reusable acid carbocatalyst for the synthesis of ester plasticizers, *RCS Advances*, 4, 57297-57307. **14**. Han Y. N., Choo Y., Lee Y. C., Moon Y. I., Kim S. D., Choi J. W. (2001), Monoamine oxidase B inhibitors from the fruits of *Opuntia ficus-indica* var. *saboten*, *Archives of Pharmacal Research*, 24(1), 51-54. **15**. Likhitayawuid K., Angerhofer C. K. (1993), Cytotoxic and antimalarial bisbenzylisoquinoline alkaloids from *Sephania evecta*, *Journal of Natural Products*, 56(1), 30-38. **16**. Skehan P., Storeng R., Scudiero D., Monks A., McMahon J., Vistica D., Warren J. T., Bokesch H., Kenney S., Boyd M. R. (1991), New colorimetric cytotoxicity assay for anticancer agents, *European Journal of Cancer*, 27, 1162-1168. **17**. Schobert R., Biersack B. (2019), Chemical and biological aspects of garcinol and isogarcinol: recent developments, *Chemistry & Biodiversity*, 16(9), e1900366.

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ANTIOXIDANT ACTIVITY OF GANODERIC ACIDS, POLYSACCHARIDES AND CRUDE EXTRACTS FROM THE *GANODERMA NEO-JAPONICUM* MUSHROOM

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Summary

Antioxidant Activity of Ganoderic Acids, Polysaccharides and Crude Extracts from the *Ganoderma neo-japonicum* Mushroom

Ganoderma neo-japonicum (GNJI) is a new mushroom recently founded in Bu Gia Map National Park on the decomposing bamboo stump (*Schizostachyum* sp.). The mushroom has been cultured and studied in the recent years. In this study, the antioxidant activities of ganoderic acids, polysaccharides, and crude extracts from GNJI mycelia and fruitbody had been determined by the antioxidant abilities against 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO), Fe^{2+}- 2,4,6-tri(2-pyridyl)-1,3,5-triazine (FRAP) free radicals. The results showed that the fruitbody (FrbExt) and biomass (MasExt) crude extracts had higher antioxidant activity than Trolox on DPPH ($EC50_{FrbExt}$ = 743.422µg/mL; $EC50_{MasExt}$ = 799.943µg/mL), NO ($EC50_{FrbExt}$ = 714.194µg/mL; $EC50_{MasExt}$ = 907.563µg/mL) and FRAP ($EC50_{FrbExt}$ = 103.313µg/mL; $EC50_{MasExt}$ = 234.814µg/mL). In multifactor anova analysis showed that crude extract, ganoderic acids showed higher antioxidant activity than polysaccharides and fruitbody had higher antioxidant effect than biomass.

Keywords: *Ganoderma neo-japonicum, Antioxidant, Ganoderic acid, Biomass, Polysaccharide.*

1. Introduction

Free radicals are produced either from normal cell metabolisms in endogenous or from external sources (pollution, cigarette smoke, radiation, medication). When overload of free radicals, the free radicals cannot gradually be destroyed, their accumulation in the body generates a phenomenon called oxidative stress. This process plays a major part in the development of chronic and degenerative illness such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases...[1]. *G. lucidum* polysaccharides exhibited the higher $DPPH^{\cdot}$, $O^{\cdot}$, and $OH^{\cdot}$ free radicals scavenging activities. It could significantly enhance the antioxidant enzyme activities (SOD, CAT and GPx), and reduce levels of IL-1β, IL-6 and TNF-α in rats with cervical cancer [2]. Triterpene group included ganoderic acids A, B, C and D, lucidenic acid B and ganodermanontriol were screened for their antioxidative effect against pyrogallol-induced erythrocyte membrane oxidation and Fe (II)-ascorbic acid induced lipid peroxidation [3].

