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Antioxidative Activities and Total Phenolic and Flavonoid Contents of Extracts from *Cordyceps millitaris* Cultivation on Streamed Jasmine Rice Mixed Rice Berry

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ABSTRACT

The objectives of this study were to determine and compare antioxidant properties between *Cordyceps millitaris* extracts and its media cultivation. The *Cordyceps millitaris* were cultivated on Jasmine rice mixed rice berry served as culture medium. The fruiting bodies and its medium were dried and blend to be powder. 93˚C and37˚C water, 50% ethanol and100% ethanol were used for extraction solutions. It was found that the radical scavenging activities of fruiting body powder extracts and its medium against 2,2' - azino- bis(3-ethylbenzthiazoline- 6- sulphonic acid) radical ($ABTS^{\bullet+}$) were 4.30±0.37 and 3.67 ±0.03 mg trolox/g extract and against 2,2-diphenylpicrylhydrazyl radical ($DPPH^{\bullet}$) were 15.04±1.06 and 10.67±0.45 mg trolox/ g extract respectively. The ferric reducing antioxidant power (FRAP) values were 6.93±0.51 and 0. 85±0. 01 mg trolox/ g DW., respectively. The total phenol content and flavonoid were found in both of fruiting bodies powder and its medium powder that of 18.12±0.49 mgGAE/ gDW., 12.37±0.32 mgQE/ gDW. and 3.88±0.27 mg GAE/ gDW., 2.58±0.03 mg QCE/ gDW., respectively. This study may conclude that the best solvents for extraction was 37 º C water. Antioxidant

properties between fruiting bodies and its medium dried powder were not significant differences at $p \leq 0.05$. Therefore, it is indicated that the waste from *Cordyceps millitaris* cultivation as its medium was still useful.

Keywords: *Cordyceps millitaris*, Antioxidant properties, Jasmin rice, Rice Berry

INTRODUCTION

The free radicals are an atom, molecule or ions with unpaired electrons. They will destroy cells resulting in cell damage which can cause the disease and may lead to human death, ie, heart disease, cirrhosis, cataracts, cerebral palsy, premature ageing, cell or cancer mutations, and other diseases (Temple, 2000). Currently, human produces and uses synthetic drugs as clinical treatments but it may be side effects in the human bodies, such as the kidney or liver work hard, headaches, shaky, sexual dysfunction, etc (Lobo, et al. 2010). Therefore, scientists have studied and researched the drug that can improve a good effect on the human body. Because the Cordyceps has active substances that are good and beneficial to the body, such as reduce blood sugar increases metabolic processes, nutrients or metabolism of the body, help in the area of the familiar system, sexual enhancement and help in other matters properties that have a beneficial effect on the body (Zuo, et al., 2015). Moreover, *Cordyceps millitaris* can enhance the immune system, hormone balance, anticancer, antiaging, etc. The Cordyceps is difficult to cultivate, therefore, scientist researched suitable material for *Cordyceps millitaris* cultivation. We found that Jasmine rice and Rice Berry that is suitable for culturing. Both types of rice are the mainstay of Thai people and in Asia. Especially,the Rice Berry is the most popular in Thailand becauseis purple colour. It is a registered rice variety from Thailand, a cross-breed of Jao Hom Nin (JHN), a local non-glutinous purple rice and Khoa Dawk Mali 105 (hom Mali rice). The nutritional properties are anthocyanin stability, and physical and cooking properties. The outcome is a deep purple whole grain rice with softness and a palatable after taste. Cordyceps is relatively quite expensive with high market demand. Currently, the Cordyceps is medicine as commercially pellets available. We confirmed that the cordyceps suit for the human body by tested in human body temperature and the solvents that non-toxic to human. *Cordyceps millitaris* can also be cultured with rice such as Jasmine rice and Rice berry. Normally, cordyceps medium is thrown away at the end of the process, after harvest.

Therefore, the researcher was interested to study the antioxidant activities of the *Cordyceps millitaris* and its medium after harvest.

MATERIAL AND METHODS

Materials

Chemicals and reagent

2,2-azino-bis (3-ethylben-zothiazoline-6-sulphonicacid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tripyridyl-s-Triazine (TPTZ). 6-Hydroxy- 2,5,7,8- tetramethylchromane- 2- carboxylic acid (Trolox) were purchased from Sigma (St. Louis, MO, USA). Folin-Ciocalteu reagent was obtained from Fisher Chemical (Leicestershire, UK). All of the chemicals were used in analytical grade. Ferrous chloride was obtained from Fluka (Germany). Ethanol was purchased from BDH (UK).

