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Effect of total phenolic content on free radical scavenging activities of Boletes mushroom extracts

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Introduction

Phenolic compounds are powerful antioxidants that can protect the human body from free radicals by acting as hydrogen donors, reducing agents and radical scavengers[1]. So, phenolic compounds have positive correlation with antioxidant activities[2]. Edible mushrooms have long been known as medicine and tonics. Recently, they have become increasingly attractive as functional foods for their health benefits due to their high level of proteins, polysaccharides, dietary fibers, and phenolic compounds[3]. Boletes mushroom is one of several edible mushrooms. There are many studies reported that it had high content of polyphenols and showed good antioxidant activities when compared with other mushrooms[4-6]. However, there is a little information about antioxidant activity of Boletes mushroom grown in Thailand[7]. In this study, extracts from Boletes mushrooms in Thailand were determined for total phenolic content and free radical scavenging activities, and their correlation was also investigated. This data will supply new information regarding the antioxidant potency and phenolic content of these Boletes mushroom extracts for further utilization especially in health and cosmetic products.

Materials and methods

Chemicals 2,2 Diphenyl-1-picrythydrazyl (DPPH), 2,2-azino-bisethylbenzthiazoline-6 sulfonic acid (ABTS), gallic acid and potassium persulfate were purchased from Sigma (Sigma-Aldrich, USA). Folin-Ciocalteau reagent was purchased from Merck, Germany. Sodium carbonate was purchased from Ajax Finechem, Australia. L-(+)-ascorbic acid was purchased from Carlo Erba, Italy. 95% Ethanol was purchased from the Liquor Distillery Organization, Thailand.

Mushroom materials and extraction: Seven Boletes mushrooms were collected from the central (TISTR Boletes A31) and the northeastern parts (TISTR Boletes A36, TISTR Boletes C14, TISTR Boletes 37, TISTR Boletes 50, TISTR Boletes 55, TISTR Boletes 56) of Thailand. After harvesting, the Boletes mushrooms were cleaned and extracted with 95% ethanol for 7 days. Then, the extracts were concentrated by rotary evaporator and dried by freeze-drying method. All of samples were kept in airtight container at -20ºC prior to further analysis.

Determination of total phenolic content:The content of total phenols was determined using Folin-Ciocalteu method by UV-VIS microplate reader[8,9]. Gallic acid was used as a standard compound. Twenty microliters of each mushroom extract solution was reacted with 100 µl of 1:10 Folin-Ciocalteu's regent in a 96-well plate. Then, 80 µl of 7.5% of sodium carbonate solution was added. The mixture was allowed to stand in darkness at room temperature for 60 min before the absorbance at 765 nm was measured against water. The concentration of polyphenols in mushroom extracts was calculated from a standard curve of gallic acid ranging from 4 to 16 µg/ml. The content of total phenols was expressed as gallic acid equivalents in mg/g extract (mg GAE/g extract).

Determination of free radical scavenging activities: DPPH[8] and ABTS[10] scavenging activities of the mushroom extract solutions were measured at the concentration of 100 µg/ml for screening test. The IC50 values of Boletes mushroom extracts were also determined. All experiments were carried out in triplicate.

- DPPH radical scavenging assay[8] One hundred microliters of the sample solution was reacted with 100 µl of 6x10-5 M DPPH ethanolic solution in a 96-well plate, then incubated in darkness at room temperature for 60 min. The absorbance was measured at 510 nm using a UV-VIS microplate reader. Ascorbic acid and gallic acid were used as positive control.
- ABTS radical scavenging assay[10] The ABTS reagent was produced by reacting 5 ml of 7 mM ABTS stock solution with 88 µl of 140 mM potassium persulfate solution and incubating the mixture at room temperature in darkness for 16 h. The ABTS reagent was diluted with water to give an absorbance of 0.700 ± 0.05 at 734 nm for measurement. Twenty microliters of the sample solution was reacted with 180 µl of ABTS reagent in a 96-well plate, then incubated in darkness at room temperature for 60 min. The absorbance was measured at 734 nm with a UV-VIS microplate reader. A positive control was ascorbic acid.

Data analysis: All data were reported as mean ± standard deviation. Analysis of data was performed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using the SPSS software (version 17.0 for window). The correlation between the antioxidant activity and total phenolic content was examined by Pearson's correlation. Significance was determined at $p < 0.05$.

