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VIETNAMESE *Phellinus baumii* FRUITING BODY, A POTENTIAL SOURCE OF ANTIOXIDANT AND α-AMYLASE INHIBITORY AGENTS

Mai Phuong Thuy[1], Do Thi Hong[2], Le Thi Tuyet Mai[2]
and Le Thi Phuong Hoa[2,*]
[1]*Faculty of Politics Education and Basic Sciences,*
College of Urban Works Construction
[2]*Faculty of Biology, Hanoi National University of Education*

Abstract. *n*-Hexane, ethyl acetate, and butanol fractions from methanol extract of Vietnamese *Phellinus baumii* fruiting body consisted of phenolics, flavonoids, and terpenoids. Ethyl acetate and butanol fractions showed a high level of total phenolics. Ethyl acetate fractions possessed the highest phenolic content (532.96 ± 19.95 mg GAE/g), accounting for approximately 53% fraction dry weight. Free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging capacity of all fractions correlated with their total phenolic content. Ethyl acetate fraction exhibited the strongest capacity with an IC_{50} value of 0.059 mg/mL, equivalent to nearly 50% of ascorbic acid's capacity. Its ferric reducing power was off more than half of quercetin and acid ascorbic at the concentration of 0.2 mg/mL. Ethyl acetate also had remarkable α-amylase inhibitory activity (IC_{50}= 0.38 mg/mL). The results suggested *P. baumii* fruiting body as a potent source for antioxidative and α-amylase inhibitory compounds.

Keywords*: Phellinus baumii*, phenolics, DPPH scavenging activity, reducing power, α-amylase inhibition.

1. Introduction

Fungi have been used widely as functional food for protein, fiber, mineral, and vitamin supply as well as health beneficial bioactive compounds [1, 2]. *Phellinus baumii* belongs to *Phellinus* genus, Hymenochaetaceae family. Some extracts and compounds from several *Phellinus* species were reported to have various effects such as antioxidative, anticancer, anti-inflammatory, hypoglycemic, antibacterial, antivirus activities [2-9]. *P. baumii* has been used as a traditional remedy in oriental countries for diabetes, cancer, ulcer, and detoxification [10, 11]. Previous researches extracted and purified several compounds from *P. baumii* mainly polysaccharides, polyphenols, sterols.

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Contact: Le Thi Phuong Hoa, e-mail address: lphoa@hnue.edu.vn

Polysaccharides showed various activities such as antioxidant, antitumor, anti-inflammatory, hypoglycemic, hepatoprotective activities [11-13]. Polyphenols were just reported with antivirus and antitumor activity [6, 14]. Furthermore, some biological activities of *P. baumii* fruiting body have been elucidated in vivo [13, 15-18] and fruiting bodies from artificially cultured *P. baumii* have been also characterized recently [12, 13], suggesting its broad scale and feasible application in functional food development.

Although phytochemical and pharmacological properties of *P. baumii* from Korea, China have been widely characterized [2, 5-7, 11, 14], there is only one report on chemical constituents of *P. baumii* from Vietnam [19]. Besides, some reports mentioned the hypoglycemic effect of *P. baumii* fruiting body extract and polysaccharides in diabetic mice [7, 16], the mechanism of which needs further clarification. Therefore, in the current research, we prepared fractions from *P. baumii* fruiting bodies collected in North Central Vietnam and investigated their antioxidant and α-amylase inhibitory activity.

2. Content

2.1. Material and Methods

2.1.1. Material

Phellinus baumii fruiting bodies were collected in Pu Mat National Park, Nghe An province, and identified by Ngo Anh, Hue University.

Chemicals and reagents (α-amylase, DPPH, ascorbic acid, quercetin, gallic acid, Folin-Ciocalteu's phenol reagent) were of analytical grade and purchased from Sigma Chemicals (MO, USA) and Merck Chemicals (Darmstadt, Germany).

