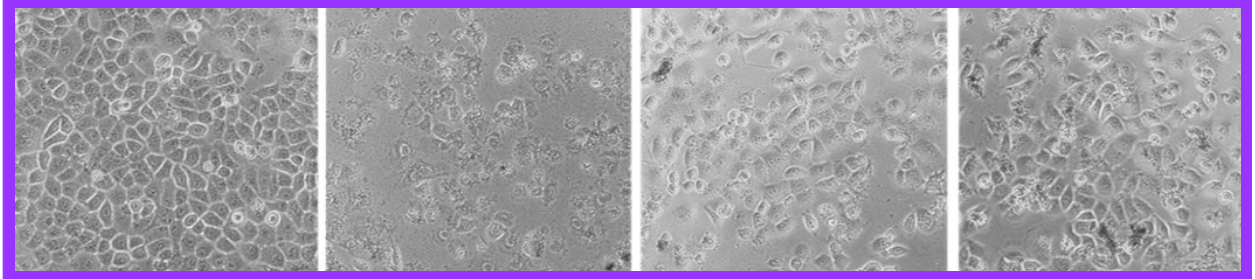

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Hepatoprotective effect of *Pluchea pteropoda* extract against CCl_4-induced hepatotoxicity

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Abstract

This study aimed to evaluate the hepatoprotective effects of *P. pteropoda* extract against carbon tetrachloride (CCl_4)-induced hepatotoxicity. The extraction condition was due to ethanol 50%, a solid-to-solvent ratio of 1:10, extraction temperature of 60°C, and extraction time of 4 hours. Phytochemical analysis indicated that total polyphenol and flavonoid contents are 76.5 ± 5.2 mg GAE/g DW and 56.8 ± 2.6 mg QE/g DW, respectively. Notably, pretreatment with the extract increased the viability of hepatic cells up to 71.5% at 200 µg/ml. Moreover, oral administration of the extract at 200 mg/kg reduced aspartate transaminase and alanine transaminase elevation levels by 65% and 59% as compared to CCl_4 treatment alone. The extract also improved body weight, maintained survival, restored hepatic GSH level, and reduced lipid peroxidation. These findings demonstrate that *P. pteropoda* exerts hepatoprotective activity against CCl_4-induced hepatotoxicity through antioxidant-mediated mechanisms.

Introduction

Liver diseases remain a major global health burden, accounting for approximately two million deaths annually and about 4% of all deaths worldwide (Devarbhavi et al., 2023). Among different forms of hepatic disorders, toxic liver injury is of particular concern because the liver is continuously exposed to xenobiotics, drugs, alcohol, environmental toxicants, and oxidative stress. Drug-induced hepatotoxicity is also an important clinical problem and remains one of the leading causes of acute liver failure in Western countries (Katarey and Verma, 2016). Although several pharmacological interventions are available for liver disorders, their long-term use may be limited by adverse effects, incomplete efficacy, high cost, or the need to remove the causative agent rather than directly restore hepatic function (Katarey and Verma, 2016). Therefore, there is growing interest in medicinal plants as potential sources of safer hepatoprotective agents, especially those rich in antioxidant and anti-inflammatory phytochemicals (Ugwu and Suru, 2021).

Therefore, there is growing interest in medicinal plants as potential sources of safer hepatoprotective agents, such as *Silybum marianum, Glycyrrhiza glabra* (Rasool et al., 2014), *Gynura procumbens* (Tithi et al., 2023), *Curcuma longa* (Karamalakova et al., 2019), *Cynara scolymus* (Colak et al., 2016), and *Ginkgo biloba* (Sherif et al., 2022).

