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POLYPRENYLATED ACYLPHLOROGLUCINOLS FROM THE FRUIT PERICARP OF *GARCINIA OBLONGIFOLIA* AND THEIR CYTOTOXICITY

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Summary

Polyprenylated Acylphloroglucinols from the Fruit Pericarp of *Garcinia oblongifolia* and their Cytotoxicity

The paper deals with chromatographic isolation, structural elucidation, and cytotoxic activity of compounds from the pericarp of *Garcinia oblongifolia* fruit. Four compounds, isogarcinol, oblongifolin A, garcinol, and 1,5-dimethyl citrate were isolated. Standard spectroscopic methods (MS, 1D and 2D NMR) were used in the evaluation of the structures of the four compounds. The SRB cytotoxicity test showed micromolar half-maximal inhibitory concentrations (IC_{50}) of isogarcinol against Hep-G2, HeLa, and Vero cell lines and garcinol against Hep-G2, HeLa, LU-1, and Vero cell lines.

Keywords: *Garcinia oblongifolia, Acylphloroglucinol, Citrate, Cytotoxicity.*

1. Introduction

The genus *Garcinia* covers nearly 260 species and is one of the largest genera of the Clusiaceae family. The highest abundance of this species is distributed in Southeast Asia [1]. A vast number of studies have been conducted on *Garcinia* phytochemistry with about 135 species investigated. Xanthones, benzophenones, biflavonoids, and triterpenoids have been isolated and the connection of their structures with antidiabetic and cytotoxic activities was disclosed in the studies of *Garcinia* species which find the habitat in Vietnam [2],[3],[4],[5]. *Garcinia oblongifolia* Champ. ex Benth. is a common edible plant in Vietnam. The fruits have been harvested from the wild or cultivated plant. Polyprenyl benzoylphloroglucinols and xanthones were isolated from different parts (bark, twig, leaves, trunk, and fruit) of *G. oblongifolia* [4],[6],[7],[8],[9]. Hydroxycitric acid (HCA) is the main constituent of *G. oblongifolia* fruit. Except for a few reports on the

isolation and application of HCA, the knowledge of the chemical metabolites in *G. oblongifolia* fruit is not significant. The present paper deals with the first attempt to isolate and structurally determine compounds from the fruit pericarp of *G. oblongifolia* in Vietnam.

2. Material and methods

2.1. Plant material

The fresh mature fruits of *Garcinia oblongifolia* Champ. ex Benth. were collected in Do Son, Hai Phong city, Vietnam in August 2021. The voucher specimen (GO082021) was identified by Dr. Nguyen Quoc Binh, Vietnam Academy of Science and Technology (VAST), and deposited at Institute of Natural Product Chemistry, VAST, Hanoi, Vietnam.

2.3. General experimental procedures

Silica gel (40-63 μm and 63-200 μm, Merck), Sephadex LH-20 (GE Healthcare, Sweden) and reversed-phase Merck RP-18 were used for column chromatography (CC). Thin-layer chromatography (TLC) was performed using *silica gel* 60 F_{254} aluminum plates (Merck). Spots were detected by UV lights (254 and 365 nm) and 5% vanillin-sulfuric acid in an absolute ethanol solution. Solvents of chromatographic grade were used for the extraction and chromatographic separation. ESI-MS spectra were obtained from a Xevo TQ-S micro mass spectrometer (Waters, USA). NMR spectra were measured on a Bruker Avance 500 or 600 using TMS as an internal standard at 500 or 600 MHz for ^{1}H NMR spectra and 125 or 150 MHz for ^{13}C NMR spectra. Optical rotations were measured on a Perkin-Elmer 141 polarimeter.

