

Antibacterial and photocatalytic effects of newly synthesized zinc oxide nanoparticles derived from Mongolian honey

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ABSTRACT

Development of bio-compatible, bio-safe and environmentally friendly nanoparticles is a matter of urgency for research in the field of nanotechnology. In this study, we aimed to prepare zinc oxide nanoparticles from Mongolian honey as raw material and to determine its biological activities. Honey-based zinc oxide nanoparticles were obtained by green synthesis method, and their characteristics and biological activities were evaluated. Developed zinc oxide nanoparticles from Khentii honey and Selenge honey were at a size of 16.02 nm and 95.23 nm, respectively. A characteristic band of Khentii honey-based zinc oxide nanoparticles was observed at 466 cm⁻¹ and a band of Selenge honey-based zinc oxide nanoparticles was also observed at 434 cm⁻¹. Antibacterial and photocatalytic effects were detected for the developed nanoparticles. The study suggested that newly synthesized honey-based zinc oxide nanoparticles might be an effective tool against bacterial infection.

Keywords: Antibacterial effect, photocatalytic effect, synthesis, nanoparticle, zinc

INTRODUCTION

Nanotechnology is one of the fastest growing fields of science and technology. It has been widely used and studied in many fields such as agriculture, food production, and medicine [1]. Recent studies have shown that the properties and potential biological applications of nanoparticles vary depending on the phase, size, and morphology of small particles. Therefore, the synthesis of new nanoparticles is a matter of great interest. Nano-sized inorganic compounds are highly biologically active, such as antibacterial and anti-cancer, depending on their unique physical and chemical properties, even at very low concentrations [2]. The development of bio-compatible, bio-safe, and ecologically friendly nanoparticles is a current requirement for research in the field of nanotechnology [3]. Recently, metal oxide nanoparticles with antibacterial properties have been studied. The inorganic metal compounds with the highest antibacterial activity are silver, gold, copper, titanium oxide, zinc oxide, and nanoparticles based on

them. Various chemical and physical methods are still being studied for the synthesis of metal (Ag, Au, Cd, Zn, etc.) nanoparticles [4].

Zinc oxide nanoparticles (ZnONPs) are considered elite nanomaterials and are widely used. ZnONPs are still synthesized and produced by conventional (physical, chemical and biological methods) and unconventional methods (reactor based method) [5]. Zinc oxide is recognized as a safe material that is non-toxic, bio-safe and bio-compatible [6]. So far, many methods, including sol-gel, combustion and autocombustion, have been applied to synthesize zinc oxide nanoparticles using honey as a reducing and stabilizing agent [7, 8].

Natural honey contains sugars such as 75% monosaccharides of glucose and fructose and 10%-15% disaccharides of sucrose, maltose, and water, enzymes, vitamins of B6, riboflavin, niacin and thiamine, minerals as well phenolic compounds [9]. The therapeutic activity of honey varies due to flower and fruit types, floral origins, beehive areas, climate

conditions and environmental pollution. A honey from the Manuka flower is highly active as an antioxidant and anti-inflammatory, and is effective against ulcers, while Jhelam tree honey is effective in suppressing respiratory tract inflammation, allergies, and asthma [10].

In Mongolia, it is estimated that 14-16 thousand colonies of bees are being bred in 5060 places in 14 provinces. In addition to honey, beekeeping produces about 10 products, including wax, pollen, nectar, rosin, mad honey, and protein substances. The honey produced in Mongolia is characterized by high mountain, multiple species of flowers and virgin nature. In other words, Mongolia produces honey from many plants, vegetables, and fruit flowers that grow in Mongolia at an altitude of 900-1500 meters above sea level [11, 12]. Therefore, it can have a promising therapeutic effect against bacterial infection and cancer compared to other honey.

In this study, we aimed to synthesize zinc oxide nanoparticles using honey obtained from various flowering plants growing in Mongolia. Zinc oxide nanoparticles differ in shape, size, and biological activity depending on the composition of the plant and original extract used as a reducing agent.

EXPERIMENTAL

Reagents: Zinc nitrate was purchased from *Tianjin heowns Biochemical Technology Co., Ltd (People's Republic of China)*, sodium hydroxide was obtained from Aladdin Reagent Database inc. (Shanghai, People's Republic of China), Selenge honey was purchased from Se Te Bi Co., Ltd (Selenge province, Mongolia), Khentii honey was obtained from Ariun honey (Khentii province, Mongolia), Muller-Hinton's agar (Thermo Scientific™), Tryptic soy broth (Sigma-Aldrich) and McFarland's solution (HiMedia, India) were kindly supported by department of Molecular biology and Genetics (Mongolian National University of Medical Sciences)

Bacterial culture: Bacterial reference strains (*S.aureus* ATCC 29213™, *E.coli* ATCC 35218™, *Klebsiella pneumoniae* (*K.pneumoniae*) ATCC baa-1706) were kindly supported by department of Molecular biology and Genetics (Mongolian National University of Medical Sciences).

Instruments: Magnetic stirrer (Iuchi: HS-4R, USA) was used for synthesis of zinc oxide nanoparticles, UV-vis spectrophotometer (Shanghai Spectrum Instruments co., Ltd, Peoples Republic of China) was used for measuring the absorbance of the developed nanoparticle, infrared spectrophotometer (FT-IR) was used to obtain an infrared spectrum from the nanoparticle. Nanodrop spectrophotometer (Thermo fisher scientific, USA) was used for DNA concentration measurement.

Synthesis method of zinc oxide nanoparticles

Preparation of honey solution: To prepare 50 mL of

honey solution with a concentration of 30% honey, 15 g of honey was added to 50 mL of distilled water and gently mixed with a magnetic stirrer for 15 minutes to dissolve the honey. The prepared honey solution was used directly in the experiment.

Preparation of zinc nitrate solution: To prepare a 50 mL solution of zinc nitrate with a ratio of 2:1, 25 g of zinc nitrate was added to 50 mL of distilled water and dissolved with a magnetic stirrer for 30 minutes. Zinc nitrate completely dissolves in distilled water to form a clear, colorless solution.

