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Biosynthesis of silver nanoparticles from *Teucrioside* and investigation of its antibacterial activity

Özlem KAPLAN [1,*], ***Nazan GÖKŞEN TOSUN*** [2]

[1]*Istanbul University, Department of Molecular Biology and Genetics, Faculty of Science, Istanbul, TURKEY*

[2]*Tokat Gaziosmanpaşa University, Graduate School of Natural and Applied Science, Department of Biomaterials and Tissue Engineering, Tokat, TURKEY*

Abstract

Teucrioside, 9′-decarboxyrosmarinic acid 4′-O-α-rhamnosyl-(1‴→6‴)-O-β-galactosyl-(1‴→4″)-Oα-rhamnoside is a natural phenolic compound. It has been isolated and identified from the genus *Teucrium*. *Teucrium* genus is widely used in traditional medicine for its antioxidant, diuretic, antiulcer, antitumor, anti-inflammatory, antispasmodic and antibacterial properties. Since silver nanoparticles have superior physicochemical properties, they have an important role in biology and medicine. In this study, the biosynthesis of silver nanoparticles was carried out using *Teucrioside* and $AgNO_3$. The effect of five independent variables (pH, $AgNO_3$ concentration, *Teucrioside* volume/total volume, microwave power and time) on nanoparticle formation was evaluated using a central composite design (CCD) based response surface methodology (RSM). Nanoparticle formation was demonstrated by UV-Vis spectroscopy and FTIR analysis. The particle size and zeta potential of silver nanoparticles were determined by dynamic light scattering method (DLS). The results showed that 5 mM $AgNO_3$, *Teucrioside* volume/total volume:0.3, 475 watt, 60 sec. and pH:7.5 were optimal reaction parameters. The antibacterial activity of biosynthesized silver nanoparticles was tested against common pathogens such as *Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Klebsiella pneumonia*. Obtained results demonstrated that biosynthesized silver nanoparticles from *Teucrioside* have great potential as a new antibacterial agent.

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1. Introduction

Silver nanoparticles (AgNPs) are very important metallic nanomaterials used in various applications due to their unique optical, electrical and biological properties [1]. The antimicrobial properties of AgNPs have made them widely used in medicine and agriculture [2, 3]. Because of the superior properties of AgNPs such as high antimicrobial activity even at low concentrations, their use reduces the environmental risk associated with excessive antibiotic or pesticide use [4-8]. Bacterial infection and resistance to antibiotics pose a serious threat to human health [9]. AgNPs exhibit adjustable structural properties and broad antibacterial spectrum advantages against antibiotic resistant bacteria. Thus, AgNPs are considered promising antibacterial agents [10]. Flavonoids, aromatic compounds, sugars, polyphenols found in plant extracts are functional groups involved in the biosynthesis of AgNPs [11, 12].

The *Teucrium* genus belongs to the *Lamiaceae* family and has about 300 species widespread all over the world [13]. Due to its pharmacological effects, various species of this genus are used widely in traditional medicine for their antioxidant, diuretic, antiulcer, antitumor, anti-inflammatory, antispasmodic and antibacterial properties [14]. Therefore, interest in *Teucrium* species has increased in recent years. One of the most studied species in the genus is *Teucrium chamaedrys* (germander). Phytochemical constituents of this species comprise flavonoids, diterpenoids and glycosides [13, 15]. Phenylethanoid glycosides are the main phenolic compounds in *Teucrium* species. Recently reports have shown the wide range of biological and pharmacological properties of these components. *Teucrioside* (9′-decarboxyrosmarinic acid 4′-O-α-rhamnosyl-(1‴→6‴)-O-β-galactosyl-(1‴→4″)-Oα-rhamnoside) is L-lyxose containing phenylethanoid glycoside found in *Teucrium* genus [13-16]. In this study, it is aimed to synthesize AgNPs in a fast, simple, environmentally friendly and low cost

