Screening of antibacterial activities of edible wild and cultivated mushrooms from Northeast Thailand against foodborne pathogenic bacteria

Amporn Srikram*, Amonrat Taochatturat and Daovini Surinpa

ABSTRACT: This study aimed to screen the antibacterial activity of methanolic extracts of eleven edible wild mushroom species—Amanita calyptroderma Ark. et Bal., Amanita princeps Corner et Bas., Astraeus odoratus, Boletus chrysenteron Bull., Boletus colossus Heim., Craterellus aureus Berk. Et Curt., Lentinus strigosus (Schw.) Fr., Russula alboareolata Hongo, Russula emetic (Schaeff. ex Fr.) S.F.Gray., Russula virescens (Schaeff.) fr., Termiomyces clypeatus Heim and five cultivated mushroom species—Auricularia auricula-judae, Lentinus polychrous Lev., Lentinus squarrosulus Mont., Pleurotus sajor-caju (Fr.) Sing, Volvariella vovacea (Bull. Ex.Fr.) Sing. The antibacterial activity from the methanol extracts of mushroom fruiting bodies were evaluated according to the agar well diffusion method on four foodborne pathogenic bacteria—Escherichia coli, Salmonella Typhimurium, Staphylococcus aureus, Bacillus cereus. All wild mushrooms tested, except R. emetic showed different inhibition degrees to the growth of B. cereus (inhibition zone 2.20-7.40 mm) and S. aureus (0.83-14.40 mm). A. princeps, A. calyptroderma, B. colossus and R. alboareolata show to inhibit the growth of E. coli (1.37-9.43 mm) and S. Typhimurium (1.80-4.20 mm). B. chrysenteron and L. strigosus show the good inhibition to S. typhimurium (8.10 mm and 8.93 mm, respectively). For five cultivated mushroom, none of them showed inhibition the growth of S. aureus. Only V. vovacea showed some inhibit the growth of E. coli with narrow inhibition zone of 0.47 mm. L. polychrous and L. squarrosulus showed to inhibit the growth of B. cereus (2.33 mm and 2.13 mm) and S. typhimurium (3.60 mm and 8.00 mm). Among all mushrooms tested, A. calyptroderma was the best study species showing high antibacterial activity.

Keywords: mushroom, antibacterial activity, agar well diffusion method, foodborne bacteria

Introduction

Food poisoning is one of the most common cause of illness and death in developing countries. Most of food poisoning reports are associated with bacterial contamination including of gram-negative bacteria (e.g. Salmonella Typhimurium and Escherichia coli) and gram-positive bacteria (e.g. Staphylococcus aureus and Bacillus cereus) (Mostafa et al., 2017). Recently, prevention of food spoilage and food poisoning pathogens is usually achieved by use of chemical preservatives which have negative impacts on human health and environment (Mostafa et al., 2017). Because of such concerns, the necessity to find a potentially effective, healthy safer and natural alternative preservatives is increased. Natural resources have been exploited in the last years and among them, mushrooms could be an alternative source of new antimicrobials.

For a long time mushrooms have been use for food. Both wild mushroom and cultivated mushrooms contain a high diversity of biomolecules beneficial to human health with nutritional (Sanmee et al., 2003; Srikram and Supapvanich, 2016) and medical properties (Borchers et al., 2004; Poucheret et al., 2006). Fruiting bodies, mycelia and spores accumulate a variety of bioactive metabolites with immunomodulatory, cardiovascular, liver protective, anti-fibrotic,
anti-inflammatory, anti-diabetic, anti-viral, anti-oxidant, antitumor, and antimicrobial properties (Mostafa et al., 2017). Ramesh and Patter (2010) reported that methanolic extract of mushroom showed antimicrobial which correlated to the phenol and flavonoids contents in the mushroom. From our previous study, cultivated and wild mushroom showed to have antioxidant activity, phenol and flavonoid content (Srikram and Supapvanich, 2016), however antimicrobial activity were not investigated yet. Therefore, the aim of this study is to screen antibacterial activity of sixteen mushrooms against foodborne pathogen; *E. coli*, *S. typhimurium*, *S. aureus* and *B. cereus*.