Synthesis of zinc oxide nanoparticles based on honey: ZnONPs were synthesized by the method of Hoseini S et al [8]. In the synthesis of ZnONPs, 50 mL of zinc nitrate $Zn(NO_3)_2$ solution was added drop by drop to the pre-prepared honey solution, and the solution was mixed continuously for 3 hrs at a speed of 400 rpm on a magnetic stirrer heated to 60 °C. 10 mL of sodium alkali was gently added dropwise to the above solution and mixed again continuously for 1 hour. The prepared solution mixture was centrifuged at 10,000 rpm for 20 minutes to remove the supernatant. The liquid part was freeze-dried to obtain honey-based zinc oxide nanoparticles (Fig. 1).

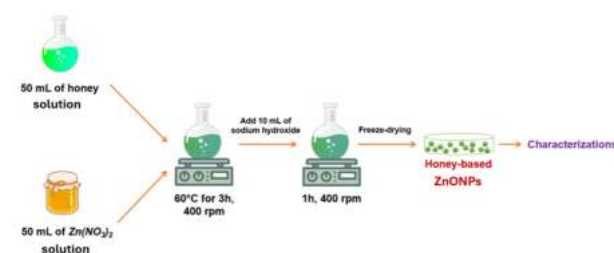


Fig. 1. Representative images for preparation of honey-based ZnONPs

Size determination of honey-based ZnONPs: A nano-size analyzer (Nanophox particle size analyzer, Sympatec, Germany) was used to determine the size of ZnONPs derived from honey. In sample preparation, the nanoparticles were diluted with distilled water, and the mean value of nanoparticles was calculated by averaging 3 measurements.

Measurement of honey-based ZnONPs by UV-vis spectrophotometry: To measure ZnONPs by UV-vis spectrophotometer, 0.03 g of synthesized honey-based ZnONPs were dissolved in 5 mL of distilled water. The spectral properties of zinc oxide nanoparticles were analyzed in the range of 200-800 nm using UV spectrophotometer. Also, the spectral properties of zinc nitrate solution and honey solution used in the reaction were determined.

Measurement of honey-based ZnONPs by FTIR spectrophotometry: Honey-based ZnONPs were evaluated using a violet-red spectrophotometer (FTIR, Shimadzu-IR Prestige-21, Germany) with a range of 500-4000 cm^{-1} . Dried powder samples were used for

the measurements.

Hemolysis assay for honey-based ZnONPs:

Hemolytic activity was determined by the method of Mollaeva MR *et al.* [13]. Rat blood (5 mL) was used for the experiment. Purified red blood cells were diluted with saline (25 mL). In the experiment, honey-based ZnONPs were calculated to be 1mg/1mL and 5mg/1mL. 0.2 μ L of each of the samples was added to 2 mL of erythrocyte solution and keep at 37 °C for 3 hrs. After that, it was centrifuged at 1600 rpm for 5 minutes, 200 μ L of supernatant was taken and oxyhemoglobin was measured at 540 nm by spectrophotometer. 5% sodium dodecyl sulfate was used as positive control and saline as negative control. The hemolysis was calculated using the following Formula 1.

$$\text{Hemolysis} = \frac{\text{Sample abs} - \text{Negative control abs}}{\text{Positive control abs} - \text{Negative control abs}} \times 100\% \quad (1)$$

Evaluation of photocatalytic activity for honey-based ZnONPs:

A method adopted by Ankamwar BG *et al.* was used to evaluate the photocatalytic activity [14]. The photocatalytic activity of honey-based ZnONPs was determined using a 0.0025 μ g/mL solution of methylene blue, using a UV-vis spectrophotometer at 298 nm and 665 nm for 0, 15, 30, and 45, 60, and 90 min. 10 mg of honey-based ZnONPs were added to the methylene blue solution, and one sample was kept in dark place and another one sample in sunlight by continuously stirring the solution. As a control, the methylene blue solution was kept in the dark and in the light environment without adding ZnONPs, and the changes at 298 nm and 665 nm were measured with spectrophotometer at time intervals.

Evaluation of antibacterial activity for honey-based ZnONPs:

Pure cultures of 3 types of gram positive and negative bacteria were used: *S. aureus* ATCC 29213TM, *E. coli* ATCC 35218TM and *K. pneumoniae* ATCC 1706TM.

Broth dilution method: Honey-based ZnONPs were dissolved in distilled water at a concentration of 52 mg/mL. 1 mL of soy broth was prepared in a vial. Then, 1 mL of honey-based ZnONPs solution was added to the first tube of soy broth, mixed, and diluted by adding 1 mL to the next tube of broth. Then, 1 mL was taken from the last vial and discarded. Finally, 1 mL of 0.5 units (1.5×10^8 colony-forming units (CFU)) of McFarland's turbidity was added to the culture after 24 h incubation. After 24 h of incubation at 37 °C, the lowest dose at which bacteria did not grow was defined as the lowest growth inhibitory dose.

Evaluation of the effect of honey-based ZnONPs on bacterial DNA concentration:

After 18-24 hours incubation of the bacteria, a colony (*S. aureus*, *E. coli*, *K. pneumoniae*) was taken with a loop and a bacterial solution was prepared, boiled at 100 °C for 10 minutes, then the cell decomposition products were centrifuged at a speed of 12000 rpm for 10 minutes. DNA in the

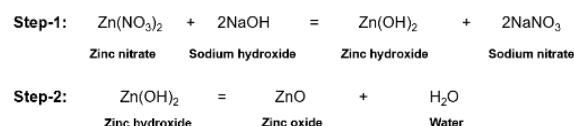
supernatant was collected. DNA concentration was determined by nanodrop spectrophotometry before starting the experiment. The sample with bacterial DNA was divided into 2 equal parts, 50 μ L of honey-based ZnONPs was added to the first sample, and 25 μ L of honey-based ZnONPs was added to the second sample. Then, it incubated at 37 °C for 24 hours. The DNA concentration in each sample was determined by nanodrop spectrophotometry at time intervals of 0, 2, 4, 6, 12 and 24 hrs.

Statistical analysis: The data for the results was carried out using SPSS-25 and graphpad prism software to analyze the numerical data. Mean and standard deviation were calculated for studies of nanoparticle size, photocatalysis and bacterial DNA stability. Statistically significant difference was evaluated as $p < 0.05$.

