

Synthesis and characterization of silver nanoparticles using *Thymbra spicata* L. herbal extract for removing methylene blue dye from aqueous solutions

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Nowadays silver nanoparticles are produced by different chemical methods. Which have disadvantages such as instability of the solution and also require advanced equipment for production. So, green synthesis routes have attracted the attention of researchers for producing nanoparticles that have the minimum environmental hazards and require simple equipment for production. Herbal extracts can be used as a green route to synthesize silver nanoparticles. In this work, synthesis of silver nanoparticles was carried out using *Thymbra spicata* L. herbal extract (with the local name of zoofai). Silver nitrate was added to the extract and the effect of silver nitrate concentration, volume of the extract, temperature and time on silver nanoparticles synthesis was investigated. The synthesized nanoparticles have been characterized by UV-Vis spectroscopy, transmission electron microscopy (TEM), XRD, DLS, scanning electron microscopy (SEM), X-ray diffraction spectroscopy (EDX), and FT-IR. At UV-Visible spectrum, the presence of a peak at 430 nm indicates the biological synthesis of this nanoparticle in the presence of extract, and TEM image showed the spherical shape for the nanoparticle. Particle size distribution and average particle size were found to be about 40 nm by DLS. XRD image confirmed face-centered cubic nanocrystals with an average size of 21.3 nm, and SEM images were also well-matched with TEM and XRD images. Many organic dyes, such as methylene blue cationic dye, are toxic, carcinogenic and non-degradable because of their complex aromatic structure and high solubility in water. Therefore, it is necessary to remove them from wastewater. In this study, the efficiency of nanoparticles in the removal of methylene blue from water was also investigated by changing some parameters such as silver nanoparticle dosage, concentration of methylene blue and time, and it was found that the removal procedure is well done.

Keywords: *Thymbra spicata* L., silver nanoparticles, green synthesis, methylene blue

INTRODUCTION

Nanotechnology is based on technological innovations in the 21st century. One of the important areas of research in nanotechnology is the synthesis of different nanoparticles. Today, the need for reliable methods for the synthesis of nanoparticles that do not hazard the environment has attracted researchers to the biological system. Metallic nanoparticles have been considered because of their unique optical properties, electrical applications, photonics, biomedical, magnetic, and catalytic properties [1]. Among the nanoparticles, silver atoms are important because of their high anti-fungal, antibacterial, and antiviral activity. In addition, these nanoparticles can play an effective role in water and industrial effluents (removing dyes or metallic pollutants) purification [8,73,74]. In fact, metal nanoparticles, such as silver, have been considered due to the high surface area, reactive surface, structural stability as an effective absorbent for water purification. The efficiency of silver nanoparticles in absorbing heavy metal ions, as well as organic pollutants (cationic and anionic ions), has been investigated [2]. Among organic pollutants, dyes pollutants are most notable because many commercial dyes are often poisonous and non-degradable because of their complex aromatic

and polymeric structure and high solubility in water [3]. These pollutants become more stable after entering organic compounds to water and their chemical decomposition become harder due to their chemical structure [4]. There is also the possibility of the formation of aromatic amines that are highly carcinogenic [5]. Cationic colors are in proportion to the toxic anionic treads [6]. Methylene blue is the most common dye which is used to color cotton, wool, and silk [7]. Exposure to methylene blue, which is a cationic color, increases heartbeat and cause shock, nausea, jaundice, and necrosis [3,8,9]. Though different methods such as biological methods, adsorption, and ion exchange are used for wastewater purification, these methods have many limitations due to high costs, poor performance, secondary pollution generation and sophisticated technology [3]. The use of green plants to biosynthesis of nanoparticles is an exciting and unknown possibility. Gold and silver nanoparticles have many biological applications because of their biocompatibility. Chemical methods typically lead to remain some toxic agents on nanoparticles. whereas synthesis of nanoparticles using plants do not have this problem, so in recent years the use of extracts of different parts of the plant such as root, stem, skin, leaf

Fruits, buds, and latex have attracted the attention of many researchers as stable and available sources for producing biocompatible nanoparticles [10-18]. In the process of synthesizing nanoparticles using plant extracts, herbal reducing agents play a major role. The main reaction in this process is oxidation and reduction. In this process, the herbal reducer, or the antioxidant present in the plant, produces silver

nanoparticles by giving electrons as a reducing agent. Silver atoms are converted into nanometric clusters with aggregation in water [19]. Synthesis of silver nanoparticles using various plant extracts is presented in many reports. Root of licorice [20], *Aloe vera* extract [21], *Centella asia* [22], Extract of cinnamon [23] and extract of *Phyllanthus emblica* [24-26] (Table 1).