*Corresponding author. *e-mail address: ozlem.kaplan@istanbul.edu.tr*

using *Teucrioside*. The effects of experimental conditions on the biosynthesis of silver nanoparticles were investigated with response surface methodology, the most widely used statistical technique for process optimization [17, 18]. There are studies in the literature regarding the application of response surface methodology in the biosynthesis of AgNPs [19, 20]. The response surface methodology is a useful method for complete, rapid optimization of conditions and the correct design of the synthesis process. Also, antibacterial activity of the synthesized AgNPs was tested against common pathogens such as *Pseudomonas aeruginosa, Klebsiella pneumonia*, *Staphylococcus aureus* and *Enterococcus faecalis.*

2. Materials and Methods

2.1. Biosynthesis of silver nanoparticles and characterization

2.1.1. Extraction and identification of *Teucrioside*

Teucrioside was extracted, isolated and identified with methods which were explained in previous study by Dr. Elmastaş and his team [13].

2.1.2. Experimental design and optimization

Response surface methodology (RSM) based on a central composite design (CCD) was used to evaluate five independent variables (pH, $AgNO_3$ concentration, *Teucrioside*/$AgNO_3$ ratio, microwave power and time) for the nanoparticle formation. Design Expert 12 software was used for regression and graphic analysis of the data. The experimental design consisted of 46 experiments for five independent variables. The absorbance data obtained from the UV-spectrum were used as optimization criteria. The experimental design along with the response was shown in Table 1.

Table 1. The central composite experimental design for *Teucrioside*&AgNPs synthesis

Run	Factor 1 A:$AgNO_3$ Conc. (mM)	Factor 2 B:Extract Vol./Total Vol.	Factor 3 C:Time(sec)	Factor 4 D:Power (Watt)	Factor 5 E:pH	Response % Yield
1	3	0.1	35	475	3	0
2	5	0.3	60	475	7.5	49.99
3	3	0.1	60	475	7.5	59.58
4	3	0.3	35	150	12	55.12
5	5	0.3	35	475	12	72.33
6	3	0.5	60	475	7.5	37.02
7	3	0.1	35	800	7.5	75.11
8	3	0.5	35	475	3	0
9	1	0.3	60	475	7.5	42.35
10	3	0.3	35	800	3	0
11	3	0.3	10	475	12	65.99
12	3	0.3	10	150	7.5	37.45
13	3	0.3	60	475	12	70.03
14	3	0.3	35	475	7.5	46.52
15	3	0.5	10	475	7.5	43.27
16	3	0.1	10	475	7.5	55.21
17	3	0.3	35	150	3	0
18	1	0.3	10	475	7.5	45.24
19	5	0.3	10	475	7.5	43.88
20	3	0.3	35	475	7.5	46.54
21	1	0.3	35	475	12	59.29
22	5	0.3	35	475	3	0
23	3	0.3	35	475	7.5	46.58
24	3	0.3	35	800	12	100
25	3	0.5	35	150	7.5	33.37
26	3	0.3	60	150	7.5	60.18
27	1	0.5	35	475	7.5	45.12
28	5	0.3	35	800	7.5	75.12
29	1	0.3	35	800	7.5	60.11
30	3	0.3	35	475	7.5	46.49
31	1	0.3	35	150	7.5	46.55
32	5	0.1	35	475	7.5	65.12
33	5	0.5	35	475	7.5	40.49
34	1	0.1	35	475	7.5	50.05
35	3	0.1	35	150	7.5	45.13
36	3	0.3	10	800	7.5	80.15
37	3	0.3	35	475	7.5	46.38
38	3	0.3	10	475	3	0
39	5	0.3	35	150	7.5	35.62
40	3	0.5	35	475	12	50.12
41	3	0.3	60	800	7.5	50.23
42	3	0.1	35	475	12	85.23
43	3	0.3	60	475	3	0
44	1	0.3	35	475	3	0
45	3	0.3	35	475	7.5	46.50
46	3	0.5	35	800	7.5	61.06