G. neo-japonicum Imazeki (GNJI) have been known as lignin-degrading mushroom by Jo et al. [4]. It was then studied to improve the cellulose degradation activity by this same author's publication [5]. The culture process of GNJI mushroom was supplemented with methionine by Lee et al. [6] to effectively increase the production of ergothioneine and was supplemented with tryptophan by Park et al. [7] to increase the efficiency of phenolic compounds. Extraction of GNJI had abilities to manage of type 2 diabetes mellitus [8]. Antioxidant activity of GNJI had found with ethanol precipitation from the mycelia [9],[10]. Active substances with strong antioxidant

capacity cryptoporic acids H and I were reported by Lin et al. [11] in fruit body.

Therefore, this study aimed to comparison antioxidant activities of crude extracts from GNJI biomass, GNJI fruit bodies and their fractions of polysaccharides and ganoderic acids.

2. Materials and methods

Mushroom materials

GNJI fruit bodies in Bu Gia Map National Park were collected, washed, and dried at 45°C until there is no change in weight (lower than 20% of humidity), finely ground, vacuum sealed and stored at room temperature. The process of morphological description and DNA sequencing for identification was carried out at the laboratory of the Institute of Biotechnology Research and Development, Can Tho University (template code GN202005). GNJI biomass was harvested from the culture process (culture medium containing 90% rice, 5% cornstarch, 5% rice bran). These biomass samples and fruit bodies were harvested to produce extracts (fruitbody extract - FrbExt, biomass extract - MasExt), polysaccharides (fruitbody polysaccharides - FrbPol, biomass polysaccharides - MasExt) and ganoderic acids (fruitbody ganoderic acids - FrbGAs, biomass ganoderic acids - MasGAs) fractions which used to test for antioxidant activity.

Preparing fruit body and biomass crude extracts (Ext)

The extract was prepared by extracting the mushroom sample in hot water at 90°C for 24 hours at a ratio of 1:10 (w/v), the extract then concentrating at 50°C until 50% volume remained. The samples were adding 30% ethanol at ratio 1:1 (v/v), evaporating at 50°C to 50% volume, cooling at 2-5°C for 8 hours, remove precipitate, continue to evaporate at 50°C until 50% volume remained [12].

Extraction polysaccharides (Pol) from the fruit body and biomass

The degreased GNJI powder was extracted with Junchen Chen's protocol [13] which had some modifications. Sample was added distilled water at ratio of 1:3 (w/v) and incubated in a water bath at 90°C for 5h, and then centrifuged at 3000 rpm for 15 min to remove the pellet. The proteins in the supernatant were removed by incubating trichloroacetic acid (TCA) at ratio 1:2 (v/v) in 1 hour and centrifuged at 5000 rpm for 15 min. The supernatant was added 96% ethanol at ratio 1:2 (v/v) then incubated at 2-8°C in 24 hours and centrifuged at 5000 rpm for 15 min. The pellet was collected and conducted by 96% ethanol. The polysaccharides were measured by using Phenol-Sulfuric Acid Method at absorbance 490 nm [13].

Preparing ganoderic acids (GAs) fraction

Every gram of sample (biomass and fruit body) was suspended in 15 mL 70% ethanol. It was extracted two times; 24 hours each. After removing biomass with cellulose filter, the supernatant was dried at 50°C under vacuum condition. The residues were suspended by water and extracted with chloroform. The GAs in the chloroform phase was further extracted by 5% (w/v) $NaHCO_3$. The pH of $NaHCO_3$ phase was then adjusted to be lower than 3.0 by 2M HCl. Then GAs was extracted by chloroform and dissolved in ethanol after chloroform evaporated. The GAs was measured at 245 nm using thymol as standard [14].