*2.3. Extraction, isolation and data of compounds **1-4***

The fresh mature fruits of *G. oblongifolia* (10 kg) were cut into two parts and the pericarp was separated and dried at 45°C for two days. The dried pericarp (957.8 g) was powdered and sonicated with 2 L of MeOH at 40°C for 30 min. The sonication was repeated three times then the extracts were filtered and solvent was evaporated under reduced pressure to obtain a concentrated methanol extract (650.2 g). The methanol extract was dissolved in water and successively partitioned with *n*-hexane (500 mL × 3) and ethyl acetate (500 mL × 3) then the organic solvents were removed under reduced pressure to obtain *n*-hexane (102 g) and ethyl acetate (80.2 g) extracts. The *n*-hexane extract was separated by *silica gel* column chromatography (CC) with gradient *n*-hexane-acetone solvent systems (80:1→10:1, v/v) to obtain 10 fractions (F1-F10). Fraction F5 was separated by *silica gel* CC with gradient *n*-hexane-acetone solvent systems (40:1→5:1, v/v) to give compound **1** (38 mg). Fraction F10 was loaded onto a *silica gel* column with gradient *n*-hexane-acetone solvent systems (20:1→7:1, v/v) to give 6 subfractions (F10.1-F10.6). Subfraction F10.1 was purified by RP-18 CC with MeOH-H_2O (5:1) and Sephadex LH-20 CC with MeOH-dichloromethane (95:5. v/v) to give compounds **2** (42 mg) and **3** (24 mg). The EtOAc extract was separated by *silica gel* CC with gradient dichloromethane-MeOH solvent systems followed by recrystallization from acetone to give compound **4** (46 mg).

Isogarcinol (1): Pale yellow solid. $[\alpha]_D^{25}$ = -159 (*c* 1.0, $CHCl_3$). ESI-MS: *m/z* 601.6 $[M-H]^-$ ($C_{38}H_{50}O_6$). ^{1}H-NMR (600 MHz, $CDCl_3$) and ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 1.

Oblongifolin A (2): Yellow oil. $[\alpha]_D^{25}$ = +25 (*c* 1.0, $CHCl_3$). ^{1}H-NMR (600 MHz, CD_3OD + 0.1% TFA) and ^{13}C-NMR (150 MHz, CD_3OD + 0.1% TFA): see Table 1.

Garcinol (3): Light yellow solid. $[\alpha]_D^{25}$ = -138 (*c* 0.1, $CHCl_3$). ESI-MS: *m/z* 603.2 $[M+H]^+$; *m/z* 601.3 $[M-H]^-$ ($C_{38}H_{50}O_6$). ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$): see Table 1.

1,5-Dimethyl citrate (4): White amorphous powder. ESI-MS: *m/z* 242.8 $[M+Na]^+$, *m/z* 218.8 $[M-H]^-$ ($C_8H_{11}O_7$). ^{1}H-NMR (500 MHz, acetone d_6) δ (ppm): 3.62 (6H, s, $2OCH_3$), 2.92 (2H, d, J = 15.0 Hz, H-2a, H-4a), 2.80 (2H, d, J = 15.0 Hz, H-2b, H-4b). ^{13}C-NMR (150 MHz, acetone d_6) δ (ppm): 174.8 (C-6), 170.8 (C-1, C-5), 73.7 (C-3), 51.8 ($2OCH_3$), 43.5 (C-2, C-4).

*2.4. SRB cytotoxicity test of compounds **1** and **3***

Cell culture: Three human cancer cell lines, including Hep-G2 (ATCC no. HB-8065), LU-1 (ATCC no. HTB-57) and HeLa (ATCC no. CCL-2), and Vero cells (ATCC no. CCL-81.4) were originated from the American Type Culture Collection (ATCC, USA) and preserved at Department of Experimental Biology, Institute of

Natural Products Chemistry, VAST. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS), 100 units/mL penicillin and 100 mg/mL streptomycin in a humidified 5% CO_2 incubator (Esco, Singapore) at 37°C. Depending on the cell line, change of culture medium was carried out every 2-3 days.

Cytotoxicity assay: Prior to the assay, compounds **1** and **3** were dissolved and serially diluted with dimethyl sulfoxide (DMSO). For determination of cytotoxicity, seeded cells (density of 4-5×10^4 cells per mL) were treated with diluted test compounds in a 96-well microtiter plate and incubated (37 °C, 5% CO_2). After 72 hours of incubation, cells were fixed with trichloroacetic acid and stained with 0.4% sulforhodamine B (SRB). The amount of dissolved cell binding dye in Tris base solution was detected by the absorbance at 510 nm using a microplate reader (Tecan F150, Switzerland). Cell survival (CS) values are presented as the mean ± SD (standard deviation) of three replicates. IC_{50} values were determined using the log(CS) *vs.* concentration function in TableCurve (Jadel Scientific, USA). Ellipticine was used as the positive control.