Table 1. Comparison of green synthesis methods for silver nanoparticles production using different plant extracts.

Plants extract	Size (nm)	Plant's part	Shape	References
Leucas aspera	7-22	Leaves	Face-Centered Cubic	25
Hyptis suaveolens	5-25	Leaves	Face-Centered Cubic	25
Lantana camara L	average diameter 425	Leaves	Spherical	26
Butea monosperma bark	average dimension 35	Bark Plant	Face-Centered Cubic	27
Dragon Fruit	26.2±8.2, 25.7±8.7 & 25.3±7.9	Peel	spherical	28
Azadirachta indica aqueous	34	leaf	spherical	29
Megaphrynium macrostachyum		leaves	Face-Centered Cubic	30
Abutilon indicum	7-17	Leaves	Spherical	31
Ziziphora tenuior	8-40	Leaves	Spherical	32
Pistacia atlantica	10-50	Seeds	Spherical	33
Vitis vinifera	30-40	Fruit		34
Alternanthera dentate	50-100	Leaves	Spherical	35
Tribulus terrestris	16-28	Fruit	Spherical	36
Boerhaavia diffusa	25	Whole plant	Spherical	37
Cocous nucifera	22	Inflorescence	Spherical	38
Acorus calamus	31.83	Rhizome	Spherical	39
Psoralea corylifolia	100-110	Seeds	-	40
Ficus carica	13	Leaves	-	41
Argyreia nervosa	20-50	Seeds	-	42
Melia dubia	35	Leaves	Spherical	43
Brassica rapa	16.4	Leaves	-	44
Pogostemon benghalensis	>80	Leaves	-	45
Thevetia peruviana	10-30	Latex	Spherical	46
Thymbra spicataL	7-33	Whole plant	Spherical & face-centered cubic	This study



Figure 1. *Thymbra spicataL* medicinal herbs.

Thymbra spicata L. is one of the medicinal and aromatic species of Kermanshah province in Iran (Figure 1). *Thymbra Spicata L.* is an Aromatic plant from the family of mint that grows up to 40 centimeters and used as a disinfectant for the respiratory tract, tonic, suppresses muscular and spasms, excretion of parasite and appetizer which is widely used for therapeutic purposes. It also has antimicrobial, antifungal and antiparasitic properties. Lamiaceae family produce a variety of terpenoid and aromatic compounds and store them in epidermal glands, leaves, stems and natal organs. The main combination of aerial parts of this plant include p-cymene, γ -terpinene, carvacrol and thymol. The major combination of *Thymbra spicata*

L. is thymol; these compounds have active agent groups that can play an important role in the biosynthesis of nanoparticles [41-47]. Iran has a wide variety of plants, however, little research has been done to investigate the potential of *Thymbra spicata L.* in the production of nanoparticles. Proving the potential of producing silver nanoparticles using this plant could be an easy and affordable solution to produce silver nanoparticles. At the same time, shows another use of this plant. In the present study, in addition, to study the biosynthesis of nanoparticles using the aqueous extract of *Thymbra spicata L.* and investigate the effective parameters, the removal of methylene blue from aqueous solutions has also been investigated by these nanoparticles.

MATERIALS AND METHODS

Preparation of aqueous extract

First, 10 g of *Thymbra spicata L.* herb was disinfected 10 minutes with sodium hypochlorite 30% then washed 3 times with distilled water once a minute. The powder was then washed with 70%

alcohol for 2 minutes and finally washed 3 times with distilled water for 2 minutes anytime. Then, sterilized water was added 5 times the weight of the powder, and then heated to 85 °C for 30 minutes in a dark environment (in the Bain Marie). After 30 minutes, the extract was filtered after cooling with Whatman 40 filter paper and the subfiltration solution was used for the next steps (Figure 2).



Figure 2. *Thymbra spicata L.* herbal extract

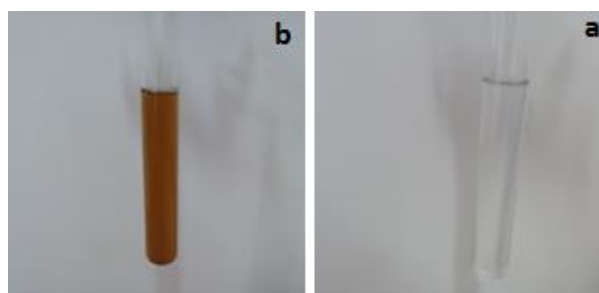


Figure 3. Herbal extracts. a) 0.01 M silver nitrate solution b) mixture of 0.01 M silver nitrate solution and *Thymbra spicata L.* extract.

