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Original Article

Hepatoprotective effects of the fruiting body extract from red *Pycnoporus sanguineus* (L.) Murrill fungus against carbon tetrachloride-induced liver damage in mice

[Efectos hepatoprotectores del extracto del cuerpo fructífero del hongo rojo *Pycnoporus sanguineus* contra el daño hepático inducido por tetracloruro de carbono en ratones]

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Abstract

Context: One of the 25 major species of medicinal fungi in the world, the red *Pycnoporus sanguineus*, has a very high therapeutic value. However, studies about the pharmacological effects on liver protection of this fungus have not been performed.

Aims: To evaluate the protective actions of the extract from the fruiting body of red *P. sanguineus* fungus in mice with impaired liver function.

Methods: Red *P. sanguineus* hydroethanolic extracts in three different doses were applied to mice 14 days before causing hepatotoxicity by a single injection of carbon tetrachloride. Blood and liver were taken to measure plasma ALT and AST levels and liver MDA and GSH contents. The gross and microscopic liver appearance was also examined.

Results: Extract of *P. sanguineus* reduced plasma liver enzyme concentrations compared to the group using only CCl_4 ($p<0.01$). MDA level in the liver also decreased in mice treated with the extract. Similarly, liver GSH concentration was also recovered when three different doses of the extract were used. These effects were dose-dependent, with higher doses demonstrating greater protection. Macroscopic observation showed that mice treated with the *P. sanguineus* extract improved liver shape, such as darker color and less congestion. Under microscopic inspection, *P. sanguineus* extract at high doses (500 and 1000 mg/kg) caused mild to moderate liver cell damage and few inflammatory foci.

Conclusions: Red *P. sanguineus* extracts displayed positive effects on preventing carbon tetrachloride-induced acute liver toxicity. These effects were dose-dependent, with the most potent actions belonging to the highest dose.

Keywords: glutathione; liver enzyme activity; macroscopic observation; malondialdehyde; microscopic observation.

Resumen

Contexto: Una de las 25 principales especies de hongos medicinales del mundo, el hongo rojo *Pycnoporus sanguineus*, posee un alto valor terapéutico. Sin embargo, no se han realizado estudios sobre sus efectos farmacológicos en la hepatopatía.

Objetivos: Evaluar la acción protectora del extracto del cuerpo fructífero del hongo rojo *P. sanguineus* en ratones con insuficiencia hepática.

Métodos: Se aplicaron extractos hidroetanólicos de *P. sanguineus* en tres dosis diferentes a ratones 14 días antes de causar hepatotoxicidad mediante una única inyección de tetracloruro de carbono. Se tomaron muestras de sangre y del hígado para medir los niveles plasmáticos de ALT y AST, así como los niveles hepáticos de MDA y GSH. También se examinó el aspecto macroscópico y microscópico del hígado.

Resultados: El extracto de *P. sanguineus* redujo las concentraciones plasmáticas de enzimas hepáticas en comparación con el grupo que solo utilizó CCl_4 ($p<0,01$). El nivel de MDA en el hígado también disminuyó en los ratones tratados con el extracto. De forma similar, la concentración hepática de GSH también se recuperó al utilizar tres dosis diferentes del extracto. Estos efectos fueron dosis-dependientes, y las dosis más altas demostraron una mayor protección. La observación macroscópica mostró que los ratones tratados con extracto de *P. sanguineus* mejoraron la forma del hígado, presentando un color más oscuro y una menor congestión. Al microscopio, el extracto de *P. sanguineus* a dosis altas (500 y 1000 mg/kg) causó daño hepático leve a moderado y pocos focos inflamatorios.

Conclusiones: Los extractos de *P. sanguineus* rojo mostraron efectos positivos en la prevención de la hepatopatía aguda inducida por tetracloruro de carbono. Estos efectos fueron dosis-dependientes, y las acciones más potentes correspondieron a la dosis más alta.

Palabras Clave: actividad enzimática hepática; glutatión; malondialdehído; observación macroscópica; observación microscópica.

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INTRODUCTION

Many edible and inedible fungi have long been used in Asia and Africa for medicinal purposes. Many pharmacologically active compounds from fungi have been discovered and isolated, including antibacterial, antioxidant, antiviral, anti-tumor, anti-allergic, immunomodulatory effects, anti-inflammatory, and hepatoprotective activities (Chen et al., 2020). The red *Pycnoporus sanguineus* (L.) Murrill fungus (family *Polyporaceae*) is one of the world's 25 main medicinal fungus species, with very high therapeutic value. It has long been used by indigenous people of countries such as Australia, Brazil, and Africa to stop bleeding and reduce symptoms of arthritis, gout, sore throat, ulcers, toothache, and fever (Chen et al., 2020). In the rural area of Santa Catarina, southern Brazil, red *P. sanguineus* is used to treat skin lesions (Téllez-Téllez et al., 2016). In some places in Vietnam, people use red *P. sanguineus* to treat ear rot (Trinh et al., 2011; 2012).

The red *P. sanguineus* has a vermilion to orange-red color, with a slightly curled concentric upper surface and a darker lower surface with small red holes. Fruiting body size ranges from 4-15 cm to 5 mm thick. Known as a wood-decomposing fungus, it is easily found on rotting tree trunks (Téllez-Téllez et al., 2016). The fungus growth is suitable for tropical and subtropical regions of Africa, America, and Asia (Jaouen et al., 2019). Research in the world and Vietnam has proven that red *P. sanguineus* fungus possesses many pharmacological effects such as antibacterial, antifungal, and antiviral activities (Smânia et al., 2003), antioxidant and liver protection, anti-inflammatory (Chen et al., 2020; Jouda et al., 2018; Lu et al., 2010), anti-cancer activity (Gambato et al., 2016; Mendoza et al., 2020; Smânia et al., 2003; Yang et al., 2020), and lipid-lowering effects (Tran et al., 2017; Von Dentz et al., 2021). It has been successfully farmed in Dong Thap Province, Vietnam, and the scientific name has been determined as *Pycnoporus sanguineus* (L: Fr) Murr (Fig. 1).

The main chemical constituents from *Pycnoporus* species, first isolated by Gripenberg (1951) and Cavill et al. (1953), were identified as phenoxazinone-framed alkaloids, including cinnabarin, cinnabaric acid, and tramesanguin. Besides, at least six different aminophenoxazine derivative compounds were also found (De Oliveira et al., 2007; Téllez-Téllez et al., 2016). The phenoxazine-3-one (nitrogen heterocycle) structure plays a significant role in the pharmacological effects and regulation of red pigmentation in *Pycnoporus* species (Achenbach and Blümm, 1991). According to research by Juliette-Ornely et al. (2019), a survey on chemical composition, antioxidant, and anticancer activities of medicinal fungi *Daedaleopsis nitida*, *Pycnoporus sanguineus*, and *Phellinus gilvus* collected from the Republic of Gabon, showed that the extracts from *Pycnoporus sanguineus* fungus are rich in reducing sugars, flavones, digitoxigenin, coumarin, and relatively abundant in alkaloids. The content of compounds such as polyphenols, sterols, and triterpenes in water and ethanol extracts of *P. sanguineus* fungus is also relatively high. Phenolic compounds are essential platforms because of their biological and pharmacological effects. In 2020, Mendoza et al. (2020) further determined that catechins and total phenolics have antioxidant, antibacterial, and antiproliferative effects in cancer cell lines.

