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Encapsulation of *Ganoderma lucidum* with Alginate and Hydroxypropylmethylcellulose polymers: Investigation of antioxidant and antimicrobial activity

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Abstract – *Ganoderma lucidum* (*G. lucidum*) is a mushroom that stands out as a functional food and is widely used in eastern folk medicine. Nowadays, microparticulate systems are a very popular field in the food industry to protect food molecules against environmental factors. The carrier material of capsules to be used in food products or processes must be food-grade and biocompatible. Alginates (Alg) are widely used in many fields such as tissue engineering, drug delivery and food science due to their excellent properties such as biocompatibility, biodegradability, and gelation. Hydroxypropylmethylcellulose (HPMC) is a biodegradable, FDA approved cellulose derived biopolymer. In this study; *G. lucidum* was extracted in water by maceration and the obtained extracts were encapsulated with Alg and HPMC polymers. The swelling of microspheres containing *G. lucidum* extracts in pH1.2, pH6.8, pH7.4 and pure water were investigated. It was observed that the highest amount of swelling in microspheres was observed in the formulation where the amount of HPMC was higher (Alg/HPMC=1/2). Total phenolic content, antioxidant and antimicrobial activity of microspheres and extracts were analyzed by Folin-Ciocalteu method, 2,2-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method and Well Diffusion Method, respectively. The antioxidant capacity of *G. lucidum* encapsulated microspheres was approximately 55%. The total phenolic contents of the extract and extract-containing microsphere formulation were found to be 15.8±3.18 and 25.4±0.7 mg GAE/g-extract, respectively. It is thought that microspheres containing *G. lucidum* extract prepared in this study may be potentially useful in functional food applications.

Keywords – G. Lucidum, Alginate, Hydroxypropylmethyl Cellulose, Microsphere, Food Supplement

I. INTRODUCTION

There is a growing interest in natural products rich in bioactive compounds such as mushrooms and medicinal plants [1, 2]. *G. lucidum* is a species of mushroom belonging to the Ganodermataceae family, which is widespread in tropical and temperate regions of Europe, North America and Asia and comprises about 200 species worldwide [3-5]. The annual global market value is reported to exceed 1.5 billion dollars [6]. Thanks to the rich bioactive compounds it contains, this interesting medicinal mushroom is intensively researched in many countries of the world and is reported to be used as a nutritional supplement [7]. Today, it is used for medicinal purposes with its immune-boosting, anti-diabetes, anticancer and anti-aging properties [8].

Nowadays, the nutraceutical and food supplement industry focuses on developing food matrices with bioactive components such as vitamins, minerals, antioxidants, phytochemicals, mycochemicals, probiotics, postbiotics [9-13]. This is because bioactive compounds have the ability to modulate many metabolic processes such as antioxidant, antibacterial and anticancer activities [14]. Literature studies show that the poor

stabilization of bioactive components in the body is preserved by encapsulation technology [15]. In encapsulation, many polymers are used as wall materials to protect the core of bioactive compounds. Natural polymers are of great interest due to their increased biocompatibility, non-toxicity and economic sustainability, especially in their application areas in the food, medicine and cosmetics sectors [16]. Alginates are an unbranched anionic, non-toxic and biodegradable polymer, usually derived from brown algae [17]. It consists of alternately arranged 1-4-linked α-l-guluronic acid (G) and β-D mannuronic acid (M). It gels easily in the presence of divalent cations [18]. HPMC is a biocompatible, non-ionic and water-soluble polymer widely used in food, pharmaceuticals and ceramics. It is also FDA (U.S. Food and Drug Administration) and EU (European Union) approved and is widely used as a binder and thickener, especially in the food industry [19, 20].

In this study, it is aimed to realize the encapsulation of extracts of *G. lucidum*, one of the important mushroom species, obtained by maceration method, with Alg and HPMC polymers via ionotropic gelation method. The development of a food supplement product based on natural ingredients with antioxidant and antibacterial properties and the creation of a prototype for the food industry are within the scope of the study.

II. Materials and Method

G. lucidum was received as a gift from Deniz Yilmaz Cosmetics in Ankara, Turkey. Alg, DPPH (2,2-Diphenyl-1-picrylhydrazyl) and methanol were supplied from Sigma Chemical Co (Germany). Calcium chloride ($CaCI_2$), di-Sodium hydrogen phosphate (Na_2HPO_4) and Sodium dihydrogen phosphate monohydrate (NaH_2PO_4), Gallic acid, Folin-Ciocalteu's phenol reagent, and sodium carbonate were purchased from Merck (Germany). Dimethylsulfoxide (DMSO) and hydrochloride acid was supplied from Carlo Erba (Italy).