2.1.3. Synthesis and characterization of *Teucrioside*&AgNPs

Teucrioside aqueous solution (5% w/v) was prepared. The biosynthesis of AgNPs was carried out using different pH, $AgNO_3$ concentration, *Teucrioside*/$AgNO_3$ ratio, microwave power and time. The mixture was centrifuged at 20.000 g for 10 minutes and AgNPs were precipitated. The AgNPs were washed with distilled water and they were dried overnight at 37°C. Biosynthesis of AgNPs were confirmed by UV-Vis spectroscopy and FTIR analysis. The particle size and zeta potential analysis of synthesized AgNPs were determined by dynamic light scattering method using HORIBA SZ-100 Nanoparticle Analyzer.

2.2. Antibacterial activity of *Teucrioside*&AgNPs

Antibacterial activity was studied with two gram negatives bacterium (*Pseudomonas aeruginosa* (ATCC 27853) and *Klebsiella pneumonia* (ATCC 15380)) and two gram positive bacterium (*Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 25923). The antimicrobial activity of AgNPs was analyzed by the minimum inhibitory concentration (MIC). Cultures were grown in exponential phase in nutrient broth at 37ºC for 16 h. The various AgNPs concentrations (250 µg/ml–3.9 µg/ml) and *Teucrioside* (250 µg/ml–3.9 µg/ml) were used for antimicrobial tests. The intensity of bacteria was standardized to equal a 0.5 McFarland standard (approximately $5x10^7$ organisms ml^{-1}) for each concentration. The bacteria were then inoculated 96 well-plates and were incubated at 37°C for 24 h. After 24 h, the optical density of each well was recorded at 600 nm using a microplate reader [21]. The experiments were repeated three times, and the mean values were used.

3. Results and Discussion

3.1. Experimental design and optimization of biosynthesis of *Teucrioside*&AgNPs

Design Expert 12 software was used to optimize the synthesis procedure of AgNPs. The experimental design consisted of 46 experiments for five independent variables (pH, $AgNO_3$ concentration, *Teucrioside*/$AgNO_3$ ratio, microwave power and time). The absorbance data obtained from the UV-spectrum were used as optimization criteria. The acquired data coincided with the quadratic polynomial model and various statistical parameters were used to fit the analysis. After data modeling which is demonstrating the existence of interaction and curvature effect was performed, polynomial equation was generated for response factor. The data obtained overlap the empirical model with correlation coefficient (r^2) values of 0.9898. The model diagnostic graphs for response are shown in Figure 1, showing that the data is parallel to the selected model.

$$\%Yield = 46{,}504 + 2{,}07969 * A + -7{,}79367 * B + -0{,}126277 * C + 11{,}5005 * D + 34{,}5181 * E + -4{,}87543 * AB + 2{,}33788 * AC + 6{,}63818 * AD + 3{,}17526 * AE + -2{,}65788 * BC + -0{,}543163 * BD + -8{,}75 * BE + -13{,}25 * CD + 1{,}00232 * CE + 10 * DE + 0{,}305598 * A^2 + 1{,}61369 * B^2 + 1{,}11769 * C^2 + 6{,}85954 * D^2 + -14{,}46 * E^2$$

