Biosynthesis, Optimization and Characterization of Zinc Chloride Nanoparticles Using Sideritis sp. Extract

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***Abstract*— Green synthesis, one of the methods used in nanoparticle synthesis, is both low-budget and environmentally friendly. In this study, after biosynthesis of endemic Sideritis plant and Zinc chloride ($ZnCl_2$) nanoparticles (ZnNPs) by distillation method, FTIR, Uv-Vis, SEM and DLS devices were used for their characterization and morphological evaluation, respectively. Optimization study, which adds originality to the study, was carried out by comparing various metal concentrations and plant extract ratios. The antibacterial and anticancer effects of these nanoparticles have been investigated. It has been concluded that ZnNPs can be prepared at a low cost and have the potential to be used as a delivery system in clinical treatments, especially for new drug formulations.**

Keywords*— Green synthesis, $ZnCl_2$ Nanoparticle, Sideritis, Anti-bacterial, Characterization, Drug Delivery System

I. INTRODUCTION

Nanotechnology is a technology that focuses on the characterization, production and processing of biological and non-biological structures smaller than 100 nm [1]. Nanoparticle synthesis include physical, chemical and biological process. Biological synthesis or known as green synthesis, is the most suitable and alternative method for obtaining NPs in a low cost and environmentally friendly manner without the need for the use of toxic chemical. Many parts of plants are used because of the rich metabolite content such as carbohydrates, amino acids, polyphenols, alkaloids, and terpenes, in the green synthesis method, which has gained popularity in recent years. The use of plants in the biogenic synthesis of nanoparticles, its rich metabolite content and the amount of biomass it will provide, its wide applicability, non-toxicity and biocompatible approach show that this study area is also open to development [2].

Sideritis plant and its species are endemic in countries with Mediterranean coast and 80% of them are endemic in Turkey and are used for medicinal purposes and belong to the Lamiaceae family. *Sideritis* plant is especially used as a homeopathic and phyto-naturotherapeutic medicine against cancer, flu or colds, urinary-digestive-respiratory tract diseases. In addition, it has many biological activities such as antivirus, antimicrobial, antioxidant, anti-inflammatory, cytotoxic, gastroprotective, neuroprotective. On the other hand, despite its known biological activity, its research in green syntheses is negligible. Namely; Nanoparticles synthesized using extracts of plants with *Sideritis* or various biological activities have less blood-brain barrier impermeability (BBB) and degradation of bioavailability, etc., than other synthetic drugs or agents. It has features. For this reason, if these nanoparticles are preferred despite the use of synthetics, the possibility of eliminating toxicity or gastrointestinal disorders is very high [3].

It is known that the leaf parts of *Sideritis* plant contain more total flavonoid and total phenolic values and the intensity of antioxidant activity is at a higher level [4]. According to studies, zinc chloride nanoparticles contain antimicrobial and anticancer properties, which is one of the main reasons why they are especially preferred in drug delivery systems [5]. In recent years, nanoparticle synthesis using plant particles and metal are preferred due to their ease of production, sustainability, biocompatibility and other mentioned properties. Although the use of nanoparticles as drug delivery systems in therapeutic applications has great potential, the development of a tumor-focused drug delivery system is of great importance for science. Chemotherapeutic pathways spread nonspecifically throughout the body and therefore have low pharmacokinetic properties [6]. However, the development of tumor-focused drug delivery systems reduces the toxic effect of drugs and plays an important role in translational medicine and targeted therapy by preventing drug resistance.

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Many characterization studies can be performed to evaluate the size, functionality, zeta potential, permeability, structure, distribution, composition and shape of nanoparticles [7]. There are many parameters such as pH, temperature, metal concentration, metal:extract ratio, incubation time that affect the results of the characterization studies [8].

There are tests to prove the antibacterial and anticancer efficacy of synthesized nanoparticles. The one for antibacterial activity is called "Antibacterial Sensitivity Test", and the one for anti-cancer activity is called "Cytotoxicity Test". Disk diffusion test is the most preferred method for antimicrobial activity. In this test, the antibacterial activity is determined by adding antibiotics to the blank paper discs, placing them on top of various microorganisms, and depending on how large a growth is seen around the disc [9]. The cytotoxicity test is necessary for the determination and evaluation of the non-toxic or toxic effect of the produced nanoparticle on cancer cells. Cytotoxicity tests are measurement methods performed in cell culture. In addition, cytotoxicity tests are used to prove the effectiveness of drugs that have a drug nature and whose toxic properties are investigated [10].

In this study, nanoparticle synthesis was realized by extracting *Sideritis* plant extract using infusion and soxhlet technique and distilling it into a zinc chloride solution with pre-adjusted pH and metal concentrations. Uv-Vis, FTIR, SEM and DLS characterization methods were used to determine the properties of the synthesized nanoparticles.

