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Lavandula Stoechas extract; Synthesis of Silver Nanoparticles (Nature-Friendly Green Synthesis Method) Characterization Antimicrobial Activity and *In Silico* Molecular Docking Study

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Abstract

Nanoparticles are structures that are applicable to a wide range of fields. The most significant characteristic of these structures is their structural and dimensional diversity, which varies the synthesis process based on the area that will be incorporated into several sections. Although there are other synthetic techniques, the green synthetic technique is the most often used. By employing only natural processes instead of chemicals, this method synthesizes products leaving no by-products. The technique is also highly preferred as it is affordable. In this respect, the green synthesis approach was utilized in this study to investigate the synthesis characteristics, phytochemical content, and evaluate the antibacterial activity of silver nanoparticles from *Lavandula stoechas*, which is commonly used as a sweetener in food and beverages. Characterization tools like FT-IR, UV-Vis, and fluorescence spectroscopy were employed. The phytochemical content was examined by HPLC. According to the HPLC analysis result; resveratrol and caffeic acid were detected as the main components. Furthermore, theoretical investigation was conducted into the glucosamine-6-phosphate synthase inhibitory characteristics of the resveratrol molecule, which was identified as the main constituent based on the HPLC outcome.

Key Words: *Lavandula stoechas,* resveratrol, glucosamine-6-phosphate synthase, HPLC, FT-IR, UV, nanoparticles.

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1. Introduction

For some decades now, silver (Ag) Agand Ag^+ have been applied in areas such as food and beverage preservation and wound treatment due to their antibacterial capabilities (Simončič & Klemenčič, 2016; Franci et al., 2015). For the synthesis of metal nanoparticles, such as AgNPs, there are three recognized or favored ways; these include physical, chemical, and green biological processes (Abdelghany et al., 2018; Malekzadeh et al., 2018). Nanomaterials (superior metallic particles), such as microbially resistant antibiotics, have attracted much attention due to their better

performance compared to ions (Theivasanthi & Alagar, 2011; Mohanta et al., 2017). These structures are highly preferred due to their unique physical, chemical and dimensional properties (McNamara & Tofail, 2017). Silver has been used in creams, solutions, medicinal and dental materials lately. Among its many iadvantageous qualities, silver is a good option for suppressing infectious germs (Theivasanthi & Alagar, 2011; Mohanta et al., 2017). As an alternative to chemical and physical synthesis methods, the biological synthesis of nanoparticles which has gained popularity recently is thought to be the most straightforward and long-lasting (Ahmed et al., 2017; Rajeshkumar & Bharath, 2017; Mohanta & Behera, 2014).

Lavandula stoecha, a member of the *Lamiaceae* family, is consumed by many Asian and European families as herbal medicine as well as preferred as a healthy diet (Hakim et al., 1991; Zuzarte et al., 2008; Canli et al., 2019). In industries like cosmetics and pharmaceuticals, *L. stoechas* is commonly used. Antibacterial properties (Teixeira et al., 2012), antioxidant activity (Ferreira et al., 2006), and numerous advantageous polyphenol components (Pereira et al., 2015; Celep et al., 2018) are all known to be present in *L. stoechas* extract. In biological systems, enzyme inhibitors function as a control mechanism. Furthermore, they are crucial in clarifying enzyme action mechanisms and metabolic pathways (Feldhammer et al., 2013). A web-based technique called molecular docking forecasts a ligand-protein complex's preferred orientation when it is bound or non-bonded together. Additionally, it is an in silico method for predicting the molecule's binding activity and affinity to protein targets (Nisha et al., 2016; Yenigün et al., 2023).

Two steps went into the design of our study. First, an environmentally friendly green synthesis approach was employed to create silver nanoparticles (AgNPs) using black pepper extract, a substance rich in polyphenols. Furthermore, various techniques were employed to examine the synthesized product's antibacterial properties and characterisation. In the second step of the chemical content analysis of the crude extract, content analysis was performed by HPLC and the main component was determined. Then, the interaction between the main component and the glucosamine-6-phosphate synthase (bacterial) enzyme was calculated theoretically by molecular docking (MolDock). The study's findings provide insight into possible medicinal applications of *L. stoecha*. There aren't many publications in the literature on the synthesis of nanoparticles with *L. stoechas* extracts. As a result, the present work will open up possibilities for future research.

