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Medicinal Mushrooms Cordyceps as a New Source of Bioactive Compounds and Their Complexation With Silver Ions

Special Issue

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Abstract Mushrooms from the genus *Cordyceps* are characterized by a wide range of biological effects due to the diverse amount of substances contained in them and are an important source of bioactive compounds. In China, mushrooms of the genus *Cordyceps* have been used as a medicinal preparation of traditional Chinese medicine for centuries. So far, a considerable number of studies have been conducted that focused on analyzing the effects of *Cordyceps*, which include their antioxidant, antibacterial, immunomodulatory, antidiabetic, antitumor, and many other effects. The ability of fungi to form complexes with various metals is also interesting. It is believed that polysaccharides are the main component of the extracts involved in the complexation with metals, after which their biological effects are improved and deepened. The work deals with the comparison of the antioxidant and antibacterial effects of *Cordyceps* extracts with extracts of this mushroom enriched with silver ions. Based on scientific studies, it is assumed that there is a complexation between the chemical compounds of the extracts and silver.

Keywords *Ophiocordyceps sinensis – DPPH radical – silver ions – antioxidant and antimicrobial activities*

Three samples of strain MFTCCB022 *Ophiocordyceps sinensis* CS_4 were tested: the first sample was cultured on maize (**1**), the second was cultured on chickpea (**2**), and the third sample was cultured on millet (**3**). Mushroom samples were cultured and processed to a final powder form by drying at 40 °C in an APT Line dryer and ground in an SM-100 mill. They were stored at 8 °C.

Six extracts were prepared from *Ophiocordyceps sinensis* samples by refluxing (**R**) and maceration (**M**). Three sample extracts were obtained by reflux (**1R**, **2R**, **3R**), and three extracts were prepared by maceration (**1M**, **2M**, **3M**). We chose reflux and maceration in order to get as much as possible in comparison with the literature studied. In both cases of **R**/**M** preparation, 5.0 g of powdered mushroom material and 50 mL of a 1:1 ethanol:water mixture were used. This different cultivation was the cause of their different activities (antioxidant and antimicrobial activities). Publications state that millet is a very good choice of substrate for cultivation. It has no distinct taste or smell and allows the full expression of secondary metabolites. *Ophiocordyceps* does not grow as fast on millet as on other cereals, but the quality of the final product is higher [1]. The prepared extracts were already visually different. Extracts **2R** and **3R** were clear, while the rest were cloudy. The extracts also differed from each other in their consistency. Samples **1R** and **2R** obtained by reflux were solid, while the other samples were oily [2-4]. These changes were caused by the decreasing ability of the extracts to scavenge DPPH radicals based on their decreasing concentration. However, samples **3R** and **3M** did not show this trend, which implies that they did not demonstrate antioxidant activity.

The antioxidant activity of the samples was compared based on their IC_{50} values. The extract of sample **2**, prepared by both reflux and maceration, showed the highest antioxidant activity. The difference between the antioxidant activities of this sample depending on its preparation was minimal. A more pronounced difference depending on the method of obtaining the extract was observed for sample **1**. It showed approximately 1.3 times higher antioxidant activity after processing by maceration with an IC_{50} value of 7.20 $mg.mL^{-1}$ compared to its preparation by reflux. A similar procedure was used in a study [4] in which an ethanolic extract of *Ageratum* plants showed antioxidant activity, determined by the DPPH radical scavenging method, with ascorbic acid as the standard for comparison. By comparing the IC_{50} values of the extracts after the addition of $AgNO_3$, we can state that the highest antioxidant activity with an IC_{50} value of 4.99 $mg.mL^{-1}$

