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Synergistic effect of water extract from combined edible mushrooms

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ABSTRACT

The synergistic effect of the water extract from Gold cordyceps (*Cordyceps militaris*), Shiitake (*Lentinus edodes*) and Lingzhi (*Garnoderma lucidum*) were studied. The results showed that the mixture of *C. militaris* and *G. lucidum* gave highest yield. However, polysaccharide content was no statistically significant difference in all extracts. Then, the extracts and mixture of extracts were test for antimicrobial activity, antioxidant activity and α-amylase inhibition. The result revealed that none of extracts showed antimicrobial activity. Interestingly, the mixture of *C. militaris* and *G. lucidum* extracts showed synergistic of antioxidant activity. The lowest IC50 of scavenging activity was 0.12±0.03 mg/ml. The mixture of three mushrooms extract exhibited α-amylase inhibition by 69.72±1.20%.

Keywords: Synergism, Extract, Shiitake, Gold cordyceps, Lingzhi

INTRODUCTION

Some edible mushrooms were used in medical therapies for a long time (Boonsong et al., 2016). It may be used in dried fruiting body, dried mycelium or the extracts. Recently, there are many solvent for the extraction of mushroom such as water, ethyl acetate, diethyl ether and methanol (Cheung et al., 2003; Boonsong et al., 2016). However, the polysaccharides in mushroom were widely bioactive compound and were extracted by water (Thetsmuang et al., 2011; Boonsong et al., 2016). Thus, the water crude extracts were performed in this study.

Gold cordyceps (*Cordyceps militaris*), Shiitake (*Lentinus edodes*) and Lingzhi (*Garnoderma lucidum*) were the popular edible mushrooms which were used as supplementary food due to contained a lot of bioactive compound. *C. militaris* is grouped in caterpillar fungus. The polysaccharide of this mushrooms have many bio-activities such as anti-virus, immune-stimulating, anti-tumor activity and antioxidant activity (Ai-li et al., 2010). Moreover, this extract was found that it can decrease glucose level in blood of diabetic rat (Dong et al., 2014). For *L. edodes*, the polysaccharide extract showed antioxidant activity and immunomodulation (Boonsong et al., 2016; Kothari et al., 2018). Finally, the water extract from *G. lucidum* contains immune adjuvant for viral vaccine, hypolipidaemic, antioxidant and anti-tumor activities (Kothari et al., 2018; Wu, 2018). Moreover, water extract of *G. lucidum* has been reported about hypoglycemic effects in diabetic mice (Seto et al., 2009).

To induce the affection of mushroom extract, the synergistic effect was characterized. The previous studied has been reported the synergistic effect of mushroom extraction in other mushrooms but not in *C. militaris*, *L. edodes* and *G. lucidum* (Queiros et al., 2009; Vieira et al., 2012). In this study, yield, antimicrobial and antioxidant activities of crude extracts were determined. In addition, the inhibition of α-amylase was also studied. Due to this enzyme involves in glucose level in blood and indirectly role in diabetic patient.

MATERIAL AND METHODS

Mushroom samples

Dried fruiting bodies of Gold cordyceps (*Cordyceps militaris*), Shiitake (*Lentinus edodes*) and Lingzhi (*Garnoderma lucidum*) were obtained from the market. Mushrooms were dried and ground into powder. The extracts were divided into 6 experiments as describe in table 1.

Table 1. Weight of each dried fruiting bodies which used for extraction

Extracts	Weight of dried fruiting bodies for extraction (g)		
	C. militaris	***L. edodes***	***G. lucidum***
C	30	0	0
L	0	30	0
G	0	0	30
C+L	15	15	0
C+G	15	0	15
C+L+G	10	10	10

Crude polysaccharides preparation

Briefly, 30 g of dried fruiting bodies were boiled twice with 600 ml of water for 3 hours. Then, the extract was filtered through Whatman filter paper no. 1 and centrifuge at 6000 rpm for 20 min. To precipitate polysaccharide, 4 volume of 95% (v/v) ethanol was added. The reactions were incubated at 4 ºC for overnight. After centrifuge at 6000 rpm for 20 min, the supernatant was discarded. The polysaccharide pellet was washed with 95% (v/v) ethanol and centrifuge at 6000 rpm for 20 min. Ethanol was removed by air dried. The dry weight of crude extract was recorded.

