

# Antioxidant and anti-*Helicobacter pylori* activities of *Hericium erinaceus* mycelium and culture filtrate

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## History

- Received: Dec 23, 2020
- Accepted: Mar 26, 2021
- Published: Mar 31, 2021

DOI: 10.15419/bmrat.v8i3.665



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## ABSTRACT

**Introduction:** *Hericium erinaceus* is known as a medicinal edible mushroom owing to its antimicrobial, antioxidant, anti-tumor and immunomodulatory effects. *Helicobacter pylori* infection is one of the major health concerns worldwide due to its high rate in global populations, frequent recurrence, and rapid emergence of drug-resistant strains. The present study aims to investigate antioxidant, anti-*H. pylori*, and urease inhibitory activities of solvent fractions from *H. erinaceus* mycelium and culture filtrate. **Methods:** *H. erinaceus* mycelium was purely cultured in a liquid medium. A polysaccharide fraction was obtained from the culture filtrate by precipitation with ethanol. The mycelium and culture filtrate were extracted by liquid extraction to obtain solvent-soluble fractions. The antibacterial effects of these fractions were determined using paper disc diffusion and broth microdilution assays. Urease inhibition was determined using the salicylate-hypochlorite method. The antioxidant activity of *H. erinaceus* was evaluated via 2,2,1-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. **Results:** The ethyl-acetate (EtOAc) fractions derived from *H. erinaceus* culture filtrate (fEtOAc Fr.) and mycelium (mEtOAc Fr.) showed the strongest anti-*H. pylori* activity with MIC (MBC) of 1.25 – 1.5 (5.0 – 7.5) mg/mL and potential urease inhibitory activity with IC<sub>50</sub> of 0.34 – 0.35 mg/mL. In addition, fEtOAc Fr. exhibited the greatest antioxidant activity (IC<sub>50</sub>, 11.83 mg/mL), which was slightly stronger than that of mEtOAc Fr. (IC<sub>50</sub>, 14.75 mg/mL). Moreover, our study also found that the water fractions from the culture filtrate (fWater Fr.) and the mycelium (mWater Fr.) displayed considerable inhibitory activities against bacterial urease (IC<sub>50</sub>, 1.26 – 1.40 mg/mL), although they showed low or no anti-*H. pylori* and antioxidant activities. **Conclusion:** The present study revealed that the EtOAc fractions derived from the *H. erinaceus* mycelium and culture filtrate potentially have anti-*H. pylori*, anti-urease and antioxidant activities. These results suggest that *H. erinaceus* mycelium and culture filtrate could be utilized to develop functional foods and nutraceuticals to prevent *H. pylori* infection. More research is needed to prove the safety of the *H. erinaceus* mycelium and culture filtrate fractions and their *in vivo* efficacy in the treatment of *H. pylori* infection.

**Key words:** Antibacterial activity, Antioxidant, Culture filtrate, *Helicobacter pylori*, *Hericium erinaceus*, Mycelium

## INTRODUCTION

*Hericium erinaceus* (Bull.) Pers., known as lion's mane mushroom, grows on old or dead hardwood trees in America and Asia and is widely consumed due to its nutritional qualities and health benefits. The mushroom has long been used as a folk medicine to treat various human diseases in several East Asian countries<sup>1</sup>. It provides numerous essential nutrients and constituents such as polysaccharides, proteins, lectins, phenols, isoindolinones, hericenones, erinacine terpenoids, and sterols, several of which possess various pharmacological properties<sup>2-4</sup>. *H. erinaceus* extracts showed to be effective in stimulating the synthesis of immune system components<sup>2,5</sup>, which contribute to inhibiting

tumor cell growth<sup>6</sup>. The mushroom displayed clinical potential in relieving inflammatory bowel disease by regulating gut microbiota and immune system<sup>7</sup>. Polysaccharide fraction derived from *H. erinaceus* has also induced immunomodulating and anti-tumor effects<sup>6</sup>, anti-gastritis activity, and significantly enhanced skin antioxidant enzymes that help retard skin aging<sup>8</sup>. In addition, the fruiting body of *H. erinaceus* has been traditionally used to ameliorate gastrointestinal disorders and treat symptoms related to gastric ulcers<sup>7,9,10</sup>. *H. erinaceus* extracts were found to have antimicrobial activities against both Gram-positive and -negative pathogenic bacteria<sup>11,12</sup>, and both antibiotic-resistant and -susceptible *Helicobacter pylori*<sup>13</sup>, a human gastrointestinal pathogen involved in gastritis, duodenal ulcers, and gastric can-

**Cite this article :** Ngan L T M, Vi N T, Tham D T M, Loan L T T, Ho P T, Hieu T T. **Antioxidant and anti-*Helicobacter pylori* activities of *Hericium erinaceus* mycelium and culture filtrate.** *Biomed. Res. Ther.*; 8(3):4267-4276.

cer<sup>14,15</sup>. Ethanol extracts from *H. erinaceus* fruiting bodies were reported to exhibit growth inhibitory effects on *H. pylori* by *in vitro* and/or *in vivo* studies<sup>9,10,13</sup>.

