

Evaluation of antioxidant and antibacterial activities of the stems of *Flammulina velutipes* and *Hypsizygus tessellatus* (white and brown var.) extracted with different solvents

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Abstract

Mushrooms are rich in pharmacologically-important phytochemicals with reported medicinal values. In this study, the antibacterial activity of *Flammulina velutipes* (Enoki), *Hypsizygus tessellatus* (brown (Buna shimeji) and white (Bunapi shimeji) variants) stem extracts prepared with different solvents (water, methanol, acetone, and ethyl acetate) was investigated against *Escherichia coli* (*E. coli* ATCC 25922), *Serratia marscescens* (*S. marscescens* ATCC14756), *Bacillus subtilis* (*B. subtilis* ATCC 23857), and *Staphylococcus aureus* (*S. aureus* ATCC 25923). Their antioxidant activities were evaluated using radical scavenging assays of 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide (H₂O₂) and ferric reducing power (FRP). The water extracts of Enoki, Buna shimeji, and Bunapi shimeji showed bacterial growth inhibition in a concentration-dependent manner. From the obtained results, all the Enoki extracts showed a significant inhibition of the gram positive bacterial species (*E. coli* and *S. marscescens* > 68%) and a reduced inhibition of the gram negative bacterial species (*B. subtilis* and *S. aureus* < 45%, $p < 0.05$) after 24 h of incubation, while water extracts of Buna shimeji showed a significantly lower bacterial growth inhibition (< 60%) against all the studied bacteria. Bunapi shimeji extract inhibited *S. marscescens*, *E. coli*, *B. subtilis*, and *S. aureus* by 54, 67, 46, and 44%, respectively. Methanol, acetone and ethyl acetate extracts showed significantly lower antibacterial activities ($p < 0.05$) compared to water extracts. Similarly, water extracts of Enoki, Bunapi shimeji and Buna shimeji showed significant antioxidant activities using DPPH (67.37 ± 0.01 , 66.30 ± 0.18 and $42.44 \pm 0.18\%$, respectively), hydrogen peroxide (67.87 ± 0.000 , 45.52 ± 0.160 and $52.08 \pm 0.000\%$ respectively), and FRP (0.891 ± 0.001 , 0.413 ± 0.001 and 0.491 ± 0.001 , respectively) at the concentration of 1 mg/mL, compared to their respective methanol, acetone and ethyl acetate fractions. Upon LC-MS analysis of the most potent fraction (Enoki water extract), several phenolic compounds were identified, of which chromogenic acid, Methyl-5-*O*-caffeoylquinic acid, Kukoamine A, Kushenol K, Methyl Kushenol C, Glabrol, Sanggenon J, Corylin, and Moracenin C were confirmed. The antioxidant activities of the water extracts of Enoki, Buna shimeji and Bunapi shimeji correlated with their total phenolic and flavonoid contents, which were (166.56 ± 1.50 , 108.13 ± 0.32 and 116.71 ± 0.01 μg gallic acid equivalent (GAE)/mg, respectively) and (96.33 ± 0.03 , 82.18 ± 0.20 and 91.37 ± 0.15 μg quercetin equivalent (QE)/mg, respectively). Collectively, the study results have shown the studied mushrooms as potential natural sources of pharmacological agents.

Keywords: Phenolic compounds; Antioxidants; Solvent polarity; Antibacterial; *H. tessellatus*; *F. velutipes*