2. Material and Methods

2.1. Reagents

Pure water, ethyl alcohol, $AgNO_3$, Filter paper (Pore Diameter: 0.22 µm) were obtained from Merck.

2.2. Preparation of *L. stoechas* Plant Extract

L. stoechas was taken, properly cleaned in clean water, and left outside in the sun for two days. Subsequently, the extracted samples were finely diced and ground utilizing a professional blender. After that, the ground samples were put in a 250 mL conical flask with 100 mL of pure water, covered with aluminum foil, and let to spin continuously at 600 rpm in a magnetic stirrer for 15-20 minutes at a temperature of between 100 and 105 ^{o}C. In previously prepared 250 mL conical flasks, the solution obtained at the conclusion of the heating procedure was filtered using filter paper with a pore size of 0.22 mm. After the filtration procedure was complete, the filtrate was kept for subsequent usage at +4 ^{o}C (Devi et al., 2019) (Figure 1).

2.3. Synthesis of AgNPs Nano Particles

A 10 mM 100 mL $AgNO_3$ solution was prepared with pure water in a 250 mL conical flask. 80 mL of *L. stoechas* plant extract, previously kept ready, was added to the $AgNO_3$ solution, covered completely with aluminum foil, and mixed in a magnetic stirrer at 600 rpm at room temperature for 12 h. At the end of the heating process, centrifugation was started (3000 rpm, 10 minutes). During this process, the washing process was carried out by adding pure water twice and then ethyl alcohol or methyl alcohol once. Finally, the solid sample obtained was completely dried every other day in an oven at 60 °C to be used in characterizations. The color of the mixture turning gray reveals that Ag^+ ions are reduced to Ag metal (Öztürk et al., 2022; Erenler et al., 2023).

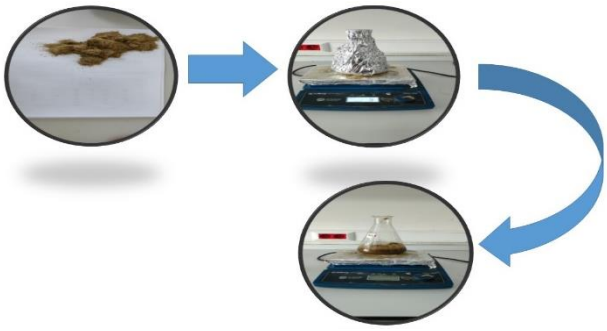

Figure 1. İmage showing the extraction of *L. stoechas* plant

2.4. Phenolic content analysis by HPLC

High-performance liquid chromatography (HPLC) was used to determine the phenolic content of the crude extract. Chromatographic conditions were optimized to achieve optimal separation for the compound and overcome imprinting effects. DAD Signal was performed at a wavelength of 300/200 nm, Ref; 500/100 nm, and chromatographic separation was performed with a reversed-phase Hi-plex (300x7.7) 8-micron analytical column. The column temperature was set at 30 ºC. Elution gradient, eluent A; 83% water (0.1 formic acid) and eluent B; 17% acetonitrile (0.1 formic acid), the solvent flow rate was set to 0.8 mL/min, and the injection volume was set to 10 µL. Also, 19 phenolic compounds were used as standards. Sample preparation: 10 mg tasted samples were dissolved with methanol and 1 mL was taken with an automatic pipette. It was then filtered through a 0.45-micrometer filter. It was diluted with water at a ratio of 1:1 and injected into the device. As a result of the calculation, it was calculated by entering the dilution factor.

2.5. Moleculer Docking Studies

The 3D structure and minimum energy of the Resveratrol molecule were made in the ChemDraw program (Başar et al., 2023; Yenigün et al., 2023). The enzymes chosen for this docking investigation were glucosamine-6-phosphate synthase [PDB ID: 1MOQ] synthase. Resveratrol interactions with glucosamine-6-phosphate were determined using the Molegro Virtual Docker (MVD) program (Y. Başar et al., 2024). 2D and 3D images of the interactions were taken with the BIOVIA Discovery Studio Visualizer program. Also, The AutoDock Vina program was used to calculate the binding affinities (Başar et al., 2024).