**Materials and Methods**

**Mushroom materials and preparation of mushroom crude extracts**

Eleven edible wild mushrooms and five commercial cultivated mushrooms were purchased from a local market (Bypass market) in Sakon Nakhon province, Thailand (Table 1, Figure 1) during June-August 2016. Approximately 2 kg of each mushroom species was purchased and collected in a separate plastic bag. All mushrooms were then delivered to the Food Technology Laboratory at the Faculty of Natural Resources and Agro-Industry, Kasetsart University campus, Chalermphrakiat Sakon Nakhon province within 3 hr after purchase. Identification was done by comparing their morphological, anatomical and physiological characteristics and monographs with descriptions given in the manual (Royal academy, 2007), except *Hed Phor Nung (Astraeus odoratus)* was identified according to Phosri et al. (2004).

Each fruiting body of mushroom was cleaned of soil with a soft brush and washing with clean water. Inedible parts and debris were removed using a sharp knife. Each mushroom sample was cut into very small pieces using a sharp knife and dry in hot air oven at 50 °C. Dried samples were ground to fine powder by blender. Twenty-five grams of mushroom power were extracted by stirring with 200 ml of 95% (v/v) methanol in shaker at 150 rpm for 24 hr at room temperature (25±2 °C). The extracts were filtered through Whatman no. 4 paper. The extract was evaporated at 40 °C in vacuum dryer. After that, crude extract was dissolved in 5% dimethylsulfoxide (DMSO) to a final concentration of 500 mg/ml and filter-sterilization was done through a 0.45µm membrane filter. Then, the extracts were stored at -18 °C until used in the test.

**Antibacterial activity**

Antimicrobial activities of methanol extracts were tested against 4 foodborne pathogens, including two strains of gram-negative bacteria; *Escherichia coli* TISTR 074 and *Salmonella enterica* serovar Typhimurium TISTR 1469 and two strains of gram-positive bacteria; *Staphylococcus aureus* TISTR 2329 and *Bacillus cereus* TISTR 1527. All the microorganisms used were obtained from the Thailand Institute of Scientific and Technological Research (TISTR).

Antibacterial activity of mushroom extract was carried out by the agar well diffusion method (Oyetayo et al., 2009) with some modifications. Briefly, bacteria were cultured in nutrient agar (NA), except *S. Typhimurium* was culture in Trypticase soy agar (TSA) at 37 °C for 24 hr. Before
experimental use, cultures from solid medium were subcultivated in nutrient broth, except S. Typhimurium was subcultivated in trypticase soy broth and incubated at 37 °C for 24 hr. To standardize the inoculum density for a susceptibility test, 0.5 MaFarland standard was used (approximately $10^8$ cfu/ml) of bacterial cells. Then, 100 µl of bacterial cell suspensions was spread on the surfaces of NA or TSA. The inoculated plates were left for 10-15 minutes at room temperature. Small well (8 mm in diameters) were made in the agar plates by sterile cork borer. One hundred microliters (equivalent to 50 mg) of the extract of each isolated of the mushroom was loaded into the difference well. DMSO was used as negative control and a paper disc contain 30 mg of chloramphenicol was used as positive control for test bacteria. The plate were incubated at 37 °C for 24 hr. After the period of incubation the zone of inhibition was measured. In this study, antimicrobial activity of each mushroom variety from three samples were analyzed separately in triplicate and the results were recorded as mean ± SD. The means diameter of inhibition zone of each mushroom variety were examined for significance difference using ANOVA with Duncan’s significant difference post-hoc test at $P<0.05$.

