Full Length Article

Antioxidant potential of rice grain processed by solid state cultivation with *Cordycep militaris*

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Abstract

Rice grain (*Oryza sativa* L) was used as the raw material for *Cordycep militaris* cultivation to investigate the effects of extract condition on the phenolic content and antioxidant activities. The 15% of ethanol concentration exhibited most effective extraction of phenolic compounds from rice grain processed by solid state cultivation with *C. militaris*. The ethanolic extracted showed highest phenolic content (1636.53±67.98 mg GAE/100g DW) and reducing ability (FRAP, 1553.41±33.24 mg Fe^{2+}/100g DW) and also high scavenging activity more than 75%. The ethanolic extracts obtained from the rice grain processed by solid state cultivation with *C. militaris* might be a potential antioxidant supplement for application in food products.

Keywords: *Cordycep militaris*, antioxidant activity, ethanolic extracted, rice grain

Introduction

Cordyceps militaris is a potential herbal drug and which contains a high nutritional value (Wasser et al., 1999). It has been widely used as a folk tonic food in Asia extensively. Besides their popular applications for tonic medicine, the constituents of *C. militaris* are now used extensively in modern systems of medicine. Contents of major bioactive components of the medicinal properties include Cordycepin, adenosine (Cunningham et al., 1950) pentastatin, carotenoids, polysaccharides, proteoglycans, terpenoids, steroids, and phenolic compounds (Bawadekji et al.,2016; Zhang et al., 2019). Cordycepin (30-deoxyadenosine), an adenosine analogue has been reported to possess various pharmacological, including immunological regulation (Noh et al., 2009;) antifungus (Sugar and McCaffrey, 1998) antileukemia (Thomadaki, Tsiapalis, and Scorilas, 2008; anticancer (Yoshikawa et al., 2008) and antioxidant ability, could terminate chain reaction, remove free radical intermediates, scavenge reactive oxygen species (ROS) (Pirakathiswaran et al., 2020). Report studies have shown that Cordyceps possesses liver protective effects (Liu et al., 2006) reduce the increase of

cholesterol and triglyceride (Kim et al., 2005) and induce the T-cell and macrophages activity (Liu et al., 2001) , decrease the level of c-Myc, c-Fos, and VEGF levels in the lungs and liver (Yang et al., 2005) [18] Besides these, Cordyceps contains some uncommon cyclic dipeptides, including cyclo-(Gly-Pro), cyclo-(Leu-Pro), cyclo-(Val-Pro), cyclo-(Ala-Leu), cyclo-(Ala-Val), and cyclo-(Tr-Leu) and small amounts of polyamines, such as 1,3-diamino propane, cadaverine, spermidine, spermine, and putrescine (Liu et al., 2006). Cordyceps was able to stabilize blood sugar and increase the liver enzymes hepatic glucokinase and glucose transporter isoform-2 (GLUT2) in hyperglycaemic diabetic rats (Kiho et al., 1999), supplementation reduced inflammatory markers in the hippocampus of the brain, it also raised increased precursor to serotonin and norepinephrine levels (Tianzhu et al., 2014). Therefore, in the present study of different ethanol extraction conditions on antioxidant activity and bioactive compounds of rice grain obtained for *C. militaris* cultivation.

Materials and Methods

Chemicals and materials

1-diphenyl-2-picrylhydrazyl radical (DPPH), Folin-Ciocalteu phenol reagent (FCR) were purchased from Sigma-Aldrich Chemical Co., (St. Louis, MO, USA). Ethanol (HPLC grade) was purchased from BDH (Poole, UK). Trolox standard (TE) (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Quercetin standard, Gallic acid standard, $FeSO_4.7H_2O$ standard and 2,4,6-Tripyridyl-5-Triazine (TPTZ) were purchased from Fluka Chemicals (Buchs, Switzerland).