2.6. Antimicrobial Assay by Agar Well Diffusion Method

Sterilized Lavandula stoechas nanoparticles were weighed and dissolved in DMSO by sonication. Stock solution was prepared at a concentration of 1024 µg/mL. Mueller Hinton Agar medium was prepared and sterilized, 20 mL was poured into each petri dish, and the agar was allowed to dry. The passages of the bacteria to be used in our study, taken from the stock at -80 °C, were cultured twice and their antimicrobial susceptibility was evaluated by the disc diffusion method from subculture. Bacteria were diluted with physiological saline and prepared to 0.5 Mc Farland turbidity and were planted equally throughout the medium. AgNP impregnated with 20 µl empty

disk was placed on the medium and the antimicrobial effect was evaluated by measuring the zone diameters at the end of the incubation period. The experiment was repeated 3 times and the averages were evaluated. The solvent DMSO was used as a negative control.

2.7. Characterizations; UV-Vis. and Fluorescence Spectrometer

The Cary 60 UV-Vis Spectrophotometer was used as a model instrument for spectroscopic investigations. In addition, fluorescence experiments were conducted using the Cary Eclipse Fluorescence Spectrometer, an Agilent model equipment. Future analysis of the plant will benefit from the obtained photographs.

2.8. Characterizations; FT-IR

Utilizing the Agilent Cary 630 FT-IR model FT-IR device, this characterization phase was carried out.

3. Results and Discussion

water extract of *L. stoechas* plant was obtained. The phytochemical content of the extract was determined by HPLC. Silver nanoparticle study was applied to the extract, and its characteristic properties were determined with UV and FT-IR. In addition, in silico molecular docking was performed to observe the interactions of the glucosamine-6-phosphate enzyme with the resevratrol molecule (Figure 3), which was determined as the main component in the HPLC analysis.

3.1. HPLC Analysis

In HPLC phenolic content analysis, 15 phenolic compounds were detected (Figure 2). According to the analysis results, resveratrol (98.597 ng/ul), caffeic acid (61.945 ng/ul) and chlorogenic acid (21.910 ng/ul) are the compounds detected in the highest amount (Table 1). The amount and types of phenolics in the plants; Vary depending on the climate where the plant grows, altitude, harvest season, as well as the extraction method.

