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Original Article

Total Phenolic and Flavonoid Contents, Antioxidant and Anti-inflammatory Activities of *Phellinus rimosus* Extracts

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ABSTRACT

Background: *Phellinus rimosus* is a medicinal mushroom with potential bioactive properties, although scientific evidence supporting its pharmacological activities remains limited. **Objective:** To investigate the phenolic and flavonoid contents, antioxidant capacity, and anti-inflammatory activity of ethanolic and aqueous extracts of *P. rimosus*. **Materials and Methods:** Total phenolic content (TPC) and total flavonoid content (TFC) were determined using colorimetric assays. Antioxidant activity was evaluated by DPPH, ABTS, and FRAP methods. Anti-inflammatory activity was assessed by measuring nitric oxide (NO) inhibition in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. Statistical analysis was performed at $P < 0.05$. **Results:** The ethanolic extract contained significantly higher TPC (361.04 ± 5.69 mg GAE/g extract) and TFC (646.55 ± 6.29 mg RE/g extract) than the aqueous extract. It exhibited strong antioxidant activity with IC_{50} values of 9.56 ± 0.47 µg/mL (DPPH) and 5.40 ± 0.06 µg/mL (ABTS), and a FRAP value of 52.39 ± 0.25 mM Fe^{2+}/100 mg extract. The ethanolic extract also significantly inhibited NO production (IC_{50} = 54.40 ± 2.35 µg/mL) without cytotoxicity, whereas the aqueous extract showed no inhibitory effect. **Conclusion:** The ethanolic extract of *P. rimosus* demonstrates notable antioxidant and anti-inflammatory activities and may serve as a promising natural source of bioactive compounds.

Keywords: *Phellinus rimosus*; antioxidant activity; anti-inflammatory activity; phenolic content; flavonoid content; nitric oxide inhibition

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INTRODUCTION

Phellinus rimosus (Berk.) Pilát (Hymenochaetaceae) is a medicinal polypore fungus that is widely distributed in tropical regions, including Southeast Asia[1]. In Thailand, this species is predominantly found in the northeastern region and has a long history of use in traditional medicine for treating conditions such as cancer, herpes infections, earaches, and various skin disorders[2]. However, despite its extensive ethnomedicinal applications, there is still relatively limited scientific evidence regarding its phytochemical composition and biological properties.

Previous investigations on *P. rimosus* have primarily focused on polysaccharide–protein complexes extracted from its fruiting bodies[3]. These macromolecular components exhibit significant antioxidant, hepatoprotective, anti-inflammatory, and radioprotective activities[4-5]. Furthermore, additional pharmacological studies have documented antimutagenic, antidiabetic, and antitumor effects[6-7]. Related species within the genus *Phellinus*, particularly *P. linteus*, have also been extensively studied for their anticancer potential and immunomodulatory properties[8-9].

Inflammation is now recognized as a central pathological process underlying many chronic diseases, including cancer, diabetes, cardiovascular disorders, and autoimmune conditions. It is closely associated with persistent oxidative stress[10-11]. Chronic activation of inflammatory pathways, particularly those mediated by pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), plays a crucial role in tissue injury, tumor progression, and metabolic dysfunction[11]. However, information regarding low-molecular-weight phytochemicals, especially phenolic and flavonoid compounds that may contribute to antioxidant and anti-inflammatory activities, remains scarce for *P. rimosus*.

Phenolic compounds are important contributors to the antioxidant capacity of medicinal plants and mushrooms due to their redox properties[12]. Flavonoids, a major class of polyphenols, also exhibit antioxidant and anti-inflammatory activities[13]. Oxidative stress caused by reactive oxygen species (ROS) is closely associated with chronic diseases[10] and is linked to inflammatory responses mediated by cytokines such as TNF-α[11]. Therefore, determining total phenolic and flavonoid contents, together with antioxidant and anti-inflammatory activities, is essential for evaluating the therapeutic potential of medicinal fungi.

Several species of the genus *Phellinus* have been reported to contain phenolic compounds and exhibit antioxidant activities. However, information regarding the phenolic and flavonoid composition of *Phellinus rimosus* remains limited. A previous study reported the antioxidant activity of *P. rimosus* using DPPH and FRAP assays; however, a comprehensive evaluation of its total phenolic and flavonoid contents and their relationship to antioxidant and anti-inflammatory activities is still lacking. Therefore, the present study aimed to determine the total phenolic

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and flavonoid contents of *P. rimosus* extracts and to evaluate their antioxidant activities using DPPH, ABTS, and FRAP assays, together with anti-inflammatory activity. The findings of this study are expected to provide scientific evidence supporting the medicinal potential of this fungus and contribute to the development of natural antioxidant and anti-inflammatory agents.