Figure 1. Red *Pycnoporus sanguineus* fungus grown in Dong Thap Province, Vietnam.

In Vietnam, Tran et al. (2017) preliminarily analyzed the presence of phenolic compounds and quantified them in red *P. sanguineus* extract. According to the results, both water and ethanol extracts from the fruiting bodies of the red *P. sanguineus* were present with a significant amount of biologically active natural compounds such as flavonoids, polyphenols, saponins, tannins, and triterpenoids. Nguyen and Ngo (2022) investigated the antioxidant and anti-inflammatory activities of red *P. sanguineus* extract isolated in Vietnam. The results showed that the fungus extract in the pre-fruiting body stage isolated in Binh Duong, Vietnam, expressed antioxidant activity with an IC_{50} of 30.0 ± 0.54 µg/mL (positive control was vitamin C with an IC_{50} of 10.32 ± 0.02 µg/mL). Dang et al. (2024) also demonstrated the concentration-dependent antioxidant effect of a standardized extract of *P. sanguineus* on DPPH, ABTS, and reducing power (RP) models, with IC_{50} values of 73.8386 ± 3.24 µg/mL, 73.7588 ± 2.24 µg/mL, and 85.1821 ± 2.28 µg/mL, respectively.

Research on cultivated red *P. sanguineus* in Vietnam mainly focused on analyzing the chemical composition (Nguyen and Ngo, 2022; Tran et al., 2017; 2018), while studies comprehensively evaluating the biological effects, especially the liver-protective activity of this fungus, are still limited. Therefore, this study aims to assess initially the hepatoprotective effect of the extract from the fruiting bodies of *P. sanguineus* fungus *in vivo*. This is an essential step in optimizing the treatment potential of this herbal fungus and orienting future research to create healthcare products using this precious medicinal source.

MATERIAL AND METHODS

Plant material

The fruiting bodies of the red *P. sanguineus* were harvested in Dong Thap province, Vietnam (10°25'15.7"N 105°38'41.9"E). Fungi were identified by Tran et al. (2018) and Tran (2019) by comparing their morphological characteristics with descriptions in standard botanical literature and determining their genetic sequences (accession number: MH225776). The fungus fruit bodies were dried, chopped, extracted, and evaluated for hepatoprotective activity.

Animals

Healthy male Swiss albino mice weighing 20-25 g, no deformities, no unusual signs. Mice were stably raised in the laboratory of the experimental animal research unit of Can Tho University of Medicine and Pharmacy under standard conditions for one week before the experiment. Mice were kept in a 25°C environment with a 12-hour light/dark cycle during the experiment period. Food made of bran pellets and clean water were provided *ad libitum*. All mice were treated following the National Institutes of Health (NIH) Guide for the Humane Care and Use of Laboratory Animals and were approved by the Council for Ethics in Biomedical Research of Can Tho University of Medicine and Pharmacy (No. 23.398.HV-ĐHYDCT dated April 12, 2023). Interventions on research animals and tissue samples collected were used for research purposes only.

Chemicals

Ethanol 70% (Vietnam) was used as the solvent for extracting the fruiting body of *P. sanguineus*. Carbon tetrachloride (CCl_4) (Guanghua, China) was used to induce liver damage model in mice. Other chemicals included formalin (Xilong, China), silymarin (Légalon® 70 Protect, Germany), reduced glutathione (GSH, Wako Pure Chemical Industries, Japan), malondialdehyde bis(dimethyl acetal) (MDA, Merck, Germany), diethyl ether (Chemsol, Vietnam), trichloroacetic acid (Merck, Germany), thiobarbituric acid (Merck, Germany), olive oil (Fragata, Spain), phosphate buffer (Merck, Germany), EDTA phosphate buffer (Merck, Germany), xylene (Xilong, China), potassium chloride (Xilong, China), and Ellman's reagent (Wako Pure Chemical Industries, Japan).

Extraction

Chopped fungus fruit bodies with determined moisture were extracted using the ultrasound-assisted extraction method (Ultrasonic Cleaner Set, Model WUC-D22H, Daihan Scientific, Korea) with a temperature of 50°C, an extraction time of 30 minutes, a sound wave amplitude of 75%, and 70% ethanol as solvent. The ratio between fungi and solvent was 1:20. The extraction process was repeated three times to extract the active ingredients fully. Combine the extract, evaporate it with reduced pressure to obtain a concentrated extract, and continue to evaporate in a water bath below 60°C to obtain the extract with a moisture content below 20%. The moisture content of fungi and extracts was determined based on the loss-on-drying method using an infrared moisture dryer, and the recovery efficiency was calculated.

Experimental design

Mice were randomly divided into six groups and treated with either saline, silymarin (100 mg/kg, p.o), or red *P. sanguineus* extracts with three different doses (250, 500, and 1000 mg/kg, p.o) once a day for 14 days. The dose of extract was identified based on previous studies (Le et al., 2019). The mice's body weight was recorded every day. On day 15, 0.2% CCl_4 dissolved in olive oil was injected intraperitoneally

(i.p). After administration of CCl_4, food and water were withdrawn. On day 16 (24 hours after CCl_4 injection), mice were anesthetized with diethyl ether, the heart blood was dissected to measure liver enzyme activity (ALT and AST), and the liver was separated for macroscopic and microscopic observations and MDA and GSH quantification (Fig. 2).

Determination of plasma liver enzyme activity

Mouse heart blood was centrifuged at 3000 rpm for 10 minutes at 25°C. Plasma was collected and used to determine liver enzyme activity, ALT (alanine aminotransferase), and AST (aspartate aminotransferase) by the enzyme kinetic measurement method using the Automation GLP-Alinity c/i machine (Abbott, USA).