Starter culture and solid-state cultivation

The healthy fruiting body of *C. militaris* was obtained from the Lungyood farm Saraburi Province, Thailand was the culture at 20˚C in dark for 7 days on potato dextrose agar (PDA) in the dark before mycelium growth after *C. militaris* mycelium was cultured continuously in potato dextrose broth (PDB) at 20˚C for 21 days, so as to obtain mycelium pellets starter culture. The solid-state media culture including rice grain and potato dextrose broth for the *C. militaris* cultivation was used by modification from Lungyood Chaemprasert farm and sterilized by autoclaving at 121 °C for 30 min. The seed starter culture with 5 mL into bottle culture and incubated in the dark at 22˚C for mycelium stage, after 14 days, controlled with a 14 h light/10 h dark cycle at 18 °C for stimulation stage. Fruiting body stage controlled with a 12 h light cycle at 22 °C. Sixty-day-old were harvested and dried at 50 °C for antioxidant and bioactive compound analysis

Preparation of the Ethanol Extracts

Rice grain powder obtained for *C. militaris* cultivation was mixed with various ethanol concentration (15, 30, 45, 60 and 75%) and then extract on ice using probe ultrasonic homogenizer with a frequency of 20 kHz. The amplitude was controlled to set the ultrasonic output power at 70% of the maximum range. After sonication was completed, the solids were separated from the mixture by centrifugation 10,000 rpm for 30 min at 4°C and collect all clear supernatant was filtered through a Whatman paper (No.1) for experimentation.

Scavenging effect on DPPH radical's assay

The DPPH radical scavenging inhibition determination using 96-well plate according to the assay (Saengha et al., 2021). DPPH reagent (0.2 mM) 200 µL in methanol mixed with clean sample (100 µL) in each 96 well plate. The reaction incubated at room temperature for 30 min and measured at 515 nm using microplate reader.

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP assay was determined according the assay (Saengha et al., 2021). FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM of 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM of HCl and 20 mM of $FeCl_3.6H_2O$ in the ratio (10:1:1 v/v/v) and incubated at 37 °C for 30 min. Sample extract (20 µL) was reacted with 180 µL of FRAP reagent, mixed, left for 30 min and measured at 593 nm. Standards of known Fe (II) concentrations and FRAP values were reported as mg Fe (II)/g DW.

Total Phenolic Content Determination (TPC)

TPC was determined using Folin-Ciocalteau assay following the assay (Saengha et al., 2021). The extract (20 µL) mixed with 100 µL of 10% Folin-Ciocalteu reagent and 80 µL of 7.5% NaHCO3, left for 30 min in a 96 well plate. The reaction mixture was measured at 765 nm using microplate reader. Results were reported as mg gallic acid equivalent (GAE)/g DW.

Total Flavonoid Content Determination (TFC)

TFC was performed according to the assay (Saengha et al., 2021). Sample extract (20 µL) added in to 96 well plate and then mixed with 60 µL of deionized water, followed by 10 µL of 5% $NaNO_3$, 10 µL of 10% $AlCl_3.6H_2O$ each well, mixed and left for a minute. Then, 100 µL of 1 M NaOH was added, mixed and kept for 30 min before measurement at 500 nm. Results were reported as mg quercetin Equivalent (QE)/g DW.

Statistical analysis

Repeat all 3 tests to find mean and standard deviation using One-way ANOVA and Duncan's Multiple Range Test with SPSS version 19.0 (IBM, Armonk, NY, US), considering the differences. Significantly when $p < 0.05$

Results and Discussion

Effect of extract condition on phenolic content and antioxidant activity

The fruiting body of *C. militaris* was grown at 60 days (Figure 1). *C. militaris* could grow on rice grain and mycelium colour will be change from white to yellow after light exposure. Then around 60 days of cultivation, fruiting body and rice grain ready to harvest.