Preparation of the Cordyceps

The Cordyceps was cultivated on jasmine rice mixed with Rice Berry, which was prepared at 1:1 ratio and streamed in broth that contains potato, peptone, glucose, yeast extract, silkworm and egg. Then, *Cordyceps millitaris* was inoculated. After that, they were stored in the dark at 20-22 °C for 5-10 days and stored in the light until flowering. This process take about 60 days before harvested. The media, jasmine rice mixed with Rice Berry, also dried after harvested. They were incubated at 40˚C. Then; they were blend to be power before which were studying in various properties.

Preparation of the Cordyceps for extraction

The Cordyceps fruiting body and its medium (Jasmine rice mixed rice berry) were extracted with hot water (93 ºC), water, 50% ethanol (ethanol: water, 50:50) and ethanol at 37ºC by using the shaking incubator at 37 ºC for 3 hours. Each extraction were done for 3 times and then filled by though filter paper What man No. 1. The solvents were removed from extraction by rotary evaporator and stored at -20 ºC until further study.

DPPH radical scavenging assay

The DPPH radical scavenging assay was carried out as described by Xu, 2003. The radical scavenging capacities of the Cordyceps extracts against DPPH radical were evaluated. Briefly, 100 µL of the extract or standard solutions mixed with 900 µL of 0.1 mM DPPH radical solution. The absorbance of the resulting was measured at 515 nm after 30 min of reaction in

the dark. The percentage inhibition was calculated by the following equation as:

$$\%\text{Inhibition DPPH radical scavenging} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

ABTS radical scavenging assay

The ABTS assay was performed according to the method of Long and Halliwell (2001). Briefly, 100 µL of different concentration of the Cordyceps extracts were mixed with 900 µL of $ABTS^{\bullet+}$ solution (0.7 mM of $ABTS^{\bullet+}$ mixed with 2.45 mM of K2S2O8 dissolved by distilled water and stored in dark for 16 h) and stored in dark for 6 min. The absorbance of the resulting was measured at 734 nm. Trolox was used as a standard solution. The radical scavenging activity was calculated using the following equation

$$\%\text{Inhibition ABTS radical scavenging} = \frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

Ferric reducing antioxidant power (FRAP)

The ferric reducing ability was performed according to the FRAP method (Benzie and Strain, 1996) FRAP reagent 900 µL was mixed with an aliquot of 100 µl of different concentrations of the Cordyceps extracts and incubated for 15 min at 37 °C. The absorbance of the resulting was measured at 595 nm. Trolox was used as a standard solution and the reducing power of the samples increased with the absorbance values.

Determination of total phenolic content

The total polyphenol content was carried out as described by Singleton et al. (1999). Briefly, 100 µL of the Cordyceps extracts were mixed with 500 µL of 0.2 N Folic-ciocaltue reagent and 400 µL of 7.5% w/v sodium carbonate stored at room temperature for 30 min. The absorbance was measured at 765 nm. The results were expressed in terms of gallic acid equivalent antioxidant activity (GAE) in mg gallic acid per dry weight.

Determination of total flavonoid content

The total flavonoid content was performed according to the aluminum chloride assay (Chang et al., 2002). Briefly, 100 µL of kernel extracts were mixed with 500 µL of 5% w/v sodium nitrile and 400 µL of 10% w/v

aluminium chloride stored at room temperature for 15 min. The absorbance was measured at 415 nm. The results were expressed in terms of quercetin equivalent (QE) in mg quercetin per dry weight (Chantiratikul et al., 2009).

Statistical analysis

The data was analyzed in triplicate. All results were expressed as mean value ±SD (n=3). The results were analyzed by one-way analysis of variance (ANOVA) and considered statistically significant ($p \leq 0.05$).

RESULTS

1. Antioxidative activities tested by DPPH, ABTS and FRAP assays.

The different assays showed the different antioxidant activity (Table 1). The results shown excellent antioxidant activity was 15.05±1.06 mg trolox/g extract by DPPH assay.

Table 1. Antioxidative activities of extract was tested by the DPPH, ABTS and FRAP assays.

