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# *In vitro* antioxidant and antimutagenic activities of different solvent extracts of *Phellinus* spp.

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**Abstract**

*Phellinus* spp. are fungi belonging to the Hymenochaetaceae family. *Phellinus* spp. is commonly grown in the Northeastern region of Thailand. The aims of this study were to investigate the antioxidant and the antimutagenic activities of *Phellinus* spp. The *Phellinus* spp. in this study are *Phellinus nigricans, Phellinus rimosus,* and *Phellinus wahlbergii*, which were extracted using water, ethanol, and alkaloid extraction procedure. The antioxidant activities were studied using the radical scavenging activity on 1,1 – diphenyl – 2 - picrylhydrazyl assay (DPPH assay) and the ferric reducing antioxidant power assay (FRAP assay). The antimutagenic activity was studied using the Ames mutagenesis assay with *Salmonella typhimurium* strains TA98 and TA100. The results showed that the ethanol extract of *P. rimosus* exhibited excellent activities among 3 spp. The $EC_{50}$ and the FRAP values were 8.26±1.20 µg/mL and 0.33±0.06 mmol of $FeSO_4$ /mg of dried extract, respectively. The antimutagenic activity showed that all of the extracts exhibited strong inhibition especially the ethanol extract of *P. rimosus* at the concentration of 1 mg/plate of TA98 and 2 mg/plate of TA100. Therefore, the ethanol extract of *P. rimosus* is great interest for further investigation in treatment and prevention of disease.



## Introduction

Currently, there is a lot of attention being exhibited in the potential and application of natural antioxidants as pharmaceuticals, nutraceuticals, and cosmeceuticals for health preservation and disease prevention. Moreover, natural antioxidants from plant extracts are remarkable due to consumer aware about the safety of the synthetic antioxidant in the product.

Mushrooms are widely consumed for their nutritional value as a food and for medicinal properties. Many studies have found that some species of mushrooms exhibit therapeutic properties such as antioxidant, antimutagenic, antimicrobial, anticancer, and immune stimulatory effects (Shon *et al*., 2003; Yuan *et al.*, 2009; Seephonkai *et al*., 2011; Wang *et al.*, 2014). Mushrooms accumulate a variety of secondary metabolites, including phenolic compounds, flavonoids, polysaccharides, and terpenes (Shon *et al.*, 2003; Song *et al*., 2003; Barros *et al*., 2007; Liu and Wang, 2007; Liang *et al*., 2009; Oyetayo, 2009; Seephonkai *et al*., 2011; Wu *et al*., 2011; Wang *et al*., 2014). *Phellinus* is a genus of mushroom belonging to the Hymenochaetaceae family. Fruit bodies are found growing on wood. Some of the species of *Phellinus* are reported in China, Japan, and Korea as medicinal mushrooms (Ajith *et al*., 2011). *P. nigricans, P. rimosus,* and *P. wahlbergii* are commonly grown in the Northeastern region of Thailand. Moure *et al.* (2001) reported that the quality of natural extracts and antioxidant activities does not only depend on storage time, geographic origin, harvesting date but also on the environment and technological factors as well. Besides that, the solvent used is one of the significant factors to extract antioxidant compounds in plant materials due to the different antioxidant ability of the compound with different polarity.

The antioxidant is a compound which reduces or prevents the oxidation of other oxidizable compounds. Phenolic compounds are the major plant compounds with antioxidant activity. In addition, other biological properties such as anticarcinogenic, antimutagenicity, anti allergenicity and antiaging activity have been reported for natural antioxidants (Yuan *et al*., 2009; Wang *et al.*, 2014; Devi *et al*., 2015).

