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Research Article

Antibacterial and Cytotoxic Activity of Silver and Zinc Oxide Nanoparticles Synthesized Using Deep Eutectic Solvent Extraction of the Sea Cucumber-*Holothuria tubulosa*

Hilal TOP HAYTAOGLU[1], Aysegul INAM[2,3], Tulay ONCU ONER[3], Murat ELIBOL[2*]

[1]Biotechnology Program, Graduate School of Natural and Applied Sciences, Ege University, Izmir, Türkiye
[2]Department of Bioengineering, Faculty of Engineering, Ege University, Izmir, Türkiye
[3]Department of Bioengineering, Faculty of Engineering and Natural Sciences, Manisa Celal Bayar University, Manisa, Türkiye

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Abstract

In this study, we synthesized these nanoparticles (NPs) utilizing a novel approach involving deep eutectic solvent (DES) extraction of the sea cucumber-*Holothuria tubulosa*. Choline chloride-urea (1:2) was used as a DES, facilitating the green synthesis without hazardous chemicals. Spectrophotometric methods were used to determine total phenolic and carbohydrate content of the extract. UV–Vis, DLS and FTIR characterized NPs. The absorption peaks in 400 nm for AgNPs and 378 nm for ZnO-NPs were observed on the UV-Vis. DLS prediction of AgNPs size at 202.2 nm and ZnO-NPs size at 269.9 nm. The cytotoxic activities of the synthesized NPs and DES extract of *H. tubulosa* were evaluated against SH-SY5Y neuroblastoma and BJ normal skin fibroblast cell lines using MTT assay. Cytotoxic effects were observed for all samples in SH-SY5Y cells. However, the IC_{50} value could not be calculated for BJ cells because it was outside the range of the highest concentration tested. Moreover, the antibacterial activities of NPs and DES extract of *H. tubulosa* were investigated against *Escherichia coli* and *Staphylococcus aureus*. The AgNPs exhibited significant inhibitory effects against both tested bacterial strains, demonstrating their antibacterial activity.

Keywords: Deep eutectic solvents, sea cucumber, nanoparticles, antibacterial, cytotoxicity

Deniz Hıyarı-*Holothuria tubulosa*'nın Derin Ötektik Çözücü Ekstraksiyonu Kullanılarak Sentezlenen Gümüş ve Çinko Oksit Nanopartiküllerinin Antibakteriyel ve Sitotoksik Aktivitesi

Öz

Bu çalışmada, deniz hıyarı-*Holothuria tubulosa*'nın derin ötektik çözücü (DES) ekstraksiyonunu içeren yeni bir yaklaşım kullanılarak nanopartiküller (NP'ler) sentezlenmiştir. DES olarak kolin klorür-üre (1:2) kullanılmış ve bu da tehlikeli kimyasallar olmadan yeşil sentezi kolaylaştırmıştır. Ekstraktın toplam fenolik ve karbonhidrat içeriğini belirlemek için spektrofotometrik yöntemler kullanılmıştır. UV-Vis, DLS ve FTIR NP'leri karakterize etmiştir. UV-Vis'te AgNP için 400 nm'de ve ZnO-NP için 378 nm'de absorpsiyon piki gözlenmiştir. DLS, AgNP boyutunu 202.2 nm ve ZnO-NP boyutunu 269.9 nm olarak tespit etmiştir. Sentezlenen NP'lerin ve *H. tubulosa* DES ekstraktının sitotoksik aktiviteleri SH-SY5Y nöroblastoma hücre hattı ve BJ normal deri fibroblast hücre hattı üzerinde MTT testi kullanılarak değerlendirilmiştir. SH-SY5Y hücrelerinde tüm örnekler için sitotoksik etkiler gözlenmiştir. Ancak, BJ hücrelerinde IC_{50} değeri test edilen en yüksek konsantrasyon aralığının dışında kaldığı için hesaplanamamıştır. Ayrıca, NP'lerin ve *H. tubulosa* DES ekstraktının antibakteriyel aktiviteleri *Escherichia coli* ve *Staphylococcus aureus*'a karşı araştırılmıştır. AgNP'ler test edilen her iki bakteri türüne karşı önemli inhibitör etkiler göstererek antibakteriyel aktivitelerini ortaya koymuştur.

Anahtar Kelimeler: derin ötektik çözücüler, deniz hıyarı, nanopartiküller, antibakteriyel, sitotoksisite

Corresponding Author: murat.elibol@ege.edu.tr

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1. Introduction

Sea cucumbers, also known as Holothurians, are slow-moving marine animals and the most species-rich group of echinoderms. Holothurians are environment-engineers since they help recycle nutrients and stimulate microalgal growth to develop primary productivity. There are more than 1400 species of sea cucumber all around the world, and more than 40 are edible. *Holothuria tubulosa* is a sea cucumber species that is among the most harvested and economically valuable echinoderms found in the Turkish Seas. It is an edible species with a high content of bioactive compounds [1].

Holothurians are of enormous importance because of their high nutritional value in East Asian countries. Although sea cucumbers' nutritional value and chemical composition often vary depending on the growing environment, most species contain a high protein, low-fat content, and no cholesterol [2]. They have attracted significant attention, especially in the pharmaceutical field, due to their high content of secondary metabolites, including triterpene glycosides (saponins), sulfated polysaccharides, sterols, peptides, phenolics, and lectins. Several studies have demonstrated the biological potential of sea cucumbers to exhibit cytotoxic, antiviral, anti-inflammatory, antimicrobial, and antioxidant activities [3].

The most common methodologies for the preparation of active ingredients are extraction, using a mixture of ethanol, methanol, and chloroform as the solvent. However, the usage of these conventional organic solvents has been identified as a significant threat to human health and the ecological environment. Deep eutectic solvents (DESs) represent a novel development in the field of green chemistry, with considerable attention being directed towards their use in a wide range of applications. To date, researchers have investigated the development of innovative DESs with the objective of improving the extraction and separation process [4].

