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Original Article

Total Phenolic, Flavonoid Content and Antioxidative, α-amylase Inhibitory Activity of *Phellinus gilvus* Fruiting Body Extracts

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Abstract: Fractions yielded from *Phellinus gilvus* fruiting bodies by liquid-liquid partition of methanol extract contained various bioactive compounds such as phenolics, flavonoids and terpenes. Butanol, water, and ethyl acetate fractions had a large amount of phenolics, 158.12 - 247.31 mg of gallic acid equivalents (GAE)/g and a low amount of flavonoids, 21.74 - 36.05 mg of quercetin equivalents (QE)/g. These values are highly correlated with their antioxidative activity including 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging potential with IC_{50} values of 84.5 -108.44 μg/mL and reducing power. These fractions showed moderate α-amylase inhibitory activity with IC_{50} values of 4.77, 10.69, and 3.9 mg/mL, respectively. *P. gilvus* fractions exhibited negligible inhibitory activity on the growth of tested Gram-positive and Gram-negative bacterial strains at the concentration of 20 mg/mL. The results suggest the utility of butanol, water, and ethyl acetate fractions from fruiting bodies of *P. gilvus* collected in Vietnam for further characterization to apply for diabetes or anti-aging prevention.

Keywords: Phellinus gilvus, antioxidative activity, α-amylase inhibition.

1. Introduction

Mushrooms have recently become a potent candidate for functional foods because of their high contents of protein, crude fiber, minerals, and vitamins [1], as well as physiologically beneficial bioactive substances [2]. Some species of mushrooms which belong to family Hymenochaetaceae (Basidiomycetes) like *P. gilvus*, *P. linteus*, *P. Baumii*, and *P. nigricans* [3] are well known for representing an unlimited source of antitumor or immuno-stimulating, anti-inflammatory polysaccharides [4, 5], and anti-inflammatory phenolic metabolites [6]. *Phellinus* species have traditionally been used as a folk medicine for a variety of human diseases such as digestive disorder, diarrhea, hemorrhage,... in several Asian countries [7, 8].

P. gilvus known as Mustard yellow polypore is a plant pathogen and mainly distributed in North America's hardwood forests. In Vietnam, the *P. gilvus* was found in

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Thua Thien Hue [9] and Cat Ba National Park [10]. The important identification features for *P. gilvus* include the yellowish-brown to orange-brown flesh, the purplish-brown pore surface composed of very tiny pores, and the red-then-black (or just black) reaction of its surfaces to KOH. *P. gilvus* has advantages over many *Phellinus* species due to its short growth period (3 months), making it cheaper to produce [11].

Previous research has found various compounds, biological activities, and benefits of *P. gilvus*. The pretreatment with the water extracts of *P. gilvus* could inhibit the increase of total white blood cells, neutrophils, and the level of interleukin-1β in bronchial lavage fluid in lipopolysaccharide (LPS)-challenged rats and might be useful in preventing acute pulmonary inflammation in humans [5]. *P. gilvus* has also significant adhesion- and abscess-reducing effects in a rat peritonitis model [12] and has significant dermal wound healing effects in clinical use [13]. In addition, some biological activities have also been investigated including anti-platelet aggregation, antioxidant, anti-xanthine oxidase, anti-cholinesterase, anti-inflammatory and immunomodulating activity for various *P. gilvus* extracts [14, 15]. Six major compounds were identified from the ethyl acetate extract of *P. gilvus*, and protocatechualdehyde was supposed to be the major phenolic compound of *P. gilvus* responsible for its DPPH free radical scavenging activity and its inhibitory effects on LPS-induced NO production in RAW264.7 cells [6]. Protocatechualdehyde significantly decreased cell viability, caused cell cycle arrest at G0/G1 phase, promoted apoptosis as well as enhanced the complement and coagulation cascades, and the p53 signaling pathway in B16-F10 melanoma cells [16]. A recent study has revealed high contents of total phenolics and flavonoids of methanol extract and hot water extract from the fruiting bodies of *P. gilvus* collected from Korea [17]. In Vietnam, there have been very little research on *Phellinus* species. In a study on the fruit body of *P. igniarius* collected from Pu Huong National Park, inoscavin A, daidzin, ergosterol, and ergosterol peroxide were isolated from its methanolic extracts [18]. A recent study on ethyl acetate fraction of *P. gilvus* fruiting body revealed two sterols and three aromatic hydrocarbons [19].