Synthesis of silver nanoparticles

Silver nitrate solution (Merck Germany with a purity of 99.99%) with the concentration of 0.01 molar (Figure 3a). Inside a test tube, 0.5 ml of extract (30% v / v) was added and 5 ml of 0.01 N silver nitrate solution was added and incubated at room temperature. Silver nanoparticles formation was monitored by observing change in color of the mixture from light yellow to dark brown (Figure. 3b). The parameters influencing the synthesis of silver nanoparticles in order to find optimal conditions were studied by UV-Vis spectroscopy in the range of 200-800 nm. Extract volume (0.1, 0.2, 0.25, 0.5, 1, 1.5 and 2 ml), concentration of silver salts (0.5, 1, 1.5, 2, 3, 5 and 8 mM), temperature (25, 35, 45, 55, 65, 75 and 85 °C) and time (10, 20, 30, 40, 50, 60, 90, 120 and 180 minutes).

Influence of *Thymbra spicata L.* extract on the synthesis of silver nanoparticles

5 ml of 0.01 M silver nitrate was added into each of the seven Erlenmeyer. Then, 0.1, 0.2, 0.25, 0.5, 1, 1.5 and 2 ml of *Thymbra spicata L.* extracts were added to them, respectively. Then all of them were placed on the stirrer with the stirring rate of 150 rpm at room temperature for 40 minutes. After centrifugation the colloidal solutions, using UV spectroscopy and obtained spectra, the optimum volume of the extract was determined, And was considered for the next steps.

Investigating the effect of silver nitrate concentration on the synthesis of silver nanoparticles using *Thymbra spicata L.* extract

For studying the effect of silver nitrate concentration, 0.5, 1, 1.5, 2, 3, 5 and 8 mM of silver nitrate solution were prepared and 5 ml of each of them were added to 7 Erlenmeyer. The optimum

volume of *Thymbra spicata L.* extract was added to them, then were placed on the shaker with the stirring rate of the 150 rpm for 40 minutes at room temperature. The obtained solutions were centrifuged. UV spectra were taken from the solution in the wavelength range of 200-800 nm, and then the best concentration was used for further experiments.

Investigating the effect of temperature on the synthesis of silver nanoparticles using Thymbra spicata L. extract

According to the previous steps, the optimum temperature was measured. 5 ml of the optimized 1.5 mM silver nitrate solution was added to 7 Erlenmeyer then 2 µl of *Thymbra spicata L.* extract was added to them. Then all samples were heated at 25, 35, 45, 55, 65, 75 and 85 °C for 40 minutes. Subsequently, all samples were centrifuged and UV spectrum was recorded to examine the optimum temperature.

Investigating the effect of time on the synthesis of silver nanoparticles using Thymbra spicata L. extract

In order to achieve the best efficiency in the synthesis of silver nanoparticles, the effect of time was also studied in this work. In fact, determining the appropriate time for synthesis of silver nanoparticles is an important step because surface plasmon resonance increases or decreases over the time. 5 ml of 1.5 M silver nitrate solution was added to 9 Erlenmeyer and then 2 ml of *Thymbra spicata L.* extract was added to them. Then, all samples were placed in the incubator shaker for 10, 20, 30, 40, 50, 60, 90, 120 and 180 minutes at optimum temperature. After centrifugation, UV spectrum of the samples was recorded. Based on the results, the best time for synthesizing nanoparticles was determined.

Characterization of synthesized silver nanoparticles

As has been mentioned in previous studies, size and shape of nanoparticles, as well as the efficiency of these nanoparticles in removing dye, mainly depend on different factors such as volume of the extract, salt concentration, adsorbent dose, dye concentration, temperature and time [48,49]. In order to characterize the synthesized silver nanoparticles, Transmission electron microscopy (TEM), X-ray diffraction (XRD), Dynamic Light Scattering (DLS), Scanning electron microscopy (SEM), X-ray diffraction spectroscopy (EDX) Fourier Transform Infrared Spectroscopy (FT-IR)

were used. Silver nanoparticles were synthesized under following optimum conditions: volume of the extract: 2 µl, silver nitrate concentration: 1.5 mM, temperature: 60 °C, and time: 60 min.