Pluchea pteropoda Hemsl. (Asteraceae) is a mangrove-

associated medicinal plant distributed in South China and Indochina, including Vietnam. It is a relatively common medicinal plant in Vietnam and is distributed in several local areas with abundant availability. In Vietnamese traditional medicine, the roots and leaves of this species have been used to treat fever, headache, chest tightness, cough, dysentery, and back pain. These ethnomedicinal uses suggest that *P. pteropoda* may represent an underexplored medicinal resource in Vietnam. Recent studies have clarified the chemical and biological properties of *P. pteropoda*. Essential oil analysis of its aerial parts revealed an oil yield of approximately 0.5%, with 2,5-dimethoxy-p-cymene, β-maaliene, and α-isocomene. The oil also showed antimicrobial activity against *Bacillus subtilis* and *Candida albicans* (The et al., 2022). Phytochemical investigations of the roots further identified phenolic constituents, including chlorogenic acid and 3,4,5-tricaffeoylquinic acid, both isolated from this species for the first time (Nguyet et al., 2024). In addition, ethanol extract of *P. pteropoda* showed no acute toxicity in mice at a single dose up to 5,000 mg/kg and significantly inhibited carrageenan-induced paw edema, with the strongest anti-inflammatory effect observed at 150 mg/kg (Tran et al., 2024). A recent study has demonstrated that the extract from *P. pteropoda* exhibits significant antioxidant properties and acetylcholinesterase-inhibitory potential, making it a promising natural candidate for managing oxidative stress and neurodegenerative disorders (Pham et al., 2025). Furthermore, the potent xanthine oxidase inhibitory activity and uric acid-lowering effects of its ethanol extract were also determined in hyperuricemic mouse models, highlighting its therapeutic and safe potential for gout management (Bui et al., 2026). These findings indicate that *P. pteropoda* contains bioactive terpenoids and phenolic compounds and possesses various pharmacological potentials. In addition to these reported biological activities, *P. pteropoda* has also been traditionally recognized in Vietnamese folk medicine as an effective herbal remedy for liver detoxification and liver health support.

Therefore, the present study aimed to evaluate the hepatoprotective effect of *P. pteropoda* extract in a CCl_4-induced hepatotoxicity model.

Materials and Methods

Plant material

P. pteropoda was collected in November 2025 from Thu Duc ward, Ho Chi Minh City, Vietnam. The plant material was authenticated based on its morphological characteristics and comparison with previously published taxonomic descriptions in Vietnam (Phung et al., 2020; Bui et al., 2026). Leaves were washed, air-dried, and oven-dried at 60°C for 1 day before being ground into powder using a specialized herbal grinder. The resulting powdered material had a moisture content of 6.2% and was directly used for extraction.

Extraction conditions

The powdered plant material was extracted by heat maceration using ethanol 50% at a ratio of 1 g of powder to 10 mL of solvent. The extraction process was repeated three times, with each cycle lasting 4 hours at 60°C. The combined extracts were concentrated under reduced pressure using a rotary evaporator. The extraction yield was 11.3%, while the moisture and ash contents of the extract were 7.2% and 6.5%, respectively.

Quantitative phytochemicals

Total polyphenol content and total flavonoid content were determined using the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively, with slight modifications (Chandra et al., 2014). Briefly, the extract solution was reacted with Folin–Ciocalteu reagent under alkaline conditions, and the absorbance was measured after color development. Gallic acid was used as the standard, and total polyphenol content was expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g extract). For total flavonoid content determination, the extract was reacted with $AlCl_3$ reagent to form a flavonoid–aluminum complex, followed by spectrophotometric measurement. Quercetin was used as the reference standard, and total flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g extract).

DPPH assay

The DPPH radical scavenging activity of the extract was evaluated according to a colorimetric assay with slight modifications (Sanchez-Moreno 2002). Briefly, the extract (100 µg/mL for screening of extraction condition or 25-200 µg/mL for determination of IC_{50} value) was mixed with freshly prepared DPPH solution (0.3 mM), followed by incubation in the dark at room temperature for 30 min. After incubation, the absorbance of the reaction mixture was measured at 517 nm using a UV–vis spectrophotometer. The reaction mixture without extract served as the control. The radical scavenging activity was calculated based on the decrease in absorbance of DPPH in the presence of the extract.

In vitro hepatoprotective assay

Cytotoxicity was evaluated using the MTT assay with slight modifications (Gonzalez et al., 2017). HepG2 hepatocytes were seeded in 96-well plates at a density of approximately 2×10^4 cells/mL and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells were maintained at 37°C in a humidified incubator

containing 5% CO_2. The cells were then treated with the extract (50, 100, 200, 400, or 600 µg/mL) or silymarin (20 µg/mL) for 24 hours. Subsequently, CCl_4 was added to each well at a final concentration of 0.4% (v/v) to induce hepatotoxicity. At the end of the treatment, the culture medium was removed, and 50 µL of MTT solution (0.5 mg/mL) was added to each well. Following incubation for 4 hours, 100 µL of DMSO was added to dissolve the formazan crystals. The absorbance was then measured at 540 nm using a microplate reader. In addition, changes in cell morphology were further examined under an inverted microscope (magnification 10x, Oxion, Euromex, Netherlands). The untreated cells, which were not exposed to either the extract or CCl_4, were used as the blank. Cells treated with CCl_4 alone or with both the extract and CCl_4 were compared with the blank to determine cell viability.