Mutagenic and antimutagenic activities have been related with the phytochemical substances, such as the flavonoids, polysaccharides, tannins, and phenolic compounds. The high antimutagenic

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activity has been presented to phenolic compounds as reported by Phadungkit *et al.* (2012) and Devi *et al.* (2015). Chemicals that persuade mutations can possibly damage the germ line leading to fertility problems and to mutations in future generations (Mortelmans and Zeiger, 2000). Mutagens are not only involved in genotoxicity and carcinogenesis but are also implied in the pathogenesis of several chronic degenerative diseases including hepatic, neurodegenerative, and cardiovascular disorders, as well as diabetes, arthritis, chronic inflammation and aging. The harmful effects of mutagens are decreased by using the natural antimutagens (Bhattacharya, 2011). Thus, the aim of this study was to investigate the antioxidant and antimutagenic activities of the extracts from *Phellinus* spp., which were extracted using ethanol, water, and alkaloid procedure.

## Materials and Methods

*Materials*

*Phellinus* spp. were *P. nigricans, P. rimosus,* and *P. wahlbergii*, kindly provided by the Natural Medicinal Mushroom Museum, Faculty of Science, Mahasarakham University. *Salmonella typhimurium* strains TA98 and TA100 were provided by Institute of Nutrition, Mahidol University. All other chemicals and reagents used were analytical reagent grade.

*Preparation of* Phellinus *mushroom extract*

Each *Phellinus* mushroom was ground to powder. The desiccator was used to store dried powdered mushrooms in a dry atmosphere.

*Water extract*

Each powdered mushroom (500 g) was extracted with 1,500 mL of distilled water using the decoction method at 90°C for 4 hr.

*Ethanol extract*

Each powdered mushroom (500 g) was macerated with 1,500 mL of 95% ethanol at room temperature for 7 days.

*Procedure for alkaloid extraction*

Each powdered mushroom (500 g) was swirled around with 500 mL of 10% ammonia solution for 5 min. Then, the extracted part was diluted with 2,500 mL of methanol and heated at 60°C for 30 min. The *Phellinus* mushroom extracts were filtrated through Whatman No.1 and concentrated using a rotary evaporator. Each *Phellinus* mushroom extract was dried by freeze dryer.

*Phytochemical screening*

Preliminary phytochemical screening of secondary metabolites such as flavonoids, alkaloids, polysaccharides, terpenoids, quinones, tannins, and phenolic compounds was carried out according to the common phytochemical methods described by Trease and Evans (1996).

*Free radical scavenging activity on 1, 1 – diphenyl – 2 - picrylhydrazyl assay (DPPH assay)*

The radical scavenging activity of the extracts was measured using DPPH as described by Devi *et al.* (2015) with slight modifications. In brief, 750 µL of each *Phellinus* extract of various concentrations (0.48 - 500 ppm) was mixed with 750 µL of DPPH (60 ppm in methanol) in 96 well flat-bottom plates. The samples were incubated at room temperature for 20 min. The absorbance was determined using a spectrophotometer at 517 nm. Ascorbic acid was used as a standard antioxidant in this experiment. The samples were analyzed five times. The radical scavenging activity was calculated as a percentage of DPPH discoloration using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_b - A_s)/A_b] \times 100$$

Where $A_s$ is the absorption of the extract sample and $A_b$ is the absorption of the blank.

The extract concentration that decreased the initial DPPH radical by 50% ($EC_{50}$) was calculated by linear regression analysis of the curve plotting the percentage of DPPH radical scavenging activity against the extract concentration.

*Ferric reducing antioxidant power assay (FRAP assay)*

The procedure to determine the ability of antioxidants to reduce ferric ions to ferrous ions was that described by Guo *et al.* (2003) with a few modifications. Briefly, the FRAP reagent contained 2.5 mL of 10 mmol/L TPTZ (2,4,6-tripyridy-s-triazine) solution in 40 mmol/L HCl plus 2.5 mL of 20 mmol/L $FeCl_3$ and 25 mL of 0.3 mol/L acetate buffer, pH 3.6 was prepared and warmed at 37°C. From the different concentrations of each *Phellinus* extract solution (0.48 – 500 µg/mL), 100 µL of each *Phellinus* extract was mixed with 100 µL of the FRAP reagent in 96 well flat-bottom plates. The absorbance of a reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37°C for 10 min. The $FeSO_4$ was used as the standard. The entire test was performed five times. The result was expressed as the concentration of antioxidants having

a ferric reducing ability equivalent to that of $FeSO_4$.