DESs are sustainable solvents derived from mixtures of naturally occurring molecules. Primary metabolites, including organic acids, sugars, amino acids, and choline derivatives, can be utilized in the DES compounds. In this instance, DES is designated as a natural deep eutectic solvent (NADES). The DES combinations utilized in this study belong to the NADES class. DES or NADES is a eutectic mixture that is formed by mixing a hydrogen bonding acceptor (HBA) with a hydrogen bonding donor (HBD). This eutectic mixture has a much lower melting point than the original HBA and HBD. Despite having equivalent properties to ionic liquids, DESs are much cheaper, safer, and biodegradable. Choline chloride (ChCl) is one of the most well-known HBAs for the synthesis of DES [5]. Nevertheless, it is water-soluble and only allows for hydrophilic extraction. In recent years, research on sea cucumbers has been divided into two main categories: the cultivation of sea cucumbers and the exploration of their biological active ingredients. Like many other marine organisms, conventional techniques and organic solvents have been used for the extraction of sea cucumbers. This study is important because it is the first time the extraction of *H. tubulosa* by DES systems has been achieved, and the synthesis of nanoparticles. In addition, a one-step sample preparation method is presented for the preparation of extracts and optimization using DES combinations. The extraction efficiency of the optimal DES-based ultrasound-assisted method is compared with traditional methods to ascertain its efficacy. Also, in the study of *H. tubulosa*, nanoparticle synthesis plays

a pivotal role in exploring the potential biomedical applications of this species. The synthesized nanoparticles are characterized using techniques such as Ultraviolet–Visible spectroscopy (UV–Vis), dynamic light scattering (DLS), and Fourier transform infrared spectroscopy (FTIR). These methods help determine the size, shape, and functional groups of the nanoparticles. The research indicates that silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnO-NPs) synthesized using deep eutectic solvent (DES) extraction from *H. tubulosa* have shown significant antibacterial properties and potential cytotoxicity against the cancer cells.

2. Materials and Methods

2.1. Chemicals

Sodium carbonate (Na_2CO_3), phenol and sulphuric acid (>37 %) were obtained from Merck (Darmstadt, Germany). Choline chloride (ChCl) (>98 %), urea (>99 %), Folin-Ciocalteu reagent, gallic acid and glucose were purchased from Sigma (St. Louis, MO, USA). $AgNO_3$ was obtained from Carlo Erba Reagents and $ZnSO_4.7H_2O$ was obtained from AFG Bioscience.

In cytotoxicity assay, DMEM medium (Gibco), FBS (Gibco), 1% penicillin/streptomycin (Gibco), 1% L-glutamine (Gibco), and 1% non-essential amino acid (Gibco), trypsin-EDTA (Gibco), MTT reagent (Sigma), PBS (Gibco), and DMSO (Merck) were used.

In the antibacterial activity test, Mueller Hinton Broth and Agar growth medium (Merck), and antimicrobial susceptibility disk (Oxoid) were used.

2.2. Sample collection and preparation

H. tubulosa is a common species of sea cucumber, particularly prevalent along the Mediterranean and Aegean Sea coasts. The specimens were collected using the bottom trawl fishing technique in Ildir Gulf, situated on the Cesme coast of Izmir, Turkey. The coordinates were 38°39'61.10″ N, 26°46'96.17″ E. Following the morphological identification of the species, the length and weight measurements of the individuals were taken according to the literature. The exact length and weight measurements are also provided in Figure 1. After freezing at -20°C, the Holothurian samples were dried in the sun for three days. Twenty-one grams of dried *H. tubulosa* body walls were cut into small pieces and stored in the dark at a temperature of +4°C until processing.

Figure 1. Preparation of sea cucumber samples

2.3. Deep eutectic solvent synthesis

The DESs were prepared by heating a two-component mixture to 80°C with constant stirring until a homogeneous liquid formed. In this study, the prepared DESs included choline chloride and urea in a 1:2 molar ratio. DES was diluted with water to 52.5% to control viscosity before extraction with sea cucumber.

2.4. Ultrasonication-assisted extraction

An ultrasonic bath system was employed for the ultrasonication-assisted extraction (Jeiotech UC-10), which was equipped with a digital control system for temperature, time, and power (ultrasonic power effective is 380W). Extraction conditions were 40°C, 35 mL/g in 100 minutes. All extractions were conducted at a constant frequency of 37 kHz. Subsequently, the extracts were centrifuged at 5000 × g for 10 minutes, and the supernatants were filtered and subjected to further analysis. The ultrasonic extraction scheme is shown in Figure 2.

Figure 2. Ultrasonic extraction of sea cucumber samples with DES

2.5. Analysis of biometabolites in extracts

Spectrophotometric methods were used to determine the total phenolic and carbohydrate content of the extract.

2.5.1. Total carbohydrates

Total carbohydrates in the extracts were measured by the Dubois method. Glucose was prepared as a standard solution to determine the sugar content of the samples. The first step was to take 0.5 mL of the extract and transfer it to the glass tube. 0.5 mL of each DES was prepared in glass tubes for the blank. 0.5 mL of 5% phenol solution was added to the prepared tubes. Then 2.5 mL of concentrated sulphuric acid was added to the glass tubes, stoppered and vortexed. After 15 minutes in a water bath, the absorbance against the blank was recorded in the spectrophotometer at a wavelength of 490 nm [6].

2.5.2. Total phenolics

The total phenolic content of the extracts was measured using the Folin-Ciocalteu method. The standard used was gallic acid. In a 100 mL erlenmeyer flask, 1 mL of the extracts was first added. Then 22 mL of distilled water and 500 µL of Folin-Ciocalteu reagent were added successively to the extracts. After 3 minutes, 1.5 mL of 3% Na_2CO_3 solution was added. The samples were incubated for a period of 2 hours at room temperature. Absorbance values were read against distilled water at 720 nm using the Optizen Pop UV/Vis spectrophotometer. DES were used instead of the control sample. The absorbance values of the total phenolic content of the sample against the standard curve were used in the equation and the results were expressed in gallic acid equivalents [7, 8].

2.6. Green synthesis of silver and zinc oxide nanoparticles

According to the results of preliminary experiments, 10 mL of extract was added dropwise to 90 ml (1:9) of 2.818 mM $AgNO_3$ solution for the AgNP synthesis. 30 mL of extract was added to 90 ml (1:3) of 4.113 mM $ZnSO_4.7H_2O$ solution for the ZnO-NP synthesis. The solutions were incubated for 2 hours in the dark. Visible color changes confirmed that the solution contained NPs. Due to the secondary metabolites in the sea cucumber, no external chemicals were required to stabilize the NPs produced by this process.