Rice grain harvested and dried at 50 ˚C in an incubator for extract with a different concentration of ethanol using probe ultrasonic homogenizer. The results clearly demonstrated that DPPH antioxidant activity increased significantly in *C. militaris* from 45% ethanol extraction (80.90±2.78%) when compared to 4 groups of ethanol concentration (Figure 2A). FRAP activity (Figure 2B) and TPC (Figure 3A) increased significantly were from 15% ethanol extraction which was significantly different from those of the 4 groups of ethanol concentration. The highest FRAP activity (1553.41±33.24 mg Fe^{2+}/100g DW) and TPC (1636.53±67.98 mg GAE/100g DW) was found in 15% ethanol extraction. Likewise, the TFC results were significantly different ($P < 0.05$) among all ethanol concentration. Ethanol extraction of 75% led to the highest TFC (356.24 ± 90.37 mg QE/100g DW) which was significantly higher than that of the 4 groups of ethanol concentrations (Figure 3B).

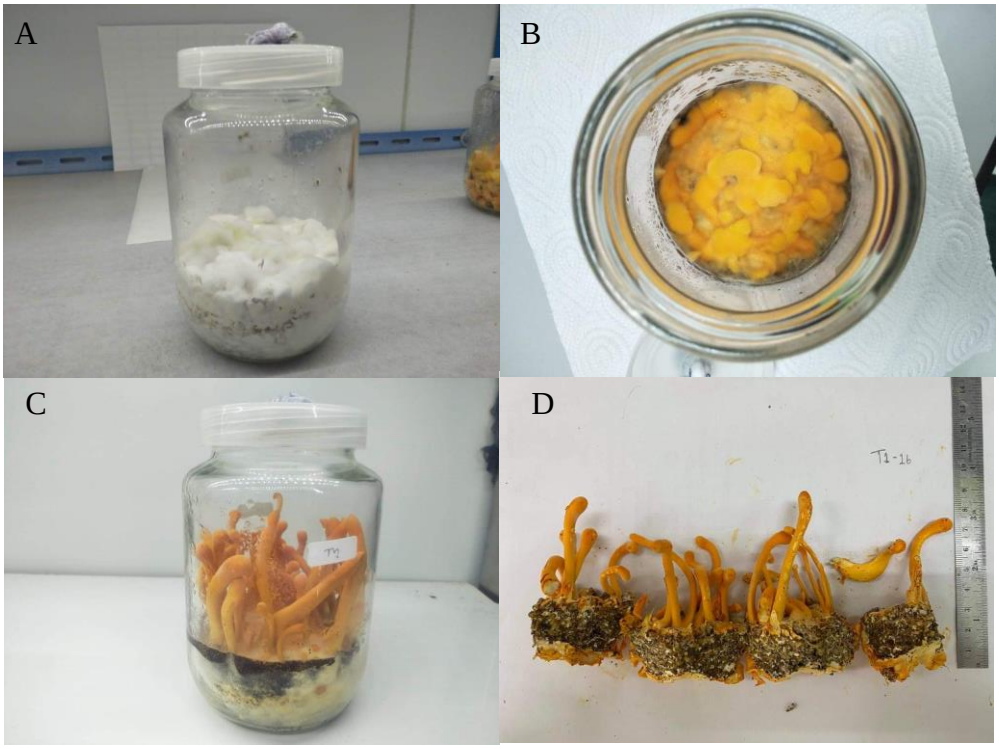

Figure 1. *C. militaris* grown on rice grain. A: 7 days after inoculated, B: *C. militaris* mycelium after light exposure, C: *C. militaris* fruiting body (60 days of cultivation), D: fruiting body and rice grain harvesting.