Investigating the effect of synthesized nano silver on removing methylene blue

To evaluate the effect of adsorbent dose on removing methylene blue, 5, 8, 20, 40, 60, 80 and 100 mg of adsorbent were weighed and added to 7 Erlenmeyer. Then 10 ml of methylene blue with the concentration of 20 ppm was added to them. All Samples were placed in the incubator shaker (temperature: 25 °C and 150 rpm) for 20 minutes. Then each sample was centrifuged. Eventually, the best absorbent value was determined according to the UV analysis.

Investigating the effect of silver nanoparticle contact time on removing methylene blue

100 mg of adsorbent was added to 7 Erlenmeyer. Then, 10 ml of methylene blue (20 ppm) was added to each of them. After that for 5, 10, 20, 30, 40, 50 and 60 minutes they were placed on incubator shaker (temperature: 25 °C and 150 rpm). After the centrifugation, the optimum time was determined using UV spectroscopy.

Effect of methylene blue concentration on synthesized nano-adsorbent

For optimizing the methylene blue concentration 100 mg of silver nano-adsorbent was added to 7 Erlenmeyer. Then 10, 20, 30, 50, 80, 120 and 150 mg / L of methylene blue were added to each Erlenmeyer, respectively. After that, they were placed on the shaker (150 rpm) for 60 minutes. Subsequently, all samples were centrifuged and UV spectra were recorded according to the previous conditions. As a result, the optimum concentration of removed methylene blue by the adsorbent was determined.

RESULT AND DISCUSSION

Using UV-Vis spectra, the effects of various parameters that were optimized for the synthesis of silver nanoparticles using *Thymbra spicata L.* extract were investigated. Metal-based nanoparticles have a lot of free electrons that move with the conductivity and balance of the strips, and when exposed to UV light, they create a surface plasmon resonance, and this collision causes the vibration of free electrons in nanoparticles. Peak in the visible area at 430 nm and changes in solution color, confirms the formation of silver nanoparticles (optimal figures).

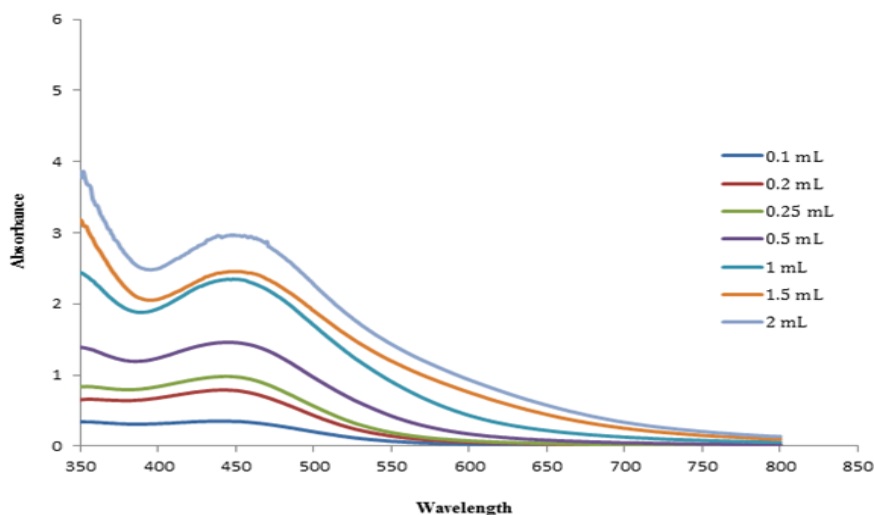


Figure 4. Influence of extract volume on silver nanoparticles synthesis. (AgNO_3 (0.01mM) =5 ml, $t=40\text{min}$, $T=\text{room}$ Tempretur, stirring rate=150 rpm)

Influence of Thymbra spicata L. volume

As shown in Figure 4, UV spectra became sharper by increasing the volume of *Thymbra spicata L.* extract. The surface Plasmon resonance appeared for all samples at 430 nm. This is because of increasing the volume of *Thymbra spicata L.* extract, concentration of groups and molecules such as tannins, phenolics, alkaloids and sugars, etc. increase[50,51]. In the solution therefore, reducing agents for silver ion and stabilization of synthesized silver nanoparticles increase, thus, according to Fig. 4, the optimal volume of *Thymbra spicata L.* was determined 2 ml.