Results and Discussion

Results of the antibacterial activity of eleven edible wild mushrooms and five commercial cultivated mushrooms extracts (50 µg) were investigated by agar well diffusion method as shown in the Table 1 and Figure 2. All wild mushroom test, except R. emetic shown inhibit different degrees to the growth of gram-positive bacteria B. cereus (clear inhibition zone diameter was 2.20-7.40 mm) and S. aureus (0.83-14.40 mm). The widest inhibition zone of 14.40 mm was obtained in the methanolic extract from A. calyptroderma against S. aureus. For gram-negative bacteria test, only A. princeps, A. calyptroderma, B. colossus and R. alboareolata show to slightly inhibit the growth of E. coli (1.37-9.43 mm) and S. Typhimurium (1.80-4.20 mm). B. chrysenteron and L. strigosus show the good inhibition to S. Typhimurium with inhibition zone of 8.10 mm and 8.936 mm, respectively. For five cultivated mushroom, none of them showed inhibit the growth of S. aureus. Only V. vovacea showed some inhibit the growth of E. coli with narrow inhibition zone of 0.47 mm. L. polychrous and L. squarrosulus showed to inhibit the growth of B. cereus (2.33 mm and 2.13 mm) and S. Typhimurium (3.60 mm and 8.00 mm). From this study, A. auricular and P. sajor did not showed any inhibit the growth of four bacteria test. However, Tambekar et al. (2006) reported that P. sajor can inhibit the growth of S. aureus, E. coli and S. Typhimurium.

The results of the current study show that methanolic extract of cultivated mushroom had lower antibacterial activity than wild mushroom. This observation is correlated with our previous study which found that the wild mushroom had more bioactive compound (total phenol and total flavonoid content) than the cultivated mushroom (Srikram and Supapvanish, 2016). Since phenolic compounds have attracted much interest recently because in vitro and in vivo studies suggest that they have a variety of beneficial biological properties which may play an important role in the maintenance of human health. Antioxidant and antimicrobial activities of Laetiporus
sulphureus were reported and correlated to the phenols and flavonoids contests (Barros et al., 2007). Moreover, Ramesh and Pattar (2010) have reported that methanolic extract of six wild edible mushrooms from India showed antimicrobial activity and correlated with the present of high phenolic compounds (the phenol and flavonoids contents).

In general, gram-negative bacteria had a higher resistance towards antimicrobial agents. Our findings are consistent with this observation that mushroom extract had higher antibacterial activity against gram-positive than gram-negative bacteria. This observation may be explained by the differences in the cell wall structure between gram-positive and gram-negative bacteria. The cell walls of gram-negative bacteria, which are more complex than of the gram-positive bacteria, act as a diffusional barrier making them less susceptible to the antimicrobial agents than gram-positive bacteria (Nostro et al., 2000).

Figure 1 Sixteen mushroom species used in this study. Five cultivated mushrooms were A. auricular (1); V. vovacea (2); P. sajo-cajur (3); L. polychrous (4); L. squarrosulus (5) and eleven wild mushroom were A. odoratus (6); A. princeps (7); A. calyptroderma (8); B. colossus (9); R. alboareolata (10); R. emetic (11); R. virescens (12); T. clypeatus (13) C. aureus (14); B. chrysenteron (15); L. strigosus (16). All mushroom samples were brought from a local market (Bypass market) in Sakon Nakhon province, Thailand during June-August 2016.
Table 1 Inhibition zone diameters of methanolic extracts from cultivated and edible wild mushrooms from Northeast Thailand.