Besides, extracts derived from the mycelium culture of *H. erinaceus* were reported to be active against pathogenic bacteria<sup>11</sup>, hepatocarcinoma cells<sup>16</sup>, stimulate nerve growth factor synthesis<sup>17</sup>, and prevent oxidative stress in human gastric mucosa epithelium cell<sup>18</sup>. The pure culture of *H. erinaceus* mycelium has been recently being studied and developed, especially for the production of nutraceuticals and pharmaceuticals. However, there is less information focusing on the antibacterial activity of *H. erinaceus* mycelium against *H. pylori*. Therefore, the present study was aimed to assess the possible antioxidant effect and growth-inhibitory, bactericidal, and urease inhibitory activities of the polysaccharide and solvent fractions extracted from mycelium and culture filtrate of *H. erinaceus* toward *H. pylori*.

## MATERIALS — METHODS

### Reagents

Brucella broth (BB), brain heart infusion broth (BHIB), and bacto-agar were purchased from Becton Dickinson, Inc. (Franklin Lakes, NJ). Newborn bovine serum (NBS) was purchased from Hyclone (Longan, UT). Tryptone and yeast extract were provided by Merck (Kenilworth, NJ) and glucose was provided by HiMedia (Kennett Square, PA). Amoxicillin ( $\geq 98\%$ ) was provided by Santa Cruz Biotechnology Inc. (Dallas, TX). Solvents, hexane 96%, ethyl acetate (EtOAc) 99.8%, absolute ethanol (EtOH), and methanol (MeOH) 99.9% were purchased from Scharlau (Barcelona, Spain). All other chemicals and reagents used in this study were of analytical grade quality and available commercially.

### Broth culture of *H. erinaceus* and solvent extraction of the mycelium and culture filtrate

*Hericium erinaceus* mycelium was provided and identified by Dr. Pham Thanh Ho, Laboratory of Bio-transformation, Faculty of Biology and Biotechnology, VNUHCM University of Science, Ho Chi Minh City, Vietnam. *H. erinaceus* mycelium could produce fruiting bodies in the laboratory conditions. The mycelium and fruiting bodies were cultured and stored at 4 °C.

Four pieces of *H. erinaceus* mycelium (1 cm<sup>2</sup>/each) from 8 day-old culture on agar medium were inoculated in 500 mL bottles containing 200 mL liquid

medium with the following composition: 20% potato extract, 2% glucose, 0.2% yeast extract, and 0.2% tryptone. The broth cultures were incubated at 25 ± 2 °C and shaken at 150 rpm for 7 days<sup>11</sup>. After that, the culture broths were filtered to obtain the mushroom mycelium and culture filtrate for solvent extraction, as shown in **Figure 1**.

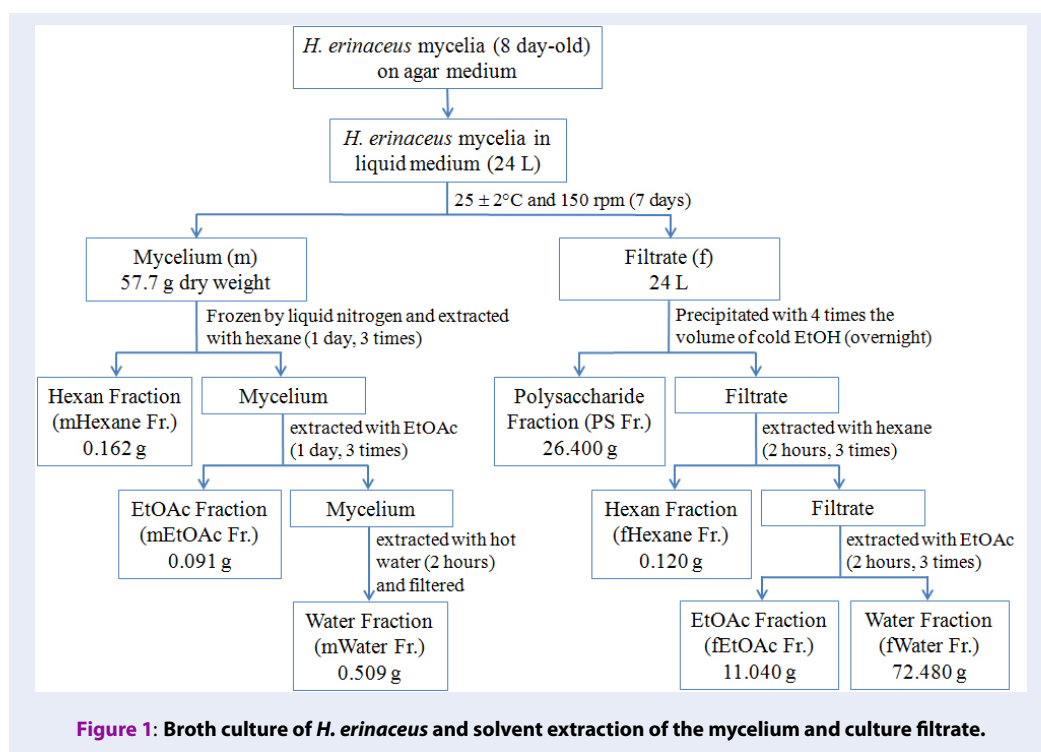
The dry mycelium was disrupted by liquid nitrogen in 30 minutes. The mycelium was then finely grounded and extracted with 200 mL hexane for 1 day (3 times) and further extracted sequentially with 200 mL EtOAc for 1 day (3 times) and 500 mL hot water for 2 hours. The extracts were filtered, and a rotary evaporator was used to remove the solvents to obtain a hexane-soluble fraction (mHexane Fr.), EtOAc-soluble fraction (mEtOAc Fr.), and water-soluble fraction (mWater Fr.) (**Figure 1**).