DPPH radical scavenging assay

Research substances with antioxidant effects will reduce the color of 1,1-diphenyl-2-picrylhydrazyl (DPPH). The free radical scavenging activity of fractions was measured by 1, 1-diphenyl-2-picrylhydrazyl using method of Sharma [15] which had some modifications. Stock solutions of DPPH were prepared by dissolving 7.89 mg in 100 mL methanol, kept 2 hours in dark. 40 µL solution of DPPH in methanol was mixed to 960µL sample (at reference compounds 100, 200, 400, 600, 800, 1000 µg/L). Trolox was used as reference material (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, CAS No 53188-07-1, Sigma aldrich). The samples were testing included MasPol, FrbPol, MasExt, FrbExt, MasGAs, FrbGAs. All the tests were performed in triplicate, wrapped in aluminum foil, incubated at 30°C. After 30 minutes, absorbance was measured at 517 nm. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. The graph was plotted with the mean of DPPH scavenging (%).

$$DPPH \text{ scavenging } (\%) = \left| \frac{A_{control} - A_{test}}{A_{control}} \right| \times 100 \ (\%) \ [16]$$

The percentage of DPPH scavenging used to make scatter chart and the trend line was deduced from the chart. The equation as y = ax + b of trend line was used to calculate the IC_{50} with y = 50. EC_{50} value (a concentration of a sample required to inhibit 50% of radical) was calculated by using the method of Piaru *et al* [17] and Nguyen Thi Thu Huong et al [18].

Nitric oxide radical scavenging assay

Inhibition of Nitric oxide radical was measured by the Griess reaction [19] and had some modifications. The reaction mixture contained 400µL sodium nitroprusside 5 mM in phosphate buffered saline (PBS, pH=7.4) and 200 µL sample (at reference compound in different concentrations 100, 200, 400, 600, 800, 1000 µg/L). The mixtures were incubated at 25°C for 5 hours then centrifuged in 11000rpm in 15 minutes to remove the pellet. The Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylene diamine dihydrochloride in 2% H_3PO_4) was added to the wells, continued incubating in 5 hours. All the tests were performed in triplicate, wrapped in aluminum foil. Trolox was used as a positive control; absorbance was measured at 546 nm. The mean of NO scavenging (%) was plotted on the graph. The percentage of inhibition of 50% (EC_{50}) were calculated [16].

Ferric reducing radical scavenging assay

Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyl triazine complex to form FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration. The inhibition of ferric reducing antioxidant was measured by the ferric reducing ability power (FRAP) assay [20] with modifications. FRAP reagent contained 2.5 mL of 10 mM tripyridyl triazine (TPTZ) in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3$ and 25 mL of 0.3M acetate buffer (pH 3.6) was freshly prepared. 10µL solution FRAP was mixed to 990µL sample (at reference concentrations 100, 200, 400, 600, 800, and 1000 µg/L). Trolox was used as control. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. All the tests were performed in triplicate, wrapped in aluminum foil, incubated at 30°C. After 150 minutes, absorbance was measured at 593 nm. The graph was plotted with percentage of Ferric reducing antioxidant effect. EC_{50} value was calculated. [16]

Statistical analysis

The experimental results were analyzed by statistical One-Way ANOVA and Multifactor ANOVA on Startgraphic 16 software.

3. Results and Discussion

Qualitative analysis of ganoderic acids and polysaccharides in crude extracts

The triterpene structure in ganoderic acids absorbed the light at wavelength 245 nm. The fruit body and biomass were determined the amount of ganoderic acids by this UV measurement method. Besides, the presenting of sulfuric acid and phenol in crude extracts liberated an orange solution composed of UV absorbing chromophore at 490 nm. Whereby polysaccharides total was determined on the Spectrophotometer at 490 nm for the wavelength.

Table 1. The content of ganoderic acids and polysaccharides in crude extracts

Crude extracts	Ganoderic acids (mg/g)	Polysaccharides (mg/g)
Fruit body	1.19	140.26
Biomass	0.62	170.04

Inhibition of DPPH radical

The DPPH free radical is a long-lived organic nitrogen radical with a deep purple color. When a DPPH solution is mixed with an antioxidant, its color turns from purple to yellow of the corresponding hydrazine.