To date, there has been no information about the biological activities of *P. gilvus* collected in Vietnam. Therefore, this study aims to evaluate some secondary metabolite compositions such as total phenolics, flavonoids, and some *in vitro* biological activities like antioxidative, antibacterial activities of *P. gilvus* to provide data for future bioactive compound characterization as well as the application of *P. gilvus* in Vietnamese medicine.

2. Materials and Methods

2.1. Materials

Phellinus gilvus fruiting bodies were collected from PuMat National Park (Nghe An province).

Bacterial strains including *Bacillus subtilis* ATCC 6633, *Staphylococus aureus* ATCC 13709, *Escherichia coli* ATCC 25922, *Samonella enterica* ATCC 13076 and *S. typhimurium* were obtained from Institute of Chemistry, Vietnam Academy of Science and Technology.

Chemicals including α-amylase, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, quercetin, gallic acid, Folin-Ciocalteau reagents as well as solvents were purchased from Sigma and Merck with analytical grade.

2.2. Methods

2.2.1. Sample Extraction and Fractionation

Fruiting bodies of *P. gilvus* were washed with distilled water to remove adhering debris and dust, dried and chopped into small pieces, and then soaked in methanol for one week and extracted in an ultrasonic bath for 30 mins at room temperature. The extraction was performed in three replicates. The extracts were mixed, filtered by filter paper, and concentrated in a rotary evaporator at 40 °C, and then

lyophilized. The crude extract was further fractionated sequentially in different solvents including n-hexane, ethyl acetate, butanol, and water. The four fractions were concentrated by vacuum evaporation and stored at -20 °C until use.

2.2.2. Thin Layer Chromatography

Extract solutions were prepared at the concentration of 10 mg/ml in absolute methanol. The solvent system used as the mobile phase includes toluene/ethyl acetate/ acetone/formic acid 5: 3: 1: 1,…). The plate was sprayed with 5% sulfuric acid solution, dried at 110 °C until the spots appeared, and observed under visible light and UV radiation at 302 nm.

2.2.3. Determination of Total Phenolics and Flavonoids

Total phenolics content was evaluated according to Waterhouse using gallic acid as the standard [20]. Absorption is measured at 765. The results were expressed as mg gallic acid equivalents (GAE) per gram dry weight of each extract by comparison with the gallic acid standard curve.

Total flavonoids were determined following the method described by Sapkota et al., [21] using quercetin as the standard. Absorption is measured at 415 nm. The flavonoid content of extracts was calculated in mg quercetin equivalents (QE) per gram dry weight of each extract.

2.2.4. Antioxidant Activity

Antioxidant activity was evaluated by determining free radical scavenging potential using DPPH according to Blois [22]. The reaction mixture contained 20 µL of extract solutions in methanol and 180 µL of 0.1 mM DPPH solution. Ascorbic acid was used as the reference. The absorbance of the solutions was measured at the wavelength of 517 nm. DPPH scavenging activity was calculated as:

DPPH scavenging activity (%) = $[(A_{control} - A_{sample})/(A_{control})]\times 100$ where $A_{control}$ represents the absorbance of the control and A_{sample} is the absorbance of the test sample. The IC_{50} value is deduced from the logarithm curve of scavenging capacity vs. sample concentration.

2.2.5. Reducing Power Assay

The reducing power of the extracts on Fe^{3+} was determined by the method of Sapkota et al., [21] using ascorbic acid and quercetin as references. The absorbance of the reaction solutions was determined at the wavelength of 700 nm. The result was calculated by the value that the sample increased the absorbance to 0.5, and high absorbance of the reaction mixture revealed high reducing power.

2.2.6. Antibacterial Activity Assay

The antibacterial activity of *P. gilvus* extracts was tested against *B. Subtilis, S. aureus*, *E. coli, S. Enterica,* and *S. typhimurium* by using the agar well diffusion method [23]. Fractions were dissolved in absolute methanol at a concentration of 20 mg/ml. Methanol served as a negative control and kanamycin 0.3% as the positive control. Antibacterial activity was determined by measuring the diameter of the inhibition zone formed around the well.

$\Delta D = D - d$

D: diameter of the sterile ring (mm)

d: diameter of the agar well (mm).