The culture filtrate was precipitated with 4 times volume of cold absolute EtOH (1 °C) overnight and then centrifuged to obtain the crude polysaccharide fraction (precipitant). The polysaccharide fraction (PS Fr.) was dissolved in sodium phosphate buffer buffer (30 mM, pH 7) to a 200 mg/mL stock concentration and stored at — 20 °C until further use. After the precipitation, the filtrate was then fractionated sequentially three times with an equal volume of hexane for 2 hours, and then three times with an equal volume of EtOAc for 2 hours using the liquid-liquid method. The solvent-soluble fractions were condensed to dryness by a rotary evaporator at 42 °C to gain the hexane fraction (fHexane Fr.), EtOAc fraction (fEtOAc Fr.), and water fraction (fWater Fr.) (**Figure 1**).

### *H. pylori* strain and culture condition

The *H. pylori* strain (ATCC 43504) was provided by the Oxford University Clinical Research Unit in Vietnam (OUCRU-VN) and identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). The bacterial strain was stored in BHIB medium supplemented with 25% glycerol and placed in a nitrogen liquid container until use.

The *H. pylori* strain was cultured on Brucella agar supplemented with 10% newborn bovine serum, and then incubated at 37 °C in 3 days under microaerophilic condition created by a 2.5 L Oxoid anaerojar and Oxoid Campygen sachet (Thermo Fisher Scientific, Waltham, MA, USA). The bacterial suspensions used for bioassay were suspended in Brucella broth supplemented with 5% NBS using a 72-h subculture of *H. pylori* on Brucella agar. Bacterial density was determined using McFarland turbidity standards.



**Figure 1: Broth culture of *H. erinaceus* and solvent extraction of the mycelium and culture filtrate.**

## Antibacterial assay

### Paper disc diffusion assay

The antibacterial activity of the fractions extracted from the *H. erinaceus* mycelium and culture filtrate was evaluated using paper-disc diffusion method, as previously described<sup>19</sup>. In brief, 100  $\mu$ L of *H. pylori* suspensions ( $10^8$  CFU/mL) were evenly spread on Brucella agar medium in petri plates ( $\varnothing$  90 mm, SPL Life Sciences, Korea). Sterile paper discs (1 mm thickness, 6 mm diameter) from Whatman/ GE Healthcare (UK) impregnated with 20  $\mu$ L of respective test material (500  $\mu$ g/ $\mu$ L) in DMSO were placed on the plate surface. DMSO (20  $\mu$ L/disc) and amoxicillin (10  $\mu$ g/disc) were similarly prepared and used as negative and positive controls, respectively. The test and control plates were incubated at 37 °C under the microaerophilic condition for 3 days. After incubation, the inhibition zone was measured. The assay was performed in duplicate three times. The inhibitory responses were classified as follows: very strong response with inhibition zone diameter > 28 mm, strong response with inhibition zone diameter of > 17 – 28 mm, moderate response with inhibition zone diameter of > 13 – 17 mm, weak response with inhibition zone diameter of 8 – 13 mm, and little or no response with inhibition zone diameter < 8 mm.

### MIC and MBC assay

The minimal inhibitory concentration (MICs) and minimal bactericidal concentration (MBCs) of the fractions extracted from *H. erinaceus* mycelium and culture filtrate towards *H. pylori* were evaluated using broth microdilution method, as reported previously<sup>19</sup>. Briefly, the fractions in DMSO (10  $\mu$ L each) at various final concentrations (0 – 10 mg/mL) were added to each well of sterile 96-well plates, which contained 40  $\mu$ L of Brucella broth medium supplemented with 5% NBS. Subsequently, 50  $\mu$ L bacterial suspension ( $5 \cdot 10^6$  CFU/mL) from cultures on Brucella agar was seeded into each well. DMSO and amoxicillin served respectively as negative and positive controls and were similarly prepared. The plates were incubated at 37 °C in a gas jar under microaerophilic conditions and shaken at 50 rpm for 48 hours. MIC values were determined as the lowest concentrations that showed bacterial growth inhibition using resazurin as an indicator. MBC values of the test fractions were performed following the MIC assays in 24-well plates with Brucella agar medium as previously described<sup>20</sup>. MIC and MBC values of each test sample were results from at least three independent experiments performed in triplicate ( $n \geq 9$ ).