2.1.2. Methods

**** Extraction and partition***

P. baumii fruiting bodies were dried at 45 °C to a constant weight and ground to powder. The powder was extracted in methanol at room temperature for 3 days and in an ultrasonic bath thereafter in three replicates for 30 mins each. The extracts were mixed, filtered, and concentrated via rotary evaporation. The crude extract was further partitioned in n-hexane, ethyl acetate, and butanol. All fractions were concentrated, freeze-dried, and stored at -20 °C until use.

**** Thin layer chromatography***

Fractions were diluted in methanol at the concentration of 10 mg/mL and subjected to thin-layer chromatography with a solvent system of toluene/ethyl acetate/acetone /formic acid 5:3:1:1. Chromatograms were visualized at ultraviolet (UV) wavelengths of 302 nm, 254 nm, 365 nm, and white light after spraying with 5% H_2SO_4 in methanol.

**** Determination of total phenolics content and flavonoid content***

Total phenolics of fractions from *P. baumii* fruiting bodies were estimated according to Waterhouse [20] as described previously for 96-well microplates [21]. Gallic acid was used as the standard. The products of the metal oxide reduction were assessed with light absorption at 765 nm. Based on the standard curve, total phenolics of

all fractions were calculated and expressed as mg gallic acid equivalents (GAE) per gram dry extract.

The total flavonoid content was evaluated employing the method described by Sapkota et al. [22] using quercetin as the standard. The absorbance of aluminum chloride-flavonoid complexes was measured at 415 nm. Flavonoid content of *P. baumii* fractions was expressed in mg quercetin equivalents (QE) per gram dry extract using a quercetin standard curve.

**** DPPH radical scavenging activity assay***

DPPH radical scavenging capacity was examined following Blois's method [23] with ascorbic acid as the reference. Samples were prepared in 2-fold dilution with methanol. Methanol was used for the control instead of fractions. DPPH radical scavenging capacity (%) was calculated as: $[(A_{control} - A_{sample})/(A_{control})] \times 100$. A stands for absorbance. The half-maximum inhibitory concentration (IC_{50}) value was estimated from the logarithm regression curve of scavenging capacity vs. sample concentration.

**** Reducing power assay***

Ferric reducing power was measured applying the method described by Sapkota et al. [22]. Ascorbic acid and quercetin were used for comparison with the fractions. Samples were prepared in 2-fold dilution with methanol. Absorbance was measured at 700 nm. Reducing power was determined by the value of sample concentration that increases the absorbance by 0.5 according to the standard curve equation.

**** Alpha-amylase inhibitory activity assay***

Alpha-amylase inhibitory activity was evaluated making use of α-amylase activity assay according to Rukhliadeva Geriacheva as described by Nguyen Van Mui [24]. In the presence of α-amylase, starch is hydrolyzed, attenuating the capacity to be colorized with iodine. The fractions were dissolved in dimethyl sulfoxide (DMSO) and then diluted by a factor of two. They were mixed with 40 IU/mL α-amylase solution (alpha-amylase bacterial, Fisher Scientific) and incubated at 30 °C for 10 minutes before being added with 0.5% starch solution for a 30-min reaction and with iodine solution thereafter. Percentage of hydrolyzed starch was calculated as $[(A - B)/A] \times 100$, where A is the absorbance of starch solution (with DMSO) at 656 nm, B is the absorbance of α-amylase containing solution at 656 nm. The α-amylase inhibition capacity (%) of different concentration solutions was measured based on the percentage of hydrolyzed starch using the control without fractions to establish a regression curve in order to estimate the IC_{50} value.

**** Statistical analysis***

The experimental data were presented as means ± standard deviation from at least triplicate experiments. The statistical analysis was performed with Microsoft Excel software and SPSS 20.0 using Dunnett T3 test and p value of 0.05. Significant differences between samples were displayed with superscript alphabetical letters.