Determination of total polysaccharide content

3 mg/ml of crude extract (0.5 ml) was mixed with 0.5 ml of 5% (v/v) phenol. Then, 2.5 ml of sulphuric acid was added. The reaction was incubated at room temperature for 10 min. After that, the tube was mixed with vortex and continue incubated for 20 min. The absorbance was measured at 490 nm using spectrophotometer.

Antimicrobial activity by agar well diffusion

Antibacterial activity. *Straphylococcus aureus* (TISTR 2399 180717), *Bacillus cereus* (TISTR 2342 180718), *Pseudomonas aeruginosa* (TISTR 1540 250718) and *Escherichia coli* (TISTR 073 190717) were used. Bacteria were prepared into $1x10^6$ CFU/ml and swab on Nutrient Agar (NA) plate. Wells (0.5 cm diameter) were created. Then, 20 mg/ml crude extract was loaded (50 µl/well). Plates were placed for 1 hr before incubated at 37 ºC for 24 hr. The diameter of clear zone was determined.

Antifungal activity. *Aspergillus fumigatus* and *Aspergillus niger* were used. Spore suspensions were prepared into $1x10^5$ spores/ml and swab on Potato Dextrose Agar (PDA) plate. Wells were generated in 0.5 cm diameter. Crude extracts were loaded (50 µl/well). After incubated at room temperature for 3 days, diameter of clear zone was measure.

DPPH scavenging activity

20 mg/ml crude extracts were doubling diluted. 500 µl of crude extract were mixed with 500 µl of 0.06 mM DPPH in ethanol. The reactions were incubated at room temperature and dark for 30 min. The absorbance was measure at 515 nm by spectrophotometer. The percentage of scavenging activity (%SA) was calculated by following formula:

$$\%SA = 100 - \left(\frac{100 \times (Abs\ sample)}{(Abs\ DPPH)}\right)$$

α-amylase inhibition

20 mg/ml crude extracts were doubling diluted. α-amylase inhibition was performed as described Bello et al. (2017). 500 µl of crude extract were mixed with 500 µl of 0.5 mg/ml α-amylase in 0.02 M sodium phosphate buffer pH 6.9 (with 0.006 M NaCl). The reactions were incubated at 25 °C for 10 min. 500 µl of 1% (w/v) starch in 0.02 M sodium phosphate buffer pH 6.9 (with 0.006 M NaCl) was added. The reactions were incubated at 25 °C for 10 min and were stopped by DNSA (500 µl). The reaction was boiled for 5 min and cooled at room temperature. The absorbance was measure at 540 nm by spectrophotometer.

RESULTS

Crude polysaccharide

Dried fruiting bodies were boiled to extract the polysaccharide. Then, the extracts were dried. All extracts were dark brown and sticky, except for crude extract from *G. lucidum* (Table 2). *G. lucidum* extract gave lowest yield. But, the mixture of *C. militaris* and *G. lucidum* gave highest yield. In term of polysaccharide content, all extracts contained similar content. However, the mixture of *G. lucidum*, *C. militaris* and *L. edodes* gave highest polysaccharide content (Table 1).

Table 2. The characteristic, yield and polysaccharide content of crude extracts.

Extracts	Color of crude	Texture	Yield (%)	Polysaccharide Content
C	Dark brown	Sticky	4.61 ^{ab} ± 0.34	207.24 ^{a} ± 16.37
L	Dark brown	Sticky	3.44 ^{ab} ± 1.57	249.65 ^{a} ± 15.58
G	Dark brown	Solid	1.70 ^{b} ± 0.72	217.80 ^{a} ± 38.63
C+L	Dark brown	Sticky	2.49 ^{ab} ± 0.07	171.78 ^{a} ± 74.38
C+G	Dark brown	Sticky	5.64 ^{a} ± 1.15	163.44 ^{a} ± 10.48
C+L+G	Dark brown	Sticky	2.85 ^{ab} ± 0.32	203.35 ^{a} ± 38.89

Note: The extract from *C. militaris* (C); *L. edodes* (L) and *G. lucidum* (G). In each column, different superscripts represent significant differences ($p \leq 0.05$). Polysaccharide content was reported in mg/ g of dry weight of crude extract.