Results and Discussion

The total phenolic content of seven Boletes mushroom extracts was determined using the Folin-Ciocalteu method. From Figure 1, their total phenolic content varied from 26.70-113.17 mg GAE/g extract. The result showed that total phenolic content of Boletes mushroom extracts could be divided into 3 groups including high (more than 80 mg GAE/g extract), medium (40-80 mg GAE/g extract) and low (less than 40 mg GAE/g extract) phenolic content groups. Their phenolic contents were higher than those reported by previous studies on Boletes mushroom extracts that showed total phenolic content between 5.67-17.50 mg GAE/g extract[3,11]. Different phenolic content of Boletes mushroom extracts may be due to type of mushroom, differences in strain, substrate, cultivation and fruiting conditions, the developmental stage of the mushroom, and the age of the fresh mushroom sample[12].

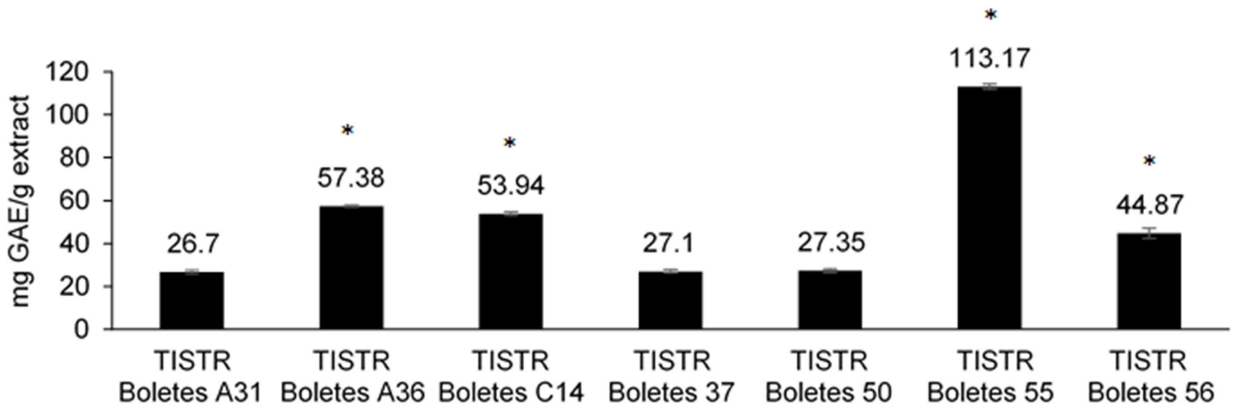

Figure 1 Total phenolic content of investigated Boletes mushroom extracts (n=3, mean ± SD) (* Significantly different from other Boletes mushroom extracts at $p < 0.05$)

The present study was found that all of the Boletes mushroom extracts obtained showed high antioxidant activities. Antioxidant activities by DPPH (43.72-93.89%) and ABTS (41.66-99.76%) scavenging activities of each extract at the concentration of 100 µg/ml are shown in Figure 2. The activities could be divided into 3 groups such as high (more than 80%), medium (60-80%) and low (less than 60%) DPPH or ABTS scavenging activity groups. TISTR Boletes 55 exhibited the highest percentage of inhibition on both assays. Some remaining magnitude of their radical scavenging effects was differently ranked in both assays. Antioxidant activities of TISTR Boletes 55 were higher than those reported by a previous study on Boletus edulis mushroom extract that showed 72.70% and 83.75% inhibition at the concentration of 1 mg/ml on DPPH and ABTS scavenging assays, respectively[9].

