The dual effect of curcumin nanoparticles encapsulated by 1-3/1-6 β-glucan from medicinal mushrooms *Hericium erinaceus* and *Ganoderma lucidum*

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Abstract
Curcumin is a polyphenol from turmeric *Curcuma longa* L that has been proved to possess numerous biological and pharmaceutical activities, including anti-cancer properties. However, curcumin has only limited clinical applications due to the aqueous insolubility characteristic that reduces its biological availability. On the other hand, using nanoparticles as drug delivery system has potential as it increases solubility of hydrophobic substances such as curcumin. Furthermore, nanoparticles can protect and control release of drug. Therefore, the objective of this project is to prepare nanoparticles by polymeric encapsulating curcumin by 1-3/1-6 β-glucan extracted from Vietnamese mushrooms to increase drug delivery efficiency and biological effect. Method of the preparation is nano-precipitation. The produced curcumin-β-glucan-nanoparticles (NanoGluCur) takes spherical shape with 60–70 nm in diameter. As expected, water solubility of curcumin increases about 180 times, from 0.6 μg ml⁻¹ to 0.11 mg ml⁻¹. Loading capacity of NanoGluCur is 18.16%. *In vitro* cytotoxicity and anti-tumor promoting effects of NanoGluCur were also investigated. Results revealed that NanoGluCur is able to inhibit the growth of two human cancer cell lines Hep-G2 and LU-1 with IC₅₀ values of 6.82 and 15.53 mg ml⁻¹, respectively, while free curcumin expresses the activity with IC₅₀ values of 7.41 and 18.82 mg ml⁻¹. At the concentration of 40 mg ml⁻¹, NanoGluCur showed anti-tumor promoting effects in reducing tumor size by 59.93% and tumor density by 40.52%, while the percentages caused by pristine curcumin were 41.36% and 29.14%, respectively. These results demonstrated dual effect of 1-3/1-6 β-glucan encapsulated curcumin nanoparticles: higher water solubility and better *in vitro* anti-cancer effects compared to free curcumin and 1-3/1-6 β-glucan, expectedly. This observation can potentially open a new approach in research and manufacture of functional foods from medicinal mushrooms.

Keywords: curcumin, 1-3/1-6 β-glucan, anticancer, nanoparticles, dual effect
1. Introduction

Curcumin (Cur) is a polyphenol compound occurring naturally in rhizomes of turmeric plant (Curcuma longa L.). This compound consists of 3 components: (i) curcumin, (ii) demethoxycurcumin and (iii) bisdemethoxycurcuminoids with ratio 77:17:3 [1]. Curcumin (1, 7-bis(4-hydroxy-3-methoxyphenyl)-1, 6 heptadiene-3, 5-dione) (figure 1) is widely known for abilities to promote wound healing, enhance blood circulation and traditionally used as a coloring agent. In recent years, curcumin has been found to have interesting pharmacological and biological activities such as anti-oxidation [2], anti-inflammation [3], anti-regression and anti-ischemia [4], anti-cancer [5] and anti-viral [6]. In addition, very recently, curcumin is demonstrated as a potential fluorescent probe for monitoring the biodistribution of drug delivery nanosystem in cancer cell and tumor [7]. However, natural curcumin has low water solubility (0.6 μg ml⁻¹) [8]. Therefore, it is introduced into the body at only low concentration, which is quickly broken down by enzymes and the liver, thus reduce bioavailability. Recently, there have been many researches on increasing solubility and bioavailability of curcumin [8–10]. Notably, natural polymers such as starch, chitosan, casein, cellulose are being investigated as delivering materials for curcumin which can enhance efficiency of curcumin usage [10].

1-3/1-6 β-glucans are polysaccharides that naturally found in cereal grains’ bran and cell walls of certain fungi, especially mushrooms [11]. Biological and pharmaceutical activities of β-glucan were well studied, proving that the polysaccharide possess a variety of activities, including nitrogen oxide synthesis of macrophage and limulus factor G activation, interferon-gamma and colony stimulating factor synthesis [11–13]. Activities of β-glucan are decided by its structure, aqueous solubility, molecular weight, degree of branching and configuration [12, 14]. According to Zhang et al [15], β-glucan extracted from mushrooms has a main chain composed of β-(1-3) and (1-6) linked D-glucopyranosyl residues (figure 2).