The DPPH radical is considered a model for a lipophilic radical. A chain reaction in lipophilic radicals was initiated by the lipid autoxidation. The radical scavenging activity of the fractions

were determined from the reduction in the optical absorbance at 517 nm due to scavenging of stable DPPH free radical [15]. Positive DPPH test suggests that was Trolox. The scavenging effects of fractions and Trolox on DPPH radical are shown in Table 2.

Table 2. DPPH scavenging ability of fractionated extracts

Testing fractions	Percentage of DPPH inhibited of fractions (%)							EC_{50} to DPPH (μg/mL)
	0 μg/mL	100 μg/mL	200 μg/mL	400 μg/mL	600 μg/mL	800 μg/mL	1000 μg/mL	
FrbPol	0	9.37	15.06	26.39	36.40	47.24	57.41	854.90^{d} ± 10.46
MasPol	0	3.28	8.03	15.09	23.15	31.28	39.14	1275.87^{g}± 4.23
FrbGAs	0	7.49	13.38	24.99	36.17	47.44	58.61	845.46^{c} ± 0.36
MasGAs	0	6.49	11.98	22.35	33.05	43.33	53.43	929.81^{e} ± 0.81
FrbExt	0	10.04	16.06	29.04	41.22	53.96	65.41	743.42^{a} ± 0.91
MasExt	0	9.07	15.12	27.33	38.51	49.98	61.39	799.94^{b} ± 0.81
Trolox	0	4.24	9.42	19.99	29.78	41.34	51.44	972.65^{f} ± 2.58

** Symbols a, b, c... after value are homogeneous groups in statistic (P<0.01).*

In this test most of fractions showed higher antioxidant ability than the control (P<0.01) except biomass polysaccharides (MasPol). By comparison of the EC_{50} showed the FrbExt, MasExt, FrbGAs, FrbPol, and MasGAs had 23.57%, 17.76%, 13.08%, 12.1%, and 4.4% higher effect than Trolox. In fruitbody had some elements which have higher antioxidant effects to DPPH than biomass because FrbExt had 5.81% higher than the MasExt (P<0.01). The extracts had polysaccharides, ganoderic acids and other elements, this made the groups of extracts had higher ability to reaction with DPPH radicals. In comparison to result of Nguyen Thi Thu Huong et al [18] on *Ganoderma lucidum*, EC_{50} effective on DPPH were 1326 μg/mL (ethanol extract) and 1708 μg/mL (hot water extract). It showed that most of fractions have higher antioxidant ability on DPPH radical. The hot water extract of wild basidiocarps had tested by Lin et al [11],[10] was 81.83μg/mL of EC_{50} on DPPH, but their result was lower than control.

Nitric oxide scavenging ability

In this assay, the compound sodium nitroprusside (SNP) is known to decompose in aqueous solution at physiological pH=7.4 producing NO. Under aerobic conditions, NO reacts with oxygen to produce stable products (nitrate and nitrite). The compound in acting wells changed to pink/red because of diazo product appearance. The absorbance at 546 nm used to determine amount of this product to calculate antioxidant activity [16]. The nitric oxide scavenging ability was showed in Table 3.

The result showed that all fractions had nitric oxide scavenging ability. Specially, FrbExt had 21.26% higher antioxidant ability to nitric oxide scavenging than control (P<0.01). MasExt had same effect to Trolox at P<0.01 while the rest of fractions had lower effective than control. In comparison to other results, methanol extract from *C. odorata* had 380 μg/mL [19] and low-density polysaccharides from *G. lucidum* had 145 μg/mL of EC_{50} [16]. It was observed that *G. neo-japonicum* have nitric oxide scavenging ability lower than *G. lucidum* and *C. odorata.*