Samples Cordyceps with	Temperature (˚C)	solvents	DPPH assay (mg trolox/g extract)	ABTS assay (mg trolox/g extract)	FRAPassay (mg trolox/g DW.)
1. medium after harvest	93	water	7.59±0.23^{d}	2.97±0.10^{a}	0.35±0.01^{e}
2. medium after harvest	37	water	15.05±1.06^{a}	3.67±0.03^{a}	0.85±0.01^{d}
3. medium after harvest	37	50% ethanol.	13.05±1.93^{b}	5.29±0.27^{a}	0.46±0.00^{e}
4. medium after harvest	37	100% ethanol	2.64±0.50^{e}	0.54±0.07^{c}	0.19±0.02^{e}
5. fruiting body	37	water	10.67±0.45^{c}	4.37±0.37^{a}	6.93±0.51^{a}
6. fruiting body	37	50% ethanol	nd	3.98±0.15^{a}	5.81±0.15^{b}
7. fruiting body	37	100% ethanol.	nd	0.69±0.67^{b}	1.29±0.13^{c}

Note: Data are given as mean values (Mean ± SE). In each column superscripts represent significant differences ($p \leq 0.05$). nd= non detected

The extract from Cordyceps with its medium had higher potential antioxidant than the Cordyceps fruiting body. The antioxidative activities by DPPH assay. The results showed that the best scavenging activity of Cordyceps with the medium extracted with water at 37ºC were 15.05±0.24 mg Trolox/g extract but by ABTS assay found the Cordyceps with the medium extracted with 50% ethanol at 37ºC showed the best scavenging activity was 5.29±0.27 mg Trolox/g extract and extracted with water at 37˚C showed the best ferric reducing ability was 3.67±0.03 mg Trolox/g DW. By FRAP assay found the best inhibition in Cordyceps with medium and the Cordyceps fruiting body extracted with water at 37ºC were 0.85±0.01 and 6.93±0.51 mg Trolox/ g DW.

2. Measured antioxidant substances by Follin-Ciocalteu assay and Aluminum choloride colorimetric.

The main antioxidant substances were total phenolic and total flavonoid as shown in table 2. The results showed that they were found in fruiting body part of Cordyceps. The suitable solubility of antioxidant substances was 37˚C water.

Table 2. The total phenolic compounds and Flavonoids found in the extract.

Sample Cordyceps with	Temperature (˚C)	solvents	Total phenolic (mg GAE/g DW.)	Total flavonoid (mg QE/ g DW.)
1. medium after harvest	93	water	0.87 ± 0.02^{de}	0.54 ± 0.015^{d}
2. medium after harvest	37	water	3.88 ± 0.27^{c}	2.58 ± 0.03^{b}
3. medium after harvest	37	50% ethanol	1.31 ± 0.19^{d}	0.95 ± 0.04^{c}
4. medium after harvest	37	100% ethanol	0.60 ± 0.02^{e}	nd
5. fruiting body	37	water	18.12 ± 0.49^{a}	12.37 ± 0.32^{a}
6. fruiting body	37	50% ethanol	10.62 ± 0.88^{b}	nd
7. fruiting body	37	100% ethanol	1.08 ± 0.07^{de}	nd

Note: Data are given as mean values (Mean ± SE). In each column superscripts represent significant differences ($p \leq 0.05$). nd= non detected

The results showed the Cordyceps with medium and Cordyceps fruiting body extracted with water at 37°C had the highest phenolic compounds were 3.88± 0.27 and 18.12±0.49 (mg GAE/g DW.) whereas Cordyceps with medium and Cordyceps fruiting body extracted with 100% ethanol had the lowest total phenolic compounds were 0.60 ± 0.02 and 1.08 ± 0.07 (mg GAE/g DW.).

Furthermore, the Cordyceps with medium and Cordyceps fruiting body extracted with water at 37°C had the highest flavonoids were 2.58±0.03 and 12.37±0.32 (mg QE/g DW).

DISCUSSION

Medicinal Cordyceps are commercially available, properly. The results showed excellent antioxidant activities. The total phenols were the major naturally occurring antioxidant components found in water extracts from Cordyceps. Overall, Cordyceps was high antioxidant activity and total phenol content (Dong and Yao, 2008). Oxidative damage caused by free radicals may be related to ageing and diseases (Vasiljevic et al, 2016) However, the antioxidants in human diets are of great interested as possible protective agents to help the human body reduce oxidative damage (Vasco, et al., 2008). This result showed fruiting body extracted with 37°C water was highest activities measured by ABTS assays. And Total phenolic compounds and flavonoids were highest in fruiting body extracted with water 37°C. Our finding showed the Cordyceps with medium and its fruiting body extracted with water at 37°C had the highest phenolic compounds than Cordyceps with medium and Cordyceps fruiting body extracted with 100% ethanol had the lowest total phenolic compounds. Mau et al. (2001) studied on antioxidant properties of methanolic extracts from several ear mushrooms. In contrast, this study showed surprisingly data that the best antioxidant activity shown in water soluble extraction means that the extract obtained from hot water gave the highest antioxidant activity. It might be caused from the suitable solubility of antioxidant substances and water. Thus, this finding showed the possibility of low cost to produce the extraction from *Cordyceps millitaris*. The best solvent was water; therefore, this study was implied that hot water was enough to extract the antioxidant compounds of *Cordyceps millitaris*. Furthermore, the best temperature was 37°C which is the same as human body temperature. Consequently, this research could be applied in the drug industry of Cordyceps culture to increase economic value.