MATERIALS AND METHODS

Mushroom Sample and Extraction

The fruiting bodies of *P. rimosus* (voucher specimen no. MSUT2615) were obtained from the Natural Medicinal Mushroom Museum, Faculty of Science, Mahasarakham University, Thailand. The mushroom specimen was taxonomically authenticated by Assoc. Prof. Dr. Khwanruan Naksuwankul, Faculty of Science, Mahasarakham University, Thailand.

Dried fruiting bodies of *Phellinus rimosus* were ground into powder prior to extraction. For aqueous extraction, 775 g of mushroom powder was mixed with 3100 mL of distilled water and subjected to decoction at 100 °C for 4 h. The extract was filtered through gauze and Whatman No. 1 filter paper and subsequently freeze-dried at −98 °C for 26 h to obtain the crude aqueous extract.

For ethanolic extraction, 1500 g of mushroom powder was macerated with 95% ethanol (6000 mL) at room temperature for 3 days. The extraction process was repeated three times. The combined extracts were filtered through gauze and Whatman No. 1 filter paper. The solvent was removed using a rotary evaporator and further concentrated in a water bath at 60 °C.

The crude extracts were weighed and the percentage yield was calculated. The yields were 3.43% for the ethanolic extract and 1.05% for the aqueous extract. The extracts were stored in sealed glass bottles covered with aluminum foil at 4 °C and used for experiments within 2 months after extraction.

Determination of Total Phenolic and Flavonoid Contents

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method with slight modifications and adapted for microscale analysis. Briefly, 100 µL of extract solution (1 mg/mL) was mixed with 1 mL of diluted Folin–Ciocalteu reagent (1:10) and incubated for 5 min at room temperature. Subsequently, 80 µL of 7.5% Na_2CO_3 was added, and the mixture was incubated for 30 min in the dark. The absorbance was measured at 630 nm using a microplate reader following the procedure described by Attard[14], which has been reported to correlate well with the conventional measurement at 760–765 nm. Gallic acid (10–125 µg/mL) was used to construct the calibration curve, and the results were expressed as mg gallic acid equivalents (GAE)/g dried extract.

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method[15]. In brief, extract or rutin standard solutions (10–80 µg/mL) were sequentially mixed with 5% $NaNO_2$, 10% $AlCl_3$, and 1 M NaOH, and adjusted to a final volume of 1 mL. The absorbance was measured at 415 nm. The results were expressed as mg rutin equivalents (RE)/g dried extract. All assays were performed in triplicate.

Antioxidant activity assays

Antioxidant activity was evaluated using DPPH, ABTS, and FRAP assays. All experiments were performed in triplicate.

DPPH radical scavenging activity

The DPPH radical scavenging activity was determined using a modified method of Amic *et al*[16]. Sample solutions were prepared at various concentrations. Briefly, 20 µL of each sample solution was mixed with 180 µL of DPPH solution (300 µM in ethanol) in a 96-well microplate. The reaction mixtures were incubated in the dark at 37 °C for 30 min, and the absorbance was measured at 517 nm using a microplate reader. Ascorbic acid was used as a positive control. The percentage of DPPH radical scavenging activity was calculated, and IC_{50} values were determined from the dose–response curves obtained by plotting percentage inhibition against sample concentration.

ABTS radical scavenging activity

The ABTS radical scavenging activity was assessed according to a modified method of Payet *et al*[17]. The $ABTS^{\bullet+}$ radical cation solution was prepared and diluted with distilled water to obtain an absorbance of 0.700 ± 0.02 at 734 nm. Sample solutions at various concentrations (20 µL) were added to 280 µL of $ABTS^{\bullet+}$ solution in a 96-well microplate and incubated at 30 °C for 5 min. The absorbance was then measured at 734 nm. Ascorbic acid and distilled water were used as positive and negative controls, respectively. The percentage inhibition was calculated, and IC_{50} values were obtained from the concentration–inhibition curves.