*Antimutagenic activity by the Ames test*

The antimutagenic activity was performed using *Salmonella typhimurium* strains TA 98 and TA 100 through the preincubation technique as described by Maron and Ames (1983) with slight modifications.

*Bacterial strains*

Two histidine-deficient (His-) tester strains, TA98 and TA100 of *S. typhimurium* were provided by Nutrition Institute, Mahidol University. Strains TA98 and TA100 are used for the detection of frameshifts and base-pair substitution mutation, respectively. The bacteria were incubated in oxoid nutrient broth no.2 at 37°C for 16 hr. The bacteria were diluted with 0.9% sodium chloride. The absorbance was determined at 620 nm.

*Nitrosated product preparation*

The nitrosated products are mutagens. The sodium nitrate ($NaNO_3$) was mixed with 1-Aminopyrene (1-AP) for preparing the nitrosated product. Briefly, TA 98 was added to 740 µL of 0.2 N hydrochloric acid (HCl), 10 µL of 1-AP, and 250 µL of 2M $NaNO_3$. TA 100 was added to 710 µL of 0.2 N HCl, 40 µL of 1-AP, and 250 µL of 2M $NaNO_3$. The mixture was shaken in a water bath at 37°C for 4 hr. After shaking, the mixture was soaked in an ice bath for 1 min. Then, 250 µL of 2M ammonium sulfate was added and mixed. The mixture was soaked in an ice bath for 10 min.

*Mutagenic test*

The sodium phosphate ($Na_3PO_4$) - KCl buffer pH 7.4 (500 µL) was mixed with 100 µL of each *Phellinus* extract of various concentration (3.5, 7.5, 15 and 30 mg) and 100 µL of *S. typhimurium* (TA 98 or TA 100). The mixture was incubated in shaking water bath at 37°C for 20 min. After shaking, 2 ml of top agar (0.6% agar, 0.5% of NaCl, 10 mL of 0.5 mM of histidine-biotin mixture) was added and mixed. The mixture was poured on a minimal glucose agar (1.5% agar with 40% glucose and VB salt) plate and incubated at 37°C for 48 hr. The number of histidine+ (His+) revertant colonies on the incubated plate were counted.

*Antimutagenic test*

The nitrosated product (100 µL) was mixed with 500 µL of $Na_3PO_4$ - KCl buffer (pH 7.4), 100 µL of each *Phellinus* extract of various concentration (3.5, 7.5, 15 and 30 mg), and 100 µL of *S. typhimurium* (TA 98 or TA 100). The method follows the details of the mutagenic test. Each sample was assayed using five plates. The inhibitory effect of each *Phellinus* extract of various concentration on mutagenicity of the standard direct mutagen was determined as a percentage of inhibition as followed:

$$\text{Percentage of inhibition} = [(A-B)/(A-C)] \times 100$$

Where A is a number of revertants per plate induced by the positive mutagen, B is a number of revertants per plate induced by positive mutagen in the presence of each *Phellinus* extract, and C is a number of spontaneous revertants per plate. The inhibition of each *Phellinus* extract of various concentration was considered according to Calomme *et al.* (1996). The antimutagenic effect was considered strong, moderate, weak, and negligible when the value was higher than 60, 40-60, 20-40, and less than 20 %, respectively.

## Results and Discussions

*The percentage of yield of* Phellinus *extracts*

The various methods used for extraction showed that the procedure for alkaloid gave the highest percentage yields of the extracts, especially *P. wahlbergii* extract (22.86 %w/w).

*Phytochemical screening*

The medicinal properties of mushrooms are owing to the type of chemical substance which they generate and reserve. Phytochemistry is concerned with the chemical study of mushrooms constituents. Extraction is the separation of medicinally active portions of mushrooms using selective solvents through standard procedures. The solvents diffuse into the powdered mushroom and solubilize compounds with similar polarity. The results obtained for the qualitative screening of phytochemicals in *Phellinus* extract are presented in Table 1.