RESULTS AND DISCUSSION

Synthesis of honey-based ZnONPs:

The zinc nitrate solution was slowly added dropwise to the prepared honey solution, heated to 60 °C, and the reaction was carried out with continuous stirring. When the addition of zinc nitrate solution was completed, the color of the reaction changed from brownish yellow (Khentii honey-based ZnONPs) and yellow (Selenge honey-based ZnONPs) to pale yellow. After continuously stirring the reaction at 60 °C for 3 hrs, 10 mL of sodium hydroxide was added dropwise. The color of the reaction started to change during the addition of sodium hydroxide, and it turned deep yellow after 1 h. As a result of this reaction, the chemical reaction for the formation of zinc oxide from zinc nitrate is represented by the following reaction equation.



Scheme 1. Chemical reaction for the formation of zinc oxide from zinc nitrate

The finished reaction product was centrifuged at 10,000 rpm for 20 minutes to separate the sample with nanoparticles. The synthesized nanoparticles were isolated by freeze-drying.

Size determination of honey-based ZnONPs: We determined the size of synthesized nanoparticles using a size determination instrument. As displayed in Table 1, The size of Khentii province honey-based ZnONPs was 16.02 ± 0.75 nm and the size of Selenge province honey-based ZnONPs was 95.23 ± 2.65 nm. The size of honey-based ZnONPs was different due to the biologically active compounds contained in the 2 types of honey.

Detection of UV-Vis spectrum characteristics for honey-based ZnONPs: The synthesized honey-based ZnONPs were dissolved in distilled water and analyzed

Table 1. Hemolytic activity for ZnONPs of each group (n=3)

Types of ZnONPs	Mean \pm St.dev	P-Value
Khentii honey-based ZnONPs	16.02 \pm 0.75 nm	P < 0.0001
Selenge honey-based ZnONPs	95.23 \pm 2.65 nm	P < 0.0001

by UV-vis 1700 spectrophotometer for absorption of 200-800 nm light. Spectral properties were evaluated by comparing the honey solution used to synthesize the two types of honey-based ZnONPs, and using the absorption determined at wavelengths around 310 nm. The wavelength of Khentii province honey produced a low, dull wave at 310 nm. However, Khentii province honey-based ZnONPs showed a high and bright light wave at 310 nm wavelength. Moreover, the wavelength of Selenge province honey produced low and dull waves at 305 nm. However, the wavelength around 310 nm of Selenge province honey-based ZnONPs showed a high and bright light wave absorption value (Fig. 2).

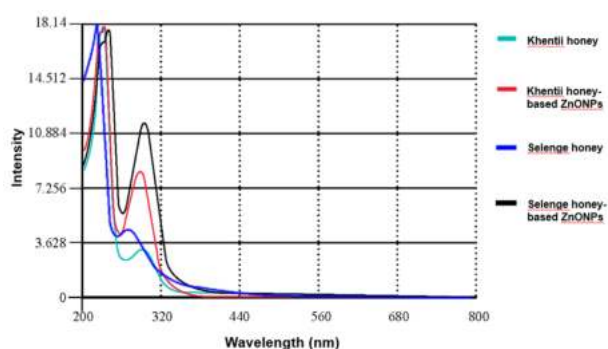
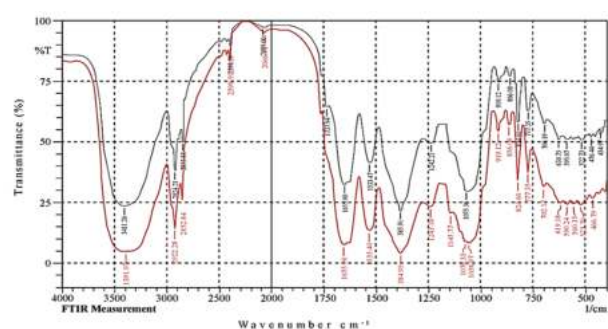


Fig. 2. UV-vis spectral characteristics of honey and honey-based zinc oxide nanoparticles.

FT-IR spectral characteristics for honey-based ZnONPs: Spectral properties of the synthesized honey-based ZnONPs were determined by FT-IR spectrophotometer at Institute of Physics and Technology, Mongolian Academy of Sciences. FTIR analysis of Khentii honey-based ZnONPs

Fig. 3. FT-IR spectral characteristics of honey-based ZnONPs. **Red** - Khentii honey-based ZnONPs and **Black** - Selenge honey-based ZnONPs

revealed stretching fluctuations in the spectral range from 466 to 3391 cm^{-1} (Fig. 3). Honey exhibits stretching fluctuations in the spectral range from 1000 to 1500 cm^{-1} and provides most characteristic stretching fluctuations associated with sugars. The spectral range of 825-466 cm^{-1} showed the stretching variation of the hydrocarbon anomeric region or C-H, C-C deformation. The spectral regions from 950 to 860 cm^{-1} were C-O stretching oscillations of C-OH group or C-C stretching oscillations in the carbohydrate structure. The additional high values due to the amide II vibration at 1535 cm^{-1} were attributed to the interaction of the compound with the honey protein. In the spectral range of 3391 cm^{-1} , there was a variation in the stretching of -OH group of carbohydrates and organic acids. The spectral region of 400-500 cm^{-1} indicated ZnO stretching oscillation, and the ZnO stretching oscillation occurred in the 466 cm^{-1} region.

FT-IR analysis of Selenge honey-based ZnONPs revealed spectral ranges from 434 to 3411 cm^{-1} (Fig. 3). In the region of 3411 cm^{-1} , O-H stretching oscillations or stretching oscillations of -OH group were formed. The bond between zinc and oxide was formed in the spectral region of 434 cm^{-1} . The two types of honey-based ZnONPs showed similar spectral characteristics in FT-IR analysis.

Hemolysis assay of honey-based ZnONPs: A hemolysis assay of honey-based ZnONPs was performed by detecting the absorption of oxyhemoglobin released from erythrocytes. Table 2 and Fig. 4 showed the hemolytic activity. It has been determined that Khentii and Selenge honey-based ZnONPs had less than 10% hemolytic activity or no hemolytic activity.