2.2.7. Alpha-amylase Inhibitory Assay

Alpha-amylase inhibitory activity of fractions was evaluated according to Geriacheva [24] based on the catalysis of α-amylase (40 IU/mL) to hydrolyze starch (0.5%) to form dextrins with different molecular masses. The fractions were dissolved by dimethyl sulfoxide (DMSO) with a concentration range from 1.25 to 20 mg/ml. The absorbance of the solutions with iodine was determined at the wavelength of 656 nm. Percentage of hydrolyzed starch was calculated as:

Percentage of hydrolyzed starch (%) = [(A - B)/A] × 100 Where A stands for the absorbance of the starch solution at 656 nm and B corresponds to the absorbance of the solution containing α-amylase at 656 nm. The α-amylase inhibition capacity (%) was measured based on percentage of hydrolyzed starch in the sample (with tested fractions) as compared to the control (without tested fractions). IC_{50} values were calculated based on the logarithm curve.

2.2.8. Statistical Analysis

Data were analyzed using Microsoft Excell software and Student's t-test. Results were

expressed as means ± standard deviation. A level of *p-value* less than 0.05 was considered to be significant.

3. Results and Discussion

3.1. Thin Layer Chromatography

Secondary metabolite composition of four fractions from *P. gilvus* has been investigated preliminarily by thin-layer chromatographic analysis using a solvent system including toluene/ethyl acetate/acetone/formic acid 5: 3: 1: 1.

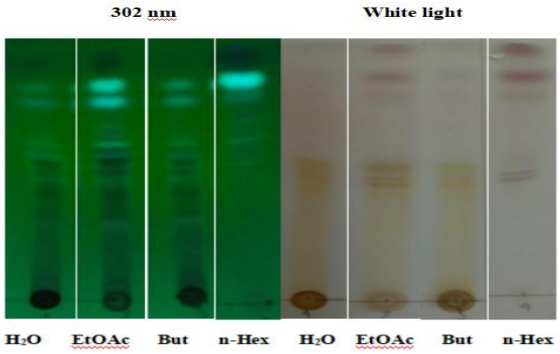

Fig. 1. Thin layer chromatogram of *P. gilvus* fractions. H_2O: water; EtOAc: ethyl acetate; But: butanol; n-Hex: n-hexane.

As a result, all *P. gilvus* fractions contained various compounds with distinct bands. Butanol, water, and ethyl acetate fractions gave more bands than n-hexane fraction especially yellow bands. However, purple bands are predominant in n-hexane fraction. The chromatogram revealed fractions from *P. gilvus* possessed terpenoids (with purple bands under white light), flavonoids (yellow or orange under white light), and phenolic acids (fluorescent bands under 302 nm radiation) (Figure 1). Previous studies showed that *Phellinus* spp. extracts (*P. rimosus, P. wahlbergii, P. nigricans*) contain a large amount of terpenoids in water fraction and ethanol fraction [25].

3.2. Total Phenolics and Flavonoids Content

Phenolic compounds play an integral role in human diets because of a wide variety of their beneficially biological activities including antioxidant, antibacterial, antiviral, antihypertensive, antilipoperoxidant, hepatoprotective, and anti-carcinogenic activities [26]. From the standard curves, the content of total phenolics and flavonoids of *P. gilvus* fractions were calculated and the results were represented in Table 1.

Table 1. Total phenolic and flavonoid content of *P. gilvus* fractions

Fraction	Total phenolic content (mg GAE/g fraction)	Total flavonoid content (mg QE/g fraction)
Water	223.45 ± 20.14^{a}	$27.28 \pm 6.93^{a, b}$
Butanol	247.31 ± 12.57^{a}	36.05 ± 3.6^{a}
Ethyl acetate	158.12 ± 27.46^{b}	21.74 ± 3.27^{b}
n-Hexane	18.04 ± 3.72^{c}	1.56 ± 0.87^{c}

GAE: gallic acid equivalents, QE: quercetin equivalents,
$^{a, b, c, d}$: significant difference among fractions ($p<0.05$).

The result showed that *P. gilvus* fractions had quite a large amount of phenolics except n-Hexane fraction. The total content of phenolics in the butanol and water fractions was the highest. The value in ethyl acetate fraction was slightly lower, about 0.7 times as compared to those of butanol and water fractions. n-Hexane fraction had very low content of phenolics. This result confirmed the detection of compounds from thin layer chromatogram. The level of phenolic compounds in ethyl acetate, butanol, and water fractions was much higher than that in methanol and hot water extracts from the fruiting bodies of *P. gilvus* collected in Korea (31.17 and 12.83 μg GAE/mg, respectively) [17].