In this paper, effects of polymeric nanoparticles-encapsulated curcumin by 1-3/1-6 β-glucans from Vietnamese medicinal mushrooms in bioavailability (water solubility) and in vitro anti-cancer activities (cytotoxicity and anti-tumor promoting effects) were investigated and evaluated. Results of our current study may provide evidences for applying the new nature-originated-nanomaterial in health caring as well as in treating acute diseases.

2. Materials and methods

2.1. Materials

2.1.1. 1-3/1-6 β-glucan. 1-3/1-6 β-glucan (Glu) used in this project is extracted from Lion’s mane mushroom (Hericium erinaceus) and Lingzhi mushroom (Ganoderma lucidum) cultured in Vietnam. Other chemicals include curcumin (Sigma-Aldrich), ethanol (Merk) and several standard chemicals for laboratory purposes.

2.1.2. Cell lines. Cell lines were supplied by Institute of Natural Product Chemistry including human hepatocellular carcinoma (Hep-G2) and human lung adenocarcinoma (LU-1).

2.2. Methods

2.2.1. Preparation of 1-3/1-6 β-glucan encapsulated curcumin nanoparticles. 1-3/1-6 β-glucan encapsulated curcumin nanoparticles (NanoGluCur) were prepared by nanoprecipitation method. In the first step of the method, 10 ml of 1 mg ml⁻¹ solution of curcumin in ethanol was dropped into 10 ml solution of 20 mg 1-3/1-6 β-glucan in deionized water. The mixture then had been stirred at room temperature for 48h. Then, solvent was evaporated by a rotary evaporator. Next, product was centrifuged at 400 rpm to eliminate free curcumin. The mixture that contains 1-3/1-6 β-glucan encapsulated curcumin was then dried by Freeze Dry System (Labconco).

2.2.2. Characterization techniques. The structure of NanoGluCur was investigated by Fourier transform infrared spectroscopy (FTIR). In addition, UV–vis absorption and emission spectroscopy were used to confirm the formation of nanoparticles. The morphology and size of nanoparticles were characterized by field emission scanning electron microscope (FESEM) and dynamic light scattering (DLS) imaging.

Loading capacity (LC) was calculated using UV–vis absorption spectroscopy. First, curcumin was separated from nanoparticles by adding ethanol. Then the mixture was centrifuged to eliminate polymer and insoluble components. Curcumin concentration in ethanol was calculated based on absorbance standard curve. LC was calculated by the
following formula:

\[ LC(\%) = \frac{\text{Encapsulated curcumin}}{\text{Total weight of components}} \times 100. \]

Solubility of NanoGluCur was determined after dissolving to saturation freeze dried NanoGluCur powder and eliminating insoluble component by centrifuging. Solubility of curcumin in the NanoGluCur solution is the concentration \( C_0 \) of the saturated solution quantified by UV–vis spectroscopy. Solubility of NanoGluCur was calculated by the following formula:

\[ S_p = \frac{C_0}{LC}, \]

with \( C_0 = kX_1 \), \( k \) is dilution factor, \( X_1 \) is concentration of curcumin after dilution.

2.2.3. Cytotoxicity assay. Cancer cells were cultured following the protocol described by Skehan et al [16]. Cytotoxicity of test samples was determined by sulforhodamine B (SRB) colorimetric method [17]. The method has been carried out in Department of Experimental Biology from Institute of Natural Product Chemistry (INPC, VAST) since 1996.