Table 2. Hemolytic activity for ZnONPs of each group

Hemolytic activity (%)				
Positive control	Negative control	Dose	Khentii honey-based ZnONPs	Selenge honey-based ZnONPs
100%	0%	1 mg	0.09%	0.06%
		5 mg	0.14%	0.15%



Fig. 4. Hemolysis assay for ZnONPs based on Mongolian honey. 1-Negative control; 2-Khentii honey-based ZnONPs (1 mg); 3-Khentii honey-based ZnONPs (5 mg); 4-Selenge honey-based ZnONPs (1 mg); 5-Selenge honey-based ZnONPs (5 mg); 6-Positive control

Compared to the negative control, ZnONPs based on honey from Khentii and Selenge at a dose of 5 mg showed a statistically significant difference ($p < 0.01$) in terms of hemolytic activity.

Evaluation of photocatalytic activity of honey-based ZnONPs: The photocatalytic activity of honey-based ZnONPs was evaluated using a 0.0025 $\mu\text{g/mL}$ solution of methylene blue. The photocatalytic activity was evaluated by UV-vis spectrophotometer using the change in absorbance at 298 nm and 665 nm after 0, 15, 30, 45, 60, and 90 minutes of addition of 10 mg/mL of honey-based ZnONPs in dark and light environments (Fig. 5). The results showed that the photocatalytic activity of the control group at 298 nm under dark conditions (Fig. 5A) was statistically significantly different ($p < 0.001$) compared to the nanoparticle group. Even though no statistically significant difference was observed between the nanoparticle groups at 0-60 minutes, Khentii honey-based ZnONPs showed high photocatalytic activity at 90 minutes ($p < 0.001$). There was a statistically significant difference in photocatalytic activity between the control and nanoparticle groups at 665 nm under dark conditions (Fig. 5B, $p < 0.001$), and the photocatalytic activity at 90 minute was higher in Khentii honey-based ZnONPs group ($p < 0.001$).

Compared to the photocatalytic activity of the control and nanoparticle groups at 298 nm and 665 nm under light conditions, statistically significant differences were found in all groups, and the photocatalytic activity of Khentii honey-based ZnONPs was high ($p < 0.001$). These results suggest that honey-based ZnONPs can be used as photocatalytically active disinfectants in the medical field and as detoxification agents in the industrial field.

Evaluation of antibacterial activity for honey-based ZnONPs: Broth dilution method was used to investigate

the minimum dose of honey-based ZnONPs to inhibit bacterial growth. The activity of two newly synthesized honey-based ZnONPs against *E. coli*, *K. pneumoniae*, and *S. aureus* standard strains of bacteria was evaluated.

52 mg/mL of honey-based ZnONPs was dissolved in distilled water. 1 mL of honey-based ZnONPs was added to 5 vials of 1 mL packed soy broth. After that, it was incubated at 37 °C for 24 hrs and evaluated. As regards Khentii honey-based ZnONPs, the minimum dose to inhibit the growth of *S. aureus*, *E. coli*, and *K. pneumoniae* was 13 mg/mL, 26 mg/mL and 26 mg/mL, respectively (Fig. 6). However, Selenge honey-based ZnONPs inhibited the growth of bacteria such as *E. coli*, *K. pneumoniae*, and *S. aureus*, with a minimum dose of 13 mg/mL (Fig. 7).

Evaluation on bacterial DNA stability by honey-based ZnONPs:

The effect of honey-based ZnONPs on DNA stabilization was evaluated by measuring DNA concentration at time intervals. In this study, bacterial DNA concentrations were determined by nanodrop spectrophotometry at 0 min, 2 h, 4 h, 6 h, 12 h and 24 h after treatment with 13 ng/mL and 6.5 ng/mL of honey-based nanoparticles. DNA samples of bacteria such as *S. aureus*, *E. coli*, and *K. pneumoniae* that were not treated with ZnONPs were used as the control group. The test was performed in triplicate and averaged (Fig. 8). The results showed that Selenge honey-based ZnONPs had a time-dependent reduction effect on *E. coli* DNA concentration at both 13 ng/mL and 6.5 ng/mL doses of nanoparticles. However, there was no direct time-dependent reduction in DNA concentration of *S. aureus* and *K. pneumoniae*, but a decrease in DNA concentration was observed compared to the control group (Fig. 8).

Khentii honey-based ZnONPs showed a time-

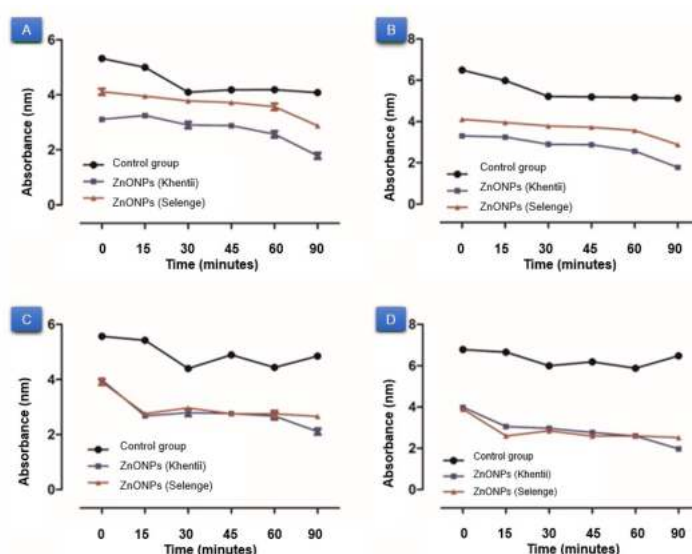


Fig. 5. Results of determination of photocatalytic activity of zinc oxide nanoparticles based on honey. (A) Dark conditions (298 nm); (B) Dark conditions (665 nm); (C) Light conditions (298 nm); (D) Light conditions (665 nm)