### Preparation of *H. pylori* urease and *in vitro* inhibition of urease activity

*H. pylori* urease crude extract was prepared as reported previously<sup>21</sup> with slight modification. Briefly, an amount of 500 mg of *H. pylori* cell mass from 72-h cultures on NBS-supplemented Brucella agar was spread as thinly as possible on the wall of a polypropylene tube and stored in a nitrogen liquid container for 15 minutes. The cell mass was then thawed at room temperature and added with 5 mL of 20 mM sodium phosphate buffer (pH 7.3) containing EDTA (1 mM). Finally, the mixture was centrifuged at  $12,000 \times g$ ,  $4^\circ C$  for 30 minutes, and the supernatant was filtered using a  $0.22 \mu m$  Millex GV Millipore filter. The protein content was determined using Bradford protein assay. Bovine serum albumin (BSA) was used as a protein standard.

*H. pylori* urease inhibitory activity of the fractions extracted from *H. erinaceus* mycelium and culture filtrate was assayed in 96-well plates using the salicylate-hypochlorite method with minor modification<sup>22</sup>. In brief,  $50 \mu L$  of  $0.25 \mu g$  *H. pylori* urease crude preparation (0.04 urease units) in EDTA-sodium phosphate buffer (pH 7.3) was added to each well containing  $50 \mu L$  of the test fraction at various concentrations. The plates were preincubated at  $37^\circ C$  and shaken at 50 rpm for 90 minutes. An amount of  $50 \mu L$  of 5 mM urea in phosphate buffer saline (PBS) was added into each well. After 30 minutes of incubation, a stop solution consisting of  $35 \mu L$  of solution A (146% Na salicylate + 0.1% sodium nitroprusside) and  $65 \mu L$  of solution B (1.78% NaOH + 11.57% Na citrate + 0.54% active NaOCl) were supplemented in sequence to each well. The plates were incubated for 30 minutes at  $37^\circ C$  for color development. The ammonia production released from the hydrolysis of urea by urease activity was quantified by measuring absorbance on the microplate reader at 625 nm using ammonium chloride as a standard. Thiourea as a standard reference was similarly prepared. Inhibition rate (I%) was calculated by the following formula:  $I (\%) = [1 - (OD_{625} \text{ test sample} - OD_{625} \text{ corresponding background}) / (OD_{625} \text{ control} - OD_{625} \text{ blank})] \times 100$ . Urease inhibition activity of the fractions was displayed as 50% inhibitory concentration ( $IC_{50}$ ), which was defined as the concentration of the fractions required to decrease urease activity to 50% of the control value. The assay was performed in duplicate three times.

### DPPH free radical scavenging assay

The antioxidant activities of the fractions extracted from *H. erinaceus* mycelium and culture filtrate

were evaluated via 2,2,1-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity with slight modification<sup>11</sup>. The experiment was performed in 96-well plates. The fraction solutions in MeOH at various concentrations from 0 – 50 mg/mL were placed in the wells, and  $150 \mu L$  of  $380 \mu M$  DPPH in MeOH was then added to each well. Blank and background wells, which contained MeOH and the fractions, respectively, without DPPH, were also prepared. The plates were placed in the dark and incubated at  $37^\circ C$  with rotation at 75 rpm for 30 minutes. Subsequently, the absorbance measurements were determined at 515 nm on a MicroLISA Plus microplate reader (Micro Lab Instruments, India). Acid ascorbic (0 – 0.5 mg/mL) served as a positive control and was similarly prepared. The percent radical scavenging activity was calculated as follows: percent scavenging effect (%) =  $[1 - (OD_{treatment} - OD_{background}) / (OD_{control} - OD_{blank})] \times 100$ , where  $OD_{control}$ ,  $OD_{blank}$ ,  $OD_{treatment}$ , and  $OD_{background}$  are absorbance values of the controls, blanks, fraction treatments, and background wells, respectively.

### Data analysis

All bioassays were repeated three to five times in triplicate. The inhibition zones of the test fractions were presented in mean values ( $\pm$  SD). DPPH  $IC_{50}$  and urease  $IC_{50}$  (half maximal inhibitory concentration) values were calculated using GraphPad Prism 5 software program (GraphPad Software, Inc., La Jolla, CA, USA). The  $IC_{50}$  values of the treatments would be declared significantly different if their 95% confidence intervals did not overlap.

## RESULTS

### Solvent extraction of the mycelium and culture filtrate of *H. erinaceus*

After 7 days of incubations, the culture broths of *H. erinaceus* were filtered to obtain 57.7 g dry weight of the mycelium and 24 L of its culture filtrate. Solvent-soluble fractions obtained from the dry mycelium and culture filtrate are presented in **Figure 1**.