Influence of metal salt concentration

According to Figure 5, results of UV spectra for the influence of metal salt concentration indicate that by increasing the concentration of metal salt, the surface plasmonic resonance spectra for all samples appear at 430 nm and silver nanoparticles become smaller and their size dispersion reduce. Similar results such as the effect of metal salt concentration on the formation of metal nanoparticles have been reported [52,53]. Based on observations the required concentration of silver nitrate was 1.5 mM.

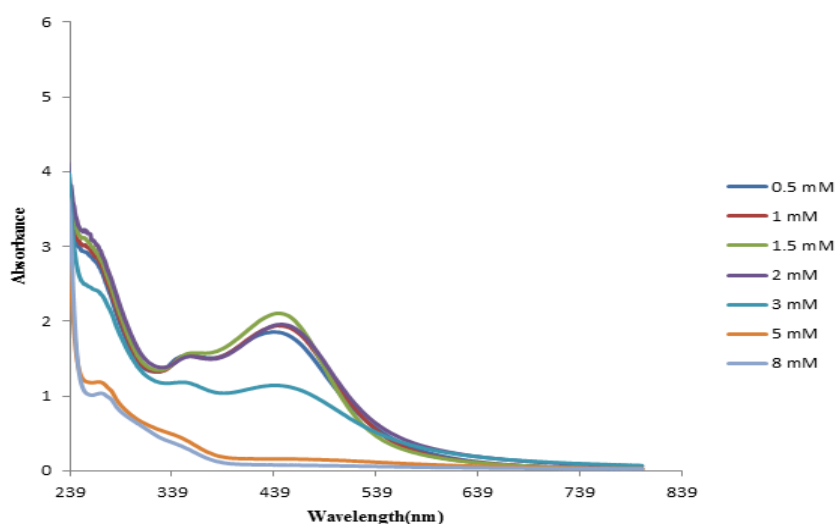


Figure. 5. Influence of silver nitrate concentration on silver nanoparticles synthesis. (Extract=2 μl , $t=40\text{min}$, $T=\text{room}$ temperature, 150 rpm)

Influence of temperature

UV spectra in Figure 6 show the effect of temperature on the synthesis of silver nanoparticles

using *Thymbra spicata L.* extract at different temperatures. It is clearly seen that by increasing reaction temperature, the surface plasmon

resonance spectra become sharper. All seven mentioned degrees show a similar response with a change in the observed maximum absorption. Similar results have been reported [52,54,55].

According to the results, 65 ° C was selected for this study.

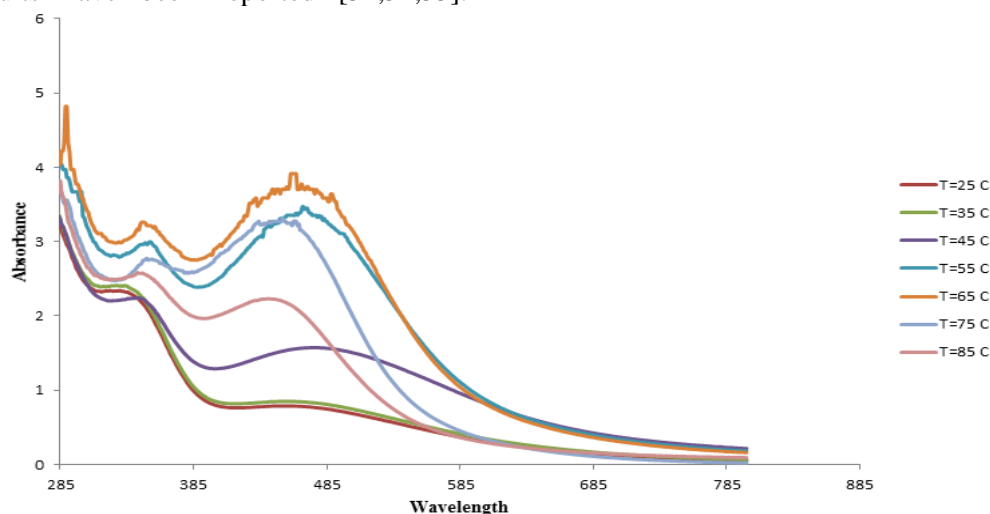


Figure. 6. Influence of temperature on silver nanoparticles synthesis. ($C_{AgNO_3}= 1.5mM, V_{Extract}=2\mu l, t=40min, 150rpm$)