II. MATERIAL & METHODS

II.I Providing the Plant and Completing The Extraction

Extraction of certain parts or all of the *Sideritis* plant using solvents such as water, alcohol or hexane is called plant extract, and in this study, green synthesis was carried out by brewing with water. The raw plant was used by passing through the grinder by binding another GH1 in the solution. The process continues until the equilibrium is reached. the equilibrium is expected to shift exponentially in relation to the GT concentration.

II.I.I Infusion Technique

Extraction of certain parts or all of the plants using solvents such as water, alcohol or hexane is called plant extract. In this study, only pure water was used for the brewing method. The infusion technique was preferred for the extraction of the raw herb, which was passed through a grinder and became powdery. For the infusion method, an assembly consisting of a magnetic stirrer and a glass beaker was set up. The magnetic stirrer in the setup was brought to 60ºC and distilled water boiling at 80ºC was transferred to a 500 mL beaker. During this process, it is mixed at 350 rpm. The infusion method was carried out between 20 and 30 minutes in order not to lose the properties of the plant.

II.I.II Soxhlet Technique

It is used to extract essential oils from sensitive plants in high quantities, in the simplest and cheapest way. In order to apply the Soxhlet extraction method, the solid material is dried, divided into small pieces, and these solid particles are filled into an extraction cartridge made of cellulose. In this technique, the ground *Sideritis* plant is wrapped in coarse filter paper, soxh. placed in. Then 200 mL with the tape measure. methanol was measured and transferred to soxhlet. The temperature of the Soxhlet assembly was set to 50ºC and a heat sink was installed. Extraction was carried out for 4 hours. The methanol was then removed with the aid of a rotary evaporator.

Fig 1. (Plant extract formed by the soxhlet method)

II.I.III Vacuum Filtration Method

A vacuum filtration device and Whatman No:1 filter paper were used to separate unwanted particles from the extract. The plant extract obtained after filtration was stored at 4 ºC to be used in nanoparticle synthesis.

II.II Nanoparticle Synthesis

The nanoparticle production process was started by establishing distillation mechanisms (Fig. 2). In the distillation setup, the magnetic stirrer was also operated using 300 rpm and above. The reason for using the magnetic stirrer is that while the plant extract is dripping into the metal solution, nanoparticles will be produced by direct interaction, thanks to the mixer, the nanoparticle sizes are smaller, they do not form aggregation and spread homogeneously. For this study, 9 runes were prepared in

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advance according to the parameters: R1. It has a pH of 4 and a metal concentration/extract ratio of 1:4, R2. It has a pH of 4 and a metal concentration/extract ratio of 2:4, R3. The plant extract ratio prepared with a pH of 4 and metal concentration/soxhlet is 2:4, R4. It has a pH of 4 and a metal concentration/extract ratio of 3:4, R5. It has a pH of 7 and a metal concentration/extract ratio of 1:4, R6. It has a pH of 7 and a metal concentration/extract ratio of 2:4, R7. Herb extract ratio prepared with pH 7 and metal concentration/soxhlet 2:4, R8. It has a pH of 7 and a metal concentration/extract ratio of 3:4, R9. It has a pH of 10 and a metal concentration/extract ratio of 1:4, R10. It has a pH of 10 and a metal concentration/extract ratio of 2:4, R11. Herb extract ratio prepared with pH 10 and metal concentration/soxhlet 2:4, R12. pH of 10 and metal concentration/extract ratio of 3:4.

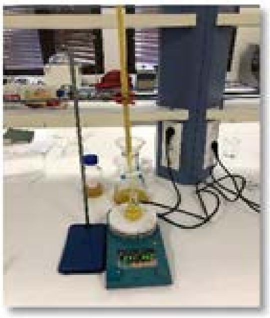

Fig 2. (NP Synthesis by Distillation Method)

According to these runs, $ZnCl_2$ solutions were distilled 1 drop per second into each flask and mixed with a magnetic stirrer to form nanoparticles. Nanoparticle formation was performed for all the prepared bottles. It was then placed in a shaking incubator to prevent aggregation, form more stable nanoparticles, and disperse them homogeneously.

III. RESULT AND DISCUSSION

As a result, nanoparticles synthesized by biological method were produced from sustainable sources and cell metabolites were used during synthesis.

The main results obtained according to characterization studies.

III.I Characterization Studies of Nanoparticles

As a result of Spectrophotometric analyzes of $ZnCl_2$ nanoparticles synthesized green with *Sideritis* plant extract were performed. According to the results obtained with the UV-vis spectrometer device, spectra were observed in the UV-visible region at wavelengths between 300-600 nm. Figure 3 shows the characteristic peaks of $ZnCl_2$ NPs. This indicates that the nanoparticles produced in the project are in the "Near UV and Visible" electromagnetic spectral regions and have the same or similar spectroscopic properties and ranges as some organic compounds with antimicrobial and anticancer properties. [11]