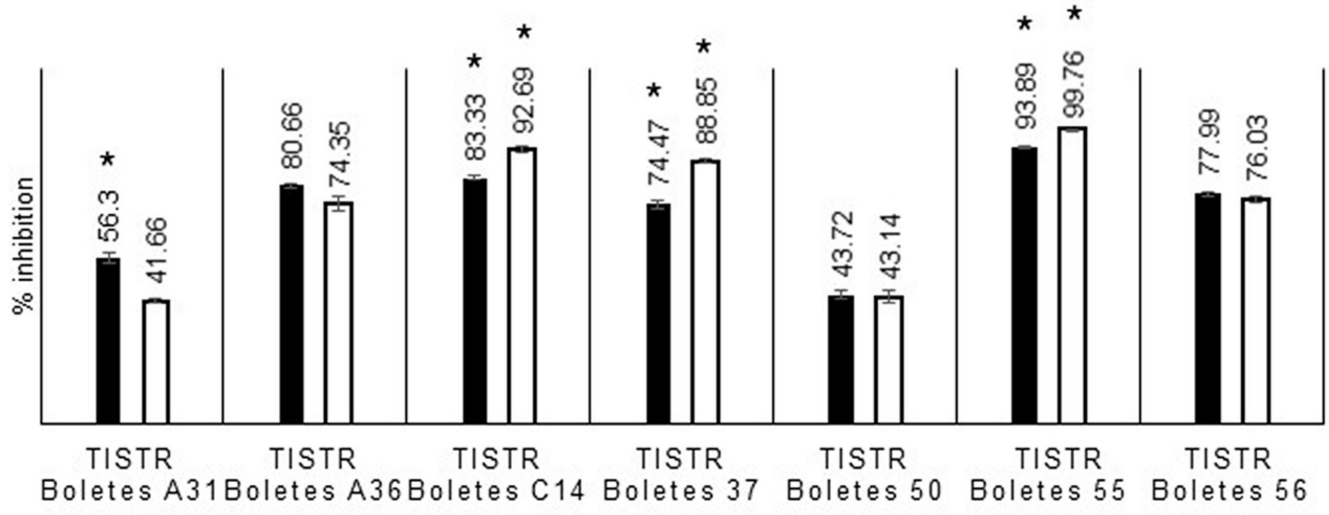

Figure 2 DPPH (▼) and ABTS (▷) free radical scavenging activities of Boletes mushroom extracts at the concentration of 100 µg/ml (n=3, mean ± SD) (* Significantly different from other Boletes mushroom extracts at $\rho < 0.05$)

IC_{50} values of Boletes mushroom extracts on DPPH (13.63-123.71 µg/ml) and ABTS (23.58-171.08 µg/ml) assays are reported in Figure 3. The sequence of IC_{50} values of DPPH and ABTS assays corresponded to their percentage of inhibition effects at the concentration of 100 µg/ml. TISTR Boletes 55 exhibited the strongest free radical scavenging activities on both scavenging assays. When compared with positive controls, TISTR Boletes 55 showed lower antioxidant activities than ascorbic acid and gallic acid.

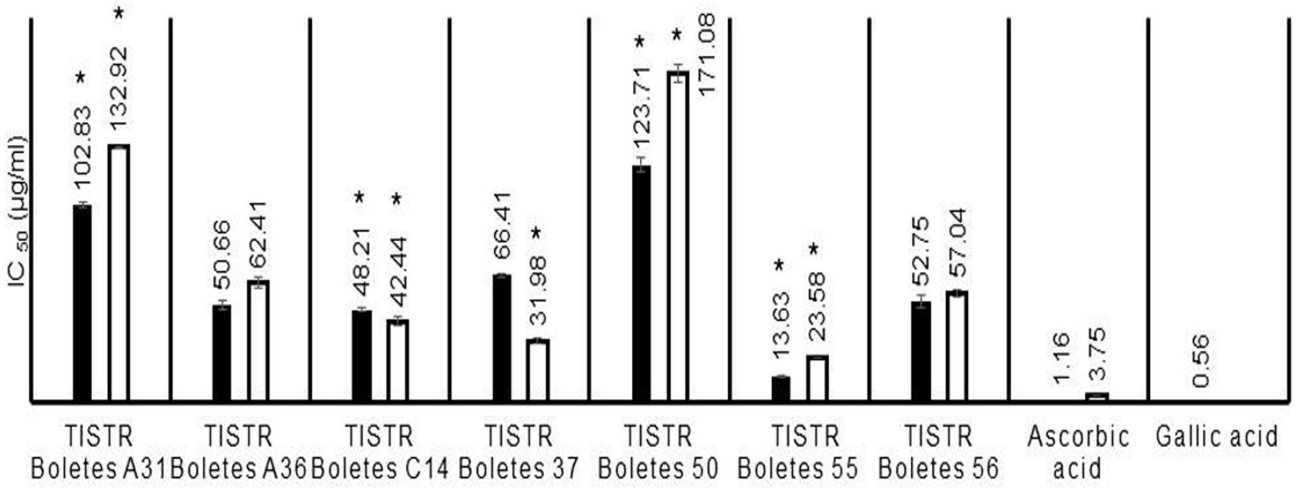

Figure 3 IC_{50} values of DPPH (▼) and ABTS (▷) scavenging activities of Boletes mushroom extracts and positive controls (n=3, mean ± SD) (* Significantly different from other Boletes mushroom extracts at $p < 0.05$)

