impurities, promoting chemical
changes that make the shift in quality of the extract
unprofitable. In contrast, the rupture of air bubbles
will be difficult when the temperature decreases because the increased environmental viscosity. Therefore, determining the suitable extraction temperature is essential to achieving high productivity and pure extracts. The results in Figure 3 showed that the extraction temperature from 35, 45 to 55°C gave the extraction ability with an enormously increasing triterpenoid content of 0.662, 0.832 and 1.358 % respectively.

![Figure 3: Effect of extraction temperature on triterpenoid content of the extract](image)

The extraction temperature increased from 65, 75 to 85°C gave triterpenoid content to increase insignificantly by 1.363, 1.365 and 1.381%, respectively. It was found that increasing the extraction temperature to 85°C gave a negligible increase in the triterpenoid extraction capacity compared with the temperature of 55°C. From this result can conclude 55°C is a suitable temperature to extract triterpenoid in red lingzhi mushrooms. An increase in temperature can enhance the permeability of triterpenoid molecules inside the cell. In the range of 30–60°C, the total triterpenoid yield increased as the temperature increased. However, the yield slightly decreased when the temperature exceeded 60°C because high temperatures (greater than 60°C) destroy the triterpenoid molecular structure of the five rings (Cai et al., 2019). The extraction temperature affected on the total phenolic content and radical-scavenging properties of the different extracts reported by Onyebuchi and DoğaKavaz (2020). Onyebuchi and DoğaKavaz (2020) also concluded that all extracts' chemical and bioactive properties showed significant dependence on the extraction temperature and solvent type. The mechanical effect of acoustic cavitation from the ultrasound increases the surface contact between solvents and samples and the permeability of cell walls. Physical and chemical properties of the materials subjected to ultrasound are altered and disrupt the plant cell wall, facilitating the release of compounds and enhancing mass transport of the solvents into the plant cells (Dhanani et al., 2013). Chew et al., (2011) concluded that extracting temperature at 65°C by 40% ethanol solvent had a significant (p<0.05) effect on phenolic contents and antioxidant compounds of crude extract from *Centellaasiatica*.

The effect of ultrasound time and ultrasonic intensity on total triterpenoid extraction: Effect of time and intensity of ultrasonic wave on ability of extraction of triterpenoid from the red lingzhi mushrooms is shown in Figure 4, the extraction without ultrasonic wave (control) for 0, 2, 3, 4, 5, 6 and 7 min obtained triterpenoids content were 0.238, 0.311, 0.367, 0.378, 0.383, 0.398 and 0.429 %, respectively.

![Figure 4: Effect of time and ultrasonic intensity on the capacity of triterpenoid extract from *G. lucidum*](image)

The extraction using ultrasonic waves at different intensities from 25 to 58 W/cm² for 2, 3, 4, 5, 6 and 7 min obtained triterpenoids content ranging from 0.55 to 2.486%. A significantly different in extracted triterpenoids content between control and samples extracted by ultrasonic wave at different intensities (P<0.05). At a different extracting times with different powers also obtained additional triterpenoids content (Figure 4). As seen in Figure 4, the extracted triterpenoids content sharply increased after 2, 3 and 5 min of extraction; after that, at 5,6 and 7 min of extraction, the extracted triterpenoids content in the extract did not increase significantly. At extracting conditions ultrasonic intensity of 58 W/cm² gave higher...
triterpenoids content when compared to the other extracting conditions with the control. At the ultrasonic intensity of 58 W/cm² with an ultrasonic extraction times of 2, 3, 4 and 5 min, triterpenoid content in extract increased by 1.458, 1.949, 2.236 and 2.461%, respectively; after 6 and 7 minutes of ultrasonic extraction, the triterpenoid content still increased at 2.474 and 2.486%, respectively, at the time level of 5, 6 and 7 min, the triterpenoid content in the extract did not increase significantly. These results justified that at an ultrasonic intensity of 58 W/cm² with an ultra-sonic extraction time of 5 min, the best condition to extract triterpenoid in red lingzhi mushrooms. Chew et al., (2011) found that extracting time for 120 min by 40% ethanol had a significant (p<0.05) effect on phenolic contents and antioxidant compounds of crude extract from Centella asiatica. The maximum yield of triterpenoids was obtained from medicinal fungus Sanghuang porussangh-uang when the ultrasonic time was 20 min. After 20 min, most of the cells were dissolved, and the triterpenoid molecules achieved equivalence between the inside and outside of the cells (Cai et al., 2019). The time and intensity of ultrasound were investigated as when the ultrasonic intensity and time increased the ability to extract triterpenoid highly increased and much higher than that of the control (without using ultrasound, for triterpenoid content in the extract is the lowest). The higher the ultrasonic intensity, the higher the effervescence, the high breaking force, which increases the mass transfer rate of the extract. In addition, the foam bubbling also creates an agitation that allows the diffusion of the extracts inside the material to escape more easily (Jian-bing et al., 2005). The longer the ultrasonic time, the more significant the intensity, the more the particles of the extracted material are destroyed and the more the viscosity and solu-bility increase, respectively. Viscosity increases with increasing ultrasonic intensity and time, the number of short-chain molecules gains, and the thickness also increases. Still, when the intensity reaches a specific value, the thickness decreases. The higher the ultrasonic intensity, which is an agent to contribute to increasing the efficiency of the extraction process, the rapid expansion of bubbles during the negative pressure cycle makes the bubbles no chance of shrinking during the positive pressure cycle. Conversely, when the ultrasonic intensity is smaller, the number of times the bubbles expand and compress will increase, and the extraction time will be longer (Herceget et al., 2010). The stronger the ultrasonic wave intensity, the higher the ability to extract the active ingredient in a shorter time. So, to save time and cost, 5 min of ultrasonic extraction at the intensity of 58 W/cm² is the most effective for the extraction of triterpenoids in red lingzhi mushrooms.