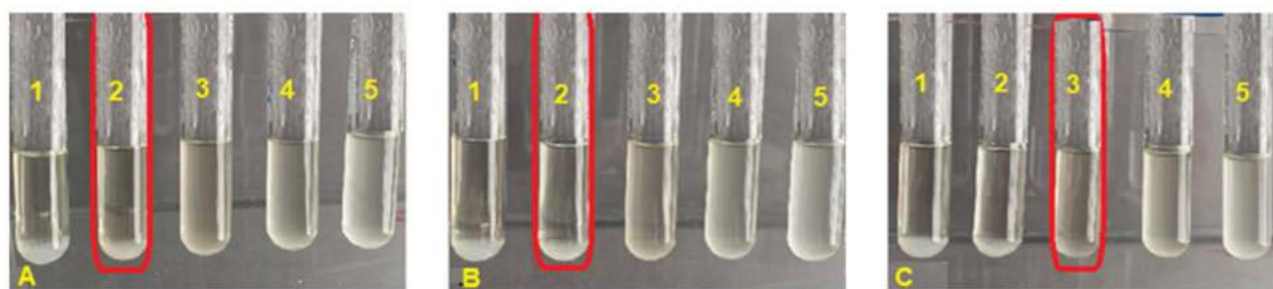


Fig. 6. Detection of the minimum dose to inhibit bacterial growth of Khentii honey-based ZnONPs. (A) *E. coli*; (B) *K. pneumoniae*; (C) *S. aureus*. Dose of honey-based ZnONPs: vial 1 - 52 mg/mL, vial 2 - 26 mg/mL, vial 3 - 13 mg/mL, vial 4 - 6.5 mg/mL, vial 5 - 3.25 mg/mL

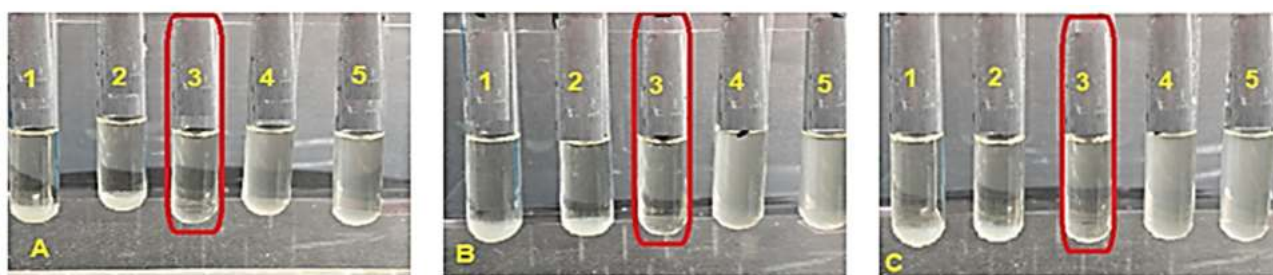


Fig. 7. Detection of the minimum dose to inhibit bacterial growth of Selenge honey-based ZnONPs. (A) *E. coli*; (B) *K. pneumoniae*; (C) *S. aureus*. Dose of honey-based ZnONPs: vial 1 - 52 mg/mL, vial 2 - 26 mg/mL, vial 3 - 13 mg/mL, vial 4 - 6.5 mg/mL, vial 5 - 3.25 mg/mL

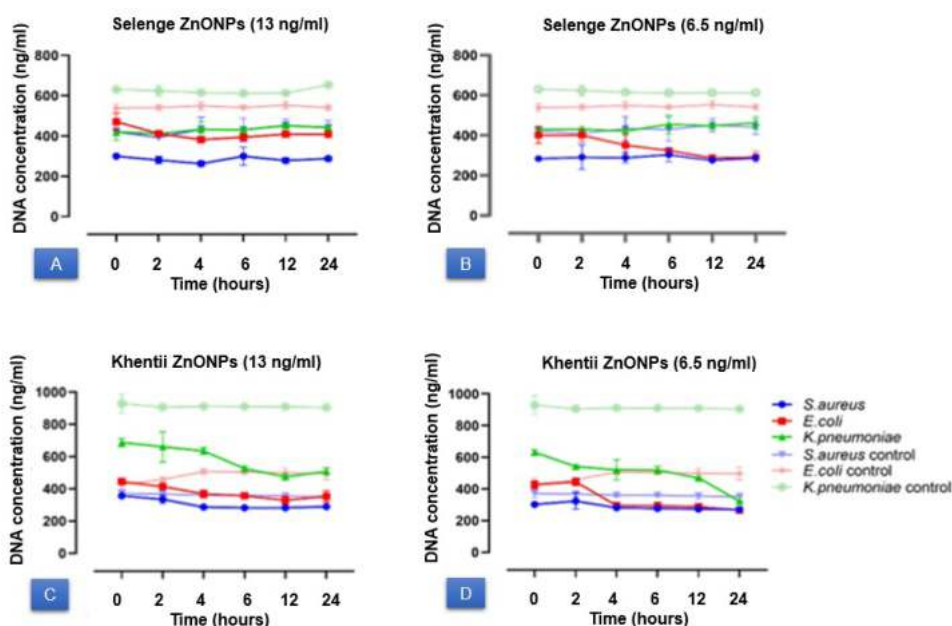


Fig. 8. Results of studies on the effects of honey-based zinc oxide nanoparticles on bacterial DNA stability. (A) Selenge honey-based ZnONPs 13 ng/mL; (B) Selenge honey-based ZnONPs 6.5 ng/mL; (C) Khentii honey-based ZnONPs 13 ng/mL; (D) Khentii honey-based ZnONPs 6.5 ng/mL

dependent reduction effect on the DNA concentration of bacteria such as *S. aureus*, *E. coli*, and *K. pneumoniae*. Also, compared to the control group, the DNA concentration of bacteria such as *S. aureus*, *E. coli*, and *K. pneumoniae* was significantly decreased in the experimental group. These results indicate that Selenge honey-based ZnONPs can inhibit *E. coli* and Khentii honey-based ZnONPs can inhibit bacterial

growth by degrading the bacterial DNA. Nanotechnology has developed rapidly in the fields of medicine, chemistry and biotechnology in the last decades. Advances in this field have opened new opportunities in nanoscience, particularly drug delivery systems, nanomedicine, and biosensors for diagnostics [15, 16]. Researchers synthesize nanoparticles using a variety of physical, chemical, and biological methods.

Various physical and chemical methods such as hydrothermal, sol-gel synthesis, laser ablation, and lithography not only require special equipment and skilled labor, but also have dangerous and harmful effects on health. However, the advantages of nanoparticles produced by green synthesis are that they are inexpensive, non-toxic, and biodegradable [17].