P. gilvus fractions had low level of flavonoids as compared to total phenolics. Butanol and water fractions had the highest content of flavonoids, which is similar as compared to methanolic extract (30.58 mg QE/g sample) that is reported in previous

research [27]. The level of flavonoid compounds of *P. gilvus* was also slightly higher than that of *P. rimosus* and *P. badius* extracts (28.1 and 26.48 mg QE/g, respectively).

3.3. DPPH Scavenging Activity

DPPH was widely used to evaluate the antioxidant activity of plant extracts because it possesses a proton free radical. In the presence of an antioxidant, its proton radical obtains one more electron, its purple color fades [28]. Table 2 illustrates IC_{50} values for DPPH scavenging activity of *P. gilvus* fractions.

Table 2. DPPH scavenging activities of *P. gilvus* fractions compared with ascorbic acid

Sample	IC_{50} (µg/ml)
Water fraction	84.5 ± 14.58^{a}
Butanol fraction	85.21 ± 15.44^{a}
Ethyl acetate fraction	108.44 ± 20.82^{a}
n-Hexane fraction	> 1600
Ascorbic acid	28.99 ± 6.11^{b}

$^{a, b, c, d}$: significant difference among fractions $p<0.05$.

Three fractions of *P. gilvus* had moderate DPPH scavenging capacity except n-hexane fraction. At the concentration of 1.6 mg/mL, the inhibitory percentage of n-hexane fraction only reached 27.01%. A previous report showed similar result, DPPH radical scavenging activity of water fraction of *P. gilvus* collected in Korea was 55% at concentration of 0.1 mg/mL [29]. However, another report showed that ethyl acetate fraction of *P. gilvus* isolated in Korea had the highest DPPH radical scavenging activity with IC_{50} value of 13.34 µg/mL (scavenging 70.38% of DPPH radical). Butanol, water and n-Hexane scavenged 20.62%, 8.61% and 5.46% at concentration of 20 µg/mL, respectively [14]. Furthermore, the various fractions of *P. gilvus* displayed weaker DPPH radical scavenging activity than that in *P. merrillii* fractions with IC_{50} values of 0.66, 0,78, 0.83, and 3.79 mg/mL for ethyl acetate, butanol, water, and n-Hexane fractions, respectively [30].

Plenty of researches have proved that biological compounds such as flavonoids, curcuminoids, phenolics play a significant role in the elimination of free radicals and expose antioxidant activity. The levels of these substances were positively correlated with the free radical scavenging activity [21, 26]. Therefore, for *P. gilvus* in this study, the large amount of phenolics and flavonoids of the water, butanol, and ethyl acetate fractions showed a high correlation to their DPPH radical scavenging capability. This result suggested the contribution of phenolic compounds, which have redox properties, adsorbing and neutralizing free radicals [26].

3.4. Reducing Power

The antioxidant activity of *P. gilvus* extracts was also evaluated through their reducing capacity on Fe^{+3} in ferric chloride to ferrous (Fe^{+2}).

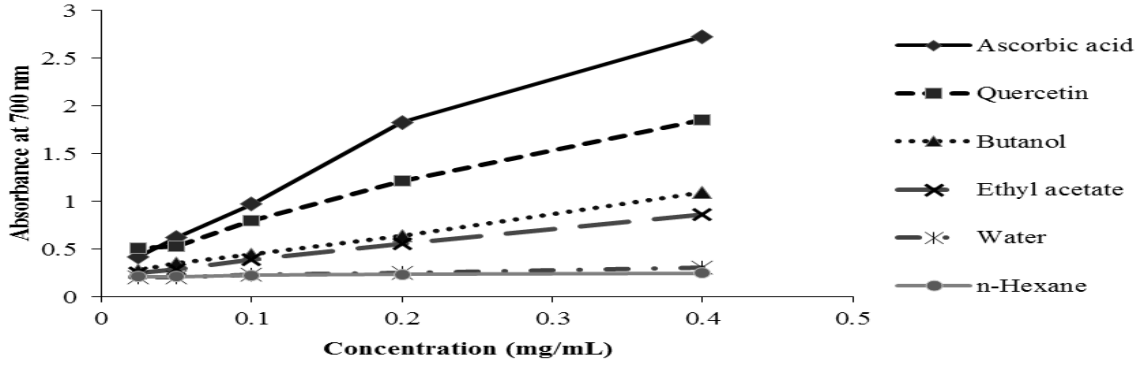

Fig. 2. Reducing power of *P. gilvus* fractions.