Antimicrobial activity by agar well diffusion

Crude extracts were tested for growth inhibition of bacteria and fungi. The results showed that all of crude extracts showed neither antibacterial nor antifungal activities (data not shown).

Scavenging activity

The antioxidant property of crude extract was determined from scavenging activity (SA). Crude extracts were diluted for doubling dilution and then mixed with DPPH. The synergistic was found in the mixture of *C. militaris* and *G. lucidum*. The IC50 was 0.12 mg/ml which was lower than IC50 of the extracts from *C. militaris* or *G. lucidum* (Table 3 and Figure 1).

Table 3. The IC50 values of DPPH scavenging activity of crude extracts.

Extracts	IC50 (mg/ml)
C	0.69±0.01 ^{d}
L	4.76±0.04 ^{a}
G	0.36±0.00 ^{e}
C+L	1.60±0.04 ^{b}
C+G	0.12±0.03 ^{f}
C+L+G	1.04±0.14 ^{c}

Note: The extract from *C. militaris* (C); *L. edodes* (L) and *G. lucidum* (G). In each column, different superscripts represent significant differences ($p \leq 0.05$).

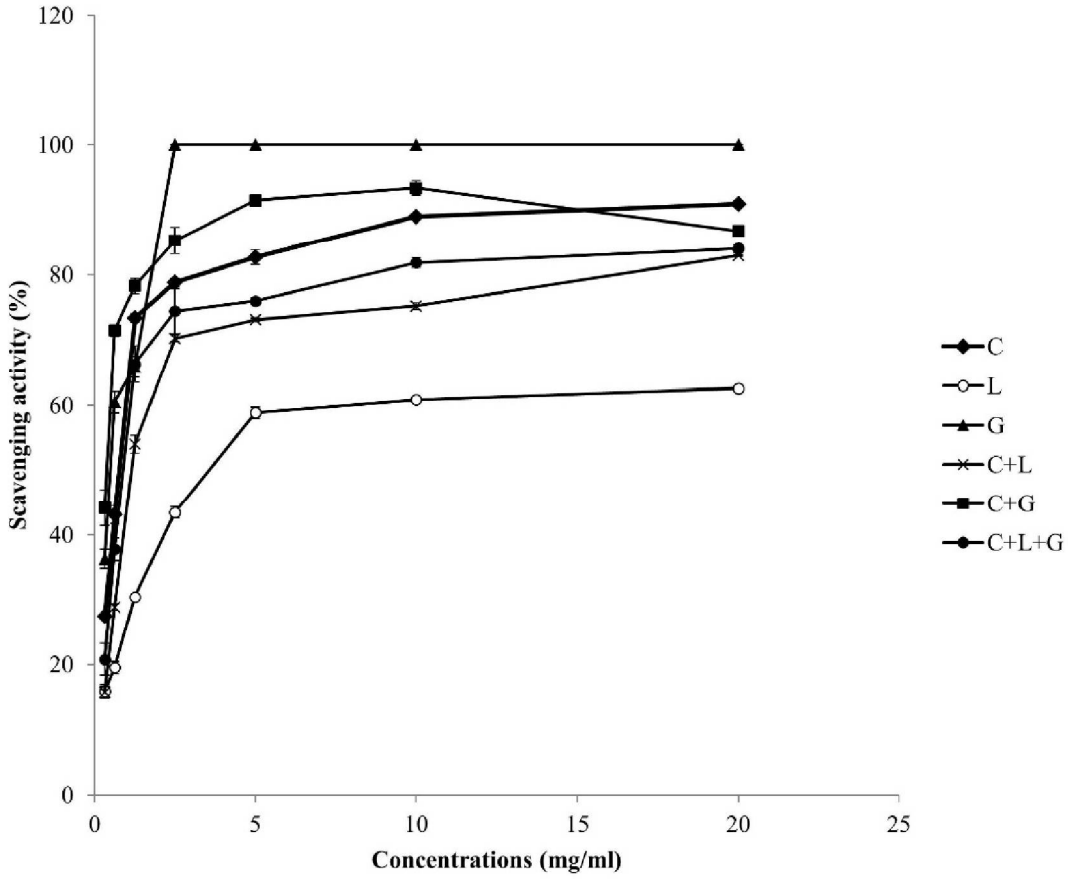

Figure 1. Scavenging activity (%) of the water extracts from *C. militaris* (C); *L. edodes* (L) and *G. lucidum* (G) at various concentrations.

α-amylase inhibition

Crude extracts were diluted and mixed with starch solution. The inhibition of α-amylase was studied. In figure 2, the results showed that the percentage of inhibition from all extracts were lower than 50%. In this study, the inhibition activities of 20 mg/ml crude extracts were compared. The highest inhibition activity (69.72±1.20%) was derived from the mixture of three mushrooms (Table 3).