Plots between total phenolic content and percentage of free radical scavenging activities of Boletes mushroom extracts were performed to determine their correlation (Figure 4). Their correlation coefficients (r) are shown in Table 1. The result showed that there was significant correlation between their total phenolic content and % inhibition of DPPH assay (r=0.762). However, the correlation was considerably higher (r=0.973) when it was conducted on only Boletes mushroom extracts with medium and high phenolic contents (more than 40 mg GAE/g extract). This result was consistent with the previous study that suggested phenolic content affected DPPH radical scavenging assay by acting as potent hydrogen donors[2]. For ABTS assay, the correlation between total phenolic content and % inhibition was low (r=0.647). This result may be due to variety of ABTS scavenging effects of low (TISTR Boletes A31, 37 and 50) and medium (TISTR Boletes 56, C14 and A36) total phenolic groups. For example, TISTR Boletes C14 and 37 showed good antioxidant effect but TISTR Boletes A31, A36, 50 and 56 did not. The reasons that could be explained these results may be 1) other bioactive compounds such as flavonoid, protein, and carbohydrate in low and medium phenolic content groups, and 2) the different presence of potent phenolic compound in crude extracts that can inhibit free radicals[4]. However, the correlation was higher (r=0.768) for medium and high phenolic content groups (more than 40 mg GAE/g extract), but it was not significant ($p > 0.05$). This result agreed with the pervious study that showed low correlation between total phenolic content and % inhibition of ABTS assay[1]. So, it assumed that total phenolic content of Boletes mushroom extracts tested did not affect their ABTS scavenging activity. These correlations corresponded to the correlations between their total phenolic content and IC_{50} values of both assays.

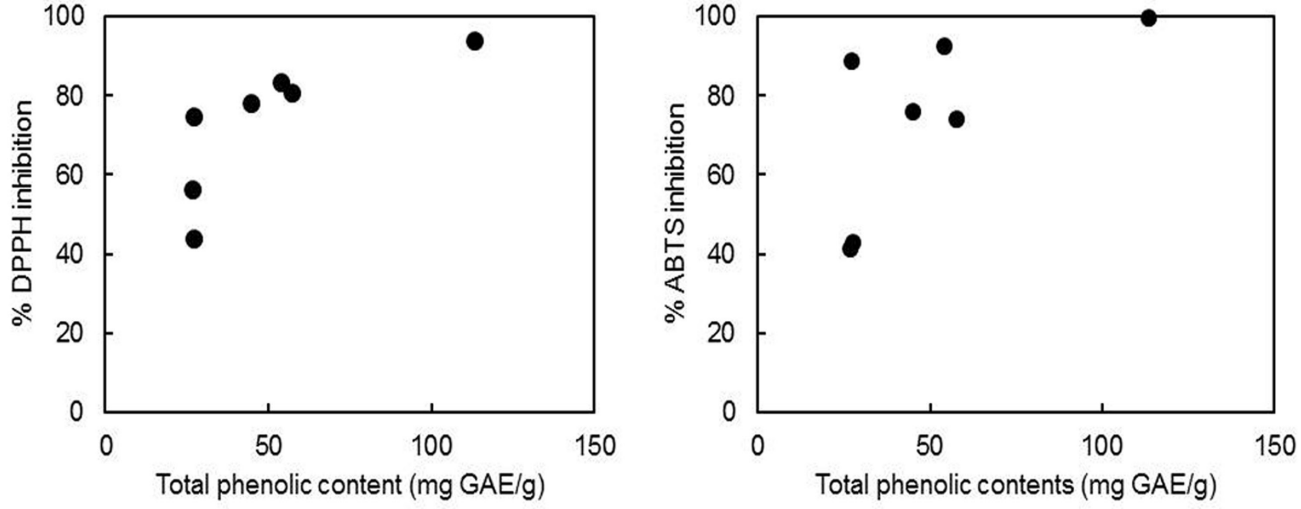

Figure 4 Plots between total phenolic content of Boletes mushroom extracts and their free radical scavenging activities (%) on DPPH assay (left) and ABTS assay (right)

Table 1 Pearson's coefficient for the correlation between total phenolic contents (mg GAE/g extract) and free radical scavenging activities of Boletes mushroom extracts

Total phenolic content	DPPH		ABTS	
	% inhibition at 100 µg/ml	IC50 (µg/ml)	% inhibition at 100 µg/ml	IC50 (µg/ml)
All groups	0.762*	-0.823*	0.647	-0.576
Medium and high groups	0.973*	-0.992**	0.768	-0.861

Conclusion

Thai Boletes mushroom extracts studied showed high phenolic content and good free radical scavenging activities. The correlation between their total phenolic content and DPPH scavenging activity was significantly high whereas there was no correlation between their total phenolic content and ABTS scavenging activity. The weak relation between their phenolic content and ABTS scavenging activity may be due to other bioactive compounds in some Boletes mushroom extracts. Therefore, the determination of other bioactive compounds in the extracts should be further studied. Moreover, based on the obtained results showing the excellent antioxidant potency of TISTR Boletes 55, it can be used as accessible source of natural antioxidant or as a new active ingredient for using in health and cosmetic products.

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