2.7. Characterization of nanoparticles

Ultraviolet–Visible spectroscopy (UV–Vis), dynamic light scattering (DLS) and Fourier transform infrared spectroscopy (FTIR) characterized AgNPs and ZnO-NPs. UV-Vis spectroscopy was used to confirm the formation of NPs. NP optical absorbance was recorded using a UV-Vis spectrophotometer (EasyPlus, Mettler Toledo) at 200-800 nm. The maximum peak absorbance values of NPs were recorded. DLS (Malvern Zetasizer Analyzer) was used to measure the average size of the NPs. The size distribution of the particles in the colloidal suspension was also characterized. The structural characteristics of the NPs were investigated using FTIR spectroscopy (Thermo Scientific, Nicolet, IS20, USA) in the wave number range of 500 cm^{-1} to 4000 cm^{-1}. Surface chemistry and functional group bonding are characterized using this method.

2.8. Biological activities of nanoparticles

2.8.1. Antibacterial analysis

For antibacterial analysis of the extract and nanoparticles, the Kirby-Bauer disk diffusion method was applied. *Staphylococcus aureus* and *Escherichia coli* bacteria were cultured. The turbidity of the bacteria was diluted to correspond to Mc Farland No.1, and then inoculated onto the Mueller Hinton Agar by spreading plate method as 1×10^6 CFU/mL. After inoculation, the samples were impregnated with sterile disks in a volume of 25 µL. The petri dishes were then incubated at 37°C for 24 hours. Then, the zone diameters around the disks were measured according to triple trials [9].

2.8.2. *In vitro* cytotoxicity analysis

The cytotoxic activities of the extract and nanoparticles were evaluated by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay. SH-SY5Y human neuroblastoma cell line and healthy BJ normal skin fibroblast cell line were used herein. The cells were cultured in DMEM medium supplemented with 10% FBS (fetal bovine serum), 1% penicillin/streptomycin, 1% L-glutamine, and 1% non-essential amino acid. Cells seeded in T75 flasks were incubated in a 37ºC incubator containing 5% CO_2. ~80 confluent cells were detached from the flask surface using trypsin-EDTA, counted using trypan blue and seeded in 96-well microplates at a concentration of 10^5 cells/ml. After 24 hours of incubation, the medium in 96-well microplates was removed and the cells were treated with different concentrations of extract, silver nanoparticles and zinc oxide nanoparticles. Then, the cells were incubated for 24

hours. At the end of the incubation period, 10 µL of 5 mg/mL MTT/PBS reagent was added to each well and incubated again for 3.5 hours. Afterwards, the culture medium was removed, and formazan crystals were dissolved using 100 µL of DMSO. Absorbance was measured at 570 nm wavelength using a microplate reader. Data were evaluated via the GraphPad Prism program [10].

3. Results and Discussion

3.1. Extraction of biometabolites

The synthesis of nanoparticles is possible with compounds such as phenols, amines, amides, carbohydrates and alkaloids, etc. The reduction of silver ions may be due to phenolic compounds and carbohydrates that may be present in sea cucumbers. For this reason, the total sugars and the total phenolics of the extract were determined by means of spectrophotometric methods. The total sugars and total phenolics were measured as 260 µg/mL and 664 mg GAE/mL, respectively (Table 1). To date, no specific research has been conducted on the metanol extraction of total glucose and the phenolic compounds in *H. tubulosa*. However in Wang, J. and their friends research, sea cucumber (Stichopus japonicus Selenka) gonads chloroform:methanol extraction total sugar was founded 69.1 g/100 g [11].

Table 1. Biometabolites in DES extract

Solvent	Glucose Concentration (µg/ml)	Phenol Concentration (mg/ml)
DES	260,00	664
Methanol	199,32	140

3.2. Characterization of silver nanoparticles

The synthesis of silver nanoparticles (AgNPs) using sea cucumber extract is indicated by a change in solution color from orange to dark brownish black upon the addition of $AgNO_3$ solution, which signifies the formation of the nanoparticles. The analysis conducted using a UV-Vis spectrophotometer corroborates this hypothesis, exhibiting a peak absorption at 400 nm, which is characteristic of AgNPs as shown in Figure 3. This absorption peak can be attributed to the excitation of electrons on the metal surface, which is influenced by the nanoparticles' shape and size.

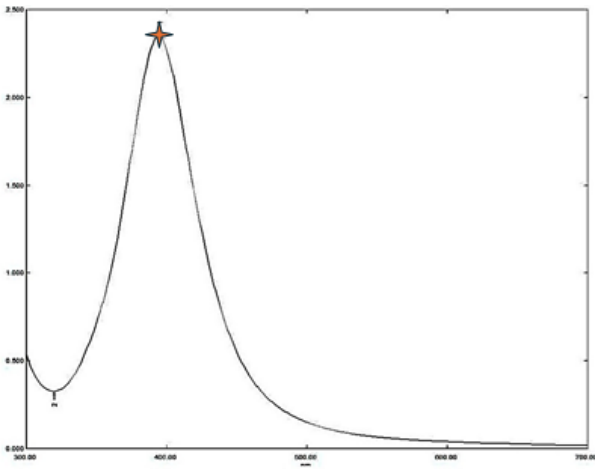

Figure 3. UV-Vis spectrophotometer of AgNPs

The study which is conducted by Srikar et al. (2016) also supports the assertion that AgNPs typically form within the 400-450 nm wavelength range [12].

The objective of the FTIR analysis in this study is to ascertain the interaction between the functional groups extracted and AgNPs. The results of the FTIR analysis of sea cucumber extract nanoparticles (AgNPs) are presented in Figure 4. As can be observed in Figure 4, the spectrum of the sea cucumber extract-AgNPs displays distinct absorption signals at wave numbers 3387 cm^{-1}, 2997 cm^{-1}, and 1649 cm^{-1} [13]. This indicates the interaction of –OH groups, -CH stretching, and C=C aromatic stretching at polyphenolic compounds stemming from the extract. Those functional group's peaks suggest that there has been an interaction between the bioactive compounds in the extract and the silver solution, resulting in the formation of AgNPs.