Since Mongolia has a vast land, mountainous terrain, and a harsh climate, each region has its own unique characteristics in terms of plants. Khentii province's land vegetation zone belongs to high mountain or taiga, mountain taiga, mountain forest-steppe belt, dry steppe and desert steppe zone. The plants in the mountain forest-steppe zone may be categorized by ecological groups such as plants of humid, humid-dry, dry, and cold environmental groups. As classified by Ölzikhutag D (1989), the flora of Mongolia is categorized into 16 regions by geographic regions. According to this classification, the Khentii region belongs to Khentii mountain taiga region, and a total of 1,276 species of tubular plants are distributed. The main plants are *Echinops latifolius* Tausch, *Trollius asiaticus*, *Orostachys malacophylla*, *Scabiosa*, *Campanula glomerata* L. Selenge province belongs to Mongolian-Dagur mountain forest-steppe region. A total of 1,310 species are distributed in the ward, and mountainous steppe plants are predominant. 50 species of plants that occur only in this circle are recorded, and the dominant plants such as *Sophora flavescens*, *Thymus vulgaris*, *Ephedra equisetina*, *Aster alpinus*, *Taraxacum officinalis* [18].

In this study, we aimed to synthesize ZnONPs using honey extracted from various flowering plants growing in Mongolia. The formation of nanoparticles was confirmed by evaluating the physicochemical properties and biological activities.

In determining the size of the newly synthesized honey-based ZnONPs, the size of Khentii honey-based ZnONPs was 16.02 nm, while the size of Selenge honey-based ZnONPs was 95.23 nm. The difference between the size of honey-based ZnONPs might be related to the type, composition, and geographical location of the honey used in our study. The size of ZnONPs varies depending on the source solution and synthesis method, as well as the analyzer used to determine the size of the nanoparticles, but they are all less than 100 nm in size. In a study by Santhoshkumar J *et al.*, ZnONPs synthesized using plant leaves were determined to be 70 nm in size [19]. This study synthesized ZnONPs by a green synthesis. Another research team obtained ZnONPs by using green synthesis method, and they were identified as 19.57 ± 1.56 nm particles in size. It is smaller than the results of our study, which may be due to the *Azadirachta indica* plant used as its source extract [20]. Also, in research of Hoseini S *et al* in 2015, honey-based ZnONPs were determined to be 30 nm in size [8, 21].

To evaluate the properties of honey-based ZnONPs, we performed UV-vis spectrophotometry and FT-IR spectral analysis. UV-vis spectrum analysis showed the highest light absorption at 305 nm. This seemed to be similar to the results of other researchers. As regards to other research, ZnONPs provided the highest light absorption at 200-400 nm, depending on the substance used as a source extract and the method for synthesis. A study conducted in India reported that ZnONPs were extracted using honey, and the nanoparticles showed the highest light absorption at 372 nm [22]. This study used honey as the original extract, similar to our study. Also, considering a study conducted in South Africa published in 2020, the antibacterial effect was determined after synthesizing ZnONPs using aqueous extracts prepared from pomegranate leaves and flowers. The ZnONPs had a crystalline structure and the highest light absorption of the nanoparticles obtained from leaves and pomegranate flower was observed at 284 nm and at 357 [23].

FT-IR spectrum analysis was performed to determine the properties of synthesized ZnONPs. In our study, we used honey collected from 2 different geographical locations in Mongolia. FT-IR analysis of samples with two types of honey revealed waves in the spectral range of 466 cm^{-1} and 434 cm^{-1} . According to other studies, it can be seen that zinc and oxide bonds show waves in the spectral range of $400\text{--}600\text{ cm}^{-1}$ [24, 25]. Hence, FT-IR analysis showed that ZnONPs were successfully synthesized due to the functional groups in the composition of honey and the detection of zinc and zinc oxide coupling waves. Scientists have confirmed that honey consists of more than 200 compounds. But according to FT-IR spectrum analysis, the compounds in the composition of honey are detected in the range above 800 cm^{-1} .

In our study, we used 3 types of bacteria, *S. aureus*, *E. coli*, and *K. pneumoniae*, to evaluate the antibacterial activity of ZnONPs. Hence, a promising effect against bacteria was confirmed in our study. Emami-Karvani Z *et al.* determined the minimum inhibition dose of ZnONPs synthesized from honey for *S. aureus* and *E. coli*, and the minimum dose was determined to be 1.5 mg/mL [26, 27]. From this result, it can be concluded that ZnONPs had a high antibacterial activity against *E. coli* and *S. aureus*. Our study showed that honey-based ZnONPs are more effective against gram-positive *S. aureus* bacteria than against gram-negative bacteria when determining the minimum lethal dose, or killing dose. The minimum dose of ZnONPs to inhibit bacterial growth was different due to the differences in the nanoparticle synthesis conditions and the source extract. Green synthesis is an environmentally friendly technology that synthesizes nanoparticles from plant leaves, roots, and flower extracts, and reduces the use of toxic chemicals used in the reaction [28].

The effects of honey-based ZnONPs on bacterial DNA content were determined. According to the results

of the experiment, the concentration of DNA in the experimental group was relatively low as compared to the control group. A study by Singh R *et al.* concluded and reported that ZnONPs penetrate bacterial cells, and damage the membrane, causing the cytotoxicity of bacteria. The antibacterial mechanism of action of ZnONPs has now been explained by increase of the reactive oxygen species (ROS), which can damage DNA, proteins, and internal components of bacterial cells [29].

Photocatalysis technology is an environmentally friendly, cost-effective, low-energy, and low-side-effect, and it is now developed for disinfection material in medicine [30]. In this study, we evaluated the photocatalytic activity of ZnONPs using methylene blue dye as a pollutant. In a study by Ankamwar BG *et al.* ZnONPs showed good photocatalytic activity or photocatalysis within 90 minutes at a concentration of 2.5 mg/L and 165 minutes at a higher concentration of 5 mg/L under direct sunlight [14]. Mahboob Alam *et al.*'s study conducted in 2021, reported that ZnONPs degrade methylene blue depending on time by decreasing the intensity of light absorption [31]. In this way, it reported that ZnONPs combine with sunlight to break down pollutants and have a purifying effect.