3. Results and discussion

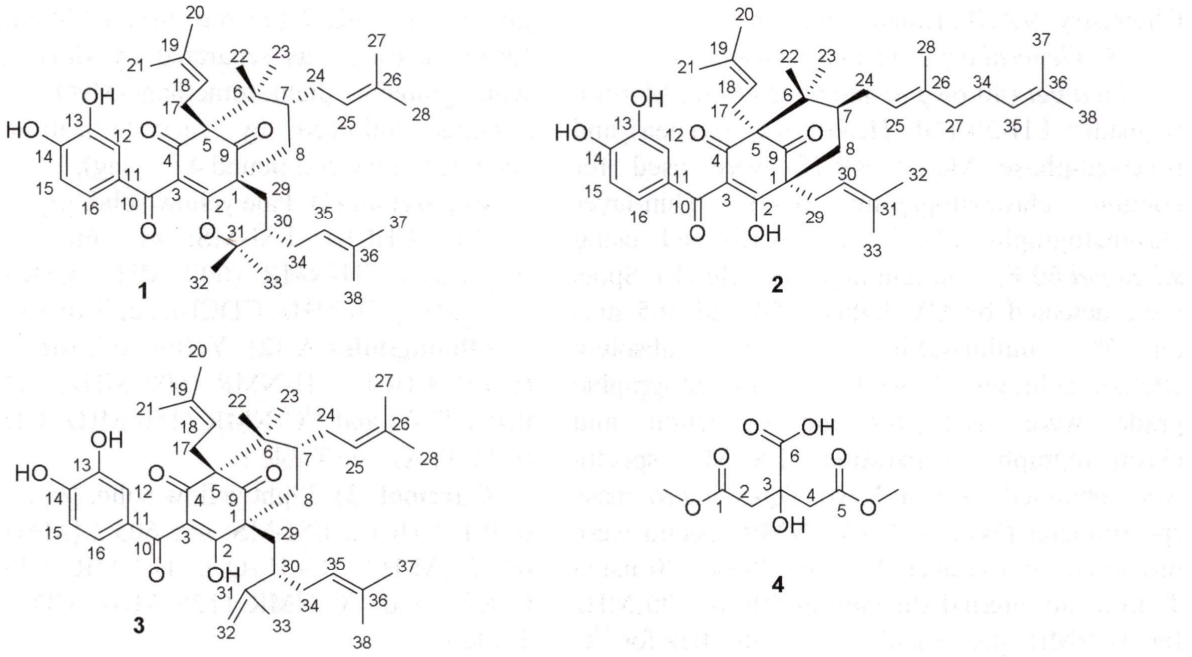

Fig. 1. Chemical structures of compounds **1-4**

Compound **1** was isolated as a white solid, $[\alpha]_D^{25}$ = -159 (c = 1.0, $CHCl_3$). Analysis of the ^{1}H-NMR spectrum of **1** revealed signals of four tertiary methyl groups at δ_H 1.15 (H-22), 0.98 (H-23), 1.24 (H-32), 0.91 (H-33), six olefinic methyl groups at δ_H 1.60 (6H, H-20, H-21), 1.68 and 1.67 (H-27, H-28), 1.77 and 1.58 (H-37, H-38), three aromatic protons at δ_H 6.75 (d, J = 7.8 Hz, H-15), 7.0 (dd, J = 7.8, 2.4, H-16), 7.35 (d, J = 2.4 Hz, H-12), and three olefinic protons at δ_H 4.89 (m, H-18), 4.91 (m, H-25), and 5.20 (m, H-35). In addition, seven methylenes and methines were visible in a range of δ_H 3.03-0.92. The ^{13}C-NMR and HSQC spectra showed the presence of six aromatic carbons (C-11 - C-16), a conjugated carbonyl group (δ_C 193.3, C-10), and a bicyclic [3,3,1]nonane-4,9-dione including three quaternary carbons [δ_C 57.2 (C-1), 68.2 (C-5), 46.2 (C-6)], a methine (δ_C 46.1, C-7), a methylene (δ_C 39.4, C-8), an isolated ketone (δ_C 207.3, C-9), a conjugated ketone (δ_C 194.4, C-4), a non-protonated olefinic carbon (δ_C 125.2, C-3), and an oxygenated olefinic carbon (δ_C 171.4, C-2). Analysis of COSY and HMBC correlations between H-29 (δ_H 3.03 and 0.92) and C-1 (δ_C 51.2), C-2 (δ_C 171.4), C-30 (δ_C 42.8), C-31 (δ_C 86.6) confirmed the fusion of the bicyclic system with a tetrahydropyran ring. The NMR data