CONCLUSION

The results showed high antioxidant properties by DPPH, ABTS and FRAP assays when the fruiting body and its medium extracted with 37°C water. The assays that can detect all samples were ABTS and FRAP assays. Antioxidant activities were not different between the fruiting body and its medium after harvest. Moreover, the total phenolic content and flavonoid in the fruiting body were higher than the medium.

REFERENCES

Benzie, I. F. F. and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry. 239*(*1): 70-76.

Chang, C. C., Yang, M. H., Wen, H. M. and Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colometric methods. Journal of Food and Drug Analysis. 10(3): 178-182.

Chantiratikul, P. et al.(2009). Antioxidant Activities and Phenolic Contents of Extracts from Salvinia molesta and Eichornia crassipes. Research Journal of Biological Sciences. 4: 1113-1117.

Dong, C.H. and Yao, Y.J. 2008. In vitro evaluation of antioxidant activities of aqueous extracts from natural and cultured mycelia of Cordyceps sinensis. LWT - Food Science and Technology. 41: 669-677.

Lobo, V. A. Phatak, P.A. and Chandra, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews. 4(8): 118–126.

Long, L. H. and Halliwell, B. 2001. Antioxidant and prooxidant abilities of foods and beverages Methods in Enzymology. 335: 181-190.

Mau, J.L., Chao, G.R. and Wu, K.T. (2001). Antioxidant properties of methanolic extracts from several ear mushrooms. Journal of Agricultural and Food Chemistry. 49: 5461-5467.

Sukanlaya L.(2015). Antioxidant activity of Caterpillar Fungus(*Cordyceps milataris*) Grown on Rice Berry and Jasmine Rice. RSU National Research Conference.

Temple, N. J. , 2000. Antioxidant and disease: more questions than anawers. Nutrition Research. 20:449-459.

Singleton, V. L., Orthofer, R. and Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by

means of folin-ciocalteu reagent. Methods in Enzymology. 299: 152-178.

Vasco, C., Ruales, J. and Kamal-Eldin, A. 2008. Total phenol compounds and antioxidant capacities of major fruits from Ecua. Food Chemistry. 111: 816-823.

Yamaguchi, Y., Kagota, S., Nakamura, K., Shinozuka, K. and Kunitomo M.. 2000. Antioxidant activity of the extracts from fruiting bodies of cultured *Cordyceps sinensis*. Phytotherapy Research. 14(8):647-9.

Vasiljevic, J.D, Zivkovic, L.P., Cabarkapa, A.M, Bajic, V.P, Djelic N.J and Spremo- Potparevic, B. M. 2016. *Cordyceps sinensis*: Genotoxic Potential in Human Peripheral Blood Cells and Antigenotoxic Properties Against Hydrogen Peroxide by Comet Assay. Alternative Therapies in Health and Medicine. 2:24-31.

Wang, Y., Wang, M., Ling, Y., Fan, W., Wang, Y. and Yin, H. 2009. Structural determination and antioxidant activity of a polysaccharide from the fruiting bodies of cultured *Cordyceps sinensis*. The American Journal of Chinese Medicine..;37(5):977-89.

Xiao, J.H., Xiao, D.M., Chen, D.X., Xiao, Y., Liang, Z.Q. and Zhong, J.J. 2012. Polysaccharides from the Medicinal Mushroom Cordyceps taii Show Antioxidant and Immunoenhancing Activities in a D-Galactose-Induced Aging Mouse Model. Alternative Therapies in Health and Medicine.2012:273-435.

Xu, J., Yang, F., Chen, L., Hu, Y. and Hu, Q. 2003. Effect of Selenium on Increasing the Antioxidant Activity of Tea Leaves Harvested during the Early Spring Tea Producing Season. Journal of Agricultural and Food Chemistry 51(4), 1081-1084.

Zou, Y., Liu,Y., Ruan, M., Xu, F., Wang, J., Chu, Z. and Zhang, Z. 2015. Cordyceps sinensis oral liquid prolongs the lifespan of the fruit fly, Drosophila melanogaster, by inhibiting oxidative stress . International Journal of Molecular Medicine. 36(4): 939–946.