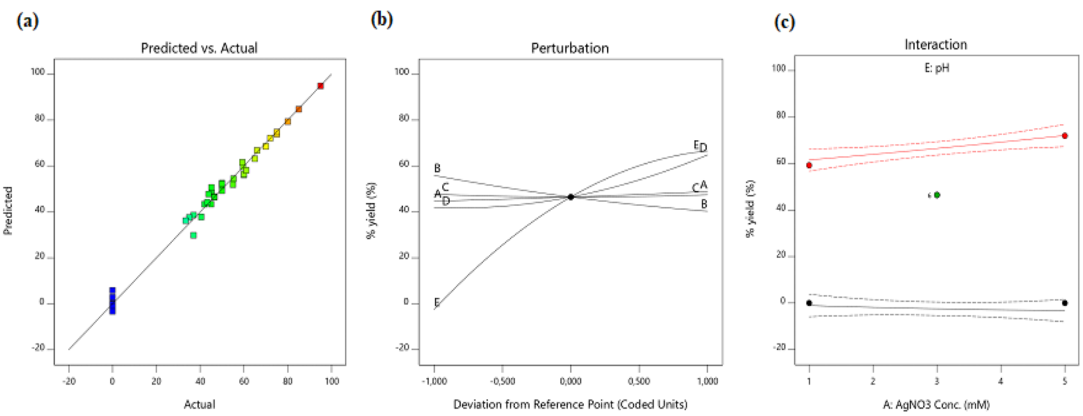

Figure 1. The graphs showing (a) predicted vs. actual plot, (b) perturbation chart, (c) interaction plot for response values

Response surface analysis was obtained using 3D response surface plots which elucidated the existence of interactions among the factors and their impacts on the response factor. 3D response surface plots of particle formation (%Yield) of synthesized AgNPs as a function of pH, $AgNO_3$ concentration, *Teucrioside*/$AgNO_3$ ratio, microwave power and time are shown in Figure 2. It is seen that the effect of pH on the yield of nanoparticle formation is quite high (Figure 2 d, g, i, j). Nanoparticle formation is very low at acidic pH in all parameters. In all experiments where pH is above 7, it is seen that the increase in $AgNO_3$ concentration, *Teucrioside*/$AgNO_3$ ratio, microwave power and time parameters increases the particle formation efficiency. The dependence of the nanoparticle formation rate on the pH of the solution has also been reported in the literature. According to these studies, nucleation of silver nanoparticles occurs in alkaline conditions, while nanoparticle aggregation is performed in acidic conditions. When examined in terms of nanoparticle efficiency, there was no nanoparticle formation in the acidic range, but it caused a gradual increase in nanoparticle production with the increase in pH [18, 22, 23].