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power (FRAP) assay was performed following a modified method of Benzie and Strain[18]. The antioxidant capacity was determined based on the reduction of the ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex to the ferrous form (Fe^{2+}–TPTZ), and the absorbance was measured at 593 nm. Ferrous sulfate was used as a reference standard.

Calculation of radical scavenging activity and IC_{50} determination

The percentage inhibition was calculated using the following equation:

$$\% \text{ inhibition} = 100 \times (A_{blank} - A_{sample}) / A_{blank}$$

Where A_{blank} = absorbance of blank, A_{sample} = absorbance of the sample

IC_{50} values were defined as the concentration of sample required to inhibit 50 % of the free radical activity and were calculated from nonlinear regression analysis of the dose–response curves.

Anti-inflammatory activity

Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 macrophages

The inhibitory effect of the extracts on nitric oxide (NO) production were determined using the Griess reaction in lipopolysaccharide (LPS)-stimulated RAW 264.7 murine macrophage cells, with slight modifications from the method previously described by Mfotie *et al*[19]. RAW 264.7 cells were cultured in RPMI-1640 medium supplemented with %10 fetal bovine serum (FBS) and %1 antibiotic–antimycotic solution at 37 °C in a humidified atmosphere containing %5 CO_2.

Cells were seeded into 96-well plates at a density of 1×10^5 cells/well and incubated for 24 h to allow cell attachment. The cells were then stimulated with LPS (5 µg/mL) and simultaneously treated with the mushroom extracts at various concentrations (12.5, 25, 50, 100 and 200 µg/mL). Stock solutions of the extracts were prepared in dimethyl sulfoxide (DMSO) and diluted with culture medium, ensuring that the final concentration of DMSO did not exceed %0.1 (v/v). Cells treated with LPS alone served as the positive control, while untreated cells served as the negative control.

After 24 h of incubation, 100 µL of culture supernatant from each well was collected and mixed with an equal volume of Griess reagent (%1 sulfanilamide and %0.1 N-(1-naphthyl)ethylenediamine dihydrochloride in %5 phosphoric acid). The reaction mixture was

incubated at room temperature for 10 min, and the absorbance was measured at 540 nm using a microplate reader. Nitrite accumulation in the supernatant was used as an indicator of NO production.

The percentage inhibition of NO production was calculated according to the following equation:

The % of inhibition = (Abs : Control – Abs : Sample)/Abs : Control x100

where Abs : Control represents the absorbance of LPS-treated cells without sample and Abs : Sample represents the absorbance of LPS-treated cells in the presence of the sample. The IC_{50} values were calculated from dose–response curves using GraphPad Prism version 8.0 software. All experiments were performed in triplicate.

Cell viability (MTT) assay

The cytotoxic effect of the extracts on RAW 264.7 cells was evaluated using the MTT colorimetric assay, following the method described by Karakas *et al* [20]. Cells were seeded into 96-well plates at a density of 1×10^5 cells/well and treated under the same experimental conditions as those used in the NO inhibitory assay. After 24 h of incubation, 10 µL of MTT solution (5 mg/mL in phosphate-buffered saline) was added to each well and further incubated at 37 °C for 4 h in the dark. The culture medium was then carefully removed, and the resulting formazan crystals were dissolved in 100 µL of dimethyl sulfoxide (DMSO). The absorbance was measured at 570 nm using a microplate reader.

Cell viability was expressed as a percentage relative to untreated control cells. Samples exhibiting cell viability greater than %70 were considered non-cytotoxic, and the observed inhibition of NO production was therefore not attributable to cell death.

Statistical analyses

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation (SD). Statistical comparisons between two groups (ethanolic and aqueous extracts) were performed using the independent Student's t-test. For comparisons involving more than two groups, such as antioxidant activity assays including the positive control, one-way analysis of variance (ANOVA) followed by Duncan's multiple range test was applied. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Total Phenolic and Flavonoid Contents

The total phenolic contents (TPC) and total flavonoid contents (TFC) of *P. rimosus* extracts are presented in Table 1. The calibration curve for gallic acid used in the TPC determination showed good linearity with the equation Y = 0.0022x ($R^2 = 0.9944$) within the tested concentration range. Likewise, the calibration curve for rutin used in the TFC assay was Y = 0.0042x ($R^2 = 0.9926$), indicating satisfactory linearity.