Table 3. Nitric oxide scavenging ability of fractionated extracts

Testing fractions	Percentage of NO inhibited of fractions (%)							EC_{50} to NO (μg/mL)
	0 μg/mL	100 μg/mL	200 μg/mL	400 μg/mL	600 μg/mL	800 μg/mL	1000 μg/mL	
FrbPol	0	10.91	14.67	20.97	27.55	33.92	40.78	1274.27^{c}± 5.65
MasPol	0	5.53	8.15	14.64	19.40	24.84	29.49	1746.08^{e}± 14.85
FrbGAs	0	12.02	14.51	21.12	29.40	35.30	41.57	1241.53^{c}± 9.40
MasGAs	0	8.36	11.87	19.31	25.92	32.19	38.71	1328.35^{d}± 7.51
FrbExt	0	19.99	24.41	34.10	44.9	53.54	64.48	714.19^{a} ± 2.65
MasExt	0	16.51	20.23	30.50	37.73	45.05	53.32	907.56^{b} ± 7.90
Trolox	0	6.01	11.20	21.82	34.44	44.08	54.67	907.00^{b} ± 4.92

** Symbols a, b, c... after value are homogeneous groups in statistic (P<0.01).*

Inhibition of Ferric reducing antioxidant

The FRAP assay relies on the reduction of Fe^{3+}-TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine) to produce Fe^{2+}-TPTZ by the antioxidants. The binding of Fe^{2+} to the ligand creates a very intense navy-blue color. The absorbance at 593 nm can be measured to test the amount of iron reduced and can be correlated with the amount of antioxidant. The ability to antioxidant of fraction on Ferric reducing was showed in Table 4.

The reducing capacity was investigated by measuring Fe^{3+} - Fe^{2+} conversion and serve as a significant indicator of its potential antioxidant activity. The antioxidant activities of putative antioxidants have been attributed to various mechanisms such as prevention of chain reaction, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued proton abstraction and radical scavenging [16]. This result also showed that FrbExt, MasExt, FrbGAs, and FrbPol had 70.22%, 34.60%, 31.87%, and 3.44% higher antioxidant activity than Trolox ($P<0.01$). In contrast, MasPol and MasGAs were lower ability than control. In nearby, ethanol-NaOH fraction of *G. lucidum* had EC_{50} value of 250µg/mL [16]. It was demonstrated that *G. neo-japonicum* have higher antioxidant ability on ferric reducing than *G. lucidum*.

Table 4. The ferric reducing capacity of fractionated extracts

Testing fractions	Percentage of FRAP inhibited of fractions (%)							EC_{50} to FRAP (µg/mL)
	0 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL	600 µg/mL	800 µg/mL	1000 µg/mL	
FrbPol	0	14.46	53.52	77.21	84.66	88.57	90.80	$347.47^{d} \pm 2.80$
MasPol	0	3.91	15.72	56.96	69.59	77.49	82.77	$510.39^{g} \pm 3.04$
FrbGAs	0	38.20	65.84	82.30	88.27	91.23	93.08	$244.59^{c} \pm 1.90$
MasGAs	0	7.79	47.78	73.70	82.31	86.62	89.55	$383.72^{f} \pm 1.64$
FrbExt	0	64.12	77.71	87.32	91.15	93.20	94.47	$103.31^{a} \pm 1.85$
MasExt	0	41.76	64.74	83.32	88.54	91.09	92.96	$234.81^{b} \pm 2.77$
Trolox	0	6.21	42.79	72.16	81.42	85.45	88.39	$359.03^{e} \pm 0.93$

** Symbols a, b, c... after value are homogeneous groups in statistic (P<0.01).*

Multifactor analysis of EC_{50}

The values of EC_{50} were analyzed by Multifactor Anova method with two factors were types of extract and sources of extract. The result of analysis was showed in Table 5 and Table 6.