(Table 1) suggested a tetrahydropyrano [3,3,1] nonane-4,9-dione system substituted with three prenyl groups and a 3,4-dihydroxybenzoyl group. The locations of the prenyl substituents at C-5, C-7, and C-30 were determined by HMBC correlations between H-17 (δ_H 2.44 and 2.63) and C-4 (δ_C 194.4), C-5 (δ_C 68.2), C-9, between H-24 (δ_H 2.16 and 2.63) and C-7, C-8, between H-34 (δ_H 2.03 and 1.79) and C-29 (δ_C 28.3), C-30. The 22- and 23-methyls were attached to C-6 and the 32- and 33-methyls were attached to C-31 (δ_C 86.6) by HMBC correlations between H-22 (δ_H 1.15), H-23 (δ_H 0.98) and C-5, C-6, C-7, and between H-32 (δ_H 1.24), H-33 (δ_H 0.91) and C-30, C-31. Thus based on the NMR spectroscopic data the planar structure of isogarcinol or 7-*epi*-isogarcinol/7-*epi*-isoxanthochymol or isoxanthochymol was proposed for compound **1**. The stereochemistry and the structure of **1** as isogarcinol were decided on the basis of the full agreement of the specific optical rotation of **1** with that of isogarcinol [10, 11]. Thus compound **1** was determined to be isogarcinol.

Compound **2** was isolated as a yellow oil, $[\alpha]_D^{25}$ = +25 (c = 1.0, $CHCl_3$). The ^1H and ^{13}C NMR spectra of **2** showed similar signals to those of compound **1** thus suggesting a bicyclic [3,3,1]nonane-2,4,9-trione linked with a 3,4-dihydroxybenzoyl group. However, the ^1H and ^{13}C NMR spectra of **2** indicated the presence of 9 methyl groups including seven olefinic methyl groups (δ_H 1.49, 1.60, 1.67, 1.68 × 2, 1.70, 1.73) and two tertiary methyl groups (δ_H 1.04 and 1.27) in comparison with 10 methyl groups of compound **1**. COSY correlations gave proton sequences of H-15-H-16, two prenyl groups, and a geranyl group. HMBC correlations between H-24 (δ_H 2.07 and 2.16) and C-6 (δ_C 48.5), C-7 (δ_C 47.9), C-8 (δ_C 40.8) implied the geranyl group was located at C-7 (δ_C 46.1). The two prenyl groups were located at C-1 (δ_C 51.2) and C-5 (δ_C 68.2) by corresponding HMBC correlations between H-29 (δ_H 2.60 and 2.75) and C-1, C-2 (δ_C 195.9), C-8 (δ_C 40.8) and between H-17 (δ_H 2.60 and 2.75) and C-4 (δ_C 195.3), C-5, C-9 (δ_C 209.9). The NMR data (in CD_3OD + 0.1% TFA) and specific optical rotation of compound **2** were superimposed with those reported for oblongifolin A isolated from the bark of *G. oblongifolia* in the literature [6]. Thus compound **2** was determined to be oblongifolin A.