Determination of liver malondialdehyde and glutathione

The contents of malondialdehyde (MDA) and glutathione (GSH) in liver tissue were determined, respectively, as described by Ohkawa et al. (1979) and Moron et al. (1979). Liver tissue was separated from the body and ground homogenously in 1.15% KCl buffer at 4°C. The homogenate (1 mL) was mixed with 0.5 mL of phosphate buffer (pH = 7.4) and incubated at 37°C for 60 minutes. The reaction was finished with 0.5 mL of 10% trichloroacetic acid and centrifuged at 13000 rpm for 10 minutes at 4°C. For MDA quantification, 1 mL of the supernatant after centrifugation was reacted with 0.5 mL of 0.8% thiobarbituric acid at 100°C for 30 minutes, and the absorbance was measured at 532 nm. MDA content (nM MDA/g tissue) was calculated based on the linear regression equation of the MDA standard (Ohkawa et al., 1979). Similarly, for GSH determination, 1 mL of the supernatant was reacted with 0.2 mL of Ellman's reagent and 3 mL of EDTA phosphate buffer. The reaction mixture was allowed to stand for 3 minutes at room temperature, and then the absorbance was measured at 412 nm. GSH content (nM GSH/g tissue) was calculated based on the linear regression equation of the GSH standard (Moron et al., 1979).

Liver macroscopic observation

After being separated from the mouse's body, the liver was washed with cold 0.9% NaCl, patted dry, and weighed to evaluate the effect of extracts on liver mass (Badr El-Din et al., 2020). The gross appearance was observed with the naked eye, and photographs were taken by iPhone 11, noting color characteristics, liver surface condition, and injury.

Liver microscopic observation

The livers were fixed in 10% neutral buffered formalin for at least 48 hours, embedded in paraffin, then cleaned in xylene, dehydrated in a succession of ethanol, and washed in distilled water for microscopic examination using hematoxylin-eosin (HE) staining (Luangmonkong et al., 2022). The structure and morphology of liver cells in the 5 µm liver slices were observed under a Nikon E100 microscope equipped with 10× and 40× objective lenses to visualize the pictures.

Statistical analysis

Data were processed using SPSS 23.0 software and Microsoft Office Excel 2016 and expressed as mean ± standard deviation (SD). The difference between specific groups was tested using the One-way ANOVA test (*post hoc* test) with a confidence level greater than 95% ($p<0.05$).

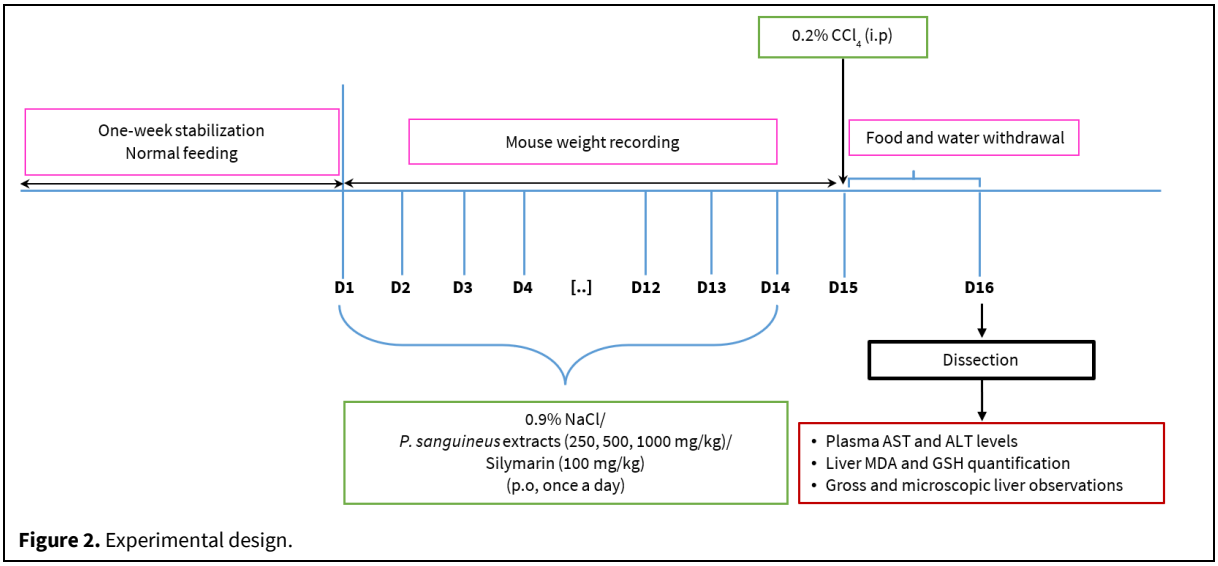

Figure 2. Experimental design.

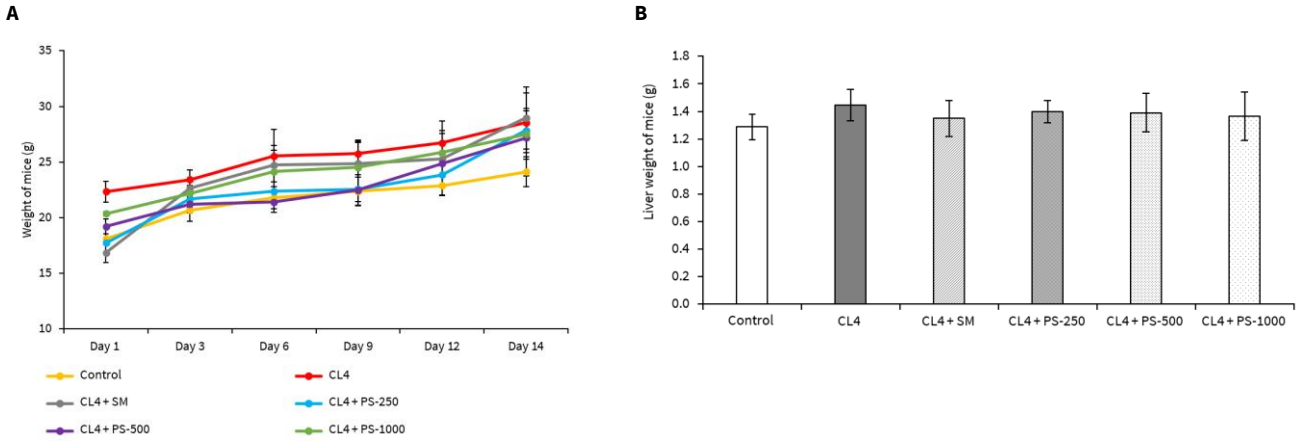

Figure 3. Mouse weight change during the experimental period **(A)** and mouse liver weight at the end of the experiment **(B)**.

Each value is the mean ± SD of six animals. CL4: CCl_4; SM: Silymarin (100 mg/kg); PS-250: *Pycnoporus sanguineus* extract (250 mg/kg); PS-500: *Pycnoporus sanguineus* extract (500 mg/kg); PS-1000: *Pycnoporus sanguineus* extract (1000 mg/kg).