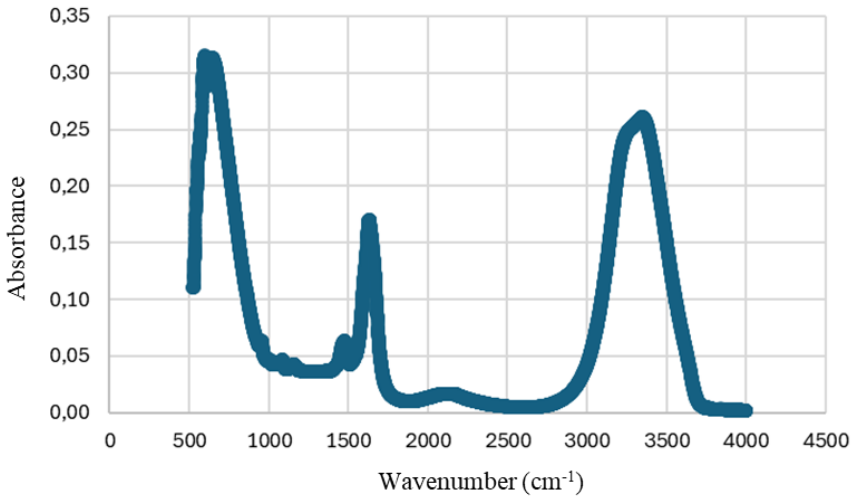

Figure 4. FTIR spectrum of AgNPs

Size distribution and polydispersity index of the nanoparticles (NPs) were analyzed by using the Zetasizer and results are shown in Figure 5. This represents the average size of AgNPs is 202.2 nm, while the polydispersity index (PDI) is 0.246. The PDI which describes the

distribution of particle size was found less than 0.5 for all nanoparticles. When the number of polydispersity index is smaller, the size of the particles is more uniform. Therefore, the polydispersity index affects the particles' characteristics, and it is expected to be less than 0.5 [14]. Previous studies have shown that nanoparticles with sizes less than 300 nm have a good ability to transport the body [15].

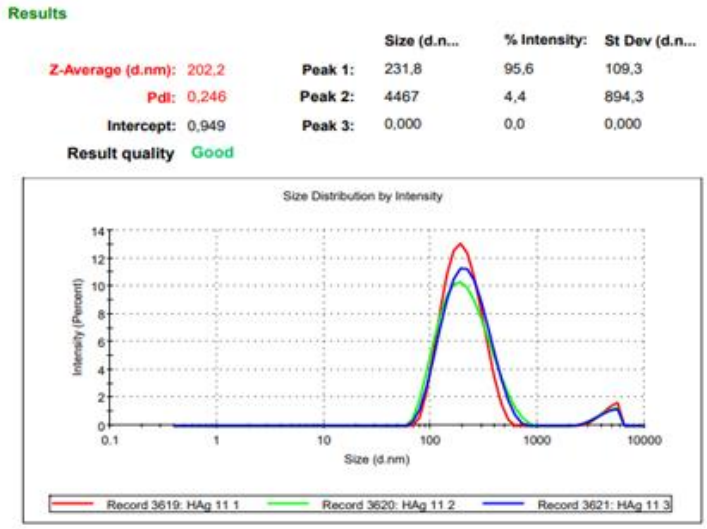

Figure 5. DLS results of AgNPs

3.3. Characterization of zinc nanoparticles

UV-Vis spectroscopy analysis was conducted on a colloidal solution containing ZnO-NPs, with measurements taken within the wavelength range of 200–800 nm. This was done to confirm the reduction of Zn^{2+} to ZnO-NPs. The UV-Vis spectra of the ZnO-NPs sample exhibited a prominent peak at 350-380 nm, which is a characteristic feature of zinc oxide nanoparticles [16,17]. As seen in Figure 6, the specific UV spectrum peak of ZnO-NPs was obtained at 378 nm in our study.

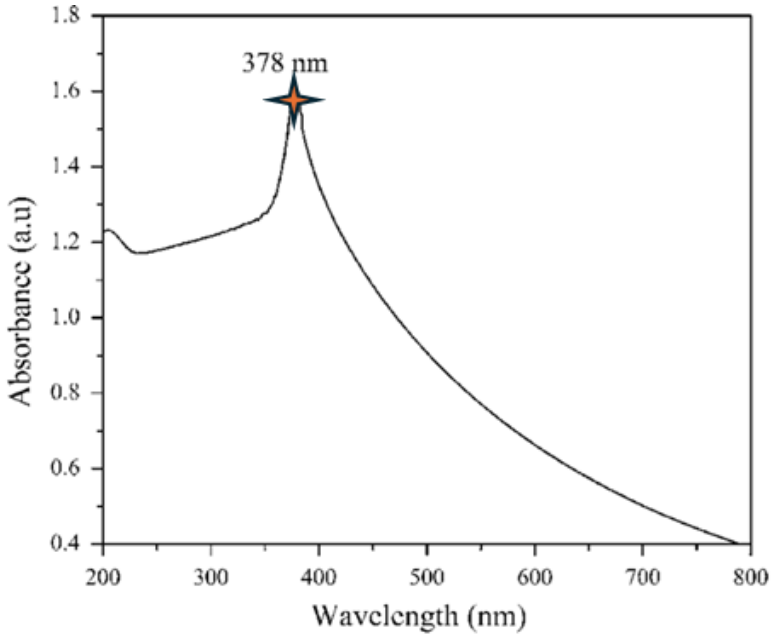

Figure 6. UV-Vis spectrophotometer of ZnO-NPs

As shown in Figure 7, the distinctive absorption band at 590 cm^{-1} (ZnO bond) substantiates the formation of ZnO. The sharp absorption peak at 1610 cm^{-1} is attributed to the amide's carbonyl (C=O) stretching vibration. The band at 1450 cm^{-1} may be attributed to the stretching of C–C groups, while the broad peak at 3450 cm^{-1} corresponds to the vibration of the O–H stretching group in alcohols and phenols. The formation of ZnO was confirmed by the characteristic absorption band at 580 cm^{-1} (ZnO bond) [18,19]. FTIR analysis confirmed the role of sea cucumber DES extract as a reducing and capping agent and the presence of some functional groups.