Table 5. The effect of extract types to EC_{50} in DPPH, NO, FRAP assays

Types of extract	EC_{50} of DPPH (µg/mL)	EC_{50} of NO (µg/mL)	EC_{50} of FRAP (µg/mL)
Crude extracts (Ext)	$771.683^{a} \pm 30.986$	$810.879^{a} \pm 106.305$	$169.063^{a} \pm 72.119$
Ganoderic acids (GAs)	$887.635^{a} \pm 46.215$	$1284.940^{b} \pm 49.343$	$314.158^{b} \pm 76.253$
Polysaccharides (Pol)	$1065.390^{b} \pm 230.626$	$1510.180^{c} \pm 259.006$	$428.929^{c} \pm 89.349$

** Symbols a, b, c after value are homogeneous groups in statistic (P<0.05).*

Table 6. The effect of extract sources to EC_{50} in DPPH, NO, FRAP assays

Sources of extract	EC_{50} of DPPH (µg/mL)	EC_{50} of NO (µg/mL)	EC_{50} of FRAP (µg/mL)
Fruit body (Frb)	$814.592^{a} \pm 53.542$	$1076.670^{a} \pm 272.399$	$231.792^{a} \pm 106.211$
Biomass (Mas)	$1001.880^{b} \pm 213.087$	$1327.330^{b} \pm 363.440$	$376.308^{b} \pm 119.518$

** Symbols a, b after value are homogeneous groups in statistic (P<0.05).*

The analyzed results in Table 5 showed the significant differences in antioxidant activity of extract types at $P<0.05$. The crude extract had average EC_{50} 771.683 µg/mL, 810.879 µg/mL, and 169.036 µg/mL (on DPPH, NO, and FRAP assays, respectively) which were lower than those of ganoderic acids and polysaccharides (Table 5). It can be explained that crude extracts may have more antioxidant compounds or synergistic activity than its ganoderic acids or polysaccharides. Comparing to Trolox in Table 2, Table 3 and Table 4 both the crude extracts and ganoderic acids had values of EC_{50} lower than Trolox. This was mean the crude extracts and

ganoderic acids had better effects on DPPH, NO, and FRAP assays. Moreover, there were some of other substances in GNJI has antioxidant activity as cryptoporic acid -H -I, ethanol precipitation... beside the ganoderic acids and polysaccharides [11].

The sources of extract were analyzed by multi-factor ANOVA. The fruit body of GNJI had higher antioxidant activity than biomass ($P<0.05$). The results in Table 6 showed that EC_{50} value of the fruit body extract lower than that of biomass extract against DPPH, NO, and FRAP free radicals, 22.97% on DPPH, 23.9% on NO and 62.7% on FRAP assays ($P<0.05$), respectively. It may be explained that fruitbody contained more ganoderic acids than biomass (Table 1). Besides, undetermined substances could be presented in spores, cap and stalk which differ from mushroom mycelia.

4. Conclusion

The crude extracts, ganoderic acids and polysaccharides from fruitbody and biomass of *Ganoderma neo-japonicum* showed significant antioxidant effects on DPPH, nitric oxide, and FRAP assays. Therein fruit body and biomass extracts possessed the EC_{50} values lower than that of control. The results suggested that *G. neo-japonicum* might be play a vital role for the prevention of some diseases related to free radicals.