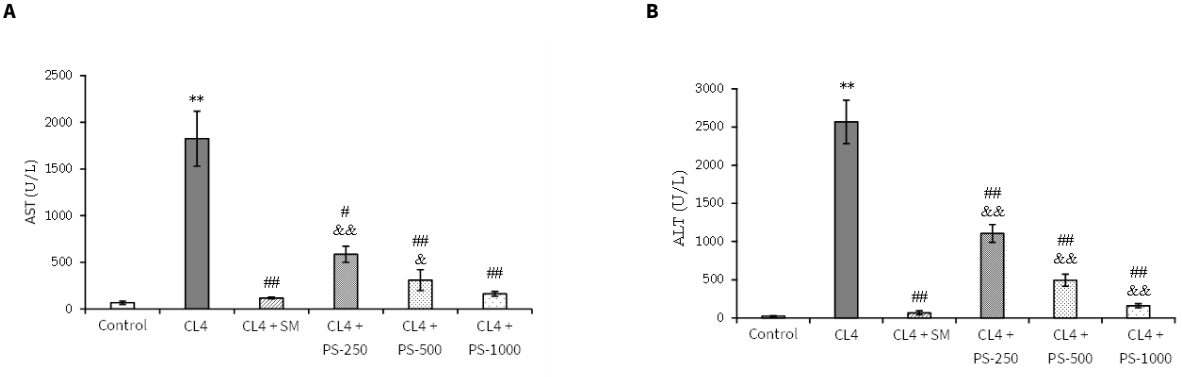

Figure 4. Effects of *Pycnoporus sanguineus* (PS) extracts and silymarin (SM) on CCl_4-induced changes in blood AST **(A)** and ALT **(B)** levels in mice.

Each value is the mean ± SD of six animals. **p<0.01 vs. control. $^{\#}$p<0.05, $^{\#\#}$p<0.01 vs. CCl_4. $^{\&}$p<0.05, $^{\&\&}$p<0.01 vs. CCl_4 + silymarin (One-way ANOVA). CL4: CCl_4; SM: Silymarin (100 mg/kg); PS-250: *Pycnoporus sanguineus* extract (250 mg/kg); PS-500: *Pycnoporus sanguineus* extract (500 mg/kg); PS-1000: *Pycnoporus sanguineus* extract (1000 mg/kg).

RESULTS

Mice and liver weight

During the experimental period, the weight of mice in all groups increased slightly over time, but the average weight between groups at each time point was not statistically different ($p>0.05$). The red *P. sanguineus* extracts and silymarin did not change the weight of mice during the test period. Similarly, the average mouse liver weight did not differ significantly between the groups (Fig. 3).

Blood liver enzyme concentrations

Carbon tetrachloride markedly increased liver enzyme activity, AST and ALT, in all groups. The highest increase in AST and ALT levels was shown in mice that used only CCl_4, and the difference was statistically significant compared to the control group ($p<0.01$). Silymarin significantly decreased liver enzyme activity, and this effect was equivalent to the group treated with the highest dose of red *P. sanguineus* extract (1000 mg/kg), which showed an insignificant difference in AST level. There were also differences in the effects of the 250 mg/kg dose compared to the 500 mg/kg and 1000 mg/kg doses (Fig. 4).

Liver malondialdehyde and glutathione levels

The results from Fig. 5A illustrate that liver MDA level was increased in all groups treated with CCl_4. This phenomenon was counteracted when treated with silymarin at 100 mg/kg. The same tendency was observed in the groups receiving *P. sanguineus* extract, with the highest dose showing the most potential protective effect. Meanwhile, GSH contents were decreased by CCl_4 treatment. This reduction was reversed in the silymarin-exposed group. The red *P.*

sanguineus extract also significantly recovered the level of GSH compared to the CCl_4 group ($p<0.01$) (Fig. 5B).

Gross appearance of the mouse liver

Mice livers in the control group were red, had smooth outer surfaces, and were not edematous and congested. There were many white dots with a diameter of 0.5-1 mm on the surface of the liver in the CCl_4-treated group. The gross appearance of the liver in mice taking silymarin was similar to that of the control group. In mice taking the red *P. sanguineus* extract, the paleness and congestion of the liver surface and the appearance of tiny dots gradually decreased as the dose increased, reflecting an improvement in the liver macroscopicity compared to the CCl_4 group (Fig. 6).

Microscopic observation of mouse liver cells

With microscopic examination, the central lobule vein was clearly seen in the control group, and the portal triad was of normal function. There was no liver cell necrosis, capillary rafts were evenly distributed, and there were no signs of cirrhosis. When inducing liver necrosis by peritoneal injection of 0.2% CCl_4, it was found that the injury was very severe, with damage to the entire lobule, leaving a shadow of denucleated liver cells and loss of all hepatocytes from the central vein to the border with adjacent lobules. In the silymarin-treated group, a few lobules were slightly damaged with scattered lesions. The high doses of red *P. sanguineus* extract (500 and 1000 mg/kg) showed mild to moderate damage with a few foci of inflammation. Liver microscopy in the group of 250 mg/kg extract showed moderate to severe necrosis with more inflammatory foci than the other two groups around the central vein. However, it was still improved compared to the liver microscopic sample of the CCl_4 group (Fig. 6).

DISCUSSION

The ultrasonic extraction method with 70% ethanol offers superior benefits to traditional extraction methods such as soaking, infusion, or Soxhlet extraction. Ultrasound-assisted extraction is an inexpensive and simple method that allows rapid, low-temperature extraction of heat-sensitive components. This is especially important for compounds that quickly decompose at high temperatures, helping to preserve their structure and activity (Pagano et al., 2021; Saborirad et al., 2024). In ultrasound-assisted extraction, ultrasonic energy (frequency above 20 kHz) creates hollow bubbles. When these bubbles burst, they make an enormous shear force, ensuring complete extraction of active ingredients from medicinal herbs. In addition, ultrasound waves increase the contact area between the two phases by dispersing them into tiny particles, disrupting the cell membrane, and enhancing the turbulence of the mixture. This process helps extract and dissolve the active ingredients in the fruiting bodies and saves time, solvents, and energy. Ultrasonic extraction equipment is suitable for small-scale laboratory use, making the extraction process more convenient and economical (Zhang et al., 2022). In this study, we successively investigated different extraction conditions, using 70% ethanol extracted at various temperatures (45°C, 50°C, and 90°C) with the ratio of fungus and solvent varying from 1:20, 1:25, and 1:30. The results showed that the extraction efficiency was optimal (12.1%) when extracted at 50°C for 30 minutes with a fungus and solvent ratio of 1:20.