2.2.4. Anti-tumor promoting assay. Anti-tumor promoting assay is a technique of three dimensional tissue culture models in cancer biology based on the formation of tumors from seed cancer cells in soft agar. The assay has been conducted by Department of Experimental Biology (INPC, VAST) since 2010 following methods that described in earlier studies of Kim [18] and Gao et al [19]. Briefly, test samples were incubated (37 °C, 10–20 days) in micro-titer plate with cancer cells (Hep-G2 cell line) in DMEM-soft agar-medium at appropriated concentrations. Effects of test samples were determined by measuring sizes and densities of forming tumors in each well by light converted microscope (Olympus MX51). Cells and tumors were stained by SRB and crystal violet dyer.

3. Results and discussion

3.1. FTIR, fluorescence and UV–vis spectra

3.1.1. FTIR spectrum. FTIR spectra and characteristic peaks of curcumin (Cur), 1,3/1,6 \( \beta \)-glucan (Glu) and NanoGluCur are shown in figure 3 and table 1.

Figure 3 and table 1 showed shifts from curcumin characteristic vibrations at 3510 cm\(^{-1} \) (O–H bond), 1628 cm\(^{-1} \) (C=O bond) and 1509 cm\(^{-1} \) (C=C bond) to 3398, 1635, 1500 cm\(^{-1} \) of curcumin contained in nanoparticles. Also, peaks at 3402 vs 860 cm\(^{-1} \), corresponding to O-H bond and \( \beta \)-bridge of free glucan, shifted to peaks of 3398 and 849 cm\(^{-1} \) of \( \beta \)-glucan in NanoGluCur. In addition, the spectrum of interval 1026–1150 cm\(^{-1} \) that characterizes the glucose link at 3-O in glucan structure shifted to that of 1014–1142 cm\(^{-1} \) of nanoparticles. These evidences induce the formation of \( \beta \)-glucan encapsulated curcumin.

3.1.2. Fluorescence and UV–vis spectra. To further confirm the formation of NanoGluCur, fluorescense and UV–vis
spectra were compared between curcumin before and after encapsulated. It was observed that emission peak of curcumin in ethanol at 540 nm is shifted to 529 nm after encapsulated by 1-3/1-6 β-glucan (figure 4(a)). Similarly, the maximum absorption wavelength of curcumin in ethanol at 430 nm was shifted to 417 nm after encapsulated by 1-3/1-6 β-glucan (figure 4(b)). These shifts in fluorescence and UV–vis spectra could be attributed to the formation of intermolecular hydrogen bonding between curcumin and 1-3/1-6 β-glucan.

3.2. Morphology and size analysis of NanoGluCur particles

Morphology and size of NanoGluCur complex were studied under FESEM and DLS. Figure 5 shows that NanoGluCur has particle shapes with average diameter of 60–70 nm and narrow size distribution.

According to Olmsted et al [20], 38–75 nm particles can easily diffuse through digestive mucosa because spacing between epithelial cells is approximately 100 nm. Furthermore, 100 nm particles are more absorbed than bigger one [21]. Also, particles with this size are suitable for drug targeting in tumor by enhanced permeability and retention effect [22]. Therefore, this nanosystem can potentially be a drug delivery system for cancer treatment.

3.3. Loading capacity and solubility of NanoGluCur

Loading capacity of curcumin in nanoparticles calculated as described in method section is 18.16%.

Solubility of NanoGluCur is determined by saturated dissolving freeze dried NanoGluCur powder, then diluting 50 times for UV–vis characterization. The maximum absorbance of this sample at 432 nm was 0.303 (a.u) (figure 6). The solubility of NanoGluCur was calculated as following:

\[ S_p = \frac{C_0}{L C} = \frac{50 \times (0.303 - 0.0436)/118.51}{0.1816} = 0.603 \text{ mg ml}^{-1}. \]

Especially, \( C_0 \), concentration of curcumin presence in saturated solution of NanoGluCur, reaches to 0.11 mg ml\(^{-1}\). Obviously, after being encapsulated, aqueous solubility of curcumin in the nano system increases about 180 times in
comparison to raw curcumin (0.6 μg ml\(^{-1}\)) [8]. The result showed that 1-3/1-6 β-glucan can carry curcumin in drug delivery nanoparticles and improve its solubility, consequently increase the bioavailability and effect of curcumin.