Compound **3** was isolated as a yellow solid, $[\alpha]_D^{25}$ = -138 (c = 0.1, $CHCl_3$). The ^1H and ^{13}C NMR spectra of **3** also showed similar signals of the bicyclic 3-(3,4-hydroxybenzoyl)[3,3,1] nonane-2,4,9-trione core skeleton to those of compounds **1** and **2**. However, the ^1H, ^{13}C NMR, and HSQC spectra of **3** indicated the presence of 9 methyl groups and a methylene group (C-31/C-32) at δ_H 3.38 (1H, s) and 4.42 (1H, s) in comparison with 10 methyl groups in compound **1**. It was easily envisioned the formation of the tetrahydropyran ring in compound **1** from compound **3** through an acid-catalyzed cyclization suggesting **3** was garcinol. The suggestion was supported by HMBC correlations between H-32 (δ_H 4.42) and C-30 (δ_C 43.6), between H-29 (δ_H 1.95 and 2.15) and C-2 (δ_C 198.7), C-31 (δ_C 148.1), and between H-33 (δ_H 1.54) and C-30. The locations of three prenyl groups were determined at the same positions as those in compound **1**, namely at C-5, C-7, and C-30 by HMBC correlations between H-17 (δ_H 2.74) and C-5 (δ_C 69.8), C-9 (δ_C 209.1), between H-24 (δ_H 1.90 and 2.14) and C-6 (δ_C 49.6), C-7 (δ_C 46.9), C-8 (δ_C 42.6), between H-34 (δ_H 1.99 and 2.07) and C-30 (δ_C 43.6), C-35 (δ_C 122.7), C-36 (δ_C 132.0). The COSY correlations of compound **3** showed structural fragments of H-15-H-16, the side chain at C-1, and the prenyl groups at C-5 and C-7 that agreed well with the proposed structure. The relative stereochemistry of compound **3** was deduced from the following NOESY correlations between β-orientated groups at C-1 (δ_H 1.95 and 2.15, H_2-29) and at C-5 (δ_H 2.59 and 2.74, H_2-17) and H-7 (δ_H 1.45). In addition H-7 and H_2-34 gave correlations with H-8 (δ_H 2.07 and 2.36). Finally by comparing the NMR data and specific optical rotation [12] the structure of **3** was determined as garcinol. Thus compound **3** was determined to be garcinol.

Compound **4** was isolated as a white amorphous powder. The structure of **4** (1,5-dimethyl citrate) was determined by NMR analysis and deduced from the NMR data of trimethyl citrate and dimethyl citrate [13],[14].

Table 1. NMR spectroscopic data of compounds **1-3**

C/H	**1** ($CDCl_3$)		**2** (CD_3OD + 0.1% TFA)		**3** ($CDCl_3$)	
	δ_H, *J* (Hz)	δ_C	δ_H, *J* (Hz)	δ_C	δ_H, *J* (Hz)	δ_C
1		51.2		61.8		57.9
2		171.4		195.9		198.7
3		125.2		117.9		115.9
4		194.4		195.3		194.7
5		68.2		67.9		69.8
6		46.2		48.5		49.6
7	1.45 *m*	46.1	1.54 *m*	47.9	1.45 *m*	46.9
8	1.98 *dd* (14.4, 7.8) 2.26 *d* (14.4)	39.4	2.17 *m* 2.08 *m*	40.8	2.36 *d* (16.8) 2.07 [a]	42.6
9		207.3		209.9		209.1
10		193.3		194.8		193.9
11		130.0		129.5		128.0
12	7.35 *d* (2.4)	114.2	7.20 *d* (1.8)	117.4	6.97 *m*	116.5
13		144.4		146.2		143.5
14		150.4		152.6		149.6
15	6.75 *d* (7.8)	114.2	6.72 *d* (8.4)	115.2	6.62 *d* (8.0)	114.4
16	7.00 *dd* (7.8, 2.4)	124.0	6.99 *dd* (8.4, 1.8)	125.1	6.98 *m*	124.2
17	2.44 *dd* (4.8, 13.2) 2.63 [a]	25.5	2.75 *dd* (9.0, 13.8) 2.60 *m*	27.1	2.74 [a] 2.59 *br d* (5.6)	26.4
18	4.89 *m*	119.6	4.95 *m*	119.9	5.09 *m*	120.2
19		134.6		135.5		135.2
20	1.60 *s*	26.0	1.73 *s*	26.3	1.80 *s*	26.1
21	1.60 *s*	18.0	1.68 *s*	18.2	1.74 *s*	18.2
22	1.15 *s*	26.7	1.04 *s*	27.3	1.16 *s*	22.7
23	0.98 *s*	22.4	1.27 *s*	23.3	1.01 *s*	27.0
24	2.16 *br d* (12.0) 2.63 [a]	29.2	2.16 *m* 2.07 *m*	30.0	2.14 [a] 1.90 *m*	36.2
25	4.91 *m*	124.9	4.90 *m*	125.6	4.93 *m*	123.9
26		133.0		137.4		132.9
27	1.68 *s*	25.9	1.97 *m*	40.8	1.70 s	25.7
28	1.67 *s*	17.9	1.49 *s*	16.4	1.54 s	18.0
29	3.03 *dd* (3.6, 14.4) 0.92 [a]	28.3	2.54 *dd* (8.4, 14.4) 2.47 *m*	32.1	2.15 [a] 1.95 *m*	29.0
30	1.40 *m*	42.8	5.18 *m*	120.7	2.75 [a]	43.6
31		86.6		135.7		148.1
32	1.24 *s*	21.2	1.70 *s*	26.3	4.42 *s* 4.38 *s*	112.7
33	0.91 *s*	28.6	1.68 *s*	18.2	1.54 *s*	17.7
34	2.03 *br d* (12.0) 1.79 *m*	29.6	2.07 *m*	27.5	2.07 [a] 1.99 *m*	32.7
35	5.20 *m*	121.4	5.08 *m*	125.4	5.04 *m*	122.7
36		133.6		132.1		132.0
37	1.77 *s*	25.7	1.68 *s*	26.0	1.67 *s*	25.7
38	1.58 *s*	17.9	1.50 *s*	17.8	1.60 *s*	18.0