The result showed that the butanol and ethyl acetate fractions of *P. gilvus* exhibited moderate reducing power as compared with ascorbic acid and quercetin. Butanol fraction revealed the strongest reducing power among the *P. gilvus* fractions. Ethyl acetate fraction exhibited slightly lower reducing power. At the same concentration of 0.4 mg/mL, the reducing power of butanol fraction reached 1.09, followed by ethyl acetate, water, and n-Hexane fractions, 0.86, 0.31, and 0.25, respectively. The concentration of butanol and ethyl acetate fractions of *P. gilvus* at the absorbance of 0.5 was calculated.

Table 3. Concentration of *P. gilvus* fractions at the absorbance of 0.5 compared with ascorbic acid and quercetin

Sample	Concentration mg/ml (Absorbance 0.5)
Butanol fraction	0.137 ± 0.0031^{a}
Ethyl acetate fraction	0.163 ± 0.0017^{b}
Ascorbic acid	0.037 ± 0.0008^{c}
Quercetin	0.053 ± 0.0066^{d}

$^{a, b, c, d}$: significant difference among fractions ($p<0.05$).

The concentration at absorbance 0.5 of butanol fraction and ethyl acetate fraction was approximately 3.7 and 4.4 times higher than that of ascorbic acid, respectively, and 2.6 and 3.1 times higher than that of quercetin, respectively. However, the data showed that reducing power of butanol and ethyl acetate fractions is nearly similar to methanol fraction and hot-water fraction from fruiting bodies of *P. gilvus* collected from Korea (1.88 and 2.24 at concentration of 0.5 mg/mL) in a previous report [8]. Data demonstrated the high correlation between reducing power and DPPH scavenging activity of butanol and ethyl acetate fractions, which probably possess antioxidant compounds with both hydrogen and electron-donating ability.

3.5. Antibacterial Activity

The antibacterial activity of *P. gilvus* fractions was assessed by using agar well diffusion method. The result showed that *P. gilvus* fractions displayed negligibly antibacterial activity at the concentration of 20 mg/mL. There was no inhibition observed on the growth of *S. enterica* and *S. typhimurium*. Figure 3 shows very slight inhibitory activity of *P. gilvus* fractions on the growth of *E. coli* and *S. aureus*.

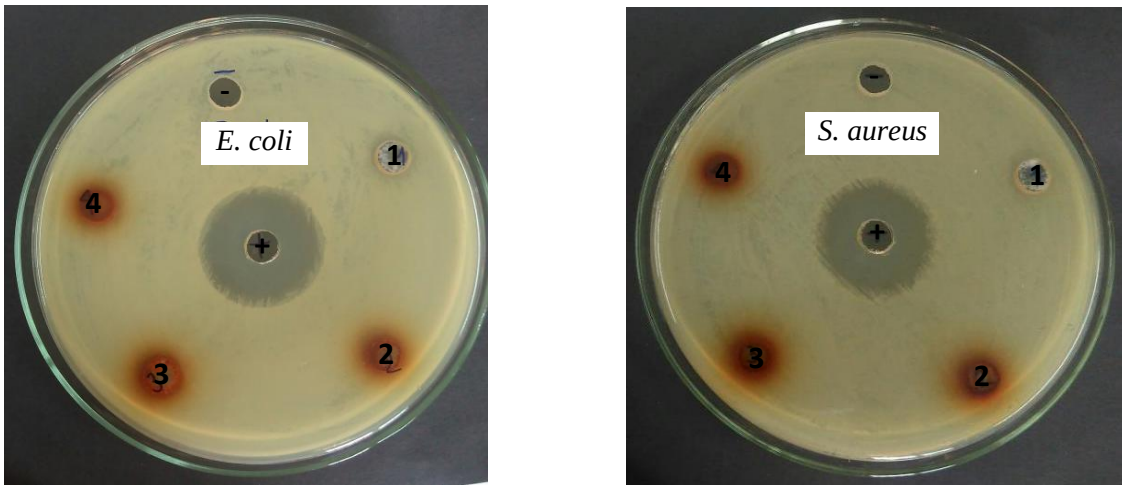

Fig 3. Antibacterial activity of *P. gilvus* fractions
(-): negative control; (+): positive control; 1: n-Hexane; 2: Butanol; 3: Ethyl acetate; 4: Water.