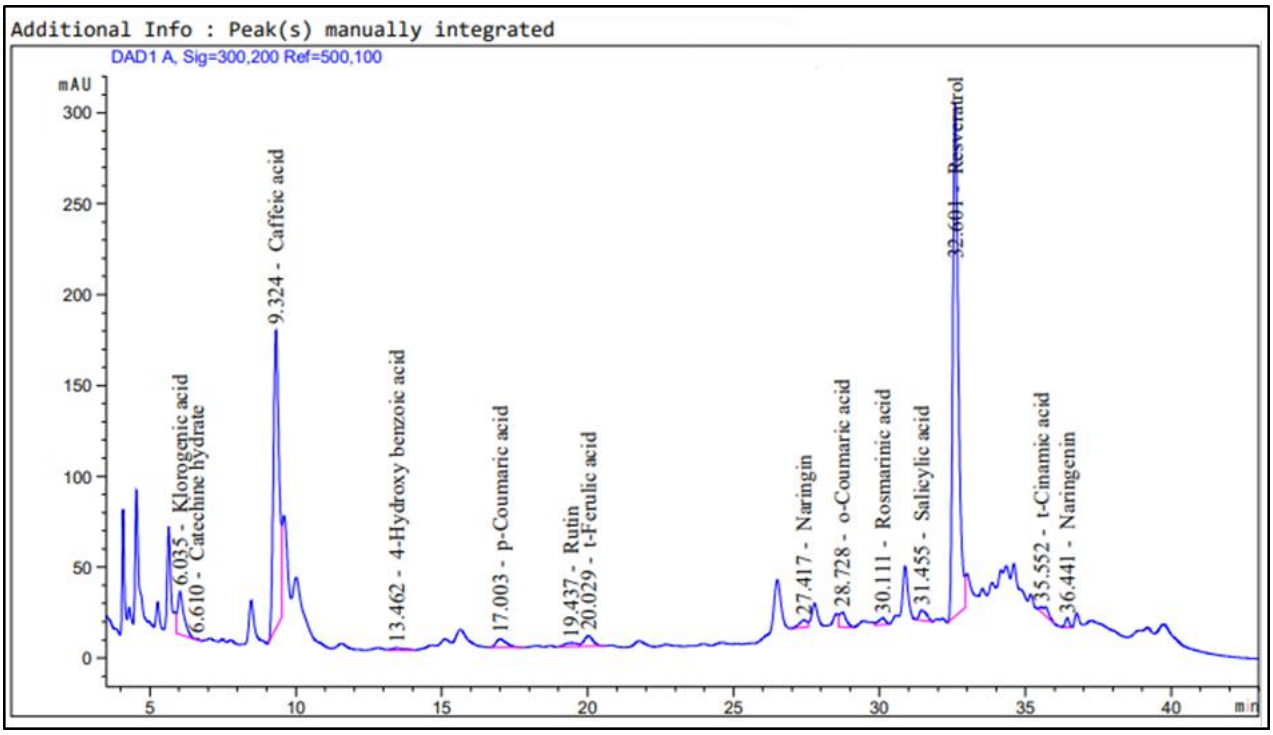

Figure 2. HPLC chromatogram of the crude extract

Table 1. HPLC phenolic content analysis

No	Retention time	Compounds	Amount (ng/ul)
1	6.035	chlorogenic acid	21.910
2	6.610	catechine hydrate	0.172
3	9.324	caffeic acid	61.945
4	13.462	4-hydroxy benzoic acid	0.991
5	16.382	vanillin	-
6	17.003	p-coumaric acid	3.110
7	19.437	rutin	3.705
8	20.029	t-ferulic acid	4.316
9	23.378	hydroxycinnamic acid	-
10	27.417	naringin	7.131
11	28.728	o-coumaric acid	2.898
12	30.111	rosmarinic acid	1.870
13	31.455	salicylic acid	4.160
14	32.601	resveratrol	98.597
15	34.787	quercetin	-
16	35.552	t-cinamic acid	2.295
17	36.441	naringenin	1.820
18	39.384	chrysin	-
19	40.770	flavones	-

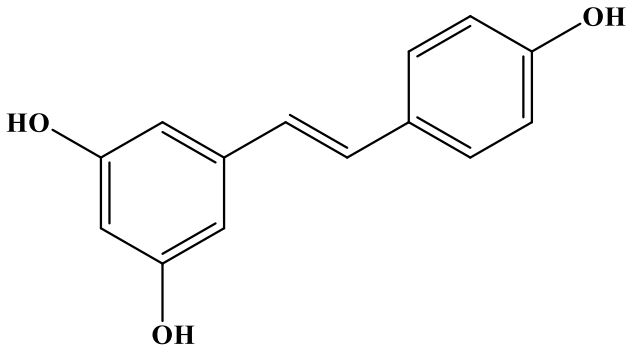

Figure 3. Structure of the resveratrol molecule

3.2. FT-IR:

The –OH and –NH groups for both samples were matched with the peaks seen 3242 and 3321 cm^{-1}, the carboxyl group's C=O group was matched with the peaks seen 1625 and 1617 cm^{-1}, and Ag-OH groups were matched with the peaks seen at about 1013 cm^{-1}. (Geoprincy et al., 2013; Salunke et al., 2014; Nasir et al., 2016; Rani et al., 2017; Bhagyaraj and Krupa, 2020; Dua et al., 2023; Mahmoudi et al., 2020; Şuică- Bunghez et al., 2024) (Figure 4).

3.3. UV-Vis

Two peaks are seen to be formed for AgNPs and the 287 nm extract. Furthermore, it is noted that the extraction yields an additional 327 nm peak (Kumar et al., 2016; Mahmoudi et al., 2020). Analysis in the 350–800 nm wavelength region of emission was achieved using fluorescence spectroscopy. It is noted that the pictures acquired using fluorescence spectroscopy exhibit some shift. It is believed that a contact brought about by AgNP production is the cause of this condition (Rasheed et al., 2023; Chelly et al., 2024) (Figure 5).