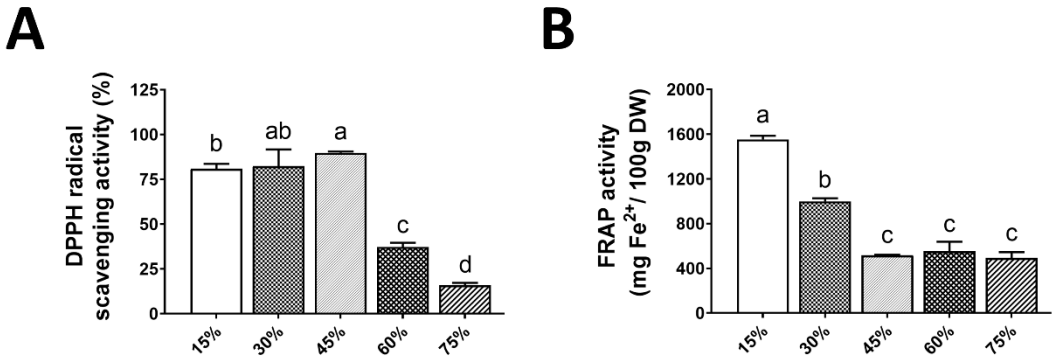

Figure 2. Effect of *C. militaris* from different concentration of ethanol extract on antioxidant activity. A) Antioxidant activity by DPPH assay. B) Antioxidant activity by FRAP assay, each value is the mean ± sd of three experiments. $P < 0.05$.

Ethanol extraction efficiency was the best on the grounds of safety and efficiency (Wang at al., 2014). The solvent polarity and extraction time affected the cordycepin content of *C. militaris* fruiting body (Xuan et al., 2019) and phenolic compounds were more efficiently extracted by high polar solvents. The phenolic compound is obviously related to the percentage of ethanol and its polarity index (Abarca-Vargas et al., 2016). The antioxidant potential of *C. militaris* extracts has stronger activity in the system of scavenging ability on DPPH• radicals and the chelating ability on ferrous ions. Antioxidant components in *C. militaris*, including total phenolic and flavonoid compounds, are important constituents because of their

scavenging inhibition due to their hydroxyl groups. It was reported that the content of total phenolic and flavonoid compound was the key component accounting for the antioxidant activity in many mushrooms (Cheung et al., 2003; Elmastas et al., 2007)

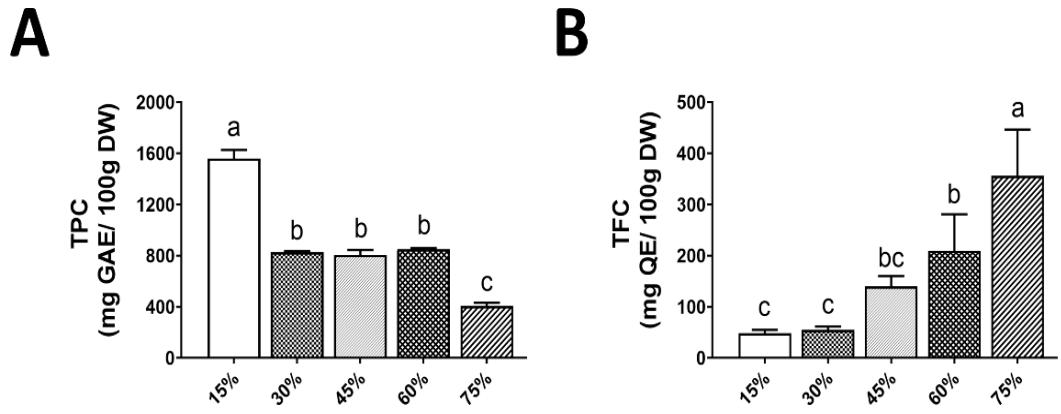

Figure 3. Effect of *C. militaris* from different concentration of ethanol extract on TPC and TFC, each value is the mean ± sd of three experiments $P < 0.05$.

Conclusion

Based on the results obtained, the higher antioxidant properties that the ethanolic extracts displayed might be somewhat beneficial to the antioxidant protection system of the human body against oxidative damage. The 15% of ethanol concentration exhibited most effective extraction of phenolic compounds from rice grain processed by solid state cultivation with *C. militaris*. Therefore, this extract condition might be a potential antioxidant supplement for application in food products. The study on the contribution of phenolic compounds to antioxidant properties of ethanolic extracts is still in progress. Due to the fact that most of the nature antioxidant compounds are relatively unstable, different solvent concentration could be taken as different antioxidant alternatives.

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Author’s contribution

ST designed, conducted the experiments, analyzed data, and wrote the manuscript. LB anchored the review, revisions and approved the article submission. All authors listed have read and approved the manuscript for publication.

Conflict of Interest Statement

The authors agree that this research was conducted in the absence of any self- benefits, commercial or financial conflicts and declare absence of conflicting interests with the funders.

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