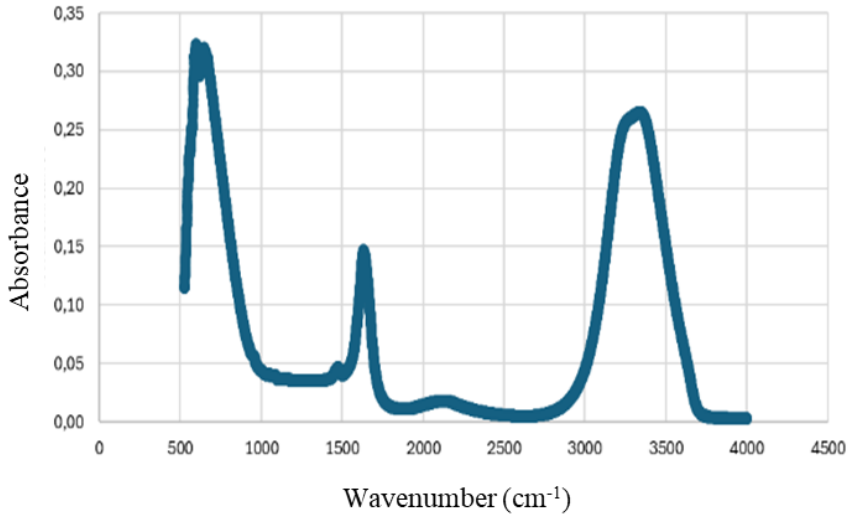

Figure 7. FTIR spectrum of ZnO-NPs

Dynamic Light Scattering (DLS) is an emerging and extensively utilized technique for the calculation of the hydrodynamic diameter of nanoparticle suspensions based on the Brownian

motion of the particles. Jamdagni et al. found that the DLS value was 74.36 nm of ZnO-NP from the flower extract of *Nyctanthes arbor-tristis,* while the PDI value was 0.488. The value in our study was found to be higher compared to the literature; DLS: 269.9 nm, PDI: 0.711 (Figure 8). The polydisperse nature of nanoparticles may explain the variation in nanoparticle size. The degree of "non-uniformity" of a distribution is called polydispersity, so this is an indication of the agglomeration of nanoparticles [20]. These results should be confirmed with the size range of nanoparticles obtained upon synthesis at optimum conditions by using Transmission electron Microscopy (TEM) or Scanning Electron Microscope (SEM) analysis in future research.

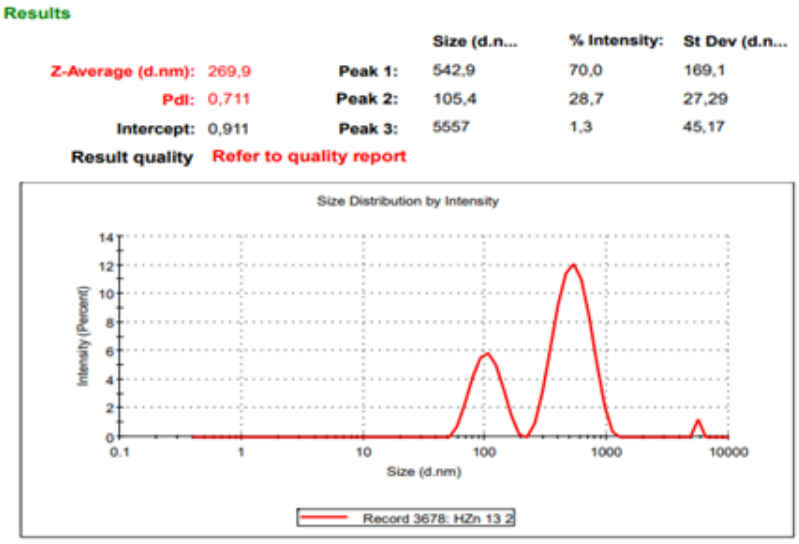

Figure 8. DLS results of ZnO-NPs

3.4. Biological activities of nanoparticles

3.4.1. Antibacterial activity test

Based on an antimicrobial effect analysis, the antibacterial activity of DES extract and synthesized nanoparticles against Gram-positive *S. aureus* and Gram-negative *E. coli* has been investigated according to triple trials. Zones formed around the disks are observed in Figure 9.