The ethanolic extract showed significantly higher TPC (361.04 ± 5.69 mg GAE/g extract) compared to the aqueous extract (117.14 ± 6.11 mg GAE/g extract) ($P < 0.05$). Similarly, the total flavonoid content of the ethanolic extract (646.55 ± 6.29 mg RE/g extract) was significantly greater than that of the aqueous extract (129.11 ± 5.29 mg RE/g extract) ($P < 0.05$).

Statistical analysis was performed using Student's t-test, and values are expressed as mean ± SD (n = 3). These results suggest that ethanol was more effective than water for extracting phenolic and flavonoid compounds from *P. rimosus*.

Antioxidant activity

The antioxidant activities of *P. rimosus* extracts were evaluated using DPPH, ABTS, and FRAP assays, and the results are shown in Table 2. Significant differences among samples were observed by one-way ANOVA ($P < 0.05$).

In the DPPH assay, the ethanolic extract exhibited significantly stronger radical scavenging activity (IC_{50} = 9.56 ± 0.47 µg/mL) than the aqueous extract (IC_{50} = 37.81 ± 0.15 µg/mL), while ascorbic acid showed an IC_{50} value of 4.53 ± 0.30 µg/mL. Similarly, in the ABTS assay, the ethanolic extract (IC_{50} = 5.40 ± 0.06 µg/mL) demonstrated greater activity than the aqueous extract (IC_{50} = 14.41 ± 0.13 µg/mL) and was comparable to ascorbic acid (IC_{50} = 5.30 ± 0.22 µg/mL).

In the FRAP assay, the ethanolic extract showed the highest ferric reducing antioxidant power (52.39 ± 0.25 mM Fe^{2+}/100 mg extract), followed by ascorbic acid (38.37 ± 1.55 mM Fe^{2+}/100 mg), whereas the aqueous extract exhibited the lowest value (12.53 ± 0.42 mM Fe^{2+}/100 mg).

Anti-inflammatory activity

The anti-inflammatory activity of *P. rimosus* extracts was evaluated based on their inhibitory effects on nitric oxide (NO) production in LPS-stimulated RAW 264.7 macrophages (Table 3). The ethanolic extract significantly suppressed NO production in a concentration-dependent manner, reaching 97.95 ± %2.29 inhibition at 200 µg/mL and 92.08 ± %4.60 at 100 µg/mL, with an IC_{50} value of 54.40 ± 2.35 µg/mL. In contrast, the aqueous extract did not exhibit inhibitory activity within the tested concentration range and showed negative inhibition values; therefore, its IC_{50} could not be determined. Cell viability assessed by the MTT assay indicated that the observed NO inhibitory effect of the ethanolic extract was not attributable to cytotoxicity, as

Table 1. Total phenolic and total flavonoid contents of *Phellinus rimosus* extracts

Samples	Total phenolic (mg GAE/g of extract)	Total flavonoid (mg RE/g of extract)
Ethanol extract	361.04 ± 5.69^a	646.55 ± 6.29^c
Water extract	117.14 ± 6.11^b	129.11 ± 5.29^d

Values are expressed as mean ± SD (n = 3). Different superscript letters within the same column indicate statistically significant differences ($P < 0.05$) according to the independent Student's t-test.

Table 2. In vitro antioxidant activity of *Phellinus rimosus* extracts

Samples	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)	FRAP (mM Fe^{2+}/ 100 mg)
Ethanolic extract	9.56 ± 0.47^b	5.40 ± 0.06^d	52.39 ± 0.25^h
Aqueous extract	37.81 ± 0.15^c	14.41 ± 0.13^e	12.53 ± 0.42^f
Ascorbic acid	4.53 ± 0.30^a	5.3 ± 0.22^d	38.37 ± 1.55^g

Values are expressed as mean ± SD (n = 3). Different superscript letters within the same column indicate statistically significant differences ($P < 0.05$) based on one-way ANOVA followed by Duncan's multiple range test. Ascorbic acid was used as a positive control.

Table 3. Inhibitory effect of *Phellinus rimosus* extracts on nitric oxide production in LPS-stimulated RAW 264.7 macrophages

Samples	Concentration (µg/mL)	NO inhibition (%)	IC_{50} (µg/mL)
Ethanolic extract	200	97.95 ± 2.29	54.40 ± 2.35
	100	92.08 ± 4.60	
	50	46.21 ± 0.43	
	25	32.16 ± 2.46	
	12.5	13.54 ± 2.53	
Aqueous extract	200	-20.98 ± 4.30	ND
	100	-9.21 ± 3.70	

Values are expressed as mean ± SD (n = 3). Nitric oxide (NO) production was measured in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. IC_{50} values were calculated from dose–response curves. ND indicates not determined due to the absence of 50% inhibition within the tested concentration range.

cell viability remained above %70 at all tested concentrations (data not shown).