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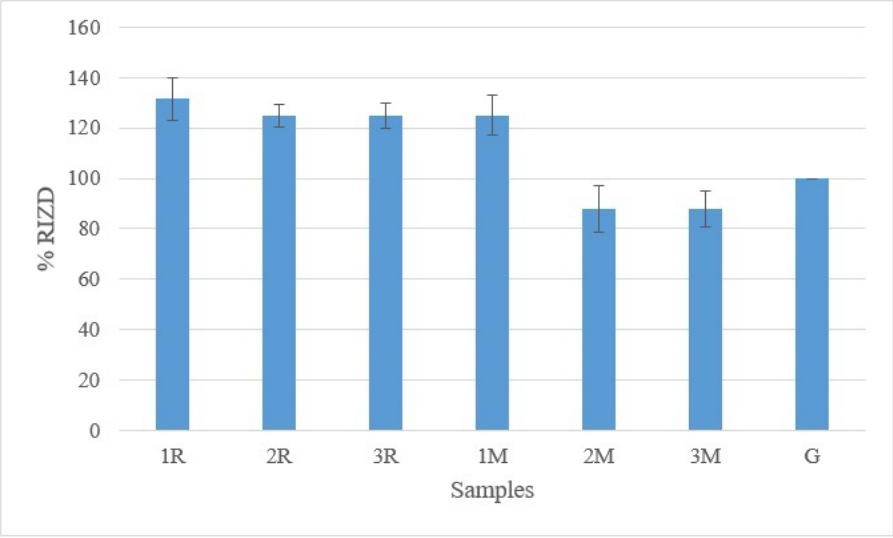

Figure 1. Antibacterial activity of 1R-3M extracts after addition of $AgNO_3$ on the Escherichia coli strain.

was shown by the **2M** sample processed by maceration. On the other hand, this sample treated with reflux **2R** showed the lowest antioxidant activity, while its IC_{50} value was up to two times higher compared to sample **2M**. In the case of sample **1**, from the point of view of the method of its preparation, no significant difference in its activity was noted; however, the **3M** sample had 1.3 times higher antioxidant activity compared to the **3R** sample. From a comparison of the antioxidant activity of extracts without $AgNO_3$ and extracts with the addition of 10.1 mmol.L^{-1} $AgNO_3$, it can be concluded that there is no significant change in the antioxidant activity of sample **1R** processed by reflux, while when this sample was processed by maceration, there was approximately 1.3 times decrease in its antioxidant activity after complexation with silver ions. In sample **2R** prepared by reflux, there was also a significant decrease in its antioxidant activity after the addition of $AgNO_3$, and its IC_{50} value increased more than 1.6 times with the addition of silver nitrate. However, in the case of its processing by maceration, on the contrary, after complexation with silver ions, its antioxidant activity increases with a decrease in IC_{50} from the value of 6.33 mg.mL^{-1} to the value of 4.99 mg.mL^{-1}. Other methods for measuring antioxidant activity will be used in the near future.

The results showed that when testing the extract of sample **3** without the addition of $AgNO_3$, it did not show any antioxidant activity regardless of the method of its preparation; after the addition of $AgNO_3$, we can observe that by complexing with silver, antioxidant activity was detected in sample **3**, which was more pronounced when the sample was processed by maceration compared to reflux. From the point of view of the preparation method of the extract samples, we can conclude that the extracts processed by maceration showed better antioxidant activity than the extracts prepared by reflux. The author of the study [5], points out the relationship between maceration and extraction yield from Chinese medicinal herbs. The work shows that the swelling of the tissue of herbs by maceration increases the extraction yield of the chemical marker of the subsequent extraction because water-soluble substances are dissolved in the water during maceration, which increases the availability of the marker, which is extracted with a mixture of ethanol and water [5].

When determining the antimicrobial activity by the disc diffusion method, the effects of *Ophiocordyceps sinensis* extracts **1**-**3** alone before the addition of $AgNO_3$, as well as the extracts with the addition of $AgNO_3$, were evaluated. Gentamycin (G) at a concentration of 30 mg.mL^{-1} was used as a positive control, and distilled water was used as a negative control. No antimicrobial activity was observed when the extracts alone were tested on *Staphylococcus aureus* and *Escherichia coli* strains. The pure silver nitrate solution inhibited the growth of the *Escherichia coli* strain but did not effect on the *Staphylococcus aureus* strain. The antibacterial activity of the extracts became apparent only after complexation with silver ions.

The highest antimicrobial activity on the *Escherichia coli* strain was exhibited by sample **1R** (prepared by reflux), which had an average value of relative inhibition zone (RIZD) of 131.5 % (Fig. 1). Consequently, this sample prepared by maceration **1M** along with **2R** and **3R** samples prepared by reflux showed 1.05-fold lower antimicrobial activity compared to the **1R** sample. Samples **2M** and **3M**, whose mean %RIZD was 87.5%, showed the lowest activity among the extracts tested.