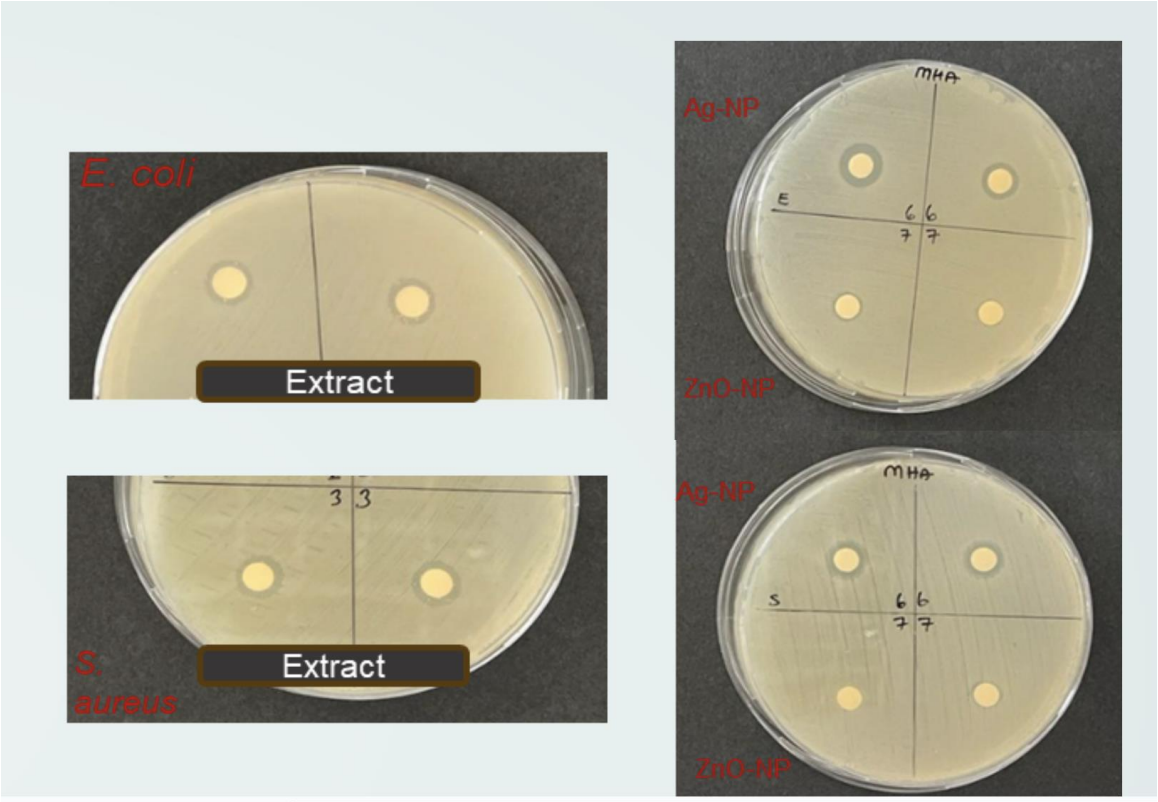

Figure 9. Results of disk diffusion test based on the extract, AgNPs and ZnO-NPs

According to the Kirby-Bauer test, the average inhibition diameters for *E. coli* can be ranked from small to large as ZnO-NPs (7±0.00 mm), extract (8.5±0.50 mm) and AgNPs (11±1.00 mm). On the other hand, the average diameters for *S. aureus* bacterium were measured as ZnO-NPs (7±0.00 mm), extract (10.33±0.57 mm) and AgNPs (11±0.00 mm) from small to large (Table 2). As with silver metal, the antibacterial effect of AgNPs was also detected here. In addition, it is very pleasing that the sea cucumber-DES extract itself also has an antibacterial effect. Similarly, Rosman et al., synthesized AgNPs using the aqueous extract of *Marphysa moribidii* (marine polychaete) and determined significant antibacterial activities against different bacteria [21]. Additionally, Willian et al. studied silver nanoparticles from marine plant as mangrove *Rhizophora stylosa* through green synthesis and found good activity against *E. coli* and *S. aureus* with 4.1-7.2 mm inhibition zones [22]. In contrast to the studies reported to have the remarkable antibacterial effect of zinc nanoparticles, the activity of ZnO-NPs in this concentration was found to be lower in this study. AgNPs are more potent than ZnO-NPs in terms of antibacterial activity, due to their higher rate of ion release capacity and interaction with the bacterial membrane [19]. Among the various physical and chemical factors, nanoparticle size was identified as the parameter that most influences the antimicrobial activity of ZnO-NPs. Generally, smaller particles are more toxic to microorganisms [22]. Therefore, it is important to synthesize AgNPs with the help of sea cucumbers and DES to enhance the antibacterial effect.

Table 2. The inhibition zone diameters of extract, AgNPs and ZnO-NPs

Inhibition zones (mm)	*E.coli -1*	*E.coli-2*	*E.coli-3*	*E. coli average*	*S.aureus-1*	*S.aureus-2*	*S.aureus-3*	*S. aureus average*
Extract	9	8	8.5	8.5±0.50	10	10	11	10.33±0.57
AgNPs	11	12	10	11±1.00	11	11	11	11±0.00
ZnO-NPs	7	7	7	7±0.00	7	7	7	7±0.00

3.4.2. *In vitro* cytotoxicity analysis

To date, different traditional solvents, including chloroform [23], water or methanol [24] were used for extraction from *H. tubulosa*. Herein the effect of DES, a more environmentally friendly solvent, was evaluated. Also, because of their potential for a wide range of biological and medicinal uses, biogenic nanoparticle synthesis made from plants, marine algae, vegetative organs, cells, and extracts has been the subject of numerous research recently [25].

Due to serious complications in the body, cancer remains one of the leading causes of mortality globally. Conventional treatments for cancer, such as chemotherapy, laser therapy, and surgery, which target tumor cells, can also destroy normal healthy cells in addition to having several negative side effects. These days, safe, inexpensive, and environmentally friendly biological sources provide an extensive range of reliable and potent anticancer drugs [25].

In this study, the cytotoxic effects of both DES extract of sea cucumber *H. tubulosa,* and nanoparticles (AgNPs, and ZnO-NPs) produced using DES extract on neuroblastoma cells and BJ normal skin fibroblast cells were tested. For the SH-SY5Y neuroblastoma cell line, the IC_{50} values of DES extract, AgNPs, and ZnO-NPs are 433.8±25.1 µg/mL, 361.8±3.2 µg/mL, and 405.8±5.7 µg/mL, respectively (Figure 10 and 11). According to these results, the cytotoxic effect of AgNPs on SH-SY5Y cells appears to be greater. This is followed by ZnO-NPs and DES extract. On the other hand, IC_{50} values could not be calculated for BJ healthy skin fibroblast cells. Because it was outside the range of the highest concentration tested. In future studies, higher concentrations should be tested in this cell line. Its cytotoxic effects on healthy cells are less than cancer cells. So, when we compare the two different cell lines, both extract and nanoparticles have more cytotoxic activity on the cancer cell line.

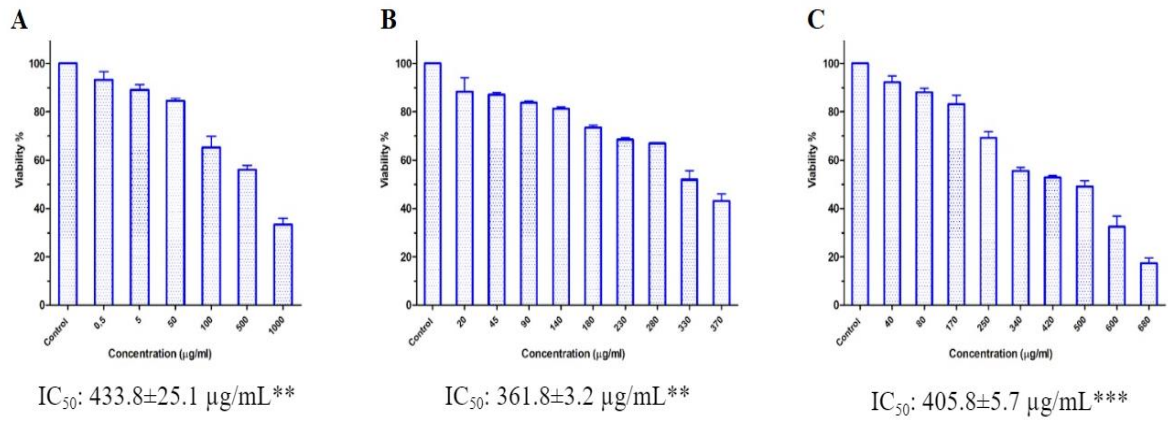

Figure 10. Cell viability values obtained as a result of MTT test in SH-SY5Y neuroblastoma cell line. **A**. DES extract of *H. tubulosa*, **B**. AgNPs, **C.** ZnO-NPs. (**p<0.01, ***p<0.001).