In vivo hepatoprotective assay

Swiss albino mice (7–8 weeks old, 22–25 g) were obtained from the Pasteur Institute, Ho Chi Minh City, Vietnam. The animals were housed under standard laboratory conditions at 26 ± 2°C and 70–80% relative humidity, with free access to a standard pellet diet and clean drinking water. All mice were acclimatized for at least one week before the experiment. Their health status and behavior were monitored daily throughout the study.

The hepatoprotective activity of *P. pteropoda* extract was evaluated in a CCl_4-induced hepatotoxicity model (Joshi et al., 2015). Mice received the extract or silymarin orally 2 hours before intraperitoneal administration of CCl_4 (1 mL/kg in olive oil, 1:2, v/v) for 7 consecutive days. Mice were randomly allocated into six groups (n=6/group): Blank receiving distilled water; CCl_4 control receiving distilled water plus CCl_4; positive control receiving silymarin (100 mg/kg) plus CCl_4; and three treatment groups receiving *P. pteropoda* extract at 100, 200, or 400 mg/kg plus CCl_4. On day 8, blood samples were collected from the tail vein, and serum aspartate transaminase and alanine transaminase activities were measured using commercial diagnostic kits (HUMAN Diagnostics Worldwide) and a Screen Master 3000 biochemical analyzer (Biochemical, Italy).

Determination of liver antioxidant and oxidative stress markers

A 1 g liver sample collected from each experimental group was homogenized with 2 mL of cold phosphate buffer (100 mM, pH 7.0). The homogenate was centrifuged at 10,000 rpm for 10 min, and the supernatant was used for the determination of oxidative stress-related biochemical markers using spectrophotometric assays (Ullah et al., 2020). Reduced glutathione (GSH) content was quantified by reacting 0.1 mL of liver homogenate with 0.5 mL of 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB), followed by adjustment of the final volume to 3 mL with phosphate buffer; absorbance was recorded at 412 nm. Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) formation. Briefly, 0.25 mL of liver homogenate was incubated at 37°C for 1 hour, followed by the addition of 5% trichloroacetic acid and 0.5 mL of 0.7% thiobarbituric acid. The absorbance of the resulting chromogenic product was measured at 535 nm.

Statistical analysis

All experiments were performed in triplicate. Data were processed using Excel and expressed as mean ± SD. Statistical analyses were conducted using SPSS software, with one-way ANOVA followed by Tukey's post hoc test.

Results

Quantitative phytochemicals and DPPH radical scavenging activity

The total polyphenol and flavonoid contents were found to be up to 76.5 ± 5.2 mg GAE/g DW and 56.8 ± 2.6 mg QE/g DW, respectively. Moreover, the optimized extract exhibited DPPH radical scavenging activity with an IC_{50} value of 104.3 ± 6.1 µg/mL.

Hepatoprotective activity of P. pteropoda extract on cell line model

As shown in Figure 1A, CCl_4 markedly reduced HepG2 cell viability to approximately 23% compared with untreated cells. However, pretreatment with *P. pteropoda* extract significantly attenuated CCl_4-induced toxicity, increasing cell viability up to 44.3%, 61.2%, and 71.5% at 50, 100, and 200 µg/mL, respectively. The protective effect was maintained at 400 µg/mL, whereas a slight decline was observed at 600 µg/mL, suggesting that 200–400 µg/mL was the most effective concentration range. Likewise, microscopic observation showed severe cell shrinkage and detachment after CCl_4 exposure, while extract-treated cells displayed improved cell density and morphology (Figure 1B).

Hepatoprotective activity of P. pteropoda extract on mice model

In the mouse model, CCl_4 administration caused a pronounced increase in aspartate transaminase and alanine transaminase (205 ± 12 and 300 ± 13 U/L) as compared with the distilled water group (38 ± 3 and 50 ± 6 U/L), respectively (Figure 2). Conversely, treatment with *P. pteropoda* extract at 200 mg/kg reduced these elevations by up to 65% for aspartate transaminase and 59% for alanine transaminase. The reduction in aspartate transaminase and alanine transaminase levels at 200 and 400 mg/kg was relatively comparable,