3.6. Alpha-amylase Inhibitory Activity

Salivary and pancreatic α-amylase is a fundamental enzyme in the digestive system and catalyzes the reaction converting starch to a mixture of smaller oligosaccharides including maltose, maltotriose, and some α-(1-6) and α-(1-4) oligoglucans. These are then reacted by α-glucosidases and further hydrolyzed to glucose which is absorbed into the blood-stream. The hydrolysis of this dietary starch proceeds rapidly and leads to raised post-prandial hyperglycemia. It has been shown that activity of human pancreatic α-amylase in the small intestine correlated to an increase in post-prandial glucose content, so the control of which is an essential part in the treatment of type 2 diabetes [31].

Thus, the delay of starch assimilation by α-amylase inhibition plays an important role in the control of diabetes.

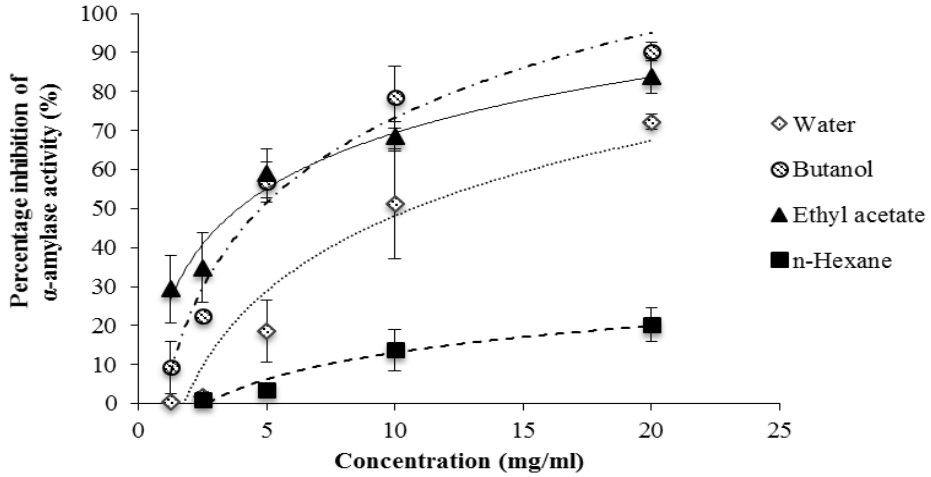

Fig 4. Alpha-amylase inhibitory activity of *P. gilvus* fractions.

The half maximal inhibitory concentration of *P. gilvus* fractions was calculated by constructing the graph of correlation between inhibitory percentage and sample concentration. Ethyl acetate and butanol fractions exhibited stronger inhibition on α-amylase enzyme than other fractions with IC_{50} values of 3.9 and 4.77 mg/mL, respectively. Water fraction had slightly lower inhibitory potential (IC_{50} value of 10.69) while low inhibitory capacity was observed for n-hexane fraction. At the highest concentration of 20 mg/mL, significant and strong α-amylase inhibition was observed for butanol fraction (90.37%) and ethyl acetate fraction (83.91%) whereas moderate and weak inhibition was observed for water fraction (72.24%) and n-Hexane fraction (20.33%), respectively. The result suggests that butanol and ethyl acetate fractions of *P. gilvus* act effectively as α-amylase inhibitors leading to a decrease in starch hydrolysis hence eventually to reduce glucose levels. This is the first report demonstrating potential inhibitory effect of *P. gilvus* on α-amylase activity. Therefore, this study supports the use of *P. gilvus* for further characterization to assess its potential for type II diabetes management.

4. Conclusions

P. gilvus fruiting body fractions possessed a variety of bioactive compounds such as phenolics, flavonoids, and terpenes. *P. gilvus* fractions had a high content of phenolics except for n-hexane fraction, in correlation with antioxidative activity including DPPH scavenging capacity and reducing power. Among of fractions, butanol fraction exposed

the strongest antioxidant activity. It also exerted the highest inhibitory activity on α-amylase. Further characterization of butanol fraction for other biological activities and bioactive compounds, *P. gilvus* fruiting bodies will be a potential source for the production of nutraceuticals.