2.2. Results and discussion

2.2.1. Second metabolite composition

**** Thin layer chromatogram analysis***

Second metabolites in fractions of *P. baumii* fruiting bodies were preliminarily investigated by thin-layer chromatography (Figure 1).

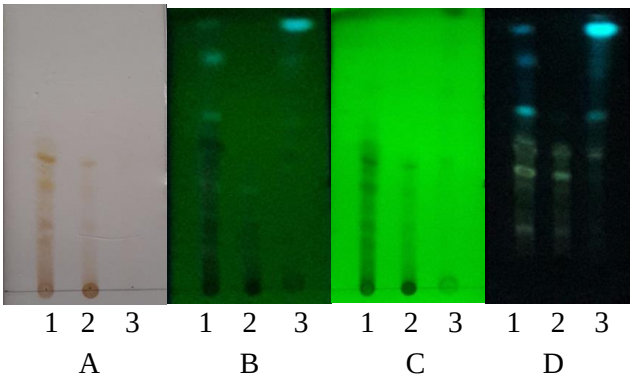

Figure 1. Thin layer chromatograms of ethyl acetate (1), butanol (2) and n-hexane (3) fractions from P. baumii fruiting bodies under white light (A), UV rays of 254 nm (B), 302 nm (C), 366 nm (D)

Figure 1 shows that each chromatogram has a different number of coloured bands corresponding to different organic compounds of the fractions. Specifically, under white light, after spraying with 5% H_2SO_4, the ethyl acetate and butanol fractions have more coloured bands than *n*-hexane fraction, indicating that many compounds were better separated in these fractions. The ethyl acetate and butanol fractions have yellow or orange-yellow bands characteristic of flavonoids. In addition, both fractions have purple bands, proving that they contain terpenoids [25]. Laovachirasuwan et al (2016) also reported that water fraction and ethanol fraction from some Phellinus species (*P. rimosus, P. wahlbergii, P. nigricans*) contained large amount of terpenoids [8], suggesting these compounds should be further characterized. Under the wavelength of 365 nm, the appearance of phenolic compounds (blue bands) is clearly seen in ethyl acetate fraction. Some polyphenols were also isolated from *P. baumii mycelia* and fruiting bodies [14,19].

**** Total phenolics content and flavonoids content***

Based on the results of thin layer chromatography, experiments were conducted to estimate phenolics and flavonoid levels in the fractions from *P. baumii*.

Table 1. Total phenolics and flavonoid content of P. baumii fruiting body fractions

Sample	Total phenolics content (mg GAE/g fraction)	Total flavonoid content (mg QE/g fraction)
Ethyl acetate fraction	532.96 ± 19.95^{a}	58.49 ± 8.06^{a}
Butanol fraction	350.88 ± 10.56^{b}	32.91 ± 7.92^{b}
n-Hexane fraction	25.46 ± 5.18^{c}	2.06 ± 0.94^{c}

GAE: gallic acid equivalents; QE: quercetin equivalents;
^{a,b,c}: Significant difference among fractions at $p < 0.05$

The results showed that the total phenolic content of the ethyl acetate fraction was the highest, accounting for around 53% fraction dry weight and about 1.5 times higher than that of butanol fraction and up to about 21 times that of n-hexane fraction. Compared with the results of the previous study, total phenolic content in ethyl acetate and butanol fractions was much higher than ethyl acetate and butanol fractions from the methanolic extract of *P. baumii* fruiting bodies collected in Korea, 32 and 28 μg caffeic acid equivalents/mL, respectively [10].

Flavonoid content in the ethyl acetate fraction was also the highest, which differs significantly from the butanol and *n*-hexane fractions. This result is consistent with the results of thin-layer chromatography, in which ethyl acetate fraction had more yellow-orange bands than butanol and n-hexane fractions. The total flavonoid content of ethyl acetate and butanol fractions of *P. baumii* was much higher than that of the two extracts from *P. igniarius*, 4.04 and 5.21 mg QE/g, respectively [4]. Some polyphenols isolated from *P. baumii mycelia* and fruiting bodies exhibited anti-influenza activity [6] and anticancer activity against human liver, colon, breast, prostate, epidermis cancer cell lines [14, 19]. It is suggested that *P. baumii* fruiting body, especially ethyl acetate fraction is a potential source for bioactive compounds.