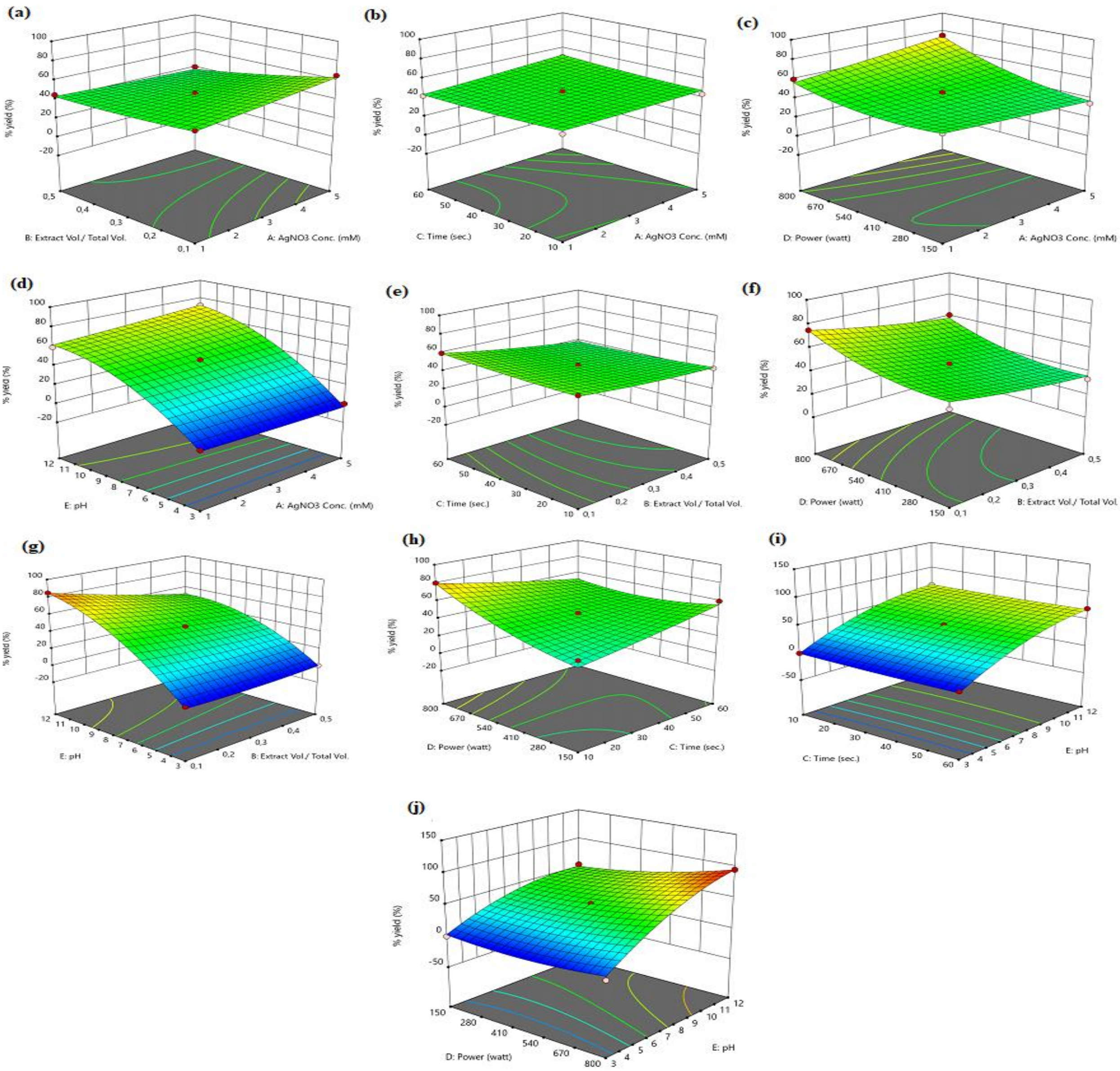

Figure 2. 3D response surface plots of particle formation (%Yield) of synthesized silver nanoparticles as a function of pH, $AgNO_3$ concentration, *Teucrioside*/$AgNO_3$ ratio, microwave power and time. (a) $AgNO_3$ concentration and Extract vol./Total vol. (b). $AgNO_3$ concentration and time (c). $AgNO_3$ concentration and power (d) $AgNO_3$ concentration and pH (e) Extract vol./Total vol. and time (f) Extract vol./Total vol. and power (g) Extract vol./Total vol. and pH (h) Time and power (i) pH and time (j) pH and power.

The AgNPs were optimized and then their values were evaluated to identify with numerical optimization. It was seen that the desirability function value was close to 1 and the goal for the response variable was achieved. As the optimized silver nanoparticles synthesis setting, the overlay plot showed the yellow color area as the optimized area along with the flagged point displaying 5 mM $AgNO_3$, *Teucrioside* volume/total volume:0.3, 475 watt, 60 sec. and pH:7.5 were optimal reaction parameters in Figure 3.