[a] *overlapping signals*

In the present study two compounds **1** (isogarcinol) and **3** (garcinol) were tested for cytotoxic activity against three human cancer cell lines, Hep-G2 (human hepatoma cancer cell line), LU-1 (human lung cancer line), HeLa (human cervical cancer cell line) and Vero cells (monkey kidney epithelial cells) according to the SRB method [15],[16]. The test results were shown in Table 2. The most potent cytotoxicity was detected for compound **1** against Vero cell line (4.68 μM) and compound **3** against LU-1 cell line (6.17 μM). In addition, compounds **1** and **3**

inhibited Hep-G2 (9.64 and 9.82 μM, respectively) and HeLa cell lines (9.87 and 10.08 μM, respectively). Some cytotoxic selectivity was observed against LU-1 and Vero cell lines pinpointing the influence of the phenolic hydroxyl group at C-2 and the conformational rigidity of the six-membered tetrahydropyran ring. The effects of garcinol and isogarcinol were studied in various types of cancer [17]. Our results are a new update on cytotoxic activity of the two compounds. Previously compound **2** (oblongifolin A) was determined to be cytotoxic against A549 and A431 cancer cells [9]. Further studies towards cancer cell lines of three benzophenones **1-3** and other compounds from *Garcinia* genus are ongoing.

Table 2: Cytotoxic activity of compounds **1** and **3**

No	samples	IC_{50} (μM)			
		Hep-G2	LU-1	HeLa	Vero
1	Ellipticine	1.26	1.38	0.69	8.61
2	Compound **1**	9.64	>20	9.87	4.68
3	Compound **3**	9.82	6.17	10.08	10.93

4. Conclusion

By chromatographic separation four compounds were isolated from the *n*-hexane and ethyl acetate extracts from the fruit pericarp of *Garcinia oblongifolia*. Spectroscopic (MS and NMR) analysis identified their structures as three acylphloroglucinols: isogarcinol (**1**), oblongifolin A (**2**) and garcinol (**3**), and a citrate: 1,5-dimethyl citrate (**4**). Isogarcinol and garcinol showed cytotoxicity against Hep-G2 and HeLa cancer cell lines with IC_{50} in the range of 9.64-10.08 μM. Garcinol was found active on LU-1 cancer cell line with IC_{50} 6.17 μM. Although compounds **1-3** were reported from *Garcinia* species, this is the first report on the occurrence of the phytochemicals **1-4** in the fruits of *G. oblongifolia* growing in Vietnam as well as cytotoxicity of garcinol and isogarcinol against Hep-G2, LU-1, HeLa and Vero cell lines.

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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