A. *Extraction of G.lucidum*

Water extraction maceration method was used in this study. 15 g of G. *lucidum* was placed in 200 mL of distilled water. Extraction was carried out at 95°C at 200 rpm for 3 hours. At the end of the extraction, the solvent was evaporated using a rotary evaporator (Onilab RE100-Pro, Türkiye). The extract obtained was stored in the refrigerator (Vestel, Türkiye) at +4°C until use [21]. Figure 1 shows the extraction process of G. *lucidum.*

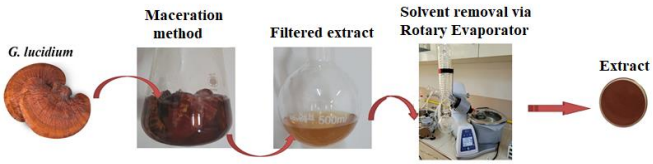

Fig. 1 Extraction process of *G.lucidum*

B. *Preparation of microspheres*

Empty microspheres containing G. *lucidum* extract were prepared by esterification reaction and ionic crosslinking between HPMC and Alg. For this purpose, the polymers were kept in distilled water overnight and the polymer was allowed to swell. Alg-HPMC solutions were prepared at 40°C at different polymer ratios. The homogeneous solution was dropped from a glass material apparatus equivalent to 30G diameter into 4% $CaCl_2$ solution from a certain height with the help of an air hose and a peristaltic pump. This step was performed at room temperature and at a certain speed (low rpm). After the last drop of polymer, the microspheres were allowed to crosslink for 30 min and collected by filtration followed by distilled water washing. The microspheres were allowed to dry in an oven for 24 hours. For microspheres containing *G.lucidum* extract, the extract was added to the polymer mixture and stirred overnight to obtain a homogeneous solution. Subsequent processes were carried out as described above. Figure 2 shows the microsphere preparation step [22].

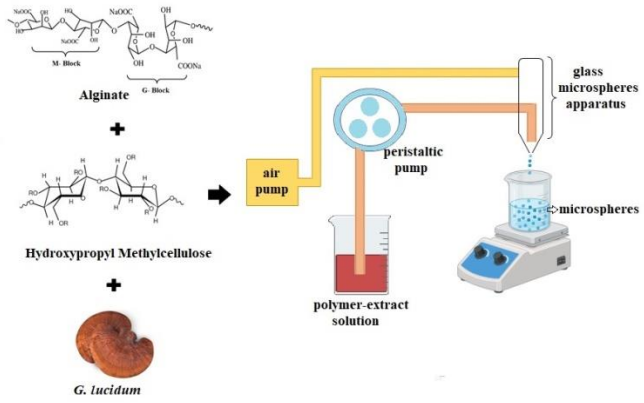

Fig. 2 Microsphere preparation process

C. *Investigation of swelling degree*

The swelling degree of the developed microspheres was studied by measuring their

swelling at different pHs using the immersion method. Microspheres (0.1 g) were immersed in 50 mL of freshly prepared buffer solutions of SGF (pH1.2), SIF (pH6.8), SCF (pH7.4) and distilled water at 37°C. At predetermined time intervals, excess surface-adherent liquid drops were removed by blotting and the swollen microspheres were immediately reweighed [23]. Swelling rates were calculated using the following equation (Eq. 1).

$$\%SD = \frac{Ws - Wd}{wd} x100 \quad \text{Eq.1}$$

where Ws is the weights of microspheres at time t and Wd is the weights of microspheres at zero time of swelling in microspheres.

D. *Determination of total phenolic content*

Folin-Ciocalteu method was used to determine the total phenolic content. A certain amount of Folin and sodium carbonate was added to the *G. lucidum* extract and microsphere medium and kept in the dark for a certain period of time and the absorbance value was measured at 760 nm. The total amount of phenolic compounds in the samples was given as mgGAE/100g and all studies were carried out in 3 replicates [24].

E. *2,2-Diphenyl-2-picrylhydrazyl (DPPH˙) radical scavenging capacity*

Antioxidant activity was determined following the method of Blois in 1958. DPPH˙ stock solution was prepared, added to a certain amount of *G. lucidum* extract solution and microsphere medium and kept at room temperature and in the dark for a while. The absorbance values were determined by reading at 517 nm via UV-Vis Spectrophotometer (Thermo Scientific, Genesys-150, The U.S.A). The radical scavenging effects (%RSA) of the compounds were calculated by the following relation compared to DPPH˙ solution used as control [25]. DPPH· radical scavenging capacity is calculated using the following equation (Eq.2)

$$\%\ RSA = \frac{A0 - A1}{A0} x100 \quad \text{Eq. 2}$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the test extracts.