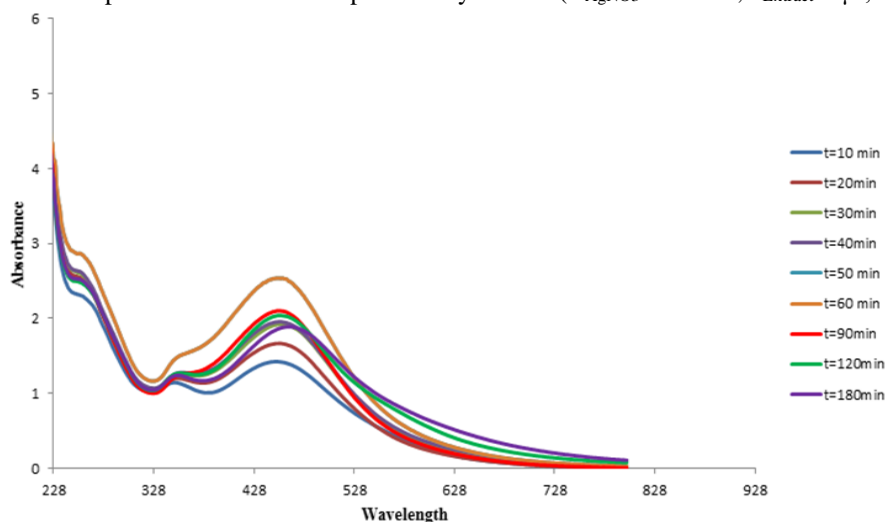


Figure. 7. Influence of time on silver nanoparticles synthesis. ($C_{AgNO_3}= 1.5mM, V_{Extract}=2\mu l, T=60\dot{C}, 150rpm$)

Influence of time

UV-Vis spectra related to the effect of time on the green synthesis of silver nanoparticles using *Thymbra spicata L.* extract is shown in Figure7. After 10 minutes, a small and weak peak appeared at 430 nm, indicating the formation of a small amount of silver nanoparticles. By increasing time from 10 minutes to 20, 30, 40, 50, 60, 90, 120 and 180 minutes, the absorbing bands become sharper and the maximum absorbing band appears after 60 minutes in the 430 nm region (Figure 7), which results from the formation of silver nanoparticles. As a result, the optimal time to improve the reaction was 60 minutes, which is similar to the previously reported in this area [56].

STUDYING THE EFFICIENCY OF METHYLENE BLUE ADSORPTION BY SYNTHESIZED NANO-ADSORBENT

Influence of adsorbent dosage

UV-Vis spectra (Figure 8) for the influence of metallic nano-adsorbent dosage show that with increasing the amount of silver nano-adsorbent, the maximum absorption for all samples appears at 665 nm. The results in the figure show that methylene blue removing is greatly increased by increasing the adsorbent dosage to 100 mg because when the adsorbent amount increase, more sites will be available for color absorption. Similar results such as the effect of nano-metal adsorbent dosage, on removal methylene blue have been reported [57-59]. The required dosage of synthesized nano silver

absorbent using *Thymbra spicata L.* extract for reaction was 100 mg.

The effect of contact time on the removal of methylene blue dye

Figure 9 shows the effect of contact time (5, 10, 20, 30, 40, 50 and 60 minutes) on adsorption capacity and methylene blue dye absorption by synthesized nano-silver adsorbent using *Thymbra*

spicata L. extract. Accordingly, the adsorption capacity and removal of methylene blue increased by increasing time. Based on UV-Vis spectra (Figure 9), after 60 minutes, the remained methylene blue of the solution was lower than the other solutions. Color of the solution also confirms the obtained result from the UV spectra. Similar results have been reported [57-59].