Phytochemical screening revealed the presence of alkaloids, polysaccharides, terpenoids, and phenolic compounds in all of *Phellinus* extracts. In additional, the ethanolic extract in all of *Phellinus* extracts contained flavonoids. On the other hand, tannins were not found in any *Phellinus* extract. Phytochemical analysis of the *Phellinus* extracts was successful, and ethanol was determined to be a good solvent for the extraction of the active constituents. The high activity of the ethanolic extracts can be attributed to the presence of high amounts of phenolic compounds. Tiwari *et al.* (2011) mentioned that alcoholic solvents are more efficient extraction in cell walls than water and cause phytochemical substances especially

Table 1. Preliminary phytochemical screening of *Phellinus* spp

| Extraction | *Phellinus* | Flavonoids | Alkaloids | Polysaccharides | Terpenoids | Quinones | Tannins | Phenolic |
|---|---|---|---|---|---|---|---|---|
| Water | *P. rimosus* | - | + | + | + | + | - | + |
| | *P. wahlbergii* | - | + | + | + | + | - | + |
| | *P. nigricans* | - | + | + | + | + | - | + |
| Ethanol | *P. rimosus* | + | + | + | + | + | - | + |
| | *P. wahlbergii* | + | + | + | + | + | - | + |
| | *P. nigricans* | + | + | + | + | - | - | + |
| Alkaloid procedure | *P. rimosus* | + | + | + | + | + | - | + |
| | *P. wahlbergii* | - | + | + | + | + | - | + |
| | *P. nigricans* | - | + | + | + | + | - | + |

Where + is the presence of constituents, but - is the absence of constituents.

Table 2. DPPH radical scavenging activity of the *Phellinus* extracts (n=5)

| *Phellinus* | $EC_{50}$ (µg/mL) | | |
|---|---|---|---|
| | Water | Ethanol | Alkaloid procedure |
| *P. rimosus* | 20.12±3.60 | 8.26±1.20 | 94.15±9.09 |
| *P. wahlbergii* | 72.90±8.98 | 9.64±5.29 | 81.11±9.09 |
| *P. nigricans* | 22.52±1.44 | 7.03±1.77 | 34.49±4.26 |

$EC_{50}$ of ascorbic acid = 5.02±0.46 µg/mL

Table 3. FRAP value of *Phellinus* extracts (n=5)

| *Phellinus* | FRAP value (Fe II equivalent, mmol/mg) | | |
|---|---|---|---|
| | Water | Ethanol | Alkaloid procedure |
| *P. rimosus* | 0.11±0.05 | 0.33±0.06 | 0.13±0.04 |
| *P. wahlbergii* | 0.06±0.02 | 0.28±0.01 | 0.16±0.01 |
| *P. nigricans* | 0.07±0.01 | 0.28±0.01 | 0.16±0.02 |

Fe II equivalent of ascorbic acid = 0.34±0.02 mmol/mg

phenolic compounds to be released from cells. These results agreed with the *Phellinus* literature, which showed these chemical constituents to be present (Shon *et al*., 2003; Liang *et al.*, 2009; Seephonkai *et al.*, 2011; Wang *et al*., 2014).

The flavonoids, polysaccharides, and phenolic compounds in plant have been reported to various biological effects including antioxidant and antimutagenicity (Javanmardi *et al*., 2003; Saikia and Upadhyaya, 2011; Seephonkai *et al.*, 2011; Wang *et al.*, 2014; Devi *et al*., 2015; Jiang *et al*., 2015; Roleira *et al*., 2015). The mechanisms that contribute to the antioxidant activities of phenolic compounds include free radical scavenging, hydrogen or electron donation, chelation of redox-active metal ions, modulation of gene expression, and interaction with the cell signaling pathway (Arouma, 2003).