The use of UV/Vis spectrophotometry showed that after adding $AgNO_3$ to the extracts, an increase in absorbance was observed in the 415-nm region (Fig. 2), which indicates the formation of complexes between the extracts and $AgNO_3$. The region from 400 to 500 nm represents the wavelength range with maximum absorption for silver nanoparticles [6].

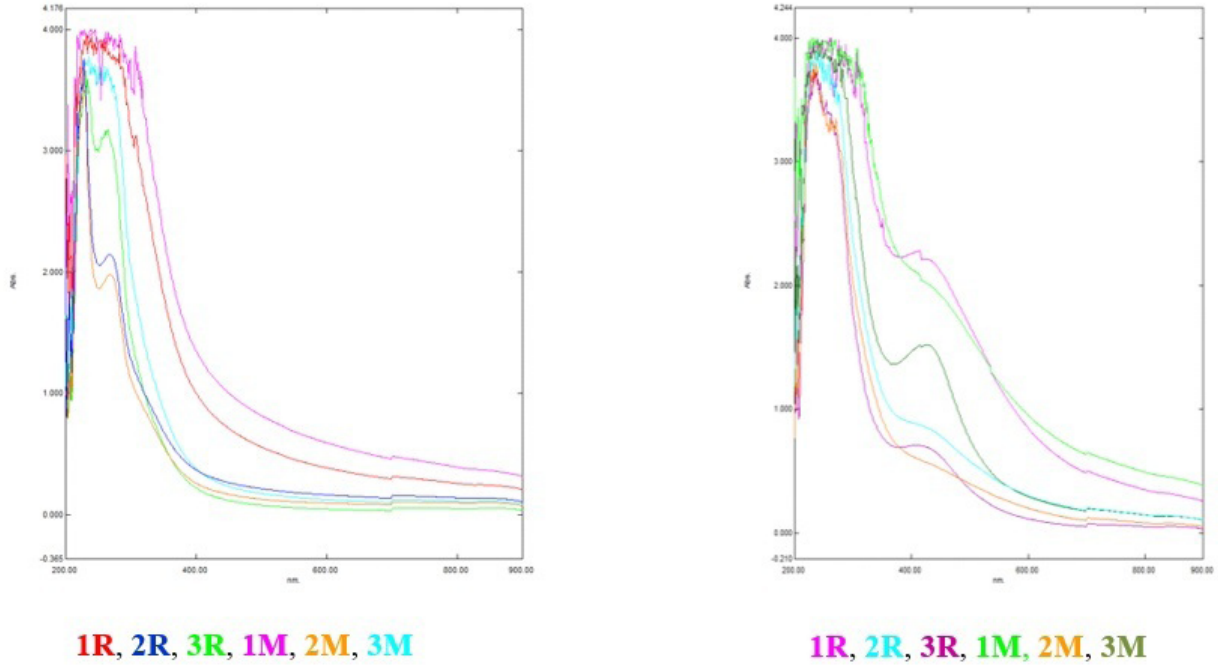

Figure 2. Left (UV/Vis spectra of 1R-3M extracts without $AgNO_3$). On the right, the predicted formation of complexes with silver ions at 415 nm (UV/Vis spectra of 1R-3M extracts with $AgNO_3$ after 100 minutes of heating at 80 °C).

Gradually heating the extracts increased the absorbance value at a wavelength of 415 nm. According to the Lambert–Beer law, an increasing concentration causes an increasing absorbance, and this represents an increase in the ability of the extracts to form complexes with $AgNO_3$.

The extract samples were heated in a water bath (80 °C) for 20, 40, and 100 minutes. After heating, the UV/Vis spectra were measured in the wavelength interval from 200 to 900 nm. The most significant increase in absorbance at 415 nm was observed after 100 minutes of heating for samples **1R** and **1M**. At all time intervals, the **1M** and **3M** samples that were prepared by maceration had higher absorbance values compared to the samples prepared by reflux. We assume that the formation of complexes with $AgNO_3$ was avoided. The exception was sample **1M**, which had a significantly lower absorbance maximum even after 100 minutes heating compared to sample **1R**. Sample **2R**, at all three heating times, entered complexation with $AgNO_3$ most readily. Sample **2M** exhibited the lowest absorbance value even after 100 minutes of heating.

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