In this study, we evaluated the hemolytic activity of honey-based ZnONPs by spectrophotometrically measuring oxyhemoglobin released from erythrocytes. Khentii and Selenge honey-based ZnONPs were found to have less than 10% hemolytic activity. A study of the biological activity of ZnONPs by Rajapriya M (2019) found 0.5% hemolysis or hemolytic activity at a dose of 100 ng/mL [32]. Biomaterials should be hemolytically inactive. Researchers suggested that up to 5% hemolytic active for biomaterials can be allowed as safe for use in medicine, including treatment [33, 34].

Advances in the field of nanotechnology have created many advantages and achievements in medicine in recent years. One of its representatives is ZnONPs, which are used in basic research for the treatment of many diseases such as cancer, infectious diseases, diabetes, and allergies. Our research is a fundamental study of the technology of synthesizing honey-based ZnONPs in Mongolia. In the future, it is possible to continue this research by investigating using electron microscopy, making additional confirmation by X-ray diffraction analysis, and determining the activity against tumor cells, antioxidant activity, and antifungal activity.

CONCLUSIONS

The newly synthesized Khentii and Selenge province honey-based ZnONPs were 16.02 nm and 95.23 nm in size, and UV spectrum at 310 nm had the highest absorption value. Based on zinc oxide stretching oscillations in the infrared spectrum region of 466 cm^{-1} and the additional high values of honey caused by the amide II vibration at 1535 cm^{-1} , it is confirmed

that honey-based ZnONPs were successfully synthesized. Moreover, it has been determined that newly synthesized honey-based ZnONPs were non-hemolytic, active against bacteria such as *S. aureus*, *E. coli*, and *K. pneumoniae*, as well as active in affecting photocatalytic and DNA concentrations.

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AUTHOR CONTRIBUTION

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REFERENCES

1. Dhandapani K.V., Anbumani D., Gandhi A.D., Annamalai P., Muthuvenkatachalam B.S., *et al.* (2020) Green route for the synthesis of zinc oxide nanoparticles from *Melia azedarach* leaf extract and evaluation of their antioxidant and antibacterial activities. *Biocatalysis and Agricultural Biotechnology*, **24**, 101517. <https://doi.org/10.1016/j.bcab.2020.101517>
2. Siddiqi K.S., ur Rahman A., Tajuddin N., Husen A. (2018) Properties of zinc oxide nanoparticles and their activity against microbes. *Nanoscale Res. Lett.*, **13**, 1-13. <https://doi.org/10.1186/s11671-018-2532-3>
3. Heideman G., Noordermeer J.W., Datta R.N., van Baarle B., *et al.* (2006) Various ways to reduce zinc oxide levels in S-SBR rubber compounds. *Macromol. Symp.*, **245-246**(1), 657-667. <https://doi.org/10.1002/masy.200651393>
4. Husen A. (2017) Gold nanoparticles from plant system: synthesis, characterization and their application. *Nanoscience and plant-soil systems*, **17**, 455-79. https://doi.org/10.1007/978-3-319-46835-8_17
5. Deka B., Baruah C., Babu A., Kalita P. (2022) Biological and non-conventional synthesis of zinc oxide nanoparticles (ZnO-NPs): Their potential applications. *J. Nanotech. Nanomat.*, **3**(2), 79-89. <https://doi.org/10.33696/Nanotechnol.3.034>
6. Frederickson C.J., Koh J.Y., Bush A.I. (2005) The neurobiology of zinc in health and disease. *Nat. Rev. Neurosci.*, **6**(6), 449-62. <https://doi.org/10.1038/nrn1671>
7. Sharmila M., Jothi Mani R., Kader A., Ahmad A., Eldesoky G.E., *et al.* (2021) Photocatalytic and biological activity of ZnO nanoparticles using honey. *Coatings*, **11**(9), 1046. <https://doi.org/10.3390/coatings11091046>
8. Hoseini S.J., Darroudi M., Oskuee R.K., Gholami L., Zak A.K. (2015) Honey-based synthesis of ZnO nanopowders and their cytotoxicity effects. *Adv. Powder Tech.*, **26**(3), 991-6.