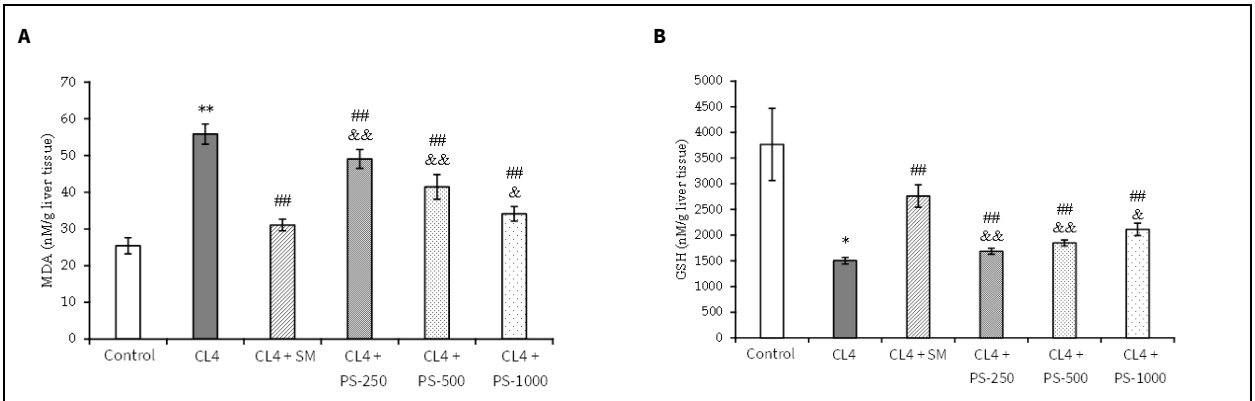

Figure 5. Effects of *Pycnoporus sanguineus* (PS) extracts and silymarin (SM) on CCl_4-induced changes in liver MDA **(A)** and GSH **(B)** contents in mice.

Each value is the mean ± SD of six animals. $^{*}p<0.05$, $^{**}p<0.01$ vs. control. $^{\#\#}p<0.01$ vs. CCl_4. $^{\&}p<0.05$, $^{\&\&}p<0.01$ vs. CCl_4 + silymarin (One-way ANOVA). CL4: CCl_4; SM: Silymarin (100 mg/kg); PS-250: *Pycnoporus sanguineus* extract (250 mg/kg); PS-500: *Pycnoporus sanguineus* extract (500 mg/kg); PS-1000: *Pycnoporus sanguineus* extract (1000 mg/kg).

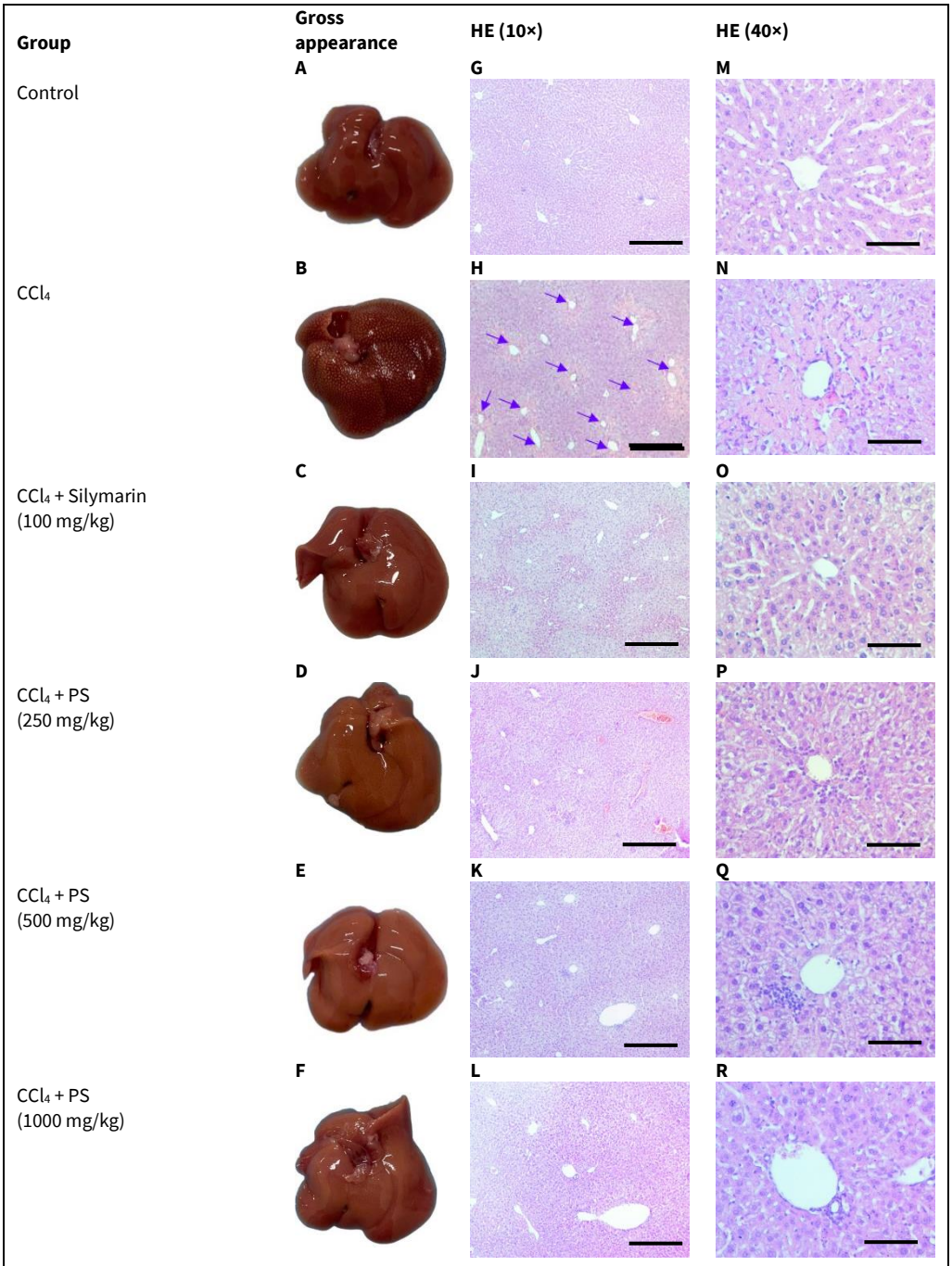

Figure 6. Macroscopic and microscopic observations after treatment with ethanolic extract of *Pycnoporus sanguineus* (PS) and silymarin in a model of CCl_4-induced toxicity.
Representative gross appearances **(A-F)**, HE staining at 10× (**G-L**, scaled bar = 200 µm), and at 40× (**M-R**, scaled bar = 50 µm) in the liver of mice. Control **(A, G, M)**; CCl_4 **(B, H, N)**; CCl_4 + silymarin **(C, I, O)**; CCl_4 + PS 250 mg/kg **(D, J, P)**; CCl_4 + PS 500 mg/kg **(E, K, Q)**; and CCl_4 + PS 1000 mg/kg **(F, L, R)**. Blue arrows indicate the necrotic region.