2.2.2. Biological activities of *Phellinus baumii fruiting body*

**** DPPH radical scavenging capacity***

DPPH radical scavenging capacity assay is a popular assay to evaluate the antioxidant activity of plant extracts and compounds. It was shown that ethyl acetate fraction of *P. baumii* fruiting body had a strong DPPH radical scavenging capacity, roughly half as strong as pure ascorbic acid (Table 2).

Table 2. DPPH radical scavenging activity of P. baumii fruiting body fractions

Sample	IC_{50} (mg/mL)
Ethyl acetate fraction	0.059 ± 0.008^{a}
Butanol fraction	0.154 ± 0.010^{b}
n-Hexane fraction	2.663 ± 1.584^{c}
Ascorbic acid	0.028 ± 0.004^{d}

^{a,b,c,d}: Significant difference among samples at $p < 0.05$

Ethyl acetate fraction of *P. baumii* fruiting body had weaker DPPH free radical scavenging activity than extracts from *P. gilvus, P. pini, P. torulosus, P. trivalis* in the previous report, with an IC_{50} value range of 24.56 - 49.57 μg/mL but stronger than other fungi such as *P. contiguus, P. hippophaecola, P. igniarius, P. linteus, P. tuberculosis* (IC_{50} values greater than 100 μg/mL). (Prapairat et al., 2011) [26].

DPPH radical scavenging activity of *P. baumii* fruiting body fractions followed the order of ethyl acetate > butanol > *n*-hexane in the same manner as that of total phenolics content (Table 1). Positive correlation was observed between their scavenging capacity and phenolic content, suggesting phenolic compounds were mainly attributed to DPPH scavenging capacity of *P. baumii* fruiting body fractions. Free radicals are formed due to stress, fatigue, UV radiation, polluted environment, causing aging and eyes, brain,

skin, immune diseases. Phenolic compounds have the ability to neutralize free radicals, transfer hydrogen and electrons and scavenge free oxygen radicals containing one or more unpaired electrons [27]. Our result suggested the potential exploitation of antioxidant compounds from ethyl acetate fraction in the pharmaceutical industry.

**** Reducing power***

In order to further evaluate the antioxidant activity of *P. baumii* fruiting body fractions, their ferric reducing power was examined with ascorbic acid and quercetin as positive references.

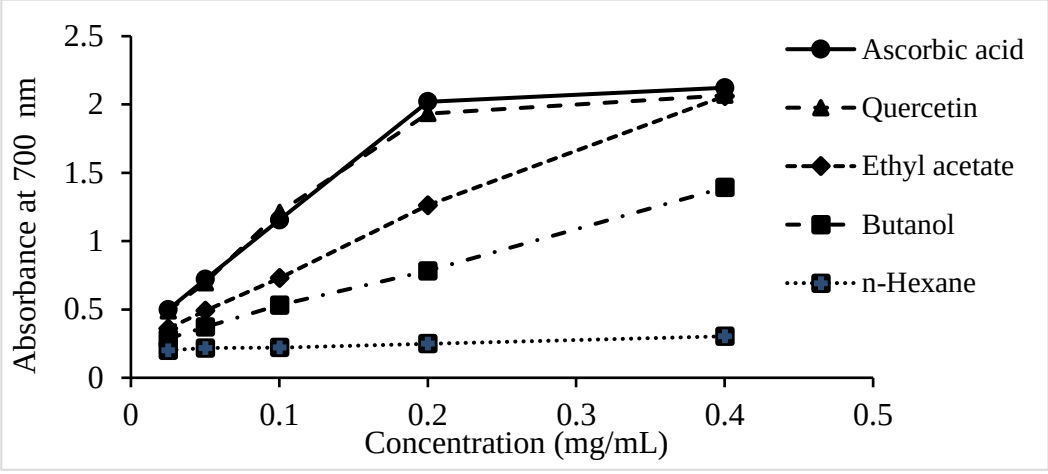

Figure 2. Reducing power of P. baumii fruiting body fractions and reference compounds