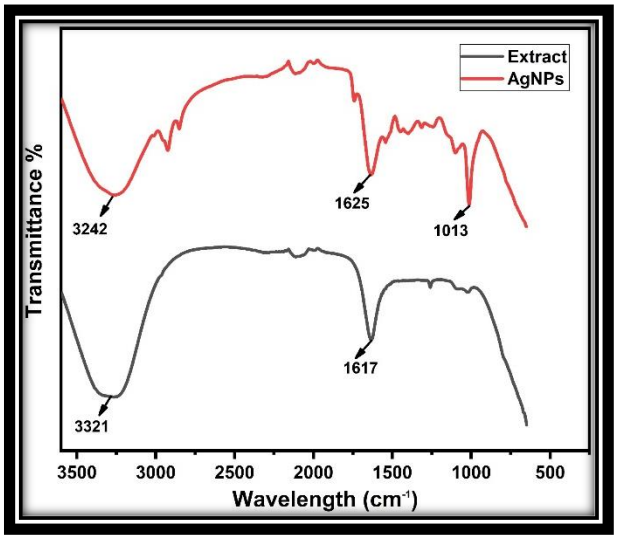

Figure 4. FT-IR image of *L. stoechas* plant extract and AgNPs

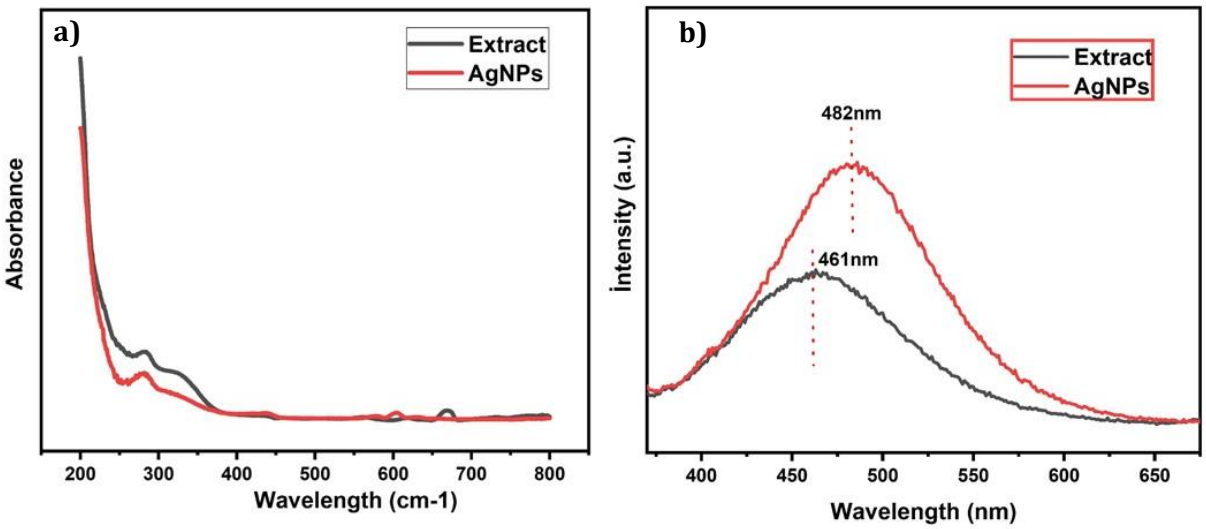

Figure 5. UV-Vis (a) and Fluorescence (b) image of *L. stoechas* plant extract and AgNPs

3.4. Molecular Docking Result

The design and development of pharmaceuticals is costly and time-consuming. With the development of new technologies, new tools are emerging to avoid this difficult process. Molecular docking is a computerised method for predicting protein-ligand interactions and calculating binding positions and energies for the target molecule. Based on the results obtained, it helps to identify the molecule (ligand) as a drug candidate and bring it to a stage that informs in vivo studies (Fan et al.,2019; Singh et al.,2022).