Most hepatotoxicants mainly cause liver damage by generating oxidative stress, which increases lipid peroxidation, reduces ATP levels, and causes oxidative damage to DNA and proteins (Allameh et al., 2023). In the mechanism of action of CCl_4, there is the formation of free radicals such as trichloromethyl and trichloromethyl peroxyl, which saturate the body's defense antioxidant system, causing lipid peroxidation, lowering the amount of cytochrome P450, leading to functional impairment due to decrease protein concentration and promote triglyceride accumulation, change water, and electrolyte balance, and increase liver enzymes in plasma (Delgado-Montemayor et al., 2015). The toxicity caused by CCl_4 depends on the dose and duration of exposure. Lipid peroxidation leads to a series of reactions, such as destroying membrane lipids and creating endogenous toxic substances, causing many liver complications and functional abnormalities. Therefore, lipid peroxidation is essential in the pathogenesis of CCl_4-induced liver

injury (Ogaly et al., 2022). A single dose of CCl_4 can reach peak concentrations in three hours. Within 24 hours, CCl_4 causes changes in biochemical and histological indicators of liver cells (Iqubal et al., 2020). The CCl_4 acute hepatitis model can be performed on both rats and mice, but the research model using mice is more applicable because the rate of CCl_4 metabolism in mice is higher than in rats.

Mice taking either saline, silymarin, or red *P. sanguineus* extract gained weight after two weeks, although there was no statistically significant difference in weight between the groups. This result is consistent with the study of Tran et al. (2017), which revealed that the ethanol extract of red *P. sanguineus* fruit bodies did not affect the average weight gain of mice after 4 weeks of the experiment. This proved that *P. sanguineus* fungus extract did not influence the weight of mice during the experiment period.

The liver enzymes AST and ALT play essential roles in the metabolism of amino acids (Delgado-Montemayor et al., 2015). Typically, AST and ALT are only present in cells, and their activity in serum is deficient (Delgado-Montemayor et al., 2015). But when liver cells are damaged, AST and ALT will be released from liver cells into the blood, causing their activity in serum to increase (Delgado-Montemayor et al., 2015). When using CCl_4, the free radical $CCl3\bullet$, a metabolic product of CCl_4 through cytochrome oxidase, is the cause of liver cell damage and increases ALT and AST activity. Prophylaxis with silymarin or extract of red *P. sanguineus* with three different doses reduced the enzyme concentrations statistically compared to the control group. Specifically, the group of mice taking silymarin reduced AST level by 10.8 times, ALT level by 35.02 times; 250 mg/kg of extract reduced AST level by 1.91 times and ALT level by 2.01 times; 500 mg/kg of extract reduced AST level by 5.02 times, ALT level by 5.04 times; the highest oral dose extract of 1000 mg/kg reduced AST level by 9.43 times and ALT level by 14.76 times. This finding is consistent with the results of Tran et al. (2017), who expressed that the ethanol extract of *P. sanguineus* fungus, with only an oral dose of 500 mg/kg, protected the liver against tyloxapol-induced oxidative stress injury. Therefore, three doses of *P. sanguineus* extract all support and protect the liver against damage caused by CCl_4, of which 1000 mg/kg was the most effective.

Malondialdehyde is the end product of cell membrane lipid peroxidation, indicating oxidative damage in cells and tissues (Moron et al., 1979; Ohkawa et al., 1979). When MDA reacts with thiobarbituric acid, it forms a pink complex with a maximum absorption peak at 532 nm. Measuring complex intensity determines the MDA content of the sample, thereby evaluating the antioxidant capacity of the research substance as shown by reducing MDA content (Ohkawa et al., 1979). The results showed that silymarin and different doses of red *P. sanguineus* extracts reduced MDA content in the liver compared to the control group by 1.14 - 1.80 times. Mice treated with the extract of *P. sanguineus* fungus were protected against damage caused by CCl_4, possibly because the fungus contains a large amount of polyphenol and flavonoid compounds. The hepatoprotective action of polyphenols and flavonoids is due to their antioxidant properties, inhibiting lipid peroxidation, increasing antioxidant enzyme activity, reducing AST and ALT enzyme content, reducing MDA content, and increasing GSH content. This is also consistent with the study of Dang et al. (2024), which illustrated that the fruit body extract of the red *P. sanguineus* showed significant *in vitro* antioxidant activity on all three methods of DPPH, ABTS•+, and RP.

A thiol molecule called glutathione is crucial in defending cells against oxidative stress-induced damage. When evaluating the oxidative and metabolic state of biochemical systems *in vitro* and *in vivo*, GSH measurement is vital. Quantification of GSH suggests a mechanism of redox damage of the sample (Rahman et al., 2006). The GSH content produced in the liver decreased by 0.40 times in the CCl_4-treated group compared to the control group, and the difference was statistically significant. Silymarin and red *P. sanguineus* fungus extract with different doses all increased liver GSH content compared to the CCl_4-treated group by 0.54-1.89 times. The results indicated that silymarin and red *P. sanguineus* reduced GSH content in the liver after the oxidative effects of free radicals generated from CCl_4.

Pathological examination is one of the most reliable methods for determining the extent of liver fibrosis. The results in Fig. 6 indicated that the mouse livers in the control group had a natural red color, smooth surface, soft density, and no congestion. In contrast, the mice in the CCl_4-treated group had a pale liver with a soft density, many white spots, and a somewhat rough surface. Most liver samples in the CCl_4-treated group showed lesions that could be seen with the naked eye. Meanwhile, mice taking red *P. sanguineus* extract at a dose of 500 mg/kg had red livers, smooth liver surface, soft density, and many white spots, while mice taking extract at a dose of 1000 mg/kg and silymarin all had red livers with smooth surface, scattered with few white spots. This proved that both silymarin and *P. sanguineus* extract (500 mg/kg and 1000 mg/kg) could reduce liver damage. Liver appearance has improved compared to the CCl_4-treated group, but has not returned to

normal. However, it is necessary to combine microscopic inspection results to help distinguish the level of liver damage in mice more clearly.

According to the microscopic findings of the liver of mice, the damage started to manifest in the form of necrotic and inflammatory cells after peritoneal injection of CCl_4 0.2% in olive oil: lobular necrosis, disorderly arrangement of venous sinuses, and central lobular hepatocytes. There was an improvement in the group that received oral administration of silymarin and red *P. sanguineus* extract (doses of 500 mg/kg and 1000 mg/kg) compared to the CCl_4-treated group. The cells were less inflamed, and the venous sinuses were less dilated. Depending on the type of cause and the length of time exposed to the cause of damage, liver cells may be significantly or minimally impacted when the liver is attacked. Liver cell injury is divided into three levels: reversible (degeneration), damage at risk of cell death, and irreversible (necrosis). Among the above cell injury levels, degenerative damage is the initial and mildest damage to cells. Cells can restore this damage and return to a normal condition.