DISCUSSION

Total Phenolic and Flavonoid Contents

The present study demonstrated that the ethanolic extract of *P. rimosus* contained significantly higher total phenolic and total flavonoid contents compared to the aqueous extract ($P < 0.05$). This finding aligns with previous research on *Phellinus* species from northeast Thailand, which reported that ethanolic extracts exhibited stronger antioxidant and cytotoxic activities than aqueous extracts, indicating a greater abundance of phenolic constituents[8]. The enhanced extraction efficiency of ethanol can be attributed to its intermediate polarity, which enables it to dissolve a broader range of phenolic and flavonoid compounds than water[21]. Additionally, the accumulation of phenolic compounds in *P. rimosus* may be influenced by ecological interactions with host trees. Wood-decay basidiomycete fungi are known to decompose lignin and other components of plant cell walls while forming close associations with wood substrates[22]. These interactions, along with various environmental factors, may play a role in the accumulation and diversity of phenolic constituents in the fruiting bodies[23].

Overall, the higher phenolic and flavonoid contents in the ethanolic extract may partly explain its stronger antioxidant and anti-inflammatory activities observed in this study. Several phenolic compounds have previously been identified in *Phellinus* species, including hispidin and related hispidin derivatives, which are well known for their antioxidant and anti-inflammatory properties[24]. These phenolic metabolites may contribute to the biological activities observed in the ethanolic extract of *P. rimosus* in the present study.

Antioxidant activity

The present study demonstrated that the antioxidant activity of *P. rimosus* extracts varied significantly based on the extraction solvent, as shown by the results from DPPH, ABTS, and FRAP assays. Similar antioxidant activities have also been reported in other medicinal mushrooms belonging to the genus *Phellinus*. Previous studies on species such as *Phellinus linteus* and *Phellinus igniarius* demonstrated strong radical scavenging and reducing activities that were closely associated with their high phenolic contents[8]. These findings are consistent with the results of the present study, suggesting that *P. rimosus*, like other *Phellinus* species, may contain phenolic constituents that contribute to its antioxidant capacity.

Among the extracts tested, the ethanolic extract consistently exhibited significantly higher antioxidant activity compared to the aqueous extract ($p < 0.05$). This indicates that ethanol is a more effective solvent for extracting antioxidant constituents from *P. rimosus*.

The DPPH radical scavenging assay indicated that the ethanolic extract had a significantly lower IC_{50} value compared to the aqueous extract, suggesting a stronger hydrogen-donating ability. This result aligns with previous studies on *Phellinus species*, which found that ethanolic extracts exhibited superior antioxidant capacity relative to water extracts[8,25]. Additionally, the ability of DPPH radicals to primarily interact with low-molecular-weight antioxidant compounds implies that ethanol may preferentially extract those compounds that possess high radical scavenging efficiency[26].

In the ABTS assay, the ethanolic extract again showed greater antioxidant activity than the aqueous extract, with IC_{50} values comparable to that of ascorbic acid. Unlike the DPPH assay, ABTS radicals can react with both hydrophilic and lipophilic antioxidants. The strong activity observed in this assay implies that the ethanolic extract of *P. rimosus* may contain a diverse range of antioxidant compounds capable of neutralizing radicals through multiple mechanisms. The consistently lower IC_{50} values obtained from the ABTS assay compared to DPPH further suggest that the extracts may be more effective against cationic radicals across multiple mechanisms[27].

The FRAP assay results supported the findings from radical scavenging assays, as the ethanolic extract exhibited markedly higher ferric reducing power than the aqueous extract. The FRAP assay reflects the electron-donating capacity of antioxidants, which is closely associated with their reducing potential[28]. The strong reducing activity observed in the ethanolic extract indicates the presence of compounds capable of participating in redox reactions, thereby contributing to overall antioxidant defense.

The lower antioxidant activity observed in the aqueous extract may result from variations in the types of compounds extracted by water. Previous research demonstrates that aqueous extracts of *Phellinus* species are primarily rich in polysaccharides and β-glucans[25], which are known to provide antioxidant effects mainly through indirect mechanisms, such as immunomodulation, rather than direct radical scavenging. Ethanolic extracts, on the other hand, are more likely to have secondary metabolites that are strong antioxidants on their own. Because of this difference, the ethanolic extracts have lower IC_{50} values and more reducing power.