*Antioxidant activity*

*Free radical scavenging activity on DPPH assay*

In the DPPH assay, the extracts were able to reduce the stable radical DPPH to the diphenyl-picrylhydrazine (Shon *et a*l., 2003). The degree of change in color from purple to yellow is a measure of the scavenging potential of antioxidant extracts. The radical scavenging activity was expressed as the $EC_{50}$ as shown in Table 2. The lower $EC_{50}$ value indicates greater antioxidant activity. The $EC_{50}$ of ascorbic acid when used as a standard reference was 5.02±0.46 µg/mL. The ethanol extracts presented the greatest potency relative to the two other methods. The highest antioxidant activity was observed in the ethanol extract of *P. nigricans* followed by *P. rimosus* and *P. wahlbergii*, respectively. However, the $EC_{50}$ of ethanol extract of *P. nigricans* was not significantly different from *P. rimosus*, and *P. wahlbergii* ($p>0.05$) by Duncan's test.

The results showed that the active antioxidant components of *Phellinus* spp. are effectively extracted with ethanol. Most likely because of the polarity of phenolic compounds contained in *Phellinus* mushrooms (Seephonkai *et al.*, 2011). The results are in agreement with finding of others that the ethanol extract had high antioxidant activity (Shyu *et al*., 2009; Prabakaran *et al*., 2011). Song *et al.* (2003) have confirmed that the ethanol extract of *P. linteus* contained potent antioxidant activity, xanthine oxidase inhibition, and anti-angiogenic activity. In addition, Seephonkai *et al.* (2011) found that the $IC_{50}$ of crude 80% ethanol extract of *P. torulosus* showed the highest scavenging ability toward DPPH radical. The ethanol fraction was the most active free radical scavenger.

Table 4. Mutagenic activity of *Phellinus* extracts (n=5)

| Extraction | *Phellinus* | Concentration (mg/plate) | Number of revertants/plate TA 98 | TA 100 |
|---|---|---|---|---|
| Water | *P. rimosus* | 0.5 | 1.00±0.70 | 62.00±4.47 |
| | | 1 | 1.60±0.89 | 64.00±7.48 |
| | | 2 | 3.20±1.09 | 63.80±5.01 |
| | | 4 | 5.00±1.00 | 82.20±13.16 |
| | *P. wahlbergii* | 0.5 | 0.80±0.44 | 26.80±10.40 |
| | | 1 | 2.00±1.00 | 36.80±9.39 |
| | | 2 | 3.20±0.83 | 48.40±8.98 |
| | | 4 | 4.80±0.83 | 65.20±9.09 |
| | *P. nigricans* | 0.5 | 1.00±1.20 | 34.00±9.74 |
| | | 1 | 2.00±1.41 | 43.20±9.17 |
| | | 2 | 3.20±1.09 | 54.00±13.96 |
| | | 4 | 4.80±0.83 | 66.40±16.50 |
| Ethanol | *P. rimosus* | 0.5 | 0.60±0.89 | 43.20±8.25 |
| | | 1 | 1.20±1.30 | 56.00±9.00 |
| | | 2 | 3.40±1.14 | 67.80±11.71 |
| | | 4 | 5.40±0.89 | 80.40±4.35 |
| | *P. wahlbergii* | 0.5 | 0.60±0.89 | 59.00±5.54 |
| | | 1 | 1.60±0.89 | 64.00±7.48 |
| | | 2 | 2.80±0.83 | 63.80±5.01 |
| | | 4 | 4.4±1.14 | 82.2±8.86 |
| | *P. nigricans* | 0.5 | 0.40±0.54 | 48.80±13.60 |
| | | 1 | 1.80±0.83 | 64.00±7.48 |
| | | 2 | 3.20±0.83 | 63.80±5.01 |
| | | 4 | 4.00±1.58 | 82.20±13.16 |
| Alkaloid procedure | *P. rimosus* | 0.5 | 1.00±1.15 | 28.80±8.28 |
| | | 1 | 2.40±0.95 | 44.80±9.57 |
| | | 2 | 3.40±1.70 | 59.80±6.53 |
| | | 4 | 5.20±1.50 | 52.60±12.81 |
| | *P. wahlbergii* | 0.5 | 0.60±0.57 | 40.80±8.04 |
| | | 1 | 2.20±0.81 | 64.40±10.71 |
| | | 2 | 3.60±0.95 | 60.20±8.70 |
| | | 4 | 5.40±1.00 | 76.40±7.92 |
| | *P. nigricans* | 0.5 | 0.80±0.83 | 50.40±13.06 |
| | | 1 | 1.80±0.83 | 64.40±10.71 |
| | | 2 | 3.20±1.30 | 76.40±7.920 |
| | | 4 | 5.20±0.83 | 82.40±11.78 |