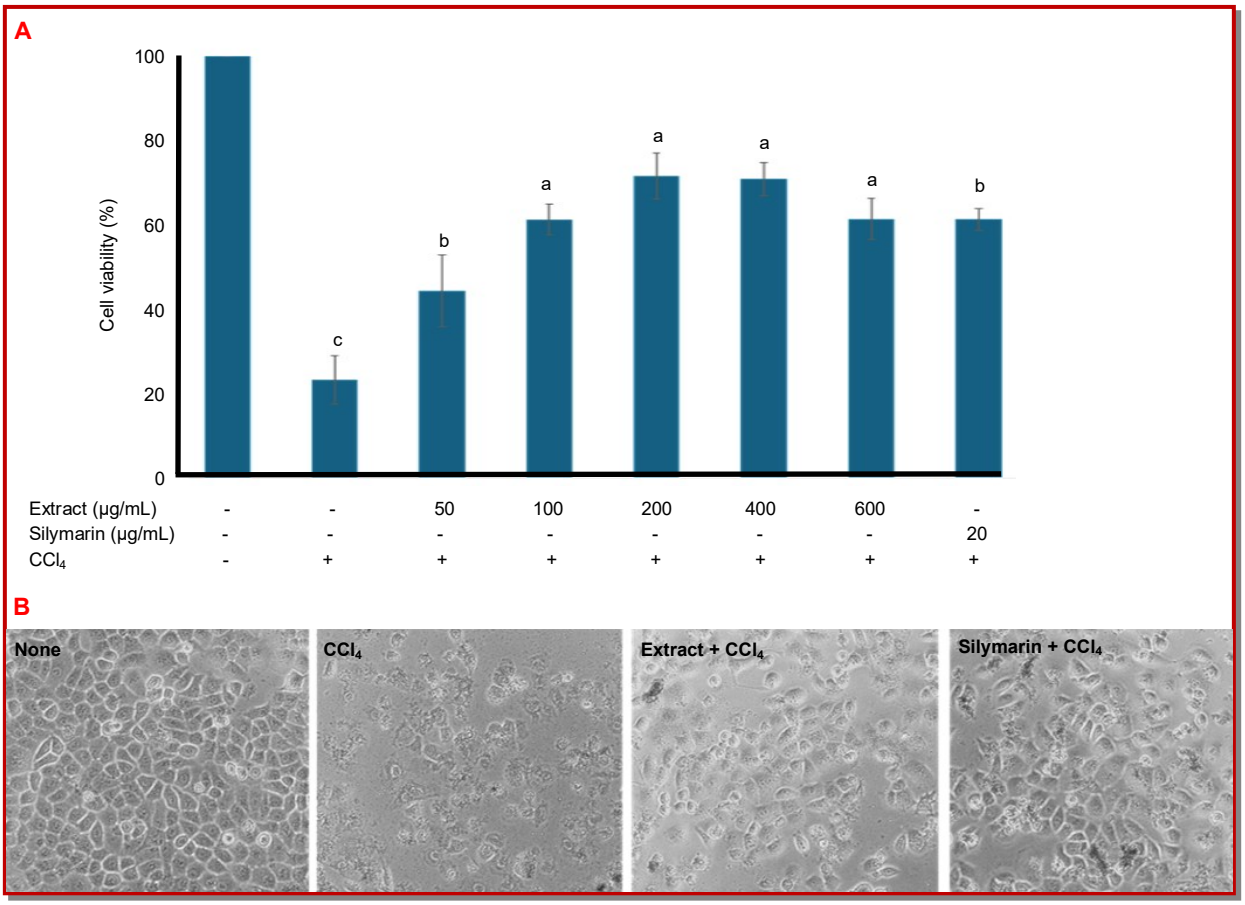

Figure 1: Protective effect of *P. pteropoda* extract against CCl4-induced cytotoxicity in HepG2 cells. Cell viability was determined using the MTT assay (A). Representative microscopy images of HepG2 cell morphology (B). Data are expressed as the mean ± SD (n=3). [a]p<0.05, [b]p<0.01, [c]p<0.001 when compared to blank group

indicating that increasing the dose beyond 200 mg/kg did not markedly enhance the response under the present experimental conditions.

Effect of P. pteropoda extract on hepatic GSH level and lipid peroxidation

As shown in Table I, CCl_4 exposure markedly decreased hepatic GSH level to 18% as compared with the distilled -water group. Meanwhile, treatment with *P. pteropoda* extract significantly restored GSH levels, particularly at 200 mg/kg (62%). In parallel, extract treatment significantly reduced lipid oxidation up to 60% as compared with the CCl_4 group.