3.4. Cytotoxicity

Cancer cells inhibitory effect of NanoGluCur complex was tested against two human cancer cell lines (Hep-G2 and LU-1). Inhibitory effects of NanoGluCur as well as pristine curcumin and 1-3/1-6 β-glucan are shown in table 2 and microscopic visualized in figures 8 and 9. As described in table 2, both NanoGluCur and pristine curcumin are toxic to Hep-G2 and LU-1 cells with low IC\(_{50}\) values, but these of NanoGluCur (6.82 μg ml\(^{-1}\) and 15.53 μg ml\(^{-1}\)) are somewhat lower than pristine curcumin’s (7.41 μg ml\(^{-1}\) and 18.82 μg ml\(^{-1}\)) (figure 7), although NanoGluCur consists of only 18.16% of curcumin, indicating that NanoGluCur is more potent in inhibiting the cancer cell lines in vitro. This may be explained by the increase in solubility of curcumin and the compatibility between glucan and curcumin.

The result suggests that curcumin encapsulated nanoparticles possess increased effectiveness in activity of curcumin in vitro.

Figures 8 and 9 show the microscopic images of Hep-G2 and LU-1.

### Table 2. Cytotoxicity of testing substances.

| Sample                   | Hep-G2 (μg ml\(^{-1}\)) | LU-1 (μg ml\(^{-1}\)) |
|--------------------------|--------------------------|------------------------|
| Positive control         | 0.28                     | 0.31                   |
| Curcumin                 | 7.41                     | 18.82                  |
| 1-3/1-6 β-glucan         | >100                     | >100                   |
| NanoGluCur               | 6.82                     | 15.53                  |

3.5. Anti-tumor promoting assay

Anti-tumor-promoting effects of test compounds, including curcumin, 1-3/1-6 β-glucan and NanoGluCur were assayed against Hep-G2 cell line in soft agar medium. The results are shown in table 3.

As shown in table 3, at concentration of 40 μg ml\(^{-1}\), NanoGluCur reduced tumor size by 59.93% and tumor density by 40.52% compared to negative control (DMSO), while the reductions of tumor size and tumor density by curcumin were 41.36% and 29.14%, respectively. Figure 10 depicts effects of test compounds in the tumor formation at concentration of 40 μg ml\(^{-1}\). There were clear changes in size and morphology of tumor between the control and all the samples tested, especially curcumin encapsulated with glucan. In the control wells, the tumor size was much larger and their surface was very rough in comparison to the tumor on the
Figure 8. Hep-G2 cells under the influence of test compounds at different concentrations: (a) negative control, (b) NanoGluCur of 10 μg ml⁻¹, (c) NanoGluCur of 2 μg ml⁻¹, (d) Cur of 10 μg ml⁻¹, (e) Cur of 2 μg ml⁻¹ and (f) Glu of 100 μg ml⁻¹. Scale bar 60 μm.

Figure 9. LU-1 cells under the influence of test compounds at different concentrations: (a) negative control, (b) NanoGluCur of 10 μg ml⁻¹, (c) NanoGluCur of 2 μg ml⁻¹, (d) Cur of 10 μg ml⁻¹, (e) Cur of 2 μg ml⁻¹ and (f) Glu of 100 μg ml⁻¹. Scale bar 60 μm.
wells. It was obvious that encapsulated curcumin had positive effects on tumor promotion of Hep-G2 cell line in vitro. The results claim enhanced activity of the nanosystem NanoGlu-Cur in comparison to pristine curcumin and 1-3/1-6 β-glucan.

4. Conclusion

The dual effect of 1-3/1-6 β-glucan encapsulated curcumin nanoparticle product (NanoGluCur) has been proven. The results showed that our system has better solubility in water and enhanced biological effects than curcumin and 1-3/1-6 β-glucan alone. However, study for better introducing into cells as well as mechanism of biological activities of the product is continuing. Nevertheless, the results suggest a new approach in the research and manufacture of food supplements from medicinal mushrooms.

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