Glucosamine-6-phosphate synthase is known as a promising target for antimicrobial agents and antidiabetics. The enzyme glucosamine-6-phosphate synthase plays an important role in the biosynthesis and synthesis of peptidoglycan in the bacterial cell wall. This enzyme is not used as an antibacterial agent, but due to its role in peptidoglycan synthesis it is of interest for the development of antibiotics. By inhibiting it with a specific ligand, glucosamine-6-phosphate synthase disrupts bacterial cell wall formation, which leads to the death of the bacterial cells (Fikrika et al., 2016; Stefaniak et al., 2022). According to the HPLC analysis result, the resveratrol molecule (Figure 3) was detected in the highest amount in the crude extract; Its interaction with the glucosamine-6-phosphate synthase enzyme was determined theoretically. Additionally, the MolDock score and binding energy of the interactions were calculated.

Resveratrol molecule interacted with glucosamine-6-phosphate synthase by six conventional-hydrogen bonds with amino acid SER316, ARG472, ASN522, VAL519, ALA520, TYR312, one pi-anion with amino acid ASP474, and one pi-alkyl with amino acid ARG472 (Figure 6-Table 2). Resveratrol molecules with glucosamine-6-phosphate synthase interactions were determined as a MolDock score of -90.81, with binding energies of -5.80 kcal/mol. According to the results, it can be said that the resveratrol molecule is effective as an inhibitor of glucosamine-6-phosphate.

3.5. Agar Well Diffusion Method

The present study aimed to evaluate the antimicrobial effects of the *L. stoechas* AgNPs and Gentamicin and antibiotic standard strains of *S. aureus* ATCC 25923, *E. coli* 25922, *P. aeruginosa* ATCC 27853, *E. feacalis* ATCC 29212 *using* the well diffusion method, the diameter of the growth inhibition zone was measured for AgNPs at a concentration of 1024 µg/mL against four bacteria. Gentamicin was used as a positive control, and a precise measurement was made for it.

Table 2. Interaction categories, types, and distances of molecular insertion of the resveratrol molecule with glucosamine-6-phosphate synthase

No	Name	Distance	Category	Type	Transmitter	From Chemistry	Receiver	To Chemistry
1	A:SER316:HG - :[001:O3	1.93855	Hydrogen Bond	Conventional Hydrogen Bond	A: SER316:HG	H-Donor	:[001:O3	H-Acceptor
2	A:ARG472:HN - :[001:O2	2.3882	Hydrogen Bond	Conventional Hydrogen Bond	A: ARG472:HN	H-Donor	:[001:O2	H-Acceptor
3	A:ASN522:HN - :[001:O1	2.27169	Hydrogen Bond	Conventional Hydrogen Bond	A: ASN522:HN	H-Donor	:[001:O1	H-Acceptor
4	:[001:H11 - A:VAL519:O	2.20973	Hydrogen Bond	Conventional Hydrogen Bond	:[001:H11	H-Donor	A:VAL519:O	H-Acceptor
5	:[001:H11 - A:ALA520:O	2.28076	Hydrogen Bond	Conventional Hydrogen Bond	:[001:H11	H-Donor	A:ALA520:O	H-Acceptor
6	:[001:H12 - A:TYR312:O	2.74218	Hydrogen Bond	Conventional Hydrogen Bond	:[001:H12	H-Donor	A:TYR312:O	H-Acceptor
7	A:ASP474:OD2 - :[001	3.47614	Electrostatic	Pi-Anion	A:ASP474:OD2	Negative	:[001	Pi-Orbitals
8	:[001 - A:ARG472	3.87475	Hydrophobic	Pi-Alkyl	:[001	Pi-Orbitals	A:ARG472	Alkyl

The values of the growth inhibition zone in millimeters are displayed in Figures, which provide the results. It showed the highest antimicrobial effect against *S. aureus* 12.00±1.00 mm. On the other hand, it showed the lowest inhibitory effect against *E. feacalis*, with a growth inhibition zone diameter of approximately 9.50±1.00 mm. These findings shed important light on the *L. stoechas* AgNPs antibacterial activity and suggest that they might be used in place of antibiotics to treat bacterial infections (Fig. 7).