Discussion

Phytochemical screening showed that phenolics and flavonoids were the predominant constituents of the optimized extract. Previous reviews on medicinal plants against CCl_4-induced hepatotoxicity have suggested that flavonoids and polyphenolic compounds contribute to hepatoprotection by enhancing antioxidant capacity, lowering hepatotoxicity markers, and supporting the restoration of hepatic architecture (Ugwu and Suru, 2021). The DPPH activity of the optimized extract is likely associated, at least in part, with its relatively high phenolic and flavonoid contents, as these compounds are commonly recognized as major contributors to the free-radical scavenging activity of medicinal plant extracts. A recent study on *P. pteropoda* reported that leaf extracts contained total phenolic contents of 48.8–52.9 mg GAE/g extract and total flavonoid contents of 15.8–20.6 mg QE/g extract, while the best DPPH activity was observed in the 45% ethanol leaf extract with an IC_{50} of 194.8 µg/mL (Pham et al., 2025). In contrast, the optimized extract in this study showed higher total polyphenol content and total flavonoid content values and a lower IC_{50} value, suggesting that the selected extraction conditions were more effective in recovering antioxidant constituents.

In the HepG2 cell model, *P. pteropoda* extract increased cell viability after CCl_4-induced cytotoxicity and improved cell morphology. The cytoprotective effect observed in this study may be related to the antioxidant constituents of the extract, particularly polyphenols and

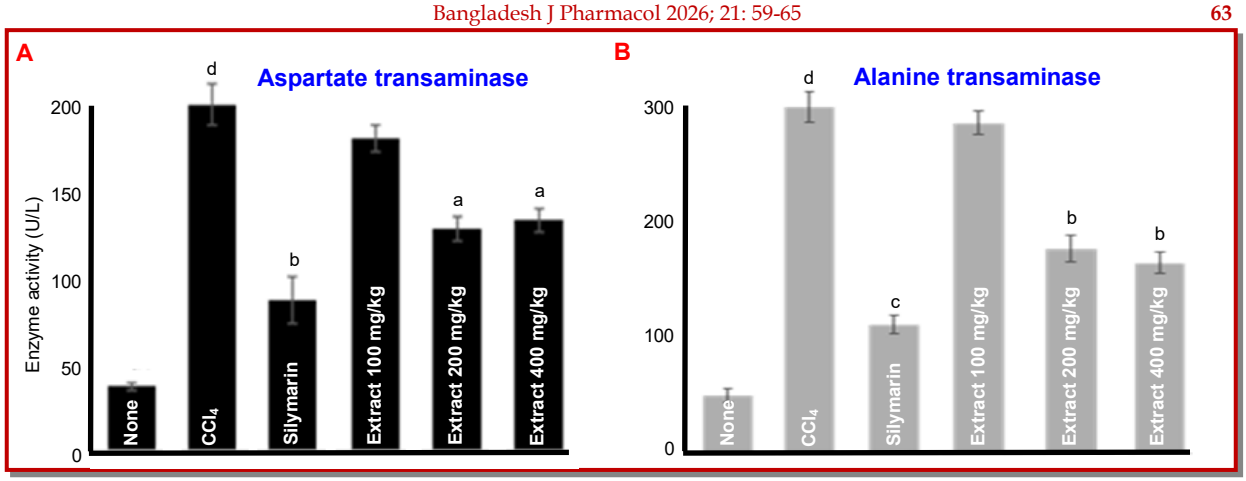

Figure 2: Effect of *P. pteropoda* extract on serum aspartate transaminase (A) and alanine transaminase (B) levels in CCl_4-induced hepatotoxicity. Mice were orally administered *P. pteropoda* extract or silymarin at 100 mg/kg before CCl_4 challenge. Data are expressed as the mean ± SD (n=6). $^{a}p<0.05$, $^{d}p<0.01$, $^{c}p<0.001$ when compared to distilled water group. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ when compared to CCl_4 group

Table I

Effect of *P. pteropoda* extract on hepatic GSH and lipid peroxidation levels in CCl_4-induced hepatotoxicity

	Distilled water	CCl_4	Silymarin	Extract (mg/kg)		
				100	200	400
GSH level (%)	100 ± 1.2	18 ± 2.8^{c}	64 ± 5.7^{b}	46 ± 3.9^{a}	62 ± 4.6^{b}	55 ± 5.1^{a}
Lipid oxidation level (%)	16 ± 1.5	100 ± 5.8^{c}	42 ± 4.1^{b}	63 ± 3.7^{a}	41 ± 4.5^{b}	45 ± 3.1^{b}

Values are mean ± SD; n=6; $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ when compared to distilled water group. $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ when compared to CCl_4 group. Statistical significance was determined within each row only

flavonoids, which can reduce free radical-mediated cellular injury. Similar HepG2/CCl_4 models have been used to demonstrate the hepatoprotective potential of antioxidant-rich natural products (Gonzalez et al., 2017).