*FRAP assay*

The principle of this method is based on the reduction of a ferric-tripyridyl triazine complex to its ferrous state, whereby intensive blue color could be measured at 593 nm (Guo *et al.*, 2003; Gohari *et al.*, 2011). The results of the FRAP assay are reported in Table 3. The antioxidant activities were expressed as the concentrations of antioxidant having a ferric reducing ability equivalent to that of 1 mM of $FeSO_4$. The ascorbic acid which was used as a standard reference calculated to be 0.34±0.02 mmol/mg. The strongest FRAP value of ethanol extract from *P. rimosus* was calculated to be 0.33±0.06 mmol of $FeSO_4$ / 1 mg of dried extract. The ethanol extract from *P. rimosus* possessed the highest degree of antioxidant activity ($P<0.05$).

The FRAP assay features a reducing agent. FRAP value is a reduction of ferric ion, where antioxidants support as the reducing agent. Antioxidants are compounds capable of donating a single electron or hydrogen atom for reduction (Rabeta and Nur Faraniza, 2013). The antioxidant activity of phenolic is mainly due to their redox properties. The hydroxyl group on the aromatic ring of phenolic compounds perform as reducing agents, singlet oxygen quenchers or hydrogen donors to radicals. Many studies have exposed that the antioxidant activity in the ethanol extracts shows the best performance in the scavenging assay of DPPH radicals and ferric reduction (Cheung *et al*., 2003; Chua *et al.,* 2008; Chen *et al*., 2010; Rabeta and Nur, 2013).

*Mutagenic activity*

The Ames *Salmonella* mutagenicity assay is a bacterial reverse mutation assay and is used to evaluate the mutagenic properties of test articles including

Table 5. Antimutagenic activity of *Phellinus* extracts (n=5)