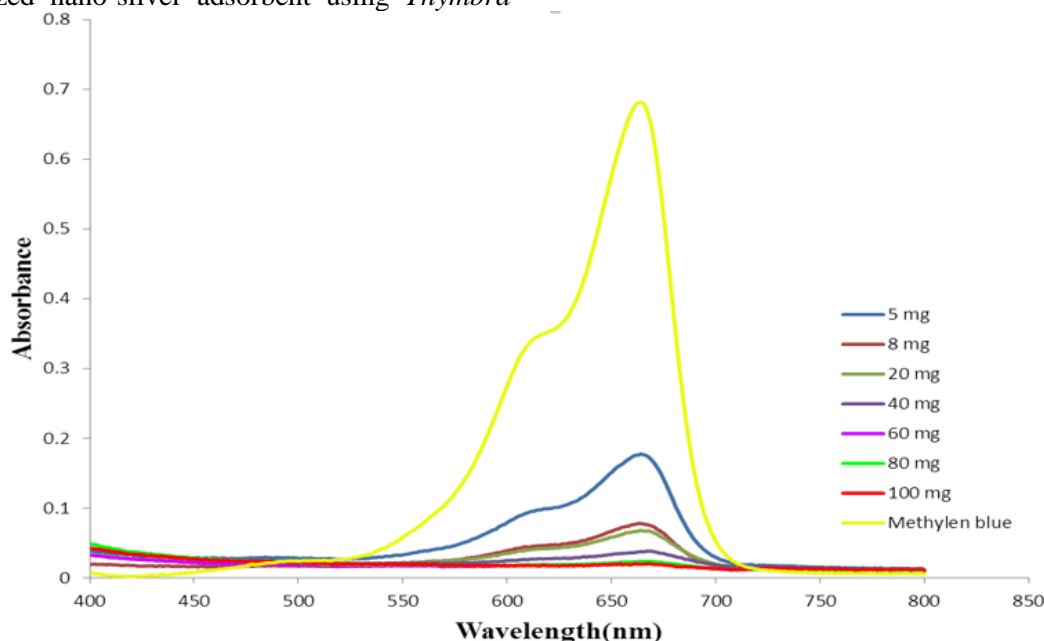


Figure 8. Influence of adsorbent dosage on methylene blue dye absorption by synthesized silver nano adsorbent using *Thymbra spicata L.* extract (10 ml of methylene blue (20 ppm), stirring time=20 min, centrifuging rate=150 rpm, T= room temperature)

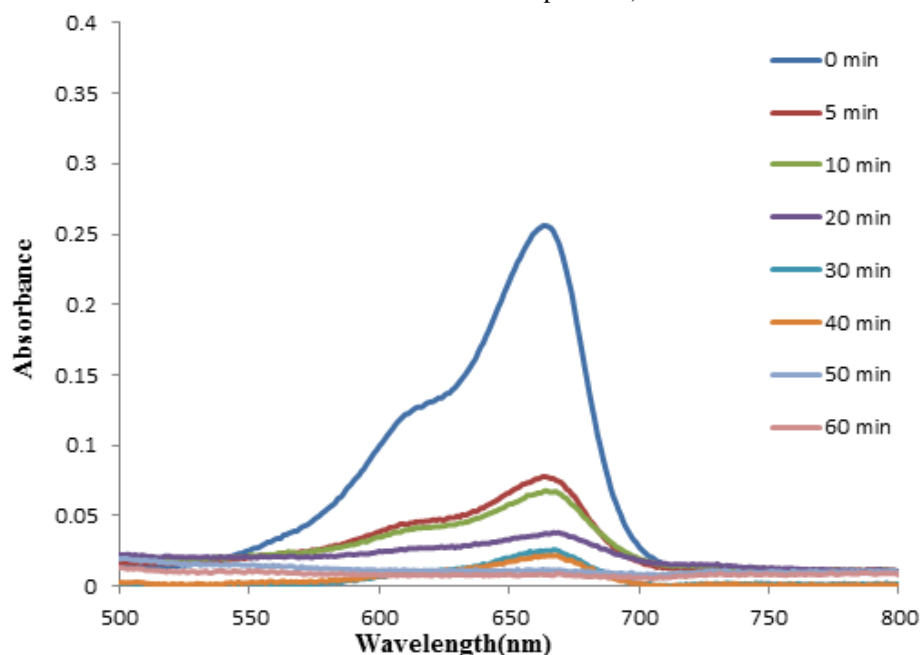


Figure 9. Influence of contact time on methylene blue dye absorption by synthesized silver nano adsorbent using *Thymbra spicata L.* extract (10 ml of methylene blue (20 ppm), adsorbent dosage =100 mg, centrifuging rate=150 rpm, T= room temperature)

Influence of methylene blue concentration on synthesized nano-adsorbent

The effect of initial concentration of methylene blue on its adsorption by synthesized nano silver using *Thymbra spicata* L. extract is shown in Figure.10. As shown in this figure, by increasing

the initial concentration of dye, the amount of absorbed dye by nano adsorbent of silver decreases (the adsorption rate increases), this is due to the blockage of the adsorbent sites, which reducing accessibility by increasing methylene blue concentration [57-59].