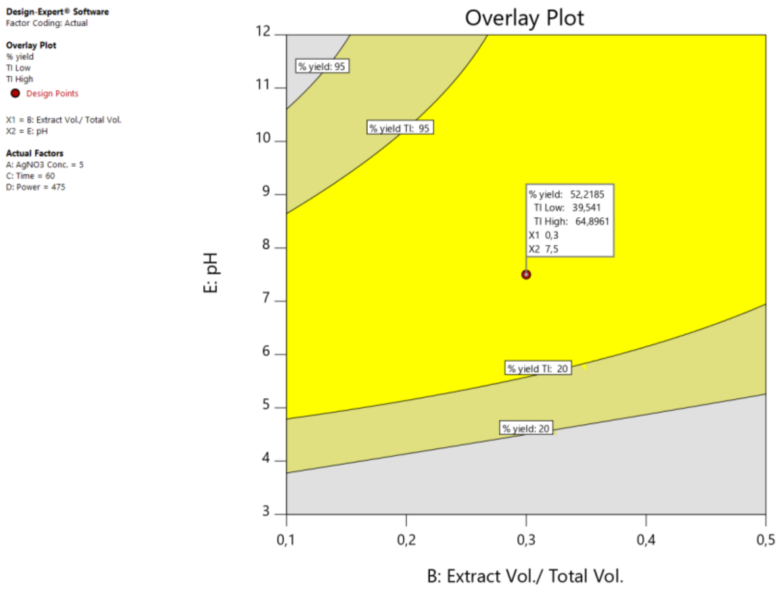

Figure 3. The graphs showing yellow color area as the optimized area and flagged point as the selected *Teucrioside*&AgNPs

3.2. Characterization of *Teucrioside*&AgNPs

The obtained AgNPs as a result of optimization were characterized by UV-VIS spectrophotometry and the result was shown in Figure 4. The spectrum indicating the peak was observed at 421 nm and this result was fitting with brownish color of the nanoparticles.

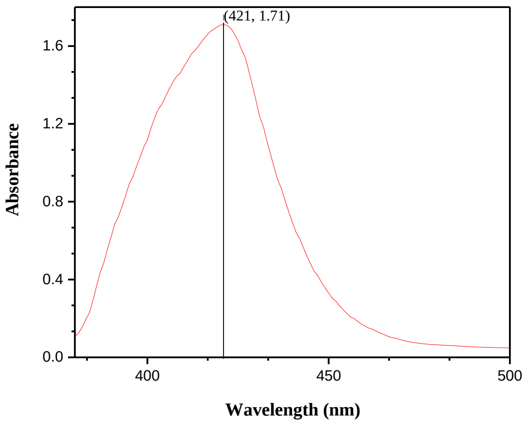

Figure 4. UV-Visible spectra of *Teucrioside*&AgNPs

FTIR spectrum of the was shown in Figure 5. The significant absorption bands for silver nanoparticles were observed at 2901.38, 1630.52 and 1399.1. The optimized silver nanoparticles were exhibited a wide absorption band of –OH groups at 3268.75. The absorption bands at 2901.38 and 2986.23 were associated with C–H stretching of aliphatic –CH, $-CH_2$ groups. The absorption peaks at 1630.52 and 1399.1 were assigned to the asymmetrical and symmetrical –COO stretching of carboxylate compounds in *Teucrioside*.

The size of the prepared silver nanoparticle was determined using dynamic light scattering as shown in Figure 6. The average size of the synthesized AgNPs was 165.9±3.1 nm. The nanoparticles showed homogeneous distribution (polydispersity index: 0.508±0.028). Also, the zeta potential value of the synthesized AgNPs was found to be -31.5±0.7 mV. A negative charge on the surface of the produced nanoparticles indicates that they have high stability.