From the dry mycelium (57.7 g), the three fractions of mHexane Fr., mEtOAc Fr. and mWater Fr. were obtained, and weighed as 0.162, 0.091 and 0.509 g, respectively (**Figure 1**).

From 24 L of the culture filtrate, the four fractions of PS Fr. (26.4 g), fHexane Fr. (0.12 g), fEtOAc Fr. (11.04 g) and fWater Fr. (72.48 g) were obtained (**Figure 1**). The yields of the Hexane and EtOAc fractions were lower than those of the PS and Water fractions. This implied that there were very few non-polar components in the mycelium and culture filtrate.

### Antibacterial assays

The results from the paper disc diffusion method are summarized in **Table 1** and **Figure 2**. The results revealed that the EtOAc fractions from both the mycelium (mEtOAc Fr.) and filtrate (fEtOAc Fr.) had strong anti-*H. pylori* activity with inhibition zones of 18 – 19 mm, while mHexane Fr. and PS Fr. showed weak activity with 12 and 9 mm inhibition zones, respectively. However, mWater Fr., fHexane Fr. and fWater Fr. showed no inhibitory activity (**Table 1**). All fractions displayed weaker inhibitory activity than the positive control amoxicillin (inhibition zone of 37.5 mm).

The MIC and MBC results also yielded similar results (**Table 1**). The fractions mEtOAc Fr. and fEtOAc Fr. showed strong anti-*H. pylori* activity with MIC (MBC) values of 1.5 (7.5) and 1.25 (5.0) mg/mL, respectively. However, mHexane Fr. and PS Fr. displayed weak anti-*H. pylori* activity with MIC (MBC) values of 7.5 ( $\geq 10$ ) mg/mL, while fHexane Fr., mWater Fr. and fWater Fr. showed no inhibitory activity (MIC  $\geq 10$  mg/mL). The *H. pylori* ATCC 43504 strain was susceptible to the positive control amoxicillin with MIC (MBC) values of 0.032 (0.063)  $\mu\text{g/mL}$ .

### Urease inhibition

Based on urease IC<sub>50</sub> data presented in **Table 2**, mEtOAc Fr. and fEtOAc Fr. displayed strong urease inhibitory activity with IC<sub>50</sub> values of 0.34 and 0.35 mg/mL, respectively, with a non-significant difference ( $p > 0.05$ ). This was followed by the inhibitory activity of mWater Fr. and fWater Fr. (IC<sub>50</sub> values of 1.26 and 1.40 mg/mL, respectively). Besides, both mEtOAc Fr. and fEtOAc Fr. caused a steep dose-response curve of the percentage of urease inhibitory activity and produced a complete inhibition against the urease at 2.5 mg/mL, whereas mWater Fr. and fWater Fr. showed a complete inhibition against the urease at 3 and  $>5$  mg/mL, respectively (**Figure 3**). Moreover, PS Fr., mHexane Fr. and fHexane Fr. gave very weak or no inhibitory activity towards the enzyme. All the fractions exhibited lower urease inhibitory activity than the positive control thiourea (IC<sub>50</sub>, 0.055 mg/mL) (**Table 2**).

### DPPH free radical scavenging

Based on DPPH IC<sub>50</sub> values presented in **Table 3**, fEtOAc Fr. displayed the greatest antioxidant activity in the DPPH assay (IC<sub>50</sub> of 11.83 mg/mL), followed by mEtOAc Fr. (IC<sub>50</sub> of 14.75 mg/mL), with a significant difference ( $p < 0.01$ ). Moreover, PS Fr., mWater Fr. and fWater Fr. showed weak or very weak antioxidant

activity (IC<sub>50</sub>, 28.66 – 34.51 mg/mL). In addition, fEtOAc Fr. caused a steep dose-response curve of percentage of DPPH radical scavenging activity and complete inhibition at 40 mg/mL (**Figure 4**). Fractions mHexane Fr. and fHexane Fr. had no radical scavenging activity. Overall, all of these fractions showed significantly weaker DPPH radical scavenging activity than the positive control acid ascorbic (IC<sub>50</sub> of 0.057 mg/mL) (**Table 3**).