Ethyl acetate fraction exhibited the strongest reducing power among *P. baumii* fruiting body fractions. At the concentration of 0.2 mg/mL, the reducing powder of ethyl acetate fraction reached 1.26 while that of ascorbic acid and quercetin were 2.02 and 1.93, respectively. It was approximately equivalent to that of quercetin and ascorbic acid at the concentration of 0.4 mg/mL when they reduced nearly all available ferric ions in the reaction mixture. The concentrations of ethyl acetate fraction and butanol fraction at the absorbance of 0.5 were 0.05 and 0.1 mg/mL, respectively, which is much lower than those of methanol extract and water extract from Korean *P. gilvus* fruiting bodies, 1.88 and 2.24 mg/mL, respectively [9]. The results on reducing the power of fractions from *P. baumii* fruiting body are consistent with the results on DPPH radical scavenging capacity, confirming the antioxidant potential of the Vietnamese *P. baumii* fruiting body, especially ethyl acetate fraction.

**** Alpha-amylase inhibitory activity***

Alpha-amylase is a basic enzyme in the digestive system, which catalyzes the first reaction that converts starch into a mixture of smaller oligosaccharides including maltose, maltotriose, and some α-1,6-linked and α-1,4-linked oligoglucans. Disorders of carbohydrate absorption and metabolism often lead to health problems such as diabetes. Therefore, amylase is one of the starch hydrolyzing enzymes of interest to many scientists in the treatment of diabetes [28].

Table 3. Alpha-amylase inhibitory activity of P. baumii fruiting body fractions

Fractions	Percentage of α-amylase inhibitory activity (%) at different fraction concentration				
	0.25 (mg/mL)	0.5 (mg/mL)	1 (mg/mL)	2 (mg/mL)	4 (mg/mL)
Ethyl acetate	36.31 ± 1.60	62.63 ± 6.54	68.24 ± 0.52	81.12 ± 0.73	91.14 ±1.56
Butanol	2.39 ± 0.36	23.38 ± 2.58	59.64 ± 0.88	85.51 ± 1.71	88.83 ± 0.69
n-Hexane	0.46 ± 0.44	1.67 ± 0.92	4.48 ± 1.3	19.28 ±0.86	24.4 ± 1.89

Ethyl acetate and butanol fractions exhibited much stronger inhibitory activity than the *n*-hexane fractions. From the data on the percentage of α-amylase inhibitory activity, their IC_{50} values were deduced as 0.38 and 0.94 mg/mL, respectively. Wang et al. reported the hypoglycemic effect of ethyl acetate fraction from *P. baumii* fruiting body on streptozotocin-induced diabetic mice. Administration of ethyl acetate fraction at the dose of 400 mg/kg efficiently reduced fasting plasma glucose level, glycated albumin level, increased the insulin level as well as insulin expression level of β-cells and their size and number in diabetic mice [7]. Our data suggest the hypoglycemic effect of ethyl acetate fraction also resulted from the capacity of α-amylase inhibition.

3. Conclusions

Fractions of *P. baumii* fruiting bodies extracts possessed a variety of second metabolites such as phenolics, flavonoids, and terpenoids. Ethyl acetate and butanol fraction contained high phenolic content. Specifically, roughly half of the ethyl acetate fraction was phenolics, which was suggested to contribute to strong DPPH free radical scavenging capacity, ferric reducing power, and α-amylase inhibitory activity. The results suggest the potential application of Vietnamese *P. baumii* fruiting bodies as a source of antioxidants and antidiabetic agents in pharmaceutical production.

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