The results indicate that the antioxidant potential of *P. rimosus* is highly dependent on the extraction solvent and the assay employed. The ethanolic extract demonstrated superior radical scavenging and ferric reducing activities in all tested models, highlighting its potential as a natural source of antioxidant compounds. These findings provide scientific support for the selection of appropriate extraction methods when developing antioxidant agents from *P. rimosus*.

Anti-inflammatory activity

The present study demonstrated that the ethanolic extract of *P. rimosus* markedly inhibited nitric oxide (NO) production in LPS-stimulated

RAW 264.7 macrophages in a concentration-dependent manner, with an IC_{50} value of 54.40 ± 2.35 µg/mL. In contrast, the aqueous extract did not exhibit inhibitory activity within the tested concentration range. The suppression of NO production without significant cytotoxicity (cell viability > %70) indicates that the observed effect was attributable to genuine anti-inflammatory activity rather than nonspecific cell death.

Previous studies have reported anti-inflammatory properties in *Phellinus* species, particularly in polysaccharide–protein complexes isolated from *P. rimosus*, which demonstrated significant inhibition of inflammatory mediators and free radical scavenging activity[3]. Moreover, related species such as *Phellinus linteus* have been shown to modulate inflammatory signaling pathways, including suppression of inducible nitric oxide synthase (iNOS) expression and pro-inflammatory cytokines[9]. These findings support the present results and suggest that bioactive constituents in *P. rimosus* may interfere with NO production pathways in activated macrophages.

The superior activity observed in the ethanolic extract may be associated with its higher phenolic and flavonoid contents, as these compounds are well recognized for their ability to scavenge reactive nitrogen species and downregulate inflammatory mediators[11]. Phenolic compounds have been reported to inhibit NF-κB activation and reduce iNOS expression, thereby decreasing NO production in macrophages[11]. Therefore, the anti-inflammatory activity of the ethanolic extract may be partly attributed to its enriched phenolic profile.

From a pharmacological perspective, inhibition of excessive NO production is relevant to the management of inflammatory disorders, as overproduction of NO by activated macrophages contributes to tissue damage and the progression of chronic inflammatory diseases. The observed activity of *P. rimosus* ethanolic extract suggests its potential as a natural source of anti-inflammatory agents and supports its traditional medicinal use. These findings also suggest that extracts of *P. rimosus* may have potential applications in the development of natural therapeutic agents for conditions associated with oxidative stress and inflammation. Since oxidative stress and excessive inflammatory responses are implicated in many chronic diseases, including cardiovascular disorders, metabolic syndrome, and neurodegenerative diseases, the antioxidant and anti-inflammatory activities observed in this study highlight the possible pharmacological significance of this medicinal mushroom.

CONCLUSION

In conclusion, the ethanolic extract of *P. rimosus* exhibited significantly higher phenolic and flavonoid contents than the aqueous extract and demonstrated superior antioxidant and anti-inflammatory activities. The extract effectively scavenged free radicals, showed strong ferric reducing power, and inhibited nitric oxide production without cytotoxicity. These findings highlight the potential of *P. rimosus* as a natural source of bioactive compounds for further pharmacological development.