- <https://doi.org/10.1016/j.appt.2015.04.003>
9. Porcza L.M., Simms C., Chopra M. (2016) Honey and cancer: current status and future directions. *Diseases*, **4**(4), 30. <https://doi.org/10.3390/diseases4040030>
 10. Sun L.P., Shi F.F., Zhang W.W., Zhang Z.H., Wang K. (2020) Antioxidant and anti-inflammatory activities of safflower (*Carthamus tinctorius* L.) honey extract. *Foods*, **9**(8), 1039. <https://doi.org/10.3390/foods9081039>
 11. Tsevegmid K., Dooshin S., Ramsey S., Chantawannakul P. (2018) Beekeeping in Mongolia. In: Chantawannakul, P., Williams, G., Neumann, P. (eds) *Asian Beekeeping in the 21st Century*. Springer, Singapore, 199-221. https://doi.org/10.1007/978-981-10-8222-1_9
 12. Kim Y.S. (2018) Introduction to beekeeping and melliferous plants in Mongolia. *Journal of Apiculture*, **33**(1), 55-62. <https://doi.org/10.17519/apiculture.2018.04.33.1.55>
 13. Mollaeva M.R., Yabbarov N., Sokol M., Chirkina M., Mollaev M.D., et al. (2021) Optimization, characterization and pharmacokinetic study of meso-tetraphenylporphyrin metal complex-loaded PLGA nanoparticles. *Int. J. Mol. Sci.*, **22**(22), 12261. <https://doi.org/10.3390/ijms222212261>
 14. Ankamwar B.G., Kamble V.B., Annsi J.I., Sarma L.S., Mahajan C.M. (2017) Solar photocatalytic degradation of methylene blue by zno nanoparticles. *J. Nanosci. Nanotech.*, **17**(2), 1185-1192. <https://doi.org/10.1166/jnn.2017.12579>
 15. Jianrong C., Yuqing M., Nongyue H., Xiaohua W., Sijiao L. (2004) Nanotechnology and biosensors. *Biotechnology Advances*, **22**(7), 505-518. <https://doi.org/10.1016/j.biotechadv.2004.03.004>
 16. Hsu J.F., Chu S.M., Liao C.C., Wang C.J., Wang Y.S., et al. (2021) Nanotechnology and nanocarrier-based drug delivery as the potential therapeutic strategy for glioblastoma multiforme: An update. *Cancers*, **13**(2), 195. <https://doi.org/10.3390/cancers13020195>
 17. Jain A.S., Pawar P.S., Sarkar A., Junnuthula V., Dyawanapelly S. (2021) Bionanofactories for green synthesis of silver nanoparticles: Toward antimicrobial applications. *Int. J. Mol. Sci.*, **22**(21), <https://doi.org/10.3390/ijms222111993>
 18. <http://nature.khe.gov.mn>. Available from: <http://nature.khe.gov.mn>.
 19. Santhoshkumar J., Kumar S.V., Rajeshkumar S. (2017) Synthesis of zinc oxide nanoparticles using plant leaf extract against urinary tract infection pathogen. *Resource-Efficient Technologies*, **3**(4), 459-465. <https://doi.org/10.1016/j.refit.2017.05.001>
 20. Sohail M.F., Rehman M., Hussain S.Z., Huma Z.E., Shahnaz G., et al. (2020) Green synthesis of zinc oxide nanoparticles by Neem extract as multi-facet therapeutic agents. *Journal of Drug Delivery Science and Technology*, **59**, 101911. <https://doi.org/10.1016/j.jddst.2020.101911>
 21. Bahari N., Hashim N., Abdan K., Md Akim A., Maringgal B., et al. (2023) Role of honey as a bifunctional reducing and capping/stabilizing agent: Application for silver and zinc oxide nanoparticles. *Nanomaterials (Basel, Switzerland)*, **13**(7), 1244. <https://doi.org/10.3390/nano13071244>
 22. Balasooriya E.R., Jayasinghe C.D., Jayawardena U.A., Ruwanthika R.W.D., Mendis de Silva R., et al. (2017) Honey mediated green synthesis of nanoparticles: New era of safe nanotechnology. *Journal of Nanomaterials*, **2017**, 5919836. <https://doi.org/10.1155/2017/5919836>
 23. Ifeanyichukwu U.L., Fayemi O.E., Ateba C.N. (2020) Green synthesis of zinc oxide nanoparticles from pomegranate (*Punica granatum*) extracts and characterization of their antibacterial activity. *Molecules (Basel, Switzerland)*, **25**(19), 5919836. <https://doi.org/10.3390/molecules25194521>
 24. Sarkhosh S., Kahrizi D., Darvishi E., Tourang M., Haghighi-Mood S., et al. (2022) Effect of zinc oxide nanoparticles (ZnO-NPs) on seed germination characteristics in two brassicaceae family species: *Camelina sativa* and *Brassica napus* L. *Journal of Nanomaterials*, **2022**, 1892759. <https://doi.org/10.1155/2022/1892759>
 25. Vijayakumar N., Bhuvaneshwari V.K., Ayyadurai G.K., Jayaprakash R., Gopinath K., et al. (2022) Green synthesis of zinc oxide nanoparticles using *Anoectochilus elatus*, and their biomedical applications. *Saudi Journal of Biological Sciences*, **29**(4), 2270-9. <https://doi.org/10.1016/j.sjbs.2021.11.065>
 26. Sirelkhatim A., Mahmud S., Seeni A., Kaus N.H.M., Ann L.C., et al. (2015) Review on zinc oxide nanoparticles: Antibacterial activity and toxicity mechanism. *Nano-micro Letters*, **7**(3), 219-42. <https://doi.org/10.1007/s40820-015-0040-x>
 27. Emami-Karvani Z., Chehrazi P. (2011) Antibacterial activity of ZnO nanoparticle on gram-positive and gram-negative bacteria. *Afr. J. Microbiol. Res.*, **5**(12), 1368-73. <https://doi.org/10.5897/AJMR10.159>
 28. Behravan M., Hossein Panahi A., Ghizadeh A., Ziaee M., Mahdavi R., et al. (2018) Facile green synthesis of silver nanoparticles using *Berberis vulgaris* leaf and root aqueous extract and its antibacterial activity. *Int. J. Biol. Macromol.*, **124**, 148-54. <https://doi.org/10.1016/j.ijbiomac.2018.11.101>
 29. Singh R., Cheng S., Singh S. (2020) Oxidative stress-mediated genotoxic effect of zinc oxide nanoparticles on *Deinococcus radiodurans*. *Biotech.*, **10**(2), 66. <https://doi.org/10.1007/s13205-020-2054-4>

30. Tahir M.B., Sohaib M., Sagir M., Rafique M. (2022) Role of Nanotechnology in Photocatalysis: *Encyclopedia of Smart Materials*, 578-89. <https://doi.org/10.1016/B978-0-12-815732-9.00006-1>
31. Alam M. (2021) Photocatalytic activity of biogenic zinc oxide nanoparticles: *In vitro* antimicrobial, biocompatibility, and molecular docking studies. *Nanotechnology Reviews*, **10**(1), 1079-91. <https://doi.org/10.1515/ntrev-2021-0069>
32. Rajapriya M., Sharmili S.A., Baskar R., Balaji R., Alharbi N.S, et al. (2020) Correction to: Synthesis and characterization of zinc oxide nanoparticles using *Cynara scolymus* leaves: Enhanced hemolytic, antimicrobial, antiproliferative, and photocatalytic activity. *Journal of Cluster Science*, **31**, 791-801. <https://doi.org/10.1007/s10876-019-01686-6>
33. Das D, Nath BC, Phukon P, Kalita A, Dolui SK. (2013) Synthesis of ZnO nanoparticles and evaluation of antioxidant and cytotoxic activity. *Colloids and surfaces B, Biointerfaces*, **111**, 556-60. <https://doi.org/10.1016/j.colsurfb.2013.06.041>
34. Vinotha V., Iswarya A., Thaya R., Govindarajan M., Alharbi N.S, et al. (2019) Synthesis of ZnO nanoparticles using insulin-rich leaf extract: Anti-diabetic, antibiofilm and anti-oxidant properties. *Journal of photochemistry and photobiology B, Biology*, **197**, 111541. <https://doi.org/10.1016/j.jphotobiol.2019.111541>