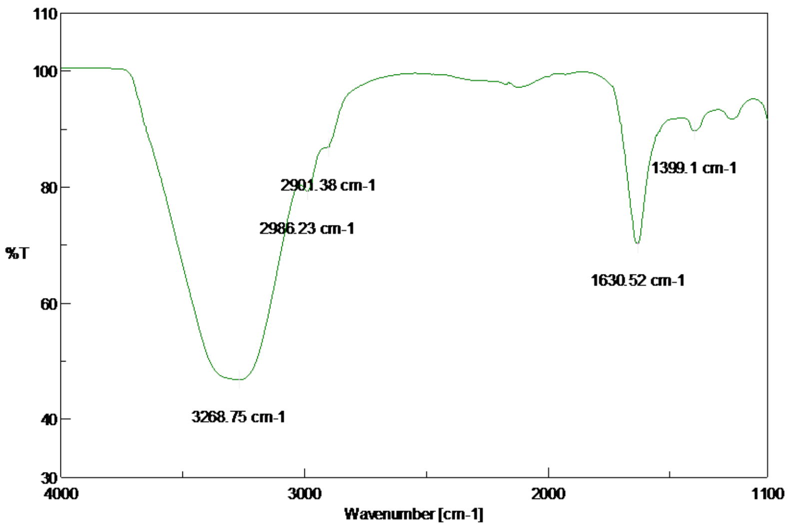

Figure 5. FTIR spectra of *Teucrioside*&AgNPs

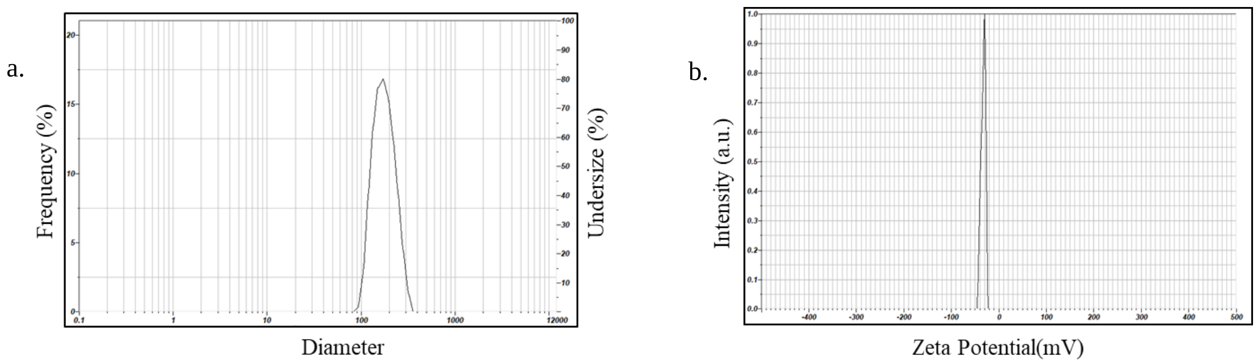

Figure 6. Dynamic light scattering (DLS) and zeta potential of *Teucrioside*&AgNPs a. DLS of *Teucrioside*&AgNPs and b. Zeta potential of *Teucrioside*&AgNPs

3.3. Antibacterial activity of *Teucrioside*&AgNPs

Growth inhibition curves of pathogenic microorganisms treated with *Teucrioside* extract and *Teucrioside*&AgNPs were shown in Figure 7. Our results show that the produced AgNPs have significant antimicrobial activity against different bacterial strains. *Teucrioside* concentration of 250 µg/ml inhibited just %15 of the *K. pneumaniae* strain, % 2 of *S. aureus* strain and *P. aeruginosa* strain and there was no influence on *E. faecalis* strain (Figure 7a). However, the optimized *Teucrioside*&AgNPs at concentration of 125 µg/ml completely inhibited all of the bacteria strains (Figure 7b).

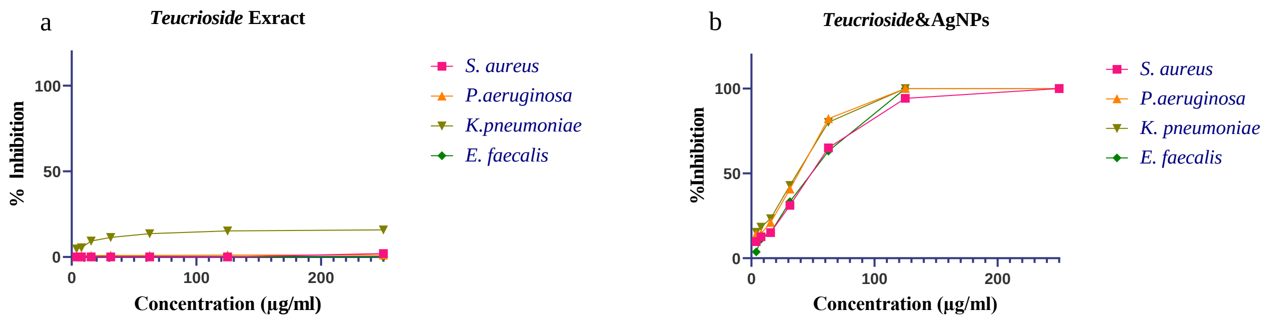

Figure 7. Growth inhibition curves of pathogenic microorganisms exposed to *Teucrioside* extract and *Teucrioside*&AgNPs a. Growth inhibition curves of pathogenic microorganisms exposed to *Teucrioside* extract b.Growth inhibition curves of pathogenic microorganisms exposed to *Teucrioside*&AgNPs