## DISCUSSION

Oxidative stress and chronic inflammation have been known to play critical roles in the pathogenesis of gastritis and gastric ulcers caused by various stimuli, including *H. pylori*<sup>23</sup>. Various physiological effects of *H. erinaceus* (lion's mane mushroom) have been presented, including anti-aging, anti-cancer, anti-gastritis, and anti-metabolic disease properties<sup>1,6-8,24</sup>. The mushroom's bioactive components, such as erinacines and hericenones, were extracted or concentrated as nutraceuticals because they have been revealed to act as the active principles for nerve growth factor synthesis and in neuroprotective function<sup>25-27</sup>. *H. erinaceus* is quite rare in the wild. However, by artificial cultivation techniques, its fruiting body and mycelium are nowadays being produced as raw materials for culinary and medicinal use. *H. erinaceus* has become attractive as a disease-preventing functional food and as a source of medicines. *H. erinaceus* aqueous and 50% EtOH extracts were demonstrated to possess immune-stimulatory activity to protect infected mice against *Salmonella* Typhimurium<sup>12</sup>. Methanolic extract from a pure culture of *H. erinaceus* mycelium was reported to produce weak inhibitory activity against *Bacillus cereus*, *B. subtilis*, *Enterococcus faecalis*, *Salmonella* sp., *Shigella* sp. and *Plesiomonas shigelloides*<sup>11</sup>. However, EtOH extract from fruiting body of *H. erinaceus* has been shown to exhibit similar growth inhibitory effects on several strains of *H. pylori*<sup>9,10,13</sup>. Petroleum ether, chloroform, and EtOAc fractions from the fruiting body extract were reported to exhibit a stronger growth inhibitory activity than the crude extract against *H. pylori*<sup>9,13</sup>. In our present study, mEtOAc Fr. and fEtOAc Fr. from mycelium and culture filtrate of *H. erinaceus* displayed stronger anti-*H. pylori* activity than the polysaccharide, hexane and water fractions.

Findings on the mechanism of *H. erinaceus* action against *H. pylori* have indicated that the mushroom extracts helped prevent oxidative stress in human gastric mucosa epithelium cell<sup>18</sup> and inhibit the adhesion ability of *H. pylori* to the host cell, thereby contributing to reduction of the bacterial infection in

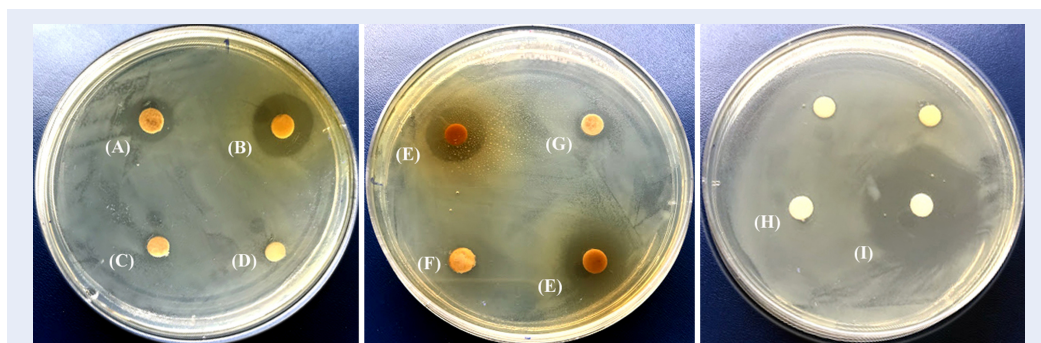
**Table 1: Antibacterial activities of *H. erinaceus* mycelium and culture filtrate fractions against *H. pylori* ATCC 43504**

Test material (10 mg/disc)	Inhibition zone* (mm)	MIC (mg/mL)	MBC (mg/mL)
fEtOAc Fr.	18.8 ± 0.75 <sup>b</sup>	1.25	5
mEtOAc Fr.	17.7 ± 1.03 <sup>b</sup>	1.5	7.5
mHexane Fr.	12.3 ± 0.82 <sup>c</sup>	7.5	10
PS Fr.	9.3 ± 0.52 <sup>d</sup>	7.5	> 10
fHexane Fr.	-	10	> 10
mWater Fr.	-	> 10	
fWater Fr.	-	> 10	
Amoxicillin**	37.5 ± 2.51 <sup>a</sup>	0.032	0.063

\*Means ± SD. <sup>a,b,c,d</sup>Mean values within a column followed by the same letter are not significantly different (P = 0.05; Bonferroni method)

\*\*positive control (10 µg/disc), MIC and MBC (µg/mL)

- No activity



**Figure 2: Antibacterial of *H. erinaceus* mycelium and culture filtrate fractions toward *H. pylori* ATCC 43504 using paper disc diffusion assay.** Inhibition zones of mHexane Fr. (A), mEtOAc Fr. (B), mWater Fr. (C), fHexane Fr. (D), fEtOAc Fr. (E), fWater Fr. (F), PS Fr. (G), negative control DMSO (H), and positive control amoxicillin (I).