Reference

1. Ai P. H. L., He H., Chuong P. H (2008), Free radicals, antioxidants in disease and health, *International Journal of Biomedical Science,* 4(2), 89-96. **2.** Chen X., Chen Y., Li S., Guo C. Y., Yun L. J., Liu L. (2009), Free radical scavenging of *Ganoderma lucidum* polysaccharides and its effect on antioxidant enzymes and immunity activities in cervical carcinoma rats, *Carbohydrate Polymers,* 77(2), 389-393. **3.** Min Z., Qi C., Leone K. W., Finny S. C., Ronald C. L. (1999), Triterpene antioxidants from *Ganoderma lucidum*, *Phytotherapy research,* 13(6), 459-548. **4.** Jo W. S., Park H. N., Cho D. H., Yoo Y. B., Park S. C. (2011), Detection of extracellular enzyme activities in *Ganoderma neo-japonicum*, *Mycobiology*, 39(2), 118-120. **5.** Jo W. S., Park H. N., Cho D. H., Yo Y,. B., Park S. C. (2011), Optimal media conditions for the detection of extracellular cellulase activity in *Ganoderma neo-japonicum*, *Mycobiology*, 39(2), 129-132. **6.** Lee W. Y., Park E. J., Ahn J. K. (2009), Supplementation of methionine enhanced the ergothioneine accumulation in the *Ganoderma neo-japonicum* mycelia, *Apllication Biochemistry Biotechnology*, 158(1), 213-221. **7.** Park E. J., Lee W. Y. (2010), Tryptophan enhanced accumulation of phenolic compounds via chorismate mutase activation in the *Ganoderma neo-japonicum* mycelia, *Journal of the Korean Society for Applied Biological Chemistry,* 53(3), 364-370. **8.** Subramaniam S., Sabaratnam V., Kuppusamy U. R. (2014), Solid-substrate fermentation of wheat grains by mycelia of indigenous *Ganoderma spp.* enhanced adipogenesis and modulated PPARγ expression in 3T3-L1 cells, *Chiang Mai Journal of Science*, 41(1) 1-13. **9.** Subramaniam S., Sabaratnam V., Rani K., Tan Y. S. (2014), Solid-substrate fermentation of wheat grains by mycelia of indigenous species of the genus *Ganoderma* (higher Basidiomycetes) to enhance the antioxidant activities, *International Journal of Medicinal Mushrooms,* 16(3), 259-67. **10.** Wee C. T., Umah R. K., Chia W. P. (2015), *Ganoderma neo-japonicum* Imazeki revisited: Domestication study and antioxidant properties of its basidiocarps and mycelia, *Scientific Repots*, 512-515. **11.** Lin J. M., Lin C. C., Chen M. F., Ujiie T., Takada A. (1995), Radical scavenger and antihepatotoxic activity of *Ganoderma formosanum*, *Ganoderma lucidum* and *Ganoderma neo-japonicum, Journal of Ethnopharmacology*, 47(1), 33-41. **12.** Tu N. M (2009), Extraction process of biologically active substances from reishi mushroom (Ganoderma lucidum) (in Vietnamese), *Vietnam Journal of Science and Technology*, 1, 45-53 **13.** J. Chen, P. Lai, H. Shen, H. Zhen, R. Fang (2013), Effect of extraction methods on polysaccharide of clitocybe maxima Stipe, *Advance Journal of Food Science and Technology*, 5(3). 370-373. **14.** Fang Q. H., Zhong J. J. (2002), Effec to finitial pH on production of ganoderic acid and polysaccharide by submerged fermentation of *Ganoderma lucidum*, *Process Biochemistry*, 37(9), 769-774. **15.** Sharma O., Bhat T. N. (2009), DPPH antioxidant assay revisited, *Food Chemistry*, 113(4), 1202-1205. **16.** Baskar R, R Lavanya, S Mayilvizhi (2008), Free radical scavenging activity of antitumour polysaccharide fractions isolated from *Ganoderma lucidum* (Fr.) P. Karst, *Natural Product Radiance*, 7(4), 320-325. **17.** Piaru S. P., Mahmud R., Majid A. M. S. A., Daoud Z. (2012), Antioxidant and antiangiogenic activities of the essential oils of Myristica fragrans and Morinda citrifolia, *Asian Pacific Journal of Tropical Medicine*, vol. 2012, 294-298. **18.** Huong N. T. T., Hang N. T. N. (2010), Study on the antioxidant effect towards protecting the liver of reishi mushroom (Ganoderma lucidum) (in Vietnamese), *Medical Journal of Ho Chi Minh City*, 14(2), 129-134. **19.** C. S. Alisi, G. O. C. Onyeze (2008), Nitric oxide scavenging ability of ethyl acetate fraction of methanolic leaf extracts of Chromolaena odorata (Linn.), *African Journal of Biochemistry Research*, 2(7), 145-145. **20.** I. Benzie, J. Strain (1996), The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay, *Analytical Biochemistry,* 239(1), 70-76.