Reference

[1] P. C. Cheung, Mini-review on Edible Mushrooms as Source of Dietary Fiber: Preparation and Health Benefits, Food Science and Human Wellness, Vol. 2, 2013, pp. 162-166.

[2] T. Anke, Basidiomycetes: A Source for New Bioactive Secondary Metabolites, Progress in Industrial Microbiology, Vol. 27, 1989, pp. 51-66.

[3] Y. C. Dai, M. Q. Xu, Studies on the Medicinal Polypore, *Phellinus baumii* and its Kin, *P. linteus*, Mycotaxon, Vol. 67, 1998, pp. 191-200.

[4] S. B. Han, C. W. Lee, Y. J. Jeon, N. D. Hong, I. D. Yoo, K. H. Yang, H. M. Kim, The Inhibitory Effect of Polysaccharides Isolated from *Phellinus linteus* on Tumor Growth and Metastasis, Immunopharmacology, Vol. 41, 1999, pp. 157-164.

[5] B. S. Jang, J. C. Kim, J. S. Bae, M. H. Rhee, K. H, Jang, J. C. Song, S. C. Park, Extracts of *Phellinus gilvus* and *Phellinus baumii* Inhibit Pulmonary Inflammation Induced by Lipopolysaccharide in Rats, Biotechnology Letters, Vol. 26, 2004, pp. 31-33.

[6] Z. Q. Chang, E. Gebru, S. P. Lee, M. H. Rhee, J. C. Kim, H. Cheng, S. C. Park, *In vitro* Antioxidant and Anti-inflammatory Activities of Protocatechualdehyde Isolated from *Phellinus gilvus*, Journal of Nutritional Science and Vitaminology, Vol. 57, 2011, pp. 118-122.

[7] I. K. Lee, B. S. Yun, Highly Oxygenated and Unsaturated Metabolites Providing a Diversity of Hispidin Class Antioxidants in the Medicinal Mushrooms *Inonotus* and *Phellinus*, Bioorganic and medicinal chemistry, Vol. 15, 2007, pp. 3309-3314.

[8] K. N. Yoon, H. S. Jang, Antioxidant and Antimicrobiol Activities of Fruiting Bodies of *Phellinus gilvus* Collected in Korea, Korean J. Clin Lab, Vol. 48, 2016, pp. 355-364.

[9] Vietnam Association for Conservation of Nature and Environment, http://www.vacne.org.vn/da-dang-cac-taxon-va-yeu-to-dia-ly-cau-thanh-khu-he-nam-lon-thua-thien-hue/21869.html/, 2010 (accessed on: September 12th, 2021) (in Vietnamese).

[10] P. T. H. Giang, Initial Research Findings of the Species Composition of Macrofungi Flora in Cat Ba National Park, Journal of Tropical Science and Technology, Vol. 5, 2013, pp. 51-58 (in Vietnamese).

[11] M. B. Ellis, J. P. Ellis, Fungi Without Gills (Hymenomycetes and Gasteromycetes): An Identification Handbook, Springer Science and Business Media, 1990, pp. 149-152.

[12] J. S. Bae, S. J. Ahn, H. Yim, K. H. Jang, H. K. Jin, Prevention of Intraperitoneal Adhesions and Abscesses by Polysaccharides Isolated from *Phellinus* spp. in a Rat Peritonitis Model, Annals of Surgery, Vol. 241, 2005, pp. 534-540.

[13] J. S. Bae, K. H. Jang, S. C. Park, H. K. Jin, Promotion of Dermal Wound Healing by Polysaccharides Isolated from *Phellinus gilvus* in Rats, Journal of Veterinary Medical Science, Vol. 67, 2005, pp. 111-114.

[14] Z. Q. Chang, M. H. Hwang, M. H. Rhee, K. S. Kim, J. C. Kim, S. P. Lee, S. C. Park, The *in vitro* Anti-platelet, Antioxidant and Cellular Immunity Activity of *Phellinus gilvus* Fractional Extracts, World Journal of Microbiology and Biotechnology, Vol. 24, 2008, pp. 181-187.

[15] N. Y. Ki, S. J. Hyung, Anti-xanthine Oxidase, Anti-cholinesterase, and Anti - inflammatory Activities of Fruiting Bodies of *Phellinus gilvus*, Korean J. Clin Lab Sci, Vol. 50, 2018, pp. 225-235.