Minimum inhibitory concentrations (MIC) of the produced AgNPs were given in Figure 8. When the MIC values are examined, it is seen that *Teucrioside*&AgNPs are effective on gram negative bacteria (*Pseudomonas aeruginosa* and *Klebsiella pneumonia*) than gram positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus)* at relatively lower concentrations. The more effective antimicrobial activity of silver nanoparticles against gram negative bacteria is likely due to their shape and size [24].

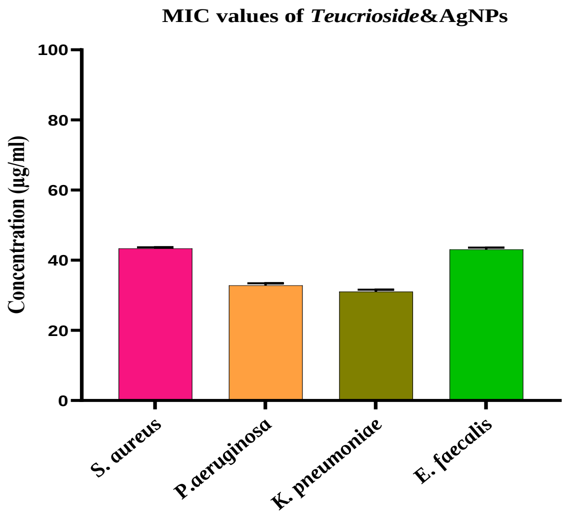

Figure 8. MIC value of *Teucrioside*&AgNPs

MIC values of *Teucrioside*&AgNPs were determined as 42.91±6.85 µg/ml, 32.03 ± 8.36 µg/ml, 30.14 ± 8.848 µg/ml and 42.30 ± 6.75 µg/ml, respectively on *S. aureus*, *P. aeruginosa*, *K. pneumonia* and *E. faecalis*. There are numerous studies on the antimicrobial effects of synthesized silver nanoparticles from various biological materials. The obtained MIC values in our study are acceptable when compared to the previously stated concentrations of 10-100 µg/ml [2, 5, 6, 8, 25]. The results clearly demonstrated that *Teucrioside*&AgNPs have strong antibacterial potential.

4. Conclusions

Silver nanoparticles have attractive physicochemical properties and are often used in biology and medicine because of these properties. Silver nanoparticles play an important role in the development of new antibacterial against pathogenic microorganisms. In this study, *Teucrioside*&AgNPs were synthesized by *Teucrioside* using as a biological reduction agent. The synthesized silver nanoparticles were systematically optimized by Design Expert 12.0 software. The experimental design consisted of 46 experiments for five independent variables (pH, $AgNO_3$ concentration, *Teucrioside*/$AgNO_3$ ratio, microwave power and time). It was observed that the pH value was highly effective in the yield of nanoparticle formation. Also, an increase in nanoparticle formation efficiency was observed with increasing $AgNO_3$ concentration, *Teucrioside*/$AgNO_3$ ratio, microwave power and time. The optimum conditions were determined as 5 mM $AgNO_3$, *Teucrioside* volume/total volume:0.3, 475 watt, 60 sec. and pH:7.5. The mean size and zeta potential of the synthesized AgNPs were 165.9 ± 3.1 nm and -31.5 ± 0.7 mV, respectively. The antibacterial activity of *Teucrioside*&AgNPs and *Teucrioside* were showed against gram positive and gram negative bacteria strains. Obtained results demonstrated *Teucrioside*&AgNPs exhibit potential as a new antibacterial agent.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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