**Table 2: *H. pylori* urease inhibitory activity of *H. erinaceus* mycelium and culture filtrate fractions**

Test material	Slope ± SE	Urease IC <sub>50</sub> , mg/mL (95% CL)
mEtOAc Fr.	0.81 ± 0.056	0.34 (0.30 – 0.39)
fEtOAc Fr.	1.25 ± 0.118	0.35 (0.31 – 0.40)
mWater Fr.	1.56 ± 0.269	1.26 (1.02 – 1.55)
fWater Fr.	0.47 ± 0.077	1.40 (1.12 – 1.74)
PS Fr.	1.59 ± 0.265	29.67 (26.12 – 33.70)
mHexane Fr.	2.13 ± 0.173	30.26 (28.53 – 32.10)
fHexane Fr.		ND
Thiourea	1.65 ± 0.215	0.055 (0.049 – 0.062)

ND: not determined

CI: confidence interval

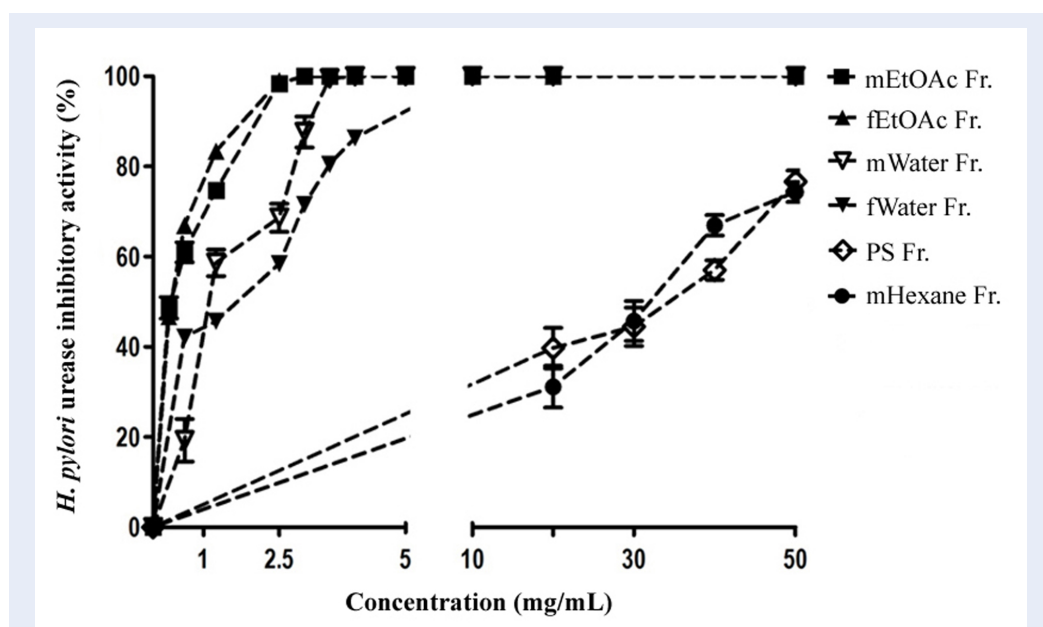


Figure 3: *H. pylori* urease inhibitory activity of *H. erinaceus* mycelium and culture filtrate fractions.

Table 3: DPPH free radical scavenging activity of *H. erinaceus* mycelium and culture filtrate fractions

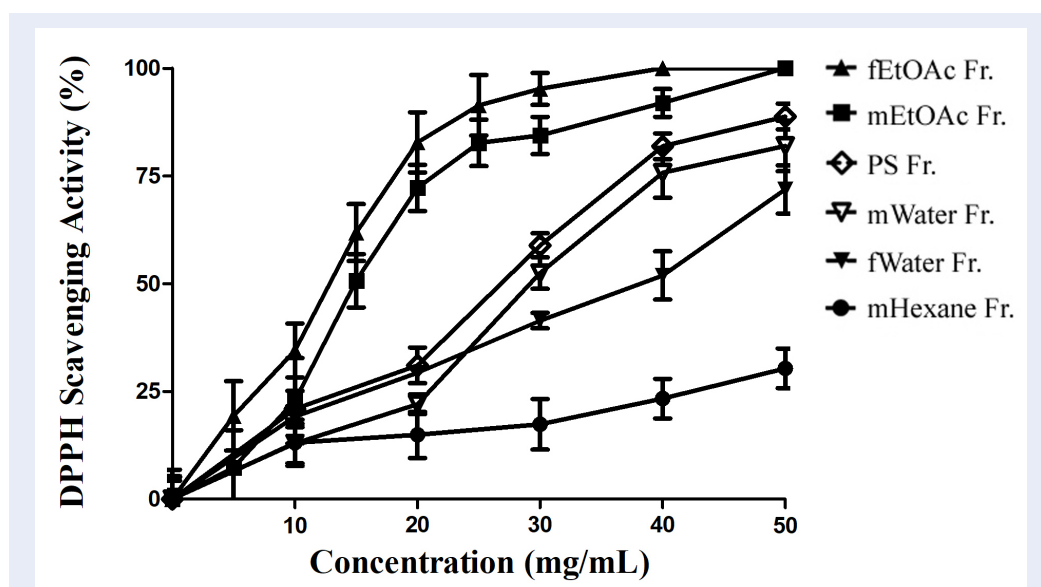
Test material	Slope ± SE	DPPH IC <sub>50</sub> , mg/mL (95% CL)
fEtOAc Fr.	2.322 ± 0.339	11.83 (10.48 – 13.36)
mEtOAc Fr.	2.972 ± 0.421	14.75 (13.52 – 16.09)
PS Fr.	3.217 ± 0.256	26.04 (24.77 – 27.38)
mWater Fr.	3.03 ± 0.365	28.66 (26.6 – 30.87)
fWater Fr.	1.879 ± 0.211	34.51 (31.90 – 37.34)
mHexane Fr.		ND
fHexane Fr.		ND
Acid ascorbic	2.712 ± 0.567	0.057 (0.048 – 0.065)