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REFERENCES

1. Parihar A, Rao YV, Padal SB, Das K. Taxonomy and diversity of the genus *Phellinus* Quél. s.s. (Basidiomycota, Hymenochaetaceae) in Koderma Wildlife Sanctuary, Jharkhand, India. *Trop Plant Res.* 2019;6(3):472–485.
2. Judprakob C, Laovachirasuwan P, Phadungkit M. Antioxidant and antimutagenic activities of *Phellinus rimosus*. Isan J Pharm Sci. 2013;9(1):1–10.
3. Janardhanan KK, Vishwakarma RA, Meera CR, Balan N. Anti-inflammatory and free radical scavenging activities of polysaccharide–protein complex isolated from *Phellinus rimosus* (Berk.) Pilát (Aphyllophoromycetideae). Int J Med Mushrooms. 2009;11(4):365–373.
4. Joseph J, Panicker SN, Janardhanan KK. Protective effect of polysaccharide-protein complex from *Phellinus rimosus* against radiation-induced oxidative stress. Redox Rep. 2012;17(1):22-27.
5. Ajith TA, Janardhanan KK. Antioxidant and antihepatotoxic activities of *Phellinus rimosus* (Berk) Pilat. J Ethnopharmacol. 2002;81(3):387-391.
6. Rony KA, Ajith TA, Kuttikadan TA, et al. *Phellinus rimosus* improves mitochondrial energy status and attenuates nephrotoxicity in diabetic rats. J Basic Clin Physiol Pharmacol. 2017;28(5):455-463.
7. Meera CR, Janardhanan KK. Antitumor activity of a polysaccharide-protein complex isolated from *Phellinus rimosus*. J Environ Pathol Toxicol Oncol. 2012;31(3):223-232.
8. Thammavong S, Phadungkit M, Laovachirasuwan P, et al. Antioxidant and cytotoxic activity of *Phellinus* mushrooms from northeast Thailand. RJP. 2021;8(1):91-99.
9. Wasser SP. Medicinal mushroom science: current perspectives, advances, evidences, and challenges. Biomed J. 2014;37(6):345–356.
10. Gabriele P, Natasha I, Mariapaola C, et al. Oxidative stress: Harms and benefits for human health. Oxid Med Cell Longev. 2017; 2017: 1-15.
11. Azab A, Nassar A, Azab AN. Anti-inflammatory activity of natural products. Molecules. 2016;21(10):1321.
12. Heleno SA, Martins A, Queiroz MJRP, Ferreira ICFR. Bioactivity of phenolic acids: metabolites versus parent compounds. Food Chem. 2015;173:501–513.
13. Al-Khayri JM, Sahana GR, Nagella P, Joseph BV, Alessa FM, Al-Mssallem MQ. Flavonoids as potential anti-inflammatory molecules: A review. Molecules. 2022;27(9):2901.
14. Attard E. A rapid microtitre plate Folin–Ciocalteu method for the assessment of polyphenols. Eur J Biol. 2013;8(1):48-53.
15. Marinova FR. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J Univ Chem Technol Metall. 2005; 40(3): 255-260.
16. Amic D, Beslo D, Trinajstić N. Structure–radical scavenging activity relationships of flavonoids. Croat Chem Acta. 2003;76(1):55–61.
17. Payet B, Shum Cheong Sing A, Smadja J. Assessment of antioxidant activity of cane brown sugars by ABTS and DPPH radical scavenging assays: determination of their polyphenolic and volatile constituents. J Agric Food Chem. 2005;53(26):10074–10079.
18. Benzie IFF, Strain JJ. Ferric reducing antioxidant power (FRAP) assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 1999;299:15–27.
19. Mfoti N, Munvera A, Mkounga P, Nkengfack A, McGaw LJ. Phytochemical analysis with free radical scavenging, nitric oxide inhibition and antiproliferative activity of *Sarcocephalus pobeguinii* extracts. BMC Complement Altern Med. 2017;17(1):1–9.
20. Karakas D, Ari F, Ulukaya E. The MTT viability assay yields strikingly false-positive viabilities although the cells are killed by some plant extracts. Turk J Biol. 2017;41(6):919–925.
21. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15:7313–7352.

22. Fukasawa Y, Kaga K. Surface area of wood influences the effects of fungal interspecific interaction on wood decomposition — a case study based on *Pinus densiflora* and selected white rot fungi. J Fungi (Basel). 2022;8(5):517.
23. Hatakka A, Hammel KE. Fungal biodegradation of lignocelluloses. In: Industrial Applications. Springer; 2010.
24. Lee IK, Yun BS. Highly oxygenated and unsaturated metabolites providing a diversity of hispidin class antioxidants in the medicinal mushrooms *Inonotus* and *Phellinus*. Bioorg Med Chem. 2007;15(10):3309-14.
25. Zhao C, Liao Z, Wu X, Liu Y, Liu X, Lin Z. Isolation, purification and structural features of a polysaccharide from *Phellinus linteus* and its hypoglycemic effect in alloxan-induced diabetic mice. J Food Sci. 2014;79(5):1002–1010.
26. Gulcin I, Alwasel SH. DPPH radical scavenging assay. Processes. 2023;11(8):2248.
27. Gulcin I. Antioxidants: a comprehensive review. Arch Toxicol. 2025;99:1893–1997.
28. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem. 2005;53(10):4290–4302.

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