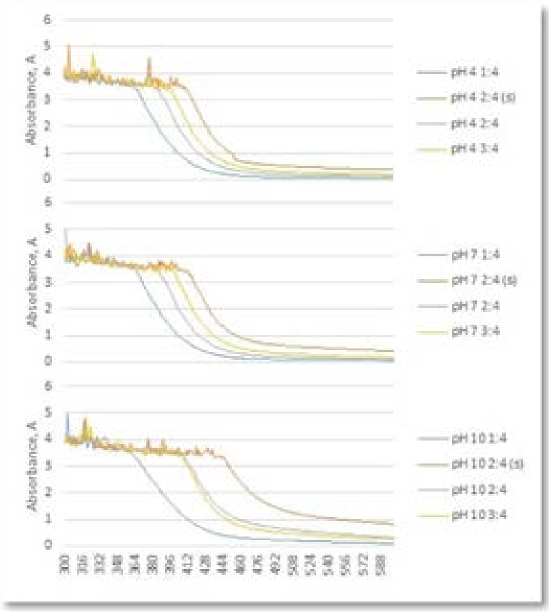

Fig 3. (UV–Vis spectra of zinc nanoparticles)

The FTIR results of the characterization study (Fig. 4.), functional groups and compounds determined from the IR spectrum table are shown relative to the absorption peaks shown in Table 1 below. The FTIR spectra of the Zinc NPs obtained with the aqueous extract of *Sideritis* and the extract from soxhlet showed the active biomolecules of the related extracts and the presence of polyphenolic compounds, and determined the O-H stretching vibrations, –C– –C-, -CH tensions and aromatic ring formations.

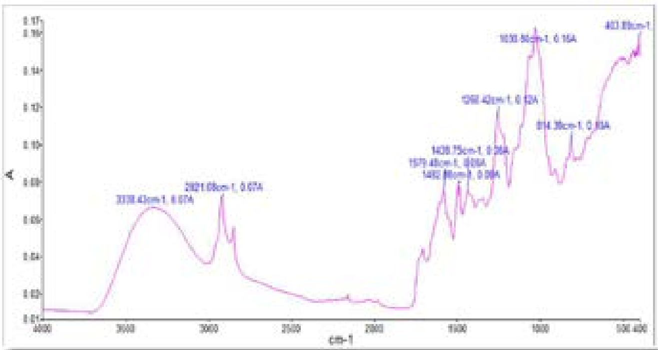

Fig 4. (FTIR spectra of Zinc Nanoparticles)

Frequency (cm^{-1})	*Bond*	*Functional Group*
3338.43	*O-H Streching* *N-H Streching*	*Alcohol* *Aliphatic Primary Amine*

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2921.08	*C-H Streching*	*Alkane*
1579.48	*None*	*None*
1492.08	*N-O Streching*	*Nitro Compound*
1260.42	*C-O Strenching*	*Aromatic Ester* *Alkyl Aryl Ether*
1030.80	*S=O Streching*	*Sulfoxide*
814.38	*C-Cl Streching* *C=C Bending*	*Halo Compound* *Alkene (trisubstituted)*

Table 1. (IR Spectrum Table by Frequency Range)

The morphology and size distribution of Zinc NPs produced by green synthesis were confirmed by SEM analysis (Fig. 5). It is widely monodisperse, with an average size of approximately 90-150 nm. These results are very successful compared to green synthesis studies.

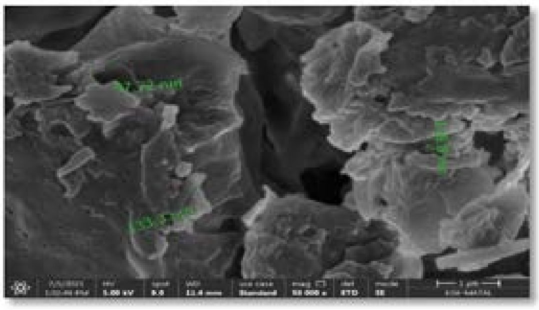

Fig 5. (SEM Images of Zinc Nanoparticles)

The zeta-potential value, low aggregation formation, nanoparticle size values and main evaluation obtained as a result of DLS characterization showed that the produced nanoparticle had "Good" quality (Figure 6). In contrast, NP sizes observed in SEM or TEM analyzes are smaller than in DLS analyses. The reason for this is that the DLS analysis shows the hydrodynamic dimension. Thus, since features such as agglomeration increase, it may not overlap with the morphological analysis of SEM. Sometimes changes due to the density in the solution can be observed [12].

Based on DLS and other characterization analyzes in our study, it can be said that the NP produced was successful in terms of monodispersity and stability.

			Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm):	289.0	Peak 1:	307.2	97,1	135.6
PdI:	0.298	Peak 2:	4654	2,9	892.1
Intercept:	0.928	Peak 3:	0.000	0,0	0.000
Result quality :	Good				

Fig 6. (DLS analysis of Zinc Nanoparticles)

CONCLUSION

It will be seen that the potential of transferring information about the effects of nanobiotechnology on social life is high, albeit partially. Although biological synthesis is a slow process compared to synthesis with other traditional chemical methods, it is thought that the commercial dimension of the biocompatible product to be obtained will be a solution to the search for new areas of use.

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