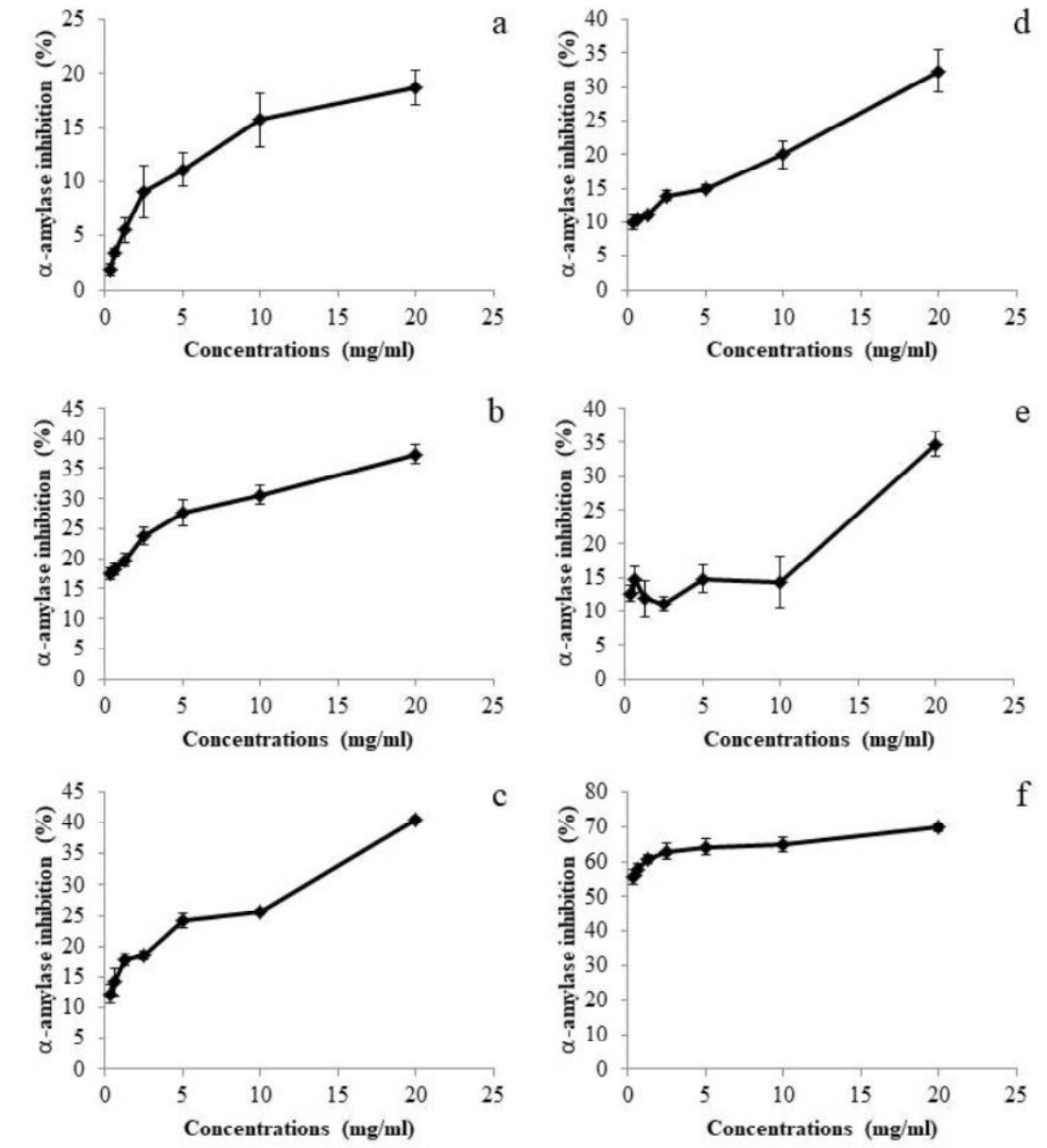

Figure 2. α-amylase inhibition (%) of water extracts from *C. militaris* (a); *L. edodes* (b), *G. lucidum* (c), *C. militaris* and *L. edodes* (d), *C. militaris* and *G. lucidum* (e), *C. militaris*, *L. edodes* and *G. lucidum* (f) at various concentrations.

Table 3. α-amylase inhibition (%) of 20 mg/ml of crude extracts.

Extracts	α-amylase inhibition (%)
C	18.70±1.59 ^{e}
L	37.35±1.54 ^{b}
G	40.49±0.42 ^{cb}
C+L	32.39±3.07 ^{d}
C+G	34.73±1.78 ^{cd}
C+L+G	69.72±1.20 ^{a}

Note: The extract from *C. militaris* (C); *L. edodes* (L) and *G. lucidum* (G). In each column, different superscripts represent significant differences ($p \leq 0.05$).

DISCUSSION

The polysaccharide of mushrooms contained many bioactive compounds such as β- glucan polymer (Friedman, 2016) . In this study, polysaccharide were extracted from the medicinal mushrooms such as Gold cordyceps (*C. militaris*), Shiitake (*L. edodes*) and Lingzhi (*G. licidum*). The results showed that the polysaccharide content in all of extract gave no significant difference. Then, bioactivities of crude extracts were studied.

For antibacterial activity, our results showed all of polysaccharide extracts exhibited no inhibition growth of bacteria and fungi. According to other studies, the polysaccharide extract gave no activity or very low activity but the methanol extract was found in antimicrobial activity (Reis et al., 2013; Ren et al., 2014).