The above results demonstrated that silymarin and extracts of red *P. sanguineus* fungus (doses of 500 and 1000 mg/kg) possessed positive effects in preventing liver damage induced by carbon tetrachloride. However, compared to mice taking red *P. sanguineus* extract, silymarin showed better liver protection effects with fewer appearances of inflammatory cells. A 1000 mg/kg dose of *P. sanguineus* extract produced more outstanding actions than 500 and 250 mg/kg doses. Many studies have proven that the red *P. sanguineus* fungus contains many compounds with potential biological effects, such as alkaloids, flavonoids, and polyphenols (De Oliveira et al., 2007; Mendoza et al., 2020; Téllez-Téllez et al., 2016). Polyphenol compounds are of particular interest due to their great antioxidant and anti-inflammatory effects (Hazafa et al., 2020; Juliette-Ornely et al., 2019; Rahman et al., 2022). In addition, oxidative stress is related to many liver diseases; most liver toxins mainly cause liver damage by inducing oxidative stress (Allameh et al., 2023). Therefore, it has been suggested that polyphenol chemicals may be responsible for the hepatoprotective activity of red *P. sanguineus* fungus.

This study demonstrated the hepatoprotective effect of red *P. sanguineus* fungus for the first time. However, the research still has some limitations that need to be considered. The study only used a total extract from the fruiting bodies of red *P. sanguineus* to examine the effects. In the future, further studies must determine precisely which compound in the fungus is primarily responsible for this beneficial activity. In addition, it is necessary to perform other models to evaluate the hepatoprotective action of this fungus and assess other indicators to support this assertion. These studies are an essential step forward in exploiting the potential of red *P. sanguineus* fungus in medicine and health, especially in liver protection and prevention of oxidative stress-related disease.

CONCLUSION

The present study indicated that the extract from fruit bodies of red *P. sanguineus* fungus showed prophylactic hepatoprotective effects on the acute mouse liver damage model using the toxic agent CCl_4. The most pronounced protective effect was found with the highest 1000 mg/kg dose. However, other *in vivo* studies should be performed to strongly demonstrate the potential actions of red *P. sanguineus*. In addition, these results are the scientific basis for further research on this valuable fungus and for developing the products that originate from it.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

Achenbach H, Blümm E (1991) Investigation of the pigments of *Pycnoporus sanguineus* - Pycnosanguin and new phenoxazin-3-ones. Arch Pharm 324(1): 3–6. https://doi.org/10.1002/ardp.19913240103

Allameh A, Niayesh-Mehr R, Aliarab A, Sebastiani G, Pantopoulos K (2023) Oxidative stress in liver pathophysiology and disease. Antioxidants 12(9): 1653. https://doi.org/10.3390/antiox12091653

Badr El-Din NK, Ali DA, Othman R, French SW, Ghoneum M (2020) Chemopreventive role of arabinoxylan rice bran, MGN-3/Biobran, on liver carcinogenesis in rats. Biomed Pharmacother 126: 110064. https://doi.org/10.1016/j.biopha.2020.110064

Cavill GWK, Ralph BJ, Tetaz JR, Werner RL (1953) The chemistry of mould metabolites. Part I. Isolation and characterization of a red pigment from *Coriolus sanguineus* (Fr.). J Chem Soc (Resumed): 525–529. https://doi.org/10.1039/JR9530000525

Chen X, Li M, Li D, Luo T, Xie Y, Gao L, Zhang Y, Chen S, Li S, Huang G, Li W, Su J, Lai X (2020) Ethanol extract of *Pycnoporus sanguineus* relieves the dextran sulfate sodium-induced experimental colitis by suppressing helper T cell-mediated inflammation via apoptosis induction. Biomed Pharmacother 127: 110212. https://doi.org/10.1016/j.biopha.2020.110212

Dang BT, Nguyen TNV, Dang DK, Duong TN, Dang TTM, Pham TT, Ly NYL, Cao YL, Tran DT, Duong XC (2024) Evaluation of *in vitro* antioxidant effects of a standardized extract from the fruiting body of red *Pycnoporus sanguineus*. Can Tho J Med Pharm 79: 142–149. https://doi.org/10.58490/ctump.2024i79.2941

De Oliveira LFC, Le Hyaric M, Berg MM, de Almeida MV, Edwards HGM (2007) Raman spectroscopic characterization of cinnabarin produced by the fungus *Pycnoporus sanguineus* (Fr.) Murr. J Raman Spectrosc 38(12): 1628–1632. https://doi.org/10.1002/jrs.1881

Delgado-Montemayor C, Cordero-Pérez P, Salazar-Aranda R, Waksman-Minsky N (2015) Models of hepatoprotective activity assessment. Med Univ 17(69): 222–228. https://doi.org/10.1016/j.rmu.2015.10.002

Gambato G, Todescato K, Pavão EM, Scortegagna A, Fontana RC, Salvador M, Camassola M (2016) Evaluation of productivity and antioxidant profile of solid-state cultivated macrofungi *Pleurotus albidus* and *Pycnoporus sanguineus*. Bioresour Technol 207: 46–51. https://doi.org/10.1016/j.biortech.2016.01.121

Gripenberg J (1951) Fungus pigments I. Cinnabarin a colouring matter from *Trametes cinnabarina* Jacq. Acta Chem Scand 5: 590–595. https://doi.org/10.3891/ACTA.CHEM.SCAND.05-0590

Hazafa A, Rehman KU, Jahan N, Jabeen Z (2020) The role of polyphenol (flavonoids) compounds in the treatment of cancer cells. Nutr Cancer 72(3): 386–397. https://doi.org/10.1080/01635581.2019.1637006

Iqubal A, Syed MA, Haque MM, Najmi AK, Ali J, Haque SE (2020) Effect of nerolidol on cyclophosphamide-induced bone marrow and hematologic toxicity in Swiss albino mice. Exp Hematol 82: 24–32. https://doi.org/10.1016/j.exphem.2020.01.007

Jaouen G, Sagne A, Buyck B, Decock C, Louisanna E, Manzi S, Baraloto C, Roy M, Schimann H (2019) Fungi of French Guiana gathered in a taxonomic, environmental and molecular dataset. Sci Data 6(1): 206. https://doi.org/10.1038/s41597-019-0218-z

Jouda JB, Mfotie Njoya E, Mbazoa CD, Zhou Z, Meli Lannang A, Wandji J, Shiono Y, Wang F (2018) Lambertellin from *Pycnoporus sanguineus* MUCL 51321 and its anti-inflammatory effect via modulation of MAPK and NF-κB signaling pathways. Bioorg Chem 80: 216–222. https://doi.org/10.1016/j.bioorg.2018.06.021

Juliette-Ornely OB, Calixte ENH, Rick-Léonid NMM, Cédric S, Prudence Y, Joseph-Privat O, Roger NAG, Louis-Clément OE (2019) Phytochemical screening, antioxidant and antiangiogenic activities of *Daedaleopsis nitida*, *Pycnoporus sanguineus* and *Phellinus gilvus* medicinal mushrooms from Gabon. Pharm Chem J 6(2): 71–80.