| Extration | *Phellinus* | Concentration (mg/plate) | TA98 | | TA100 | |
|---|---|---|---|---|---|---|
| | | | Number of revertants/plate | % Inhibition | Number of revertants/plate | % Inhibition |
| Water | *P. rimosus* | 0.5 | 384.62±17.04 | 12.76±3.53 | 844.63±32.72 | 13.98±3.81 |
| | | 1 | 309.41±21.23 | 30.19±4.92 | 762.62±20.17 | 23.52±2.34 |
| | | 2 | 174.04±13.64 | 61.60±3.16 | 620.04±22.17 | 40.12±2.58 |
| | | 4 | 107.21±14.41 | 77.09±3.34 | 425.61±15.08 | 62.74±1.75 |
| | *P. wahlbergii* | 0.5 | 391.60±17.98 | 11.13±4.17 | 886.40±39.84 | 9.11±4.64 |
| | | 1 | 291.80±18.24 | 34.28±4.23 | 689.80±62.15 | 32.00±7.23 |
| | | 2 | 173.60±21.66 | 61.69±5.02 | 444.60±40.76 | 60.53±4.74 |
| | | 4 | 115.40±17.44 | 75.19±4.05 | 342.80±19.75 | 72.37±2.30 |
| | *P. nigricans* | 0.5 | 404.40±15.57 | 8.16±3.61 | 853.40±33.01 | 12.96±3.84 |
| | | 1 | 296.40±12.58 | 33.21±2.91 | 737.60±33.01 | 26.43±3.84 |
| | | 2 | 148.00±13.32 | 61.13±3.09 | 645.80±37.91 | 37.12±4.41 |
| | | 4 | 126.20±17.02 | 72.68±3.95 | 447.00±28.91 | 60.20±3.36 |
| Ethanol | *P. rimosus* | 0.5 | 309.20±42.64 | 30.24±8.84 | 803.40±33.24 | 18.76±3.87 |
| | | 1 | 163.80±29.24 | 63.96±6.77 | 695.80±38.17 | 31.30±4.44 |
| | | 2 | 127.60 ±17.73 | 72.36±4.12 | 356.80±34.63 | 70.75±4.03 |
| | | 4 | 87.60±6.64 | 81.63±1.54 | 269.00±47.74 | 80.96±5.55 |
| | *P. wahlbergii* | 0.5 | 388.20±19.40 | 11.92±4.50 | 858.60±37.85 | 12.35±4.40 |
| | | 1 | 307.60±18.74 | 30.61±4.35 | 729.00±65.94 | 27.43±7.67 |
| | | 2 | 180.80±17.93 | 60.02±4.16 | 560.40±20.77 | 47.05±2.42 |
| | | 4 | 101.00±11.25 | 78.53±2.61 | 421.80±25.78 | 63.18±3.00 |
| | *P. nigricans* | 0.5 | 383.20±13.57 | 13.08±3.15 | 843.60±41.44 | 14.10±4.82 |
| | | 1 | 282.40±18.56 | 36.46±4.30 | 657.20±48.82 | 35.79±5.69 |
| | | 2 | 173.00±21.66 | 61.83±5.02 | 373.80±49.18 | 68.77±5.72 |
| | | 4 | 133.20±15.43 | 71.06±3.58 | 358.40±27.34 | 70.56±3.19 |
| Alkaloid procedure | *P. rimosus* | 0.5 | 393.60±16.74 | 10.67±3.47 | 890.20±35.82 | 8.68±4.17 |
| | | 1 | 305.00±28.13 | 31.22±6.52 | 702.00±31.97 | 30.58±3.72 |
| | | 2 | 174.00±12.77 | 61.60±2.96 | 543.00±48.61 | 49.08±5.66 |
| | | 4 | 114.80±14.51 | 75.33±3.36 | 361.40±45.14 | 70.21±2.50 |
| | *P. wahlbergii* | 0.5 | 393.00±15.48 | 10.81±3.59 | 888.40±50.87 | 8.88±5.92 |
| | | 1 | 314.40±40.59 | 29.04±9.41 | 749.40±42.51 | 25.06±4.95 |
| | | 2 | 236.80±23.37 | 47.03±5.42 | 545.60±47.19 | 48.78±5.49 |
| | | 4 | 141.60±12.78 | 69.10±2.96 | 379.00±49.30 | 68.16±5.74 |
| | *P. nigricans* | 0.5 | 381.20±9.10 | 13.54±2.11 | 847.60±18.41 | 13.63±2.14 |
| | | 1 | 267.00±26.90 | 40.03±6.24 | 642.40±29.89 | 37.51±3.48 |
| | | 2 | 155.60±17.16 | 65.86±3.98 | 457.40±50.96 | 59.04±5.93 |
| | | 4 | 103.20±15.79 | 78.02±3.66 | 356.20±27.73 | 70.82±3.23 |

natural product. Mutation causes DNA damage via different mechanisms. When the *Salmonella* tester strains are grown on a minimal media agar plate containing a trace of histidine, only those bacteria that revert to histidine independence ($his^+$) are able to form colonies. The number of spontaneously induced revertant colonies per plate is relatively constant (Mortelmans and Zeiger, 2000). The chemical and physical factors that induce mutations are referred to as mutagens while those that reduce mutagenicity are antimutagens (Bhattacharya, 2011).