The antioxidant activity of polysaccharide extracts from *G. lucidum*, *C. militaris* and *L. edodes* has been reported (Cheung et al., 2003; Ren et al., 2014; Boonsong et al. 2016). But, the antioxidant activity of the mixture of these mushrooms was no reported. In this study, *G. lucidum* was gave lowest IC50 when compared with *C. militaris* or *L. edodes*. However, *G. lucidum* which combined with *C. militaris* gave the better result. The IC50 of combined mushroom was lower. It showed the synergistic effect which according to previous studied. *Marasmius oreades* was found the antioxidant synergistic effect when mixed with other mushrooms (*Boletus edulis*). These results were found in both polysaccharide and phenolic extracts (Queiros et al., 2009; Vieira et al., 2012).

The sugar level in blood of diabetic patient was high due to lack or defective of insulin. This is cause of hyperglycemia and various complications in patient (Dong et al., 2014). Therefore, the control of blood sugar is necessary. The inhibition of enzyme which involve in carbohydrate metabolism such as α-amylase and α-glucosidase were determined (Bello et al., 2017). However, the water extract showed low α-amylase inhibition efficiency (<50%) but in the mixture of three mushrooms showed 69.72% inhibition.

CONCLUSION

The synergistic effect of antioxidant activity and α-amylase inhibition were found. Antioxidant activity, the mixture of *C. militaris* and *G. lucidum* gave the lowest IC50 of scavenging activity. In addition, the mixture of three mushrooms gave highest inhibition of α-amylase. From our results showed that the extract of combined mushrooms give advantage more than single mushroom extract.