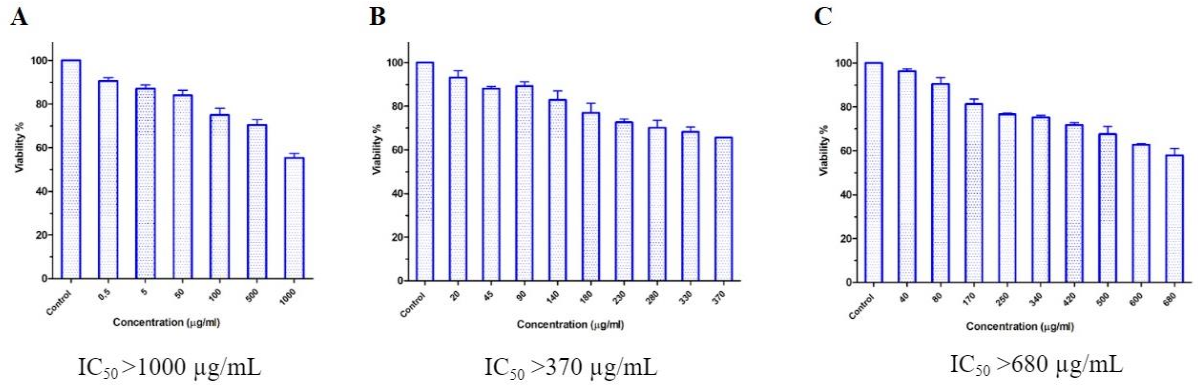

Figure 11. Cell viability values obtained as a result of MTT test in BJ skin fibroblast cell line. **A**. DES extract of *H. tubulosa*, **B**. AgNPs, **C.** ZnO-NPs.

In the study by Alper and Güneş (2020), MTT assays of water and methanol extracts of *H. tubulosa* on cancerous A549 (Human lung adenocarcinoma), HeLa (human cervix adenocarcinoma), PC-3 (human prostate adenocarcinoma), and MCF-7 (human breast adenocarcinoma), and healthy HEK-293 (human embryonic kidney) cell lines. The study determined that the lowest IC_{50} value of the water extract was in A549 cells (IC_{50}=112.8 µg/ml) for a 48-hour incubation period and in HeLa cells (IC_{50}=21.01 µg/ml) for a 72-hour incubation period. The IC_{50} value of the methanol extract was calculated as 83.73 µg/ml for HeLa cells at 48 hours and 63.12 µg/ml for A549 cells at 72 hours [24]. In another study, the cytotoxic activity of chloroform extracts of *H. tubulosa* was studied. Accordingly, IC_{50} values were found to be 709.7, 253.7, 151.6 and 132.2 at 24 hours in A549, HeLa, MCF-7 and PC-3 cell lines, respectively. This value was not determined in the HEK-293 cell line [23]. Production of NPs using extracts from natural substances is emerging as an important area in nanotechnology. The use of natural resources to produce NPs is sustainable, eco-friendly, inexpensive and free of chemical contaminants for biological and medical applications where the purity of NPs is of major concern. Furthermore, NPs synthesized via the green route are more stable and effective in comparison with those produced by physicochemical methods. The results obtained here can

be supported by further experiments. *In vivo* cytotoxic studies of *H. tubulosa* nanoparticles could be applied. Pure compounds can be isolated from *H. tubulosa* using DES instead of toxic solvents.

4. Conclusion

The innovative green synthesis approach using Deep Eutectic Solvent (DES) extraction from *Holothuria tubulosa* has demonstrated significant potential in the biomedical field. Our study successfully synthesized silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnO-NPs). The AgNPs exhibited notable antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, while both AgNPs and ZnO-NPs showed promising cytotoxic effects against the SH-SY5Y neuroblastoma cell line. These findings underscore the potential of sea cucumber-derived nanoparticles as effective agents for cancer therapy and antimicrobial applications, paving the way for further research and development in this exciting domain.

Ethics in Publishing

There are no ethical issues regarding the publication of this study. Ethical approval was not required for the study involving animals in accordance with local legislation and institutional requirements because the species used in the work (*Holothuria tubulosa*) is not included in any taxa listed in Directive 2010/63/EU that regulates the use of live animals for experimental purposes. Therefore, the experiments and animal sampling do not require authorizations.

Author Contributions

Conceptualization and design: Hilal Top Haytaoglu, Aysegul Inam, Tulay Oncu Oner, Murat Elibol; methodology and formal analysis: Hilal Top Haytaoglu, Aysegul Inam, Tulay Oncu Oner, Murat Elibol; validation: Hilal Top Haytaoglu, Aysegul Inam, Tulay Oncu Oner, Murat Elibol; investigation: Hilal Top Haytaoglu, Aysegul Inam, Tulay Oncu Oner, Murat Elibol; Writing – original draft: Hilal Top Haytaoglu, Aysegul Inam, Tulay Oncu Oner, Murat Elibol. The submitted manuscript was accepted and reviewed by all the authors.

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References

[1] Fagbohun, O. F., Joseph, J. S., Oriyomi, O. V., & Rupasinghe, H. V. (2023). Saponins of North Atlantic Sea Cucumber: Chemistry, health benefits, and future prospectives. Marine Drugs, 21(5), 262.

[2] Maskur, M., Sayuti, M., Widyasari, F., & Setiarto, R. H. B. (2024). Bioactive Compound and Functional Properties of Sea Cucumbers as Nutraceutical Products. Reviews in Agricultural Science, 12, 45-64.