The average number of revertants per plate expressed after deducting the spontaneous revertants of TA98 and TA 100 were 8.4±2.06 and 105.4±8.07, respectively. However, the range of revertant colonies of most samples was between 0.4-5.4 for TA98 and 26.8-82.2 for TA100, which was less than the spontaneous revertants. The number of revertant colonies was not greater than that of the negative control. Thus, the extracts of *Phellinus* spp. at various concentration were not mutagenic. The range for herbal extracts with regard to revertant colonies was similar to that reported previously by Ajith and Janardhanan (2011) and Taherkhani (2014).

*Antimutagenicity activity*

The bacterial reversed mutation assay is used to evaluate the mutagenic properties of the test. The test uses amino acid-dependent strains of *S. typhimurium*. In the absence of an external histidine source, the cells cannot form colonies. Colony growth is resumed if a reversion of the mutation occurs, allowing the production of histidine to be resumed. Spontaneous

reversions occur with each of the strains. The antimutagen can prevent the transformation of a mutagenic compound into mutagen, inactivate the mutagen or otherwise prevent the reaction between mutagen and DNA. Other kinds of antimutagens may induce, inactivate directly or indirectly the enzymes of the DNA repair recombination and replication pathways (Bhattacharya, 2011).

The results showed that *Phellinus* spp. lacked mutagenicity when tested with *S. typhimurium* strains TA98 and TA100. On the other hand, they showed that each *Phellinus* extract had antimutagenic activity when tested with TA98 and TA100 assay systems, especially the ethanol extract of *P. rimosus*. Table 5 shows the effect of ethanol, water, and alkaloid procedure extracts from *Phellinus* spp. at various concentration on the mutagenicity of nitrite treated 1-aminopyrene on *S. typhimurium* strains TA98 and TA100.

The results showed that all of the extracts exhibited strong inhibition (more than 60% inhibition), especially the extract of ethanol extract from *P. rimosus*. In the case of TA98, the ethanol extract of *P. rimosus* had strong activity at 1mg/plate, whereas ethanol extract of *P. rimosus* showed strong antimutagenic activity at the concentration of 2 mg/plate for TA100. The ethanol fraction had the most active antimutagenic activity. This result was supported by Taherkhani (2014); Prabakaran *et al.* (2011), and Devi *et al.* (2015). Based on these findings, the antimutagenicity activity of ethanol extract from *P. rimosus* resulted from phenolic compounds in this mushroom. The mechanism by which the extract showed antimutagenicity is not clear. However, the antimutagenic activity of the extract against direct acting mutagens is probably due to the direct inactivation of the mutagens by complex formation with the ingredients present in the extract. But the antimutagenic effect may also result by protecting the bacterial genome from the directly acting mutagens. This protection can be rendered by the rapid elimination of mutagens from bacteria before their interaction with the DNA, which may be mediated by facilitating or stimulating the transmembrane export system in bacteria (Ajith and Janardhanan, 2011).

The results of this study showed significant antioxidant and antimutagenic activity in both strains (TA98 and TA100) for ethanolic extracts of *P. rimosus*. This result was expected due to the phytochemical constituents especially the phenolic compound. A wide range of antimutagenic agents have antioxidant activity e.g. flavonoids and phenolic compounds (Bhattacharya, 2011; Phadungkit *et al.*, 2012). The results were in agreement with finding of Prabakaran *et al.* (2011); Taherkhani (2014), and Devi *et al.* (2015).

## Conclusions

This research found that the phytochemicals in the ethanol extract from *P. rimosus* are flavonoids, alkaloids, polysaccharides, terpenoids, and phenolic compounds. In addition, the ethanol extract of *P. rimosus* possessed excellent antioxidation and antimutagenic activities. Therefore, the ethanol extract of *P. rimosus*, which is rich in natural antioxidant and antimutagenic agents, may be an attractive source for promotion of health and disease prevention as well as a source of potential pharmaceuticals, nutraceuticals, and cosmeceuticals.

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