F. *Antibacterial Activity*

The antibacterial activities were determined by the disc diffusion technique against two pathogenic bacteria, *S. aureus ATCC 25923*, *E. coli ATCC 25922*. 100 µL of 10^7 CFU mL^{-1} bacterial suspension was taken and spread on the surface of Brain Heart Infusion Agar using sterile spreader. Then, samples impregnated with the Susceptibility testing disk were placed on the agar surface. The petri dish was incubated at 37°C for 24 hours to observe the area of inhibition. At the end of this period, the diameter of the inhibition zone was measured.

III. RESULTS

A. *Swelling degree of G.lucidum*

When the swelling values were examined, the highest swelling value at pH 1.2 was observed in the microsphere formulation containing a higher amount of HPMC (Alg/HPMC=1/2), followed by Alg/HPMC:2/1 and Alg/HPMC:1/1, respectively. All microspheres disintegrated at pH6.8 and pH7.4. The microspheres retained their shape at pH1.2 and showed over 100% swelling behavior. The highest swelling value at pH 1.2 was observed in the microsphere formulation containing higher amount of HPMC (Alg/HPMC=1/2), followed by Alg/HPMC:2/1 and Alg/HPMC:1/1, respectively. In the other study where the microspheres were kept in distilled water for the same period of time, the highest swelling of the microspheres was observed in the microsphere formulation with higher amount of HPMC (Alg/HPMC=1/2).

Table 1. Swelling results of microsphere formulations

	Alg/HPMC: 1/1	Alg/HPMC: 1/2	Alg/HPMC: 2/1
pH 1,2	123,48	183,56	139,14
pH 6,8	-	-	-
pH 7,4	-	-	-
distilled water	76,22	169	89,78

B. *Total phenolic content of G.lucidum*

When the total phenolic contents of the extract and the extract-containing microsphere formulation were evaluated as gallic acid equivalent, they were found to be 15.8±3.18 and 25.4±0.7 mg GAE/g-extract, respectively.

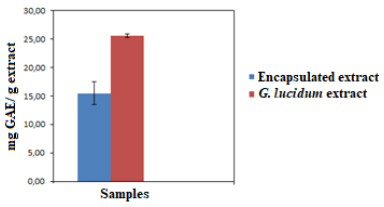

Fig. 3 Total phenolic content

C. *Antioxidant capacity of G.lucidum*

When the antioxidant results of the microspheres were examined, it was observed that the

antioxidant capacity was approximately 55% when calculated with the help of correlation.

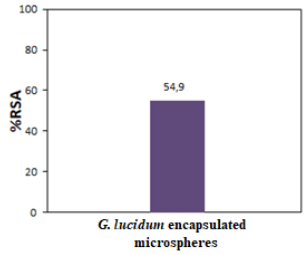

Fig. 4 Antioxidant capacity

D. Antibacterial activity of G.lucidum

G.lucidum encapsulated samples showed activity on *E.coli*, no activity was observed on *S. aureus*. Fig. 5 showed the antibacterial activity. Encapsulated extract samples showed a narrow 7.5 mm zone of inhibition against *E.coli*.

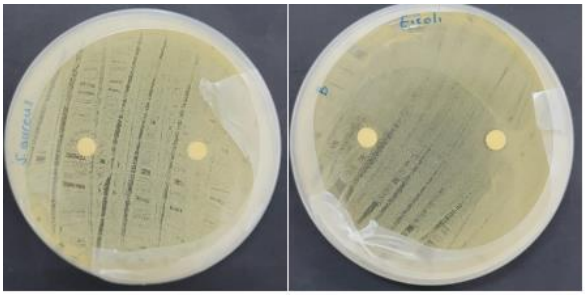

Fig. 5 Antibacterial activity

IV. DISCUSSION

The increase in the amount of HPMC was interpreted as an increase in the hydrophilic character of the microsphere structure. Bioactive components obtained from *G. lucidum* vary depending on the organ type of the fungus and the extraction condition. The total phenolic content of *G. lucidum*, extracted by hot water maceration method by Kozarski et al., was found to be 3.3 g/100 g GAE [26]. In the study conducted by Kank et al., it was found that aqueous maceration extracts showed higher DPPH radical scavenging activity than ultrasound-assisted extraction [27]. It was observed that encapsulation did not change the phenolic content and antioxidant capacity.

V. CONCLUSION

In this study, it was aimed to encapsulate *G. lucidum* with previously unstudied Alg and HPMC polymers. *G. lucidum* was extracted in water by maceration. The highest swelling degree of extract-containing microspheres was observed in the formulation with Alg/HPMC=1/2 at pH 1.2. Total phenolic content and antioxidant capacity of the extract-containing microsphere formulation were 25.4±0.7 mg GAE/g-extract and 55%, respectively. While antibacterial activity of the prepared samples was observed on *E.coli*, no activity was observed on *S. aureus*. Therefore, the new formulation with antibacterial and antioxidant activity was proposed by encapsulating *G. lucidum* with Alg and HPMC polymers.

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The heading of the Acknowledgment section and the References section must not be numbered.

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