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REFERENCES

Ai-li, Z., Jia-hui, L., Na, Z., Dan, Z., Gui-rong, Z. and Li-rong, T. 2010. Extraction, purification and anti-tumor activity of polysaccharide from mycelium of mutant *Cordyceps militaris*. Chemical Research in Chinese Universities. 26(5): 798-802.

Bello, M., Oluwamukomi, M. O. and Enujiugha, V. N. 2017. Anti-diabetic activity of three species of oyster mushroom. Annals Food Science and Technology. 18(2): 247-254.

Boonsong, S., Klaypradit, W. and Wilaipun, P. 2016. Antioxidant activities of extracts from five edible mushrooms using different extractants. Agriculture and Natural Resource. 50: 89-97.

Cheung, L. M., Cheung, P. C. K. and Ooi, V. E. C. 2003. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chemistry. 81: 249-255.

Dong, Y., Jing, T., Meng, Q., Liu, C., Hu, S., Ma, Y., Liu, Y., Lu, J., Cheng, Y., Wang, D. and Teng, L. 2014. Studies on the antidiabetic activities of *Cordyceps militaris* extract in diet-streptozotocin-induced diabetic sprague-dawley rats. Hindawi Publishing Corporation BioMed Research International. 1-11.

Friedman, M. 2016. Mushroom polysaccharides: chemistry and antiobesity, antidiabetes, anticancer, and antibiotic properties in cells, rodents, and humans. Foods. 5:1-40.

Kothari, D., Patel, S. and Kim, S. K. 2018. Anticancer and other therapeutic relevance of mushroom polysaccharides: A holistic appraisal. Biomedicine and Pharmacotherapy. 105: 377-394.

Queiros, B., Barreira, J. C. M., Sarmento, A. C. and Ferreira, I. C. F. R. 2009. In search of synergistic effects in antioxidant capacity of combined edible mushrooms. International Journal of Food Sciences and Nutrition. 60: 160-172.

Reis, F. S., Barros, L., Calhelha, R. C., Ciric, A., Griensven, L. J. L. D., Sokovic, M. and Ferreira, I. C. F. R. 2013. The methanolic extract of *Cordyceps militaris* (L.) Link fruiting body shows antioxidant, antibacterial, antifungal and antihuman tumor cell lines properties. Food and Chemical Toxicology. 62: 91-98.

Ren, L., Hemar, Y., Perera, C. O., Lewis, G., Krissansen, G. W. and Buchanan, P. K. 2014. Antibacterial and antioxidant activities of aqueous extracts of eight edible mushrooms. Bioactive Carbohydrates and Dietary Fibre. 3: 41-51.

Seto, S. W., Lam, T. Y., Tam, H. L., Au, A. L. S., Chan, S. W., Wu, J. H., Yu, P. H. F., Leung, G. P. H., Ngai, S. M., Yeung, J. H. K., Leung, P. S., Lee, S. M. Y. and Kwan, Y. W. 2009. Novel hypoglycemic effects of *Ganoderma lucidum* water-extract in obese/diabetic (+db/+db) mice. Phytomedicine 16: 426-436.

Thetsrimuang, C., Khammuang, S. and Sarnthima, R. 2011. Antioxidant activity of crude polysaccharides from edible fresh and dry mushroom fruiting bodies of *Lentinus* sp. Strain RJ-2. International Journal of Pharmacology. 7(1): 58-65.

Vieira, V., Marques, A., Barros, L., Barreira, J. C. M. and Ferreira, I. C. F. R. 2012. Insights in the antioxidant synergistic effects of combined edible mushrooms: phenolic and polysaccharidic extracts of *Boletus edulis* and *Marasmius oreades*. Journal of Food and Nutrition Research. 51(2): 109-116.

Wu, S. 2018. Hypolipidaemic and anti-lipidperoxidant activities of *Garnoderma lucidum* polysaccharide. International Journal of Biological Macromolecules. 118: 2001-2005.