Le NPT, Tran KH, Tran DT, Duong XC (2019) Study on the acute and sub-chronic toxicity of the ethanol extract of the fruiting body from red *Pycnoporus sanguineus* fungus in mice. Can Tho J Med Pharm 22-25: 556–563.

Lu MK, Lin HO, Hsu FL, Lin YL (2010) Anti-inflammatory principles of cultivated *Pycnoporus sanguineus*. J Chin Med 21(3,4): 75–83.

Luangmonkong T, Pransin C, Nopphalee L, Meechai S, Chunya S, Rattanavaraha A, Kaewnoppharat N, Khuituan T, Bunyakiat S, Parichatikanond W (2022) Carbon tetrachloride-induced acute liver toxicity: Selecting dosage and biomarkers for evaluating hepatoprotective drugs in ICR outbred mice. Pharm Sci Asia 49(6): 534–542. https://doi.org/10.29090/psa.2022.06.22.212

Mendoza WC, Dulay RMR, Valentino MJG, Reyes RG (2020) Mycelial biomass and biological activities of Philippine mushroom *Pycnoporus sanguineus* in time-course submerged culture. J Appl Biol Biotech 8(5): 88–93. https://dx.doi.org/10.7324/JABB.2020.80512

Moron MS, Depierre JW, Mannervik B (1979) Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 582(1): 67–78. https://doi.org/10.1016/0304-4165(79)90289-7

Nguyen TP, Ngo NV (2022) Investigation of anti-oxidant and anti-inflammatory properties of red *Pycnoporus sanguineus* extract isolated in Vietnam. HCMCOU J Sci Eng Technol 18(1): 45–56. https://doi.org/10.46223/HCMCOUJS.tech.vi.18.1.2357.2023

Ogaly HA, Aldulmani SAA, Al-Zahrani FAM, Abd-Elsalam RM (2022) D-Carvone attenuates CCl_4-induced liver fibrosis in rats by inhibiting oxidative stress and TGF-ß 1/SMAD3 signaling pathway. Biology 11(5): 739. https://doi.org/10.3390/biology11050739

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95(2): 351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Pagano I, Campone L, Celano R, Piccinelli AL, Rastrelli L (2021) Green non-conventional techniques for the extraction of polyphenols from agricultural food by-products: A review. J Chromatogr A 1651: 462295. https://doi.org/10.1016/j.chroma.2021.462295

Rahman I, Kode A, Biswas SK (2006) Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nat Protoc 1(6): 3159–3165. https://doi.org/10.1038/nprot.2006.378

Rahman M, Rahaman S, Islam R, Rahman F, Mithi FM, Alqahtani T, Almikhlafi MA, Alghamdi SQ, Alruwaili AS, Hossain S, Ahmed M, Das R, Emran TB, Uddin S (2022) Role of phenolic compounds in human disease: Current Knowledge and future prospects. Molecules 27(1): 233. https://doi.org/10.3390/molecules27010233

Saborirad S, Baghaei H, Hashemi-Moghaddam H (2024) Optimizing the ultrasonic extraction of polyphenols from mango peel and investigating the characteristics, antioxidant activity and storage stability of extract nanocapsules in maltodextrin/whey protein isolate. Ultrason Sonochem 103: 106778. https://doi.org/10.1016/j.ultsonch.2024.106778

Smânia A Jr, Marques CJ, Smânia EF, Zanetti CR, Carobrez SG, Tramonte R, Loguercio-Leite C (2003) Toxicity and antiviral activity of cinnabarin obtained from *Pycnoporus sanguineus* (Fr.) Murr. Phytother Res 17(9): 1069–1072. https://doi.org/10.1002/ptr.1304

Téllez-Téllez M, Villegas E, Rodríguez A, Acosta-Urdapilleta ML, O'Donovan A, Díaz-Godínez G (2016) Mycosphere Essay 11: Fungi of *Pycnoporus*: morphological and molecular identification, worldwide distribution and biotechnological potential. Mycosphere 7(10): 1500–1525. https://doi.org/10.5943/MYCOSPHERE/SI/3B/3

Tran DT (2019) Production of *Pycnoporus sanguineus* on substrates of corn and rice hulls. Sci Technol J Agric Rural Dev 19: 29–35.

Tran DT, Tang VP, Duong XC, Bui TMD (2017) Antihyperlipidemic activity of the mushroom *Pycnoporus sanguineus* (L.: Fr.) Murr. on the experimental model of endogenous tyloxapol-induced hyperlipidemia in mice. Viet Med J 461(2): 187–191.

Tran DT, Tang VP, Duong XC, Bui TMD (2018) Hypoglycemic activity of fruiting body extracts from *Pycnoporus sanguineus* (L.: Fr.) Murrill mushroom. Acad J Biol 40(3): 37–44. https://doi.org/10.15625/2615-9023/v40n3.13146

Trinh TK (2011) Mushrooms in Vietnam, Volume I. Natural Science and Technology, Hanoi.

Trinh TK (2012) Mushrooms in Vietnam, Volume II. Natural Science and Technology, Hanoi.

Von Dentz M, Gambato G, Ferrari A, Fontana RC, Rodrigues E, Salvador M, Camassola M, Jahn MP (2021) Antihyperlipidemic effect of the hydroalcoholic extract of Basidiomycete *Pycnoporus sanguineus* (Fr.) Murr. in streptozotocin-induced diabetic rats. Adv Tradit Med 21(3): 453–461. https://doi.org/10.1007/s13596-020-00459-1

Yang W, Chen X, Li Y, Guo S, Wang Z, Yu X (2020) Advances in pharmacological activities of terpenoids. Nat Prod Commun 15(3): 1–13. https://doi.org/10.1177/1934578X20903555

Zhang Y, Zhang S, Yang X, Wang W, Liu X, Wang H, Zhang H (2022) Enhancing the fermentation performance of frozen dough by ultrasonication: Effect of starch hierarchical structures. J Cereal Sci 106: 103500. https://doi.org/10.1016/j.jcs.2022.103500

AUTHOR CONTRIBUTION:

Contribution	Dang KD	Dang TB	Nguyen VTN	Duong NT	Lu TA	Dang MTT	Pham TT	Ly LNY	Cao LY	Tran TD	Duong CX
Concepts or ideas	x	x	x							x	x
Design	x	x	x							x	x
Definition of intellectual content	x	x									
Literature search	x	x									x
Experimental studies	x	x		x	x	x	x	x	x		
Data acquisition	x	x		x	x	x	x	x	x		
Data analysis	x	x									
Statistical analysis	x	x									
Manuscript preparation	x	x	x	x	x	x	x	x	x	x	x
Manuscript editing	x	x	x	x	x	x	x	x	x	x	x
Manuscript review	x	x	x	x	x	x	x	x	x	x	x

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