In the mouse model, *P. pteropoda* extract reduced the CCl_4-induced elevation of serum aspartate transaminase and alanine transaminase, especially at 200 mg/kg. CCl_4 is a classical hepatotoxicant that markedly elevates serum aminotransferases as a consequence of hepatocyte membrane disruption and enzyme leakage into the circulation; therefore, increased aspartate transaminase and alanine transaminase are commonly used as biochemical indicators of hepatotoxicity (Ugwu and Suru, 2021). The reduction in aspartate transaminase and alanine transaminase levels indicates that the extract reduced CCl_4-mediated hepatotoxicity by preserving hepatocyte integrity and limiting aminotransferase leakage. This protective effect may be associated with the antioxidant potential of the extract, as polyphenol- and flavonoid-rich extracts have been reported to protect against CCl_4-induced hepatotoxicity through suppression of oxidative damage, downregulation of liver marker enzymes, and restoration of hepatic antioxidant status. Similar findings have been described in CCl_4-induced hepatotoxicity models, where antioxidant-rich plant extracts and silymarin reduced serum aspartate transaminase and alanine transaminase levels and improved liver biochemical profiles (Pham et al., 2025; Ouassou et al., 2021).

Likewise, other species of the genus *Pluchea*, such as *P. indica and P. dioscoridis* have also been reported to exhibit hepatoprotective effects. It was determined that both the methanolic extract of *P. indica* root and the aqueous ethanolic extract of *P. dioscoridis* aerial parts exhibit potent hepatoprotective effects against CCl4-induced acute liver toxicity in animal models by significantly normalizing hepatic enzyme markers and restoring tissue architecture (Sen et al., 1993; El-Toumy et al., 2014).

The extract also restored hepatic GSH level and reduced lipid peroxidation in CCl_4-treated mice. GSH is one of the major intracellular non-enzymatic antioxidants in the liver and plays a critical role in detoxification, free-radical neutralization, and protection of hepatocytes against oxidative injury. During CCl_4 intoxication, hepatic metabolism of CCl_4 generates reactive radicals that deplete endogenous antioxidant defenses, including GSH, and promote lipid peroxidation (Di Paola et al., 2022). Thus, restoration of GSH and reduction of lipid peroxidation are important indicators of antioxidant-mediated hepatoprotection. The increase in GSH and decrease in lipid peroxidation may be associated with the phenolic and flavonoid constituents of *P. pteropoda* extract, as these compounds

are known to scavenge reactive radicals and may support endogenous antioxidant defense pathways (Liao et al., 2024). These findings are consistent with previous CCl_4-induced hepatotoxicity studies showing that antioxidant-rich natural products protect liver tissue by restoring GSH or other antioxidant markers and suppressing MDA/lipid peroxidation (Alkinani et al., 2021).

Although this study demonstrated the antioxidant and hepatoprotective effects of *P. pteropoda* extract through DPPH activity, hepatic cell protection, reduction of aspartate transaminase/alanine transaminase levels, restoration of GSH, and inhibition of lipid peroxidation, the active compounds responsible for these effects were not isolated or structurally identified. In addition, inflammatory mediators, molecular signaling pathways, and histopathological changes in liver tissue were not fully investigated.

Conclusion

This study demonstrated that *P. pteropoda* extract possesses promising hepatoprotective activities against CCl_4-induced hepatotoxicity. These findings provide preliminary scientific evidence supporting the traditional use of *P. pteropoda* for liver detoxification and suggest that its hepatoprotective effect may be mainly associated with antioxidant-mediated mechanisms.

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Ethical Issue

All procedures involving animals were performed in accordance with the 3Rs principles (Replacement, Reduction, and Refinement) and were approved by the Animal Ethics Committee of Nong Lam University, Ho Chi Minh City, Vietnam. Animal welfare was carefully monitored throughout the experiment to minimize pain, stress, and discomfort.

Conflict of Interest

Authors declare no conflict of interest

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