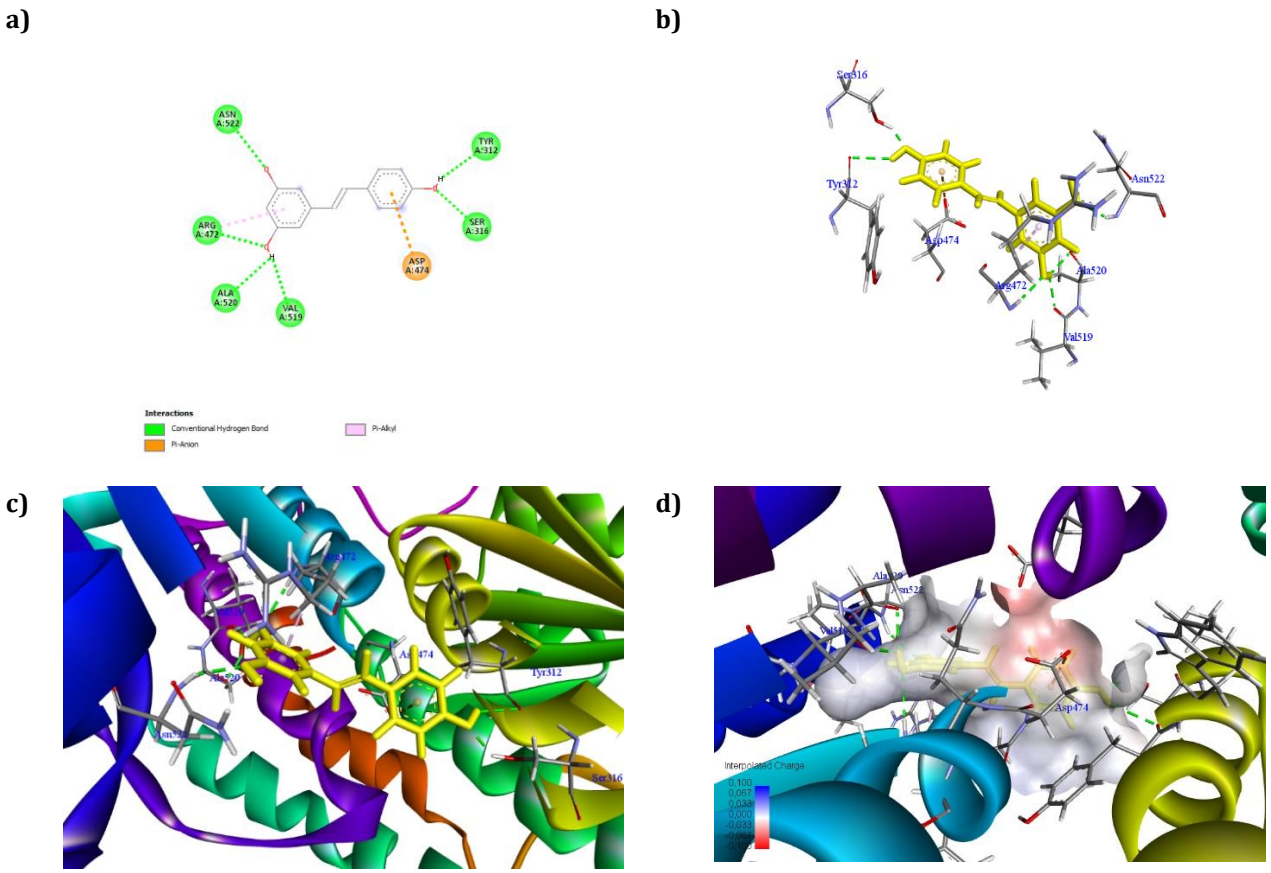

Figure 6. Resveratrol-glucosamine-6-phosphate synthase interaction a) 2D images b) general view c) 3D images d) interpolated load view

Using natural approaches, we produced AgNPs from the high polyphenol content plant, *L. stoechas*, more economically and without leaving any chemical by-products in the environment. UV-Vis spectrophotometry can be used to identify the reduction of Ag^{+} ions to metallic Ag^{0} in this situation. Additionally, it was discovered that S. Aureus was more negatively impacted by it in studies of nanoparticle antibacterial activity. Additionally, the high reduction potential of *L. stoechas* extract was linked to an increase in AgNP production via an increase in silver nitrate concentration. The extract of *L. stoechas* includes flavonoids, phenolic acids, and phytosterols. It belongs to the Lamiaceae family of mints, which also includes mint, and has numerous active metabolites that can be used as capping and reducing agents (Nitzsche et al., 2004; Gonçalves & Romano, 2013).