[3] Sanjeewa, K. K., & Herath, K. H. I. N. M. (2023). Bioactive secondary metabolites in sea cucumbers and their potential to use in the functional food industry. Fisheries and Aquatic Sciences, 26(2), 69-86.

[4] Liu, J., Li, X., & Row, K. H. (2022). Development of deep eutectic solvents for sustainable chemistry. Journal of Molecular Liquids, 362, 119654.

[5] Liu, Y., Friesen, J. B., McAlpine, J. B., Lankin, D. C., Chen, S. N., & Pauli, G. F. (2018). Natural deep eutectic solvents: properties, applications, and perspectives. Journal of natural products, 81(3), 679-690.

[6] Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. Analytical chemistry, 28(3), 350-356.

[7] Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In Methods in enzymology, 299, 152.

[8] Tüzün, S., Baş, İ., Karakavuk, E., Sanyürek, N. K., & Benzer, F. (2020). Çeşitli pekmez türlerinde farklı yöntemlerle tespit edilen antioksidan aktivitelerin karşılaştırılması. Türk Tarım ve Doğa Bilimleri Dergisi, 7(2), 323-330.

[9] Matuschek, E., Copsey-Mawer, S., Petersson, S., Åhman, J., Morris, T. E., & Kahlmeter, G. (2023). The European committee on antimicrobial susceptibility testing disc diffusion susceptibility testing method for frequently isolated anaerobic bacteria. Clinical Microbiology and Infection, 29(6), 795-e1.

[10] Khanavi, M., Gheidarloo, R., Sadati, N., Ardekani, M. R. S., Nabavi, S. M. B., Tavajohi, S., Ostad, S. N. (2012). Cytotoxicity of fucosterol containing fraction of marine algae against breast and colon carcinoma cell line. Pharmacognosy Magazine, 8(29), 60.

[11] Wang, J., Shi, S., Li, F., Du, X., Kong, B., Wang, H., & Xia, X. (2022). Physicochemical properties and antioxidant activity of polysaccharides obtained from sea cucumber gonads via ultrasound-assisted enzymatic techniques,160, 113307.

[12] Srikar, S. K., Giri, D. D., Pal, D. B., Mishra, P. K., & Upadhyay, S. N. (2016). Green synthesis of silver nanoparticles: a review. Green and Sustainable Chemistry, 6(01), 34.

[13] Thanigaivel, S., Vickram, S., Saranya, V., Ali, H., Alarifi, S., Modigunta, J. K. R., Rohini, K. (2022). Seaweed polysaccharide mediated synthesis of silver nanoparticles and its enhanced disease resistance in Oreochromis mossambicus. Journal of King Saud University-Science, 34(2), 101771.

[14] Nurkhasanah, N., Yuwono, T., Nurani, L. H., Rizki, M. I., & Kraisintu, K. (2015). The development of chitosan nanoparticles from Hibiscus sabdariffa L calyx extract from Indonesia and Thailand, 6(5), 1855-1861.

[15] Sivasankar, M., & Kumar, B. P. (2010). Role of nanoparticles in drug delivery system. International Journal of Research in Pharmaceutical and Biomedical Sciences, 1(2), 41-66.

[16] Mohammadian, M., Es'haghi, Z., & Hooshmand, S. (2018). Green and chemical synthesis of zinc oxide nanoparticles and size evaluation by UV–vis spectroscopy. J Nanomed Res, 7(1), 00175.

[17] Fakhari, S., Jamzad, M., & Kabiri Fard, H. (2019). Green synthesis of zinc oxide nanoparticles: a comparison. Green chemistry letters and reviews, 12(1), 19-24.

[18] Hasaballah, A. I., El-Naggar, H. A., Abd-El Rahman, I. E., Al-Otibi, F., Alahmadi, R. M., Abdelzaher, O. F., ... & Elbahnasawy, M. A. (2023). Surf Redfish-Based ZnO-NPs and Their Biological Activity with Reference to Their Non-Target Toxicity. Marine Drugs, 21(8), 437.

[19] Elbahnasawy, M. A., El-Naggar, H. A., Abd-El Rahman, I. E., Kalaba, M. H., Moghannem, S. A., Al-Otibi, F., ... & Hasaballah, A. I. (2023). Biosynthesized ZnO-NPs Using Sea Cucumber (Holothuria impatiens): Antimicrobial Potential, Insecticidal Activity and In Vivo Toxicity in Nile Tilapia Fish, Oreochromis niloticus. Separations, 10(3), 173.

[20] Jamdagni, P., Khatri, P., & Rana, J. S. (2018). Green synthesis of zinc oxide nanoparticles using flower extract of Nyctanthes arbor-tristis and their antifungal activity. Journal of King Saud University-Science, 30(2), 168-175.

[21] Rosman, N. S. R., Harun, N. A., Idris, I., & Ismail, W. I. W. (2020). Eco-friendly silver nanoparticles (AgNPs) fabricated by green synthesis using the crude extract of marine polychaete, Marphysa moribidii: biosynthesis, characterisation, and antibacterial applications. Heliyon, 6(11).

[22] Willian, N., Syukri, S., Zulhadjri, Z., Pardi, H., & Arief, S. (2021). Marine plant mediated green synthesis of silver nanoparticles using mangrove Rhizophora stylosa: Effect of variable process and their antibacterial activity, F1000Research, 10.

[23] Doğangüneş, M., Türker, A., Güneş, H., & Alper, M. (2021). Cytotoxic and Antioxidant Potential of Chloroform Extract of Holothuria tubulosa Gmelin, 1791. *Russian Journal of*

Marine Biology, *47*(6), 498-507.

[24] Alper, M., & Güneş, M. (2020). Evaluation of cytotoxic, apoptotic effects and phenolic compounds of sea cucumber Holothuria tubulosa (Gmelin, 1791) extracts. *Turkish Journal of Veterinary & Animal Sciences*, *44*(3), 641-655.

[25] Karmous, I., Pandey, A., Haj, K. B., & Chaoui, A. (2020). Efficiency of the green synthesized nanoparticles as new tools in cancer therapy: insights on plant-based bioengineered nanoparticles, biophysical properties, and anticancer roles. Biological trace element research, 196, 330-342.