[16] Z. Shi, J. Qinshen, Y. Taihen, Z. Jianxun, L. Yougui, *Phellinus Gilvus*-derived Protocatechualdehyde Induces G0/G1 Phase Arrest and Apoptosis in Murine B16-F10 Cells, Molecular Medicine Reports, Vol. 21, 2020, pp. 1107-1114.

[17] K. N. Yoon, H. S. Jang, Antioxidant and Antimicrobial Activities of Fruiting Bodies of *Phellinus gilvus* Collected in Korea, Korean Journal of Clinical Laboratory Science, Vol. 48, 2016, pp. 355-364.

[18] N. T. Thanh, N. N. Tuan, H. V. Trung, T. D. Thang, Phenolic Compounds and Steroid from the Fruit Body of *Phellinus igniarus* in Vietnam, VNU Journal of Science: Natural Sciences and Technology, Vol. 32, 2016, pp. 264-268 (in Vietnamese).

[19] D. X. Hung, L. H. Nga, T. D. Thang, D. N. Quang, Chemical Constituents of the Ethyl Acetae Fraction of the Fruit Bodies of *Phellinus gilvus*, Vietnam Journal of Science and Technology, Vol. 56, 2018, pp. 246-251.

[20] A. L. Waterhouse, Determination of Total Phenolics, *In* Current Protocols in Food Analytical Chemistry, 2002, pp. I1.1.1 - I1.1.8.

[21] K. Sapkota, S. E. Park, J. E. Kim, S. Kim, H. S. Choi, H. Schun, S. P. Voravuthikunchai, Antoxidant and Antimelanogenic Properties of Chestnut Flower

Extract, Biotech and Biochem, Vol. 74, 2010, pp. 1527-535.

[22] M. S. Blois, Antioxidant Determination by the Use of a Stable Free Radical, Nature, Vol. 181, 1958, pp. 1199-1200.

[23] P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, R. H. Yolken, Manual of Clinical Microbiology, 6th Edition, ASM Press, Washington, DC, 1995, pp. 15-18.

[24] N. V. Mui, Practical Biochemistry, Vietnam National University Publishing House, Hanoi, 2007, pp. 108-111 (in Vietnamese).

[25] P. Laovachirasuwan, C. Judprakob, B. Sinaphet, M. Phadungkit, *In vitro* Antioxidant and Antimutagenic Activities of Different Solvent Extracts of *Phellinus* spp, International Food Research Journal, Vol. 23, 2016, pp. 2608-2615.

[26] J. Pokomý, Natural Antioxidants for Food Use, Trends in Food Science and Technology, Vol. 2, 1991, pp. 223-227.

[27] J. F. A. Zavala, B. A. S. Espinoza, M. R. C. Valenzuela, Y. C. López, Antioxidant and Antifungal Potential of Methanol Extracts of *Phellinus* spp. from Sonora, Mexico, Revista Iberoamericana de Micologia, Vol. 29, 2012, pp. 132-138.

[28] T. Yamaguchi, H. Takamura, T. Matoba, J. Terao, HPLC Method for Evaluation of the Free Radical-scavenging Activity of Foods by Using 1,1,- diphenyl-2-picrylhydrazyl, Bioscience, Biotechnology, and Biochemistry, Vol. 62, 1998, pp. 1201-1204.

[29] S. H. Kim, J. H. Lim, C. Moon, S. H. Park, S. H. Kim, D. H. Shin, S. C. Park J. C. Kim, Antiinflammatory and Antioxidant Effects of Aqueous Extract from *Phellinus gilvus* in Rats, Journal of Health Science, Vol. 57, 2011, pp. 171-176.

[30] H. Y. Chang, Y. L. Ho, M. J. Sheu, Y. H. Lin, M. C. Tseng, S. H. Wu, G. J. Huang, Y. S. Chang, Antioxidant and Free Radical Scavenging Activities of *Phellinus merrillii* Extracts, Botanical Studies, Vol. 48, 2007, pp. 407-417.

[31] H. G. Eichler, A. Korn, S. Gasic, W. Prison, J. Businger, The Effect of New Specific α-amylase Inhibitor on Post-prandial Glucose and Insulin Excursions in Normal Subjects and Type II (Non-insulin Dependent) Diabetic Patients, Diabetologia, Vol. 26, 1984, pp. 278-281.