**Comparison of triterpenoid extraction method in red lingzhi mushrooms by using ultrasonic wave and without ultrasonic wave:** Table 1 indicated that extraction with ultrasonic waves intensity of 58W/cm² at 20 kHz frequency, at 55 ± 2°C, the ratio of ethanol 85% mushroom was 3:1 (v:w), for 5 minutes gave the highest triterpenoid extraction ability of 2.47 % and soluble dry matter of 16.30%.

<table>
<thead>
<tr>
<th>Norm</th>
<th>Unit</th>
<th>Triterpenoid extract</th>
<th>Without ultrasonic</th>
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<tr>
<td></td>
<td></td>
<td>Ultrasonic intensity 58 W/cm², 20 kHz</td>
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<td>Material size</td>
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<td>1.8</td>
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<td></td>
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<td>Proportion of ethanol and G. lucidum ingredient</td>
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<tr>
<td>Extract temperature</td>
<td>°C</td>
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<td></td>
<td></td>
<td>98 ± 2.0</td>
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<td>Extract duration</td>
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<td>Triterpenoids</td>
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<td>Dry matter</td>
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<td>16.92±0.04</td>
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While the extraction method did not use ultrasonic waves, extracted at 55 ± 2°C, the ratio of ethanol 85%/mushroom was 3:1 (v:w), for 5 min gaveless triterpenoid content (0.38%) and soluble dry matter of 2.12%. Extraction without ultrasonic wave at 98 ± 2°C (traditional method), a
ratio of water/mushroom (d=30mm) was 6:1 (v:w), for 180 min (3 h) gave the possibility of extracting triterpenoid content only of 0.81% and soluble dry matter of 16.92%. Extraction without an ultrasonic wave provides lower triterpenoid content than extraction with an ultrasonic wave. At ultrasonic wave intensity 58 W/cm², frequency 20 kHz for 5 min, triterpenoid content was about 3.06 times higher than non-ultrasound for 3h at 98 ± 2°C. There was a significant reduction in extraction temperature of 98 ± 2°C in the conventional extraction process to 55 ± 2°C during the use of ultrasonic waves. This shows that ultrasonic energy gives the effect of extracting triterpenoid in red lingzhi mushrooms.

CONCLUSIONS
Extracting triterpenoid in red lingzhi mushrooms by 85% ethanol solvent with the ratio between solvent and mushroom was 3:1 combined with ultrasonic wave assistance at ultrasonic intensity 58 W/cm², frequency 20 kHz, time 5 min at 55 ± 2°C instead of conventional extraction methods provides an attractive technological alternative for obtaining the total triterpenoid content 3.06 times higher than the traditional extraction meth-ods. This study shows the potential of using ultrasonic waves is more effective than traditional extraction methods without using ultrasonic waves, which is to shorten the extraction time and more obtain triterpenoids in red lingzhi mush-rooms.

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