ND: not determined  
 CI: confidence interval

the stomach of test mice<sup>10</sup>. The urease-producing ability of *H. pylori* has been known to play a crucial role in infection and survival of the pathogen in the stomach. Therefore, inhibition of the urease activity has also been considered as an alternative strategy for the treatment of the *H. pylori* infection. Hot water extracts, ether and EtOAc fractions of some mushrooms, such as *Ganoderma lucidum*, *Coriolus versicolor*, *A garicus bisporus* and *Gyrophora esculenta*, were reported to possess growth inhibitory activity against *H. pylori*, but these extracts and fractions showed no inhibitory effect on *H. pylori* urease activity<sup>28</sup>. Several studies have reported on the inhibitory effect of plant preparations and phytochem-

icals on *H. pylori* urease<sup>19,22,29</sup>. However, no information was reported on the inhibitory activity of *H. erinaceus* preparations or its constituents against urease activity of *H. pylori*. In our present study, the EtOAc fractions from both mycelium and culture filtrate of *H. erinaceus* exhibited growth inhibitory activity against *H. pylori*. They had a stronger inhibitory urease than the water fractions, while the polysaccharide and hexane fractions showed no effect.

MeOH extracts from fresh fruiting body and mycelium of *H. erinaceus* were found to have antioxidant activity in DPPH free radical scavenging, and the activity was not due to phenolic compounds in *H. erinaceus* extracts<sup>11</sup>. It was reported that polysac-



**Figure 4:** DPPH free radical scavenging activity of *H. erinaceus* mycelium and culture filtrate fractions toward *H. pylori* ATCC 43504.

charides purified from liquid culture of *H. erinaceus* mycelium enhanced the growth of rat adrenal nerve cells and improved the extension of neurites of PC12 cells<sup>30</sup>. The polysaccharide fraction from *H. erinaceus* mycelium was found to possess anti-ulcer and anti-gastritis activities<sup>31,32</sup>. Moreover, the antioxidant capacity of the polysaccharide fraction from *H. erinaceus* mycelium was higher than that of hot water extract from *H. erinaceus* fruiting body<sup>18</sup>. In our present study, the polysaccharide fraction from the culture filtrate of *H. erinaceus* showed a lower antioxidant effect than the EtOAc fractions from both the mycelium and culture filtrate. These results indicate that the EtOAc fractions from the mycelium and culture filtrate of *H. erinaceus* could exert growth-inhibiting and urease inhibitory effects on *H. pylori* to enhance antioxidant defense and protect the human stomach from *H. pylori* infection. More research is needed to prove the safety of the *H. erinaceus* mycelium and culture filtrate fractions and their *in vivo* efficacies in the treatment of *H. pylori* infection.

## CONCLUSION

The present study revealed that the EtOAc fractions derived from the mycelium and culture filtrate of *H. erinaceus* exhibited pronounced antioxidant and inhibitory effects against *H. pylori*. The results suggest that the *in vitro* culture of mycelium and culture filtrate of *H. erinaceus* could be further studied to de-

velop potent antibacterial products for the treatment of *H. pylori* infection.

## ABBREVIATIONS

ATCC: American Type Culture Collection

CFU: Colony forming units

MIC: Minimal inhibitory concentration

MBC: Minimal bactericidal concentration

**mHexane Fr.:** Hexane fraction derived from mycelium

**mEtOAc Fr.:** Ethyl acetate fraction derived from mycelium

**mWater Fr.:** Water fraction derived from mycelium

NBS: newborn bovine serum

**PS Fr.:** Polysaccharide fraction derived from culture filtrate

**fHexane Fr.:** Hexane fraction derived from culture filtrate

**fEtOAc Fr.:** Ethyl acetate fraction derived from culture filtrate

**fWater Fr.:** Water fraction derived from culture filtrate

## ACKNOWLEDGMENTS

All authors gratefully acknowledge the financial support from Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106-YS.06-2015.17.



## AUTHORS' CONTRIBUTIONS

LTM Ngan, TT Hieu and PT Ho conceived and designed the experiments. LTM Ngan, TT Hieu, NT Vi, DTM Tham and LTT Loan performed the experiments. LTM Ngan, TT Hieu and NT Vi analyzed and interpreted the data. LTM Ngan and TT Hieu participated in drafting and writing the article. All authors read and approved the manuscript.

## FUNDING

Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106-YS.06-2015.17.

## AVAILABILITY OF DATA AND MATERIALS

Data and materials used and/or analysed during the current study are available from the corresponding author on reasonable request.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

## CONSENT FOR PUBLICATION

Not applicable.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

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