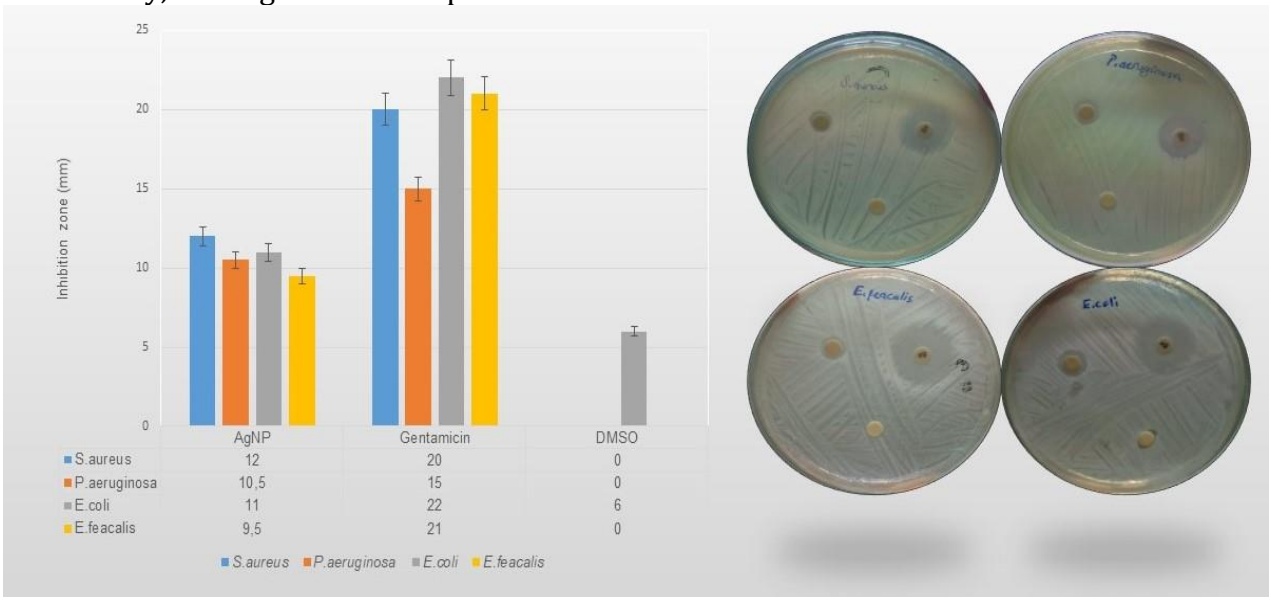

Figure 7. Image of the antimicrobial activity study performed by the Agar Well Diffusion method (inhibition zone, mm)

4. Conclusion

Since the 1990's, scientists have been calling for the synthesis of nanoparticles using the environmentally benign green synthesis approach. *L. stoechas* extract was chosen as the reducing agent in this work because it was more cost-effective, quicker, and environmentally friendlier than other methods for synthesizing silver nanoparticles. The shape of the produced AgNPs was investigated in addition to their structural characteristics. The fact that these AgNPs work well against *S. aureus, P. aeruginosa*, *E. coli*, and *E. faecalis* further supports the nanoparticle's potent antibacterial activity. As a result, in this study, resveratrol compound was determined in the highest amount as the main compound in the HPLC device. In addition, in antimicrobial activity studies, it was determined by the disk diffusion method that it showed the highest antimicrobial effect against *S. aureus* but the lowest effect against *E. feacalis*. In the interaction of glucosamine-6-phosphate synthase and resveratrol, the MolDock score was determined to be -90.81 and the binding energies -5.80 kcal/mol. It is assumed that this study will provide ideas for further research.

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Author Contribution

All authors declare equal contribution to the design and experimental work, interpretation of the resultsand editing the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest during the accomplishment of this research. None of the authors has any financial and/or personal relationships with other people or organizations that could inappropriately influence (bias) their work.

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