<table>
<thead>
<tr>
<th>No.</th>
<th>Mushroom species</th>
<th>Local (Thai) name</th>
<th>Gram positive bacteria</th>
<th>Gram negative bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>B. cereus</td>
<td>S. aureus</td>
</tr>
<tr>
<td>(1)</td>
<td>Auricularia auricular-judae</td>
<td>Hed Hoo Noo</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>(2)</td>
<td>Volvariella vovacea (Bull. Ex.Fr.) Sing</td>
<td>Hed Fang</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>(3)</td>
<td>Pleurotus sajor-caju (Fr.) Sing</td>
<td>Hed Nang Pha</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>(4)</td>
<td>Lentinus polychrous Lev.</td>
<td>Hed Bod</td>
<td>2.33±1.75cd</td>
<td>NA</td>
</tr>
<tr>
<td>(5)</td>
<td>Lentinus squarrosulus Mont.</td>
<td>Hed Khon Kaw</td>
<td>2.13±0.34ad</td>
<td>NA</td>
</tr>
<tr>
<td>(6)</td>
<td>Astraeus odoratus</td>
<td>Hed Phor Nung</td>
<td>3.00±2.22ad</td>
<td>0.83±1.18d</td>
</tr>
<tr>
<td>(7)</td>
<td>Amanita princeps Corner et Bas</td>
<td>Hed Ra Ngok Kao</td>
<td>5.17±0.58abc</td>
<td>11.50±2.29ab</td>
</tr>
<tr>
<td>(8)</td>
<td>Amanita calyptroderma Ark. et Bal.</td>
<td>Hed Ra Ngok Laung</td>
<td>7.73±1.27c</td>
<td>14.40±3.97a</td>
</tr>
<tr>
<td>(9)</td>
<td>Boletus colossus Heim</td>
<td>Hed Phung</td>
<td>4.83±2.60ab</td>
<td>6.20±2.49cd</td>
</tr>
<tr>
<td>(10)</td>
<td>Russula aboareolata Hongo</td>
<td>Hed Kao Din</td>
<td>4.50±4.09dcd</td>
<td>9.10±8.01abc</td>
</tr>
<tr>
<td>(11)</td>
<td>Russula etimic (Schaeff. ex Fr.) S.F.Gray.</td>
<td>Hed Daeng</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>(12)</td>
<td>Russula vircensc (Schaeff.) fr.</td>
<td>Hed Chai</td>
<td>2.20±1.72cd</td>
<td>2.07±2.25cd</td>
</tr>
<tr>
<td>(13)</td>
<td>Termitomyces clypeatus Heim</td>
<td>Hed Chone</td>
<td>3.50±2.86abcd</td>
<td>8.83±4.17abcd</td>
</tr>
<tr>
<td>(14)</td>
<td>Craterellus aureus Berk. et Curt.</td>
<td>Hed kamin noi</td>
<td>4.00±2.76abcd</td>
<td>8.67±3.36abcd</td>
</tr>
<tr>
<td>(15)</td>
<td>Boletus chrysenteron Bull.</td>
<td>Hed Tub Taw Kha Daeng</td>
<td>4.93±2.37b</td>
<td>8.40±4.66b</td>
</tr>
<tr>
<td>(16)</td>
<td>Lentinus strigosus (Schw.) Fr.</td>
<td>Hed Phai</td>
<td>7.40±0.73a</td>
<td>2.67±3.77ad</td>
</tr>
</tbody>
</table>

Positive control Chloramphenicol 30 mg 17.57±0.33 24.73±0.60 20.50±0.24 12.80±0.75
Negative control 5% DMSO NA NA NA NA

Each value is a mean of three replicates ± Standard Error. The means diameter of inhibition zone of each mushroom variety were examined for significance difference using ANOVA. In each row, different lowcase superscript letters in the same column indicate a statistical difference at P<0.05.

NA = indicate no antibacterial effect.
Figure 2 Inhibition zone of methanol extracts of mushrooms against *B. cereus*, *S. aureus*, *E. coli* and *S. Typhimurium* by agar-well diffusion method. The well (8 mm in diameter) were loaded with 100 µl (equivalent to 50 mg) of mushroom extracts: (1) *A. auricular*; (2) *V. vovacea*; (3) *P. sajo-cajur*; (4) *L. polychrous*; (5) *L. squarrosulus*; (6) *A. odoratus*; (7) *A. princeps*; (8) *A. calyptroderma*; (9) *B. colossus*; (10) *R. alboareolata*; (11) *R. emetic*; (12) *R. virescens*; (13) *T. clypeatus*; (14) *C. aureus*; (15) *B. chrysenteron*; (16) *L. strigosus*. One hundred microliters of 5% DMSO were used for a negative control (N) and a paper disc of 30 mg Chloramphenicol was used for positive control (P) in each plate.
Conclusions

It has been concluded from the results of this investigation that the extract of cultivate mushroom are weak inhibitors of bacterial growth than wild mushrooms. Among mushroom test, A. calyptroderma is the best study species. For further study, the identification of the bioactive compounds and study of mechanisms of mushroom activity against bacteria pathogens are necessary prior to use as food additives.

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References