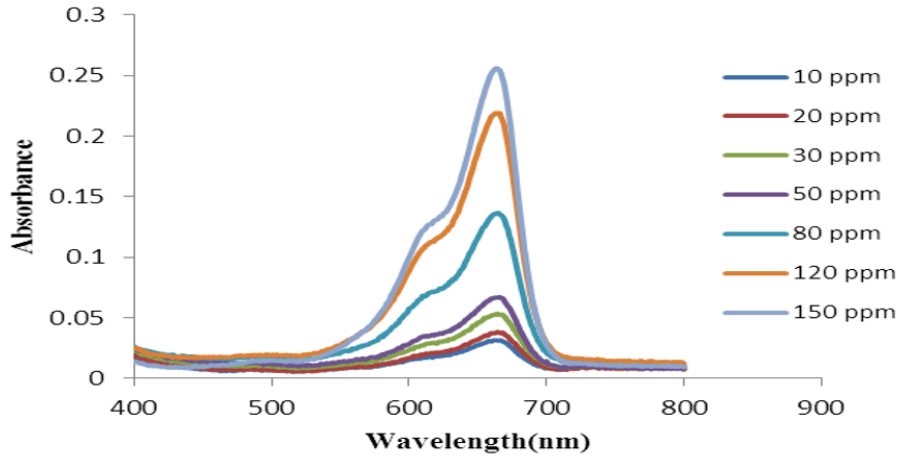


Figure 10. Influence of methylene blue concentration on absorption by synthesized nano silver adsorption using *Thymbra spicata*L extract (contact time=60 min, adsorbent dosage =100 mg, centrifuging rate=150 rpm, T= room temperature)

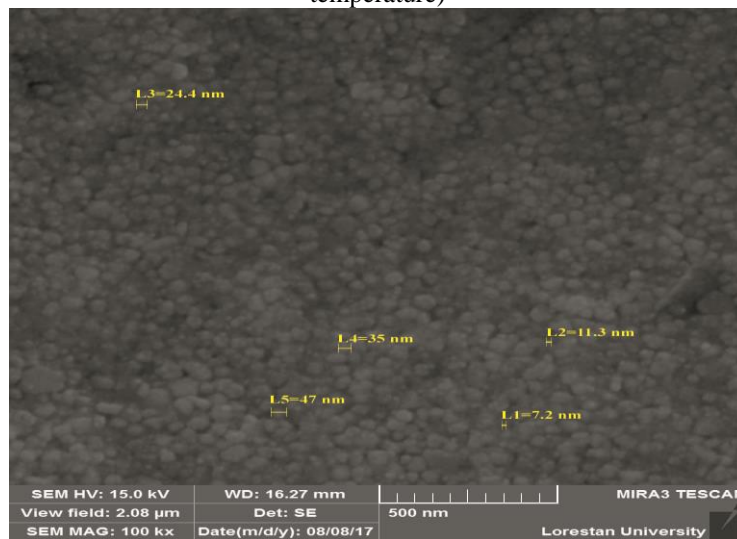


Figure 11. SEM image of AgNPs using *Thymbra spicata*L extract

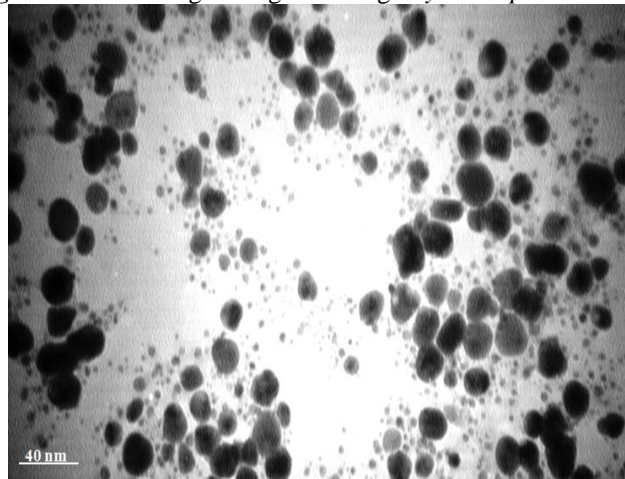


Figure. 12. TEM image of AgNPs using *Thymbra spicata*L extract

The maximum concentration of methylene blue, which was removed by nanosilver absorbent, was 150 ppm (similar to previous reports).

SEM analysis

Surface morphology of silver nanoparticles was investigated using SEM. In Figure 11, the SEM image clearly confirmed the formation of cubic silver nanoparticles with an average particle size of

about 20 nm. The results of SEM analysis are very similar to reports of other researchers [75,76].

TEM analysis

Figure 12 shows Transmission electron microscopy image of synthesized silver nanoparticles by applying all optimized conditions. According to the image of the nanoparticles, most of them are face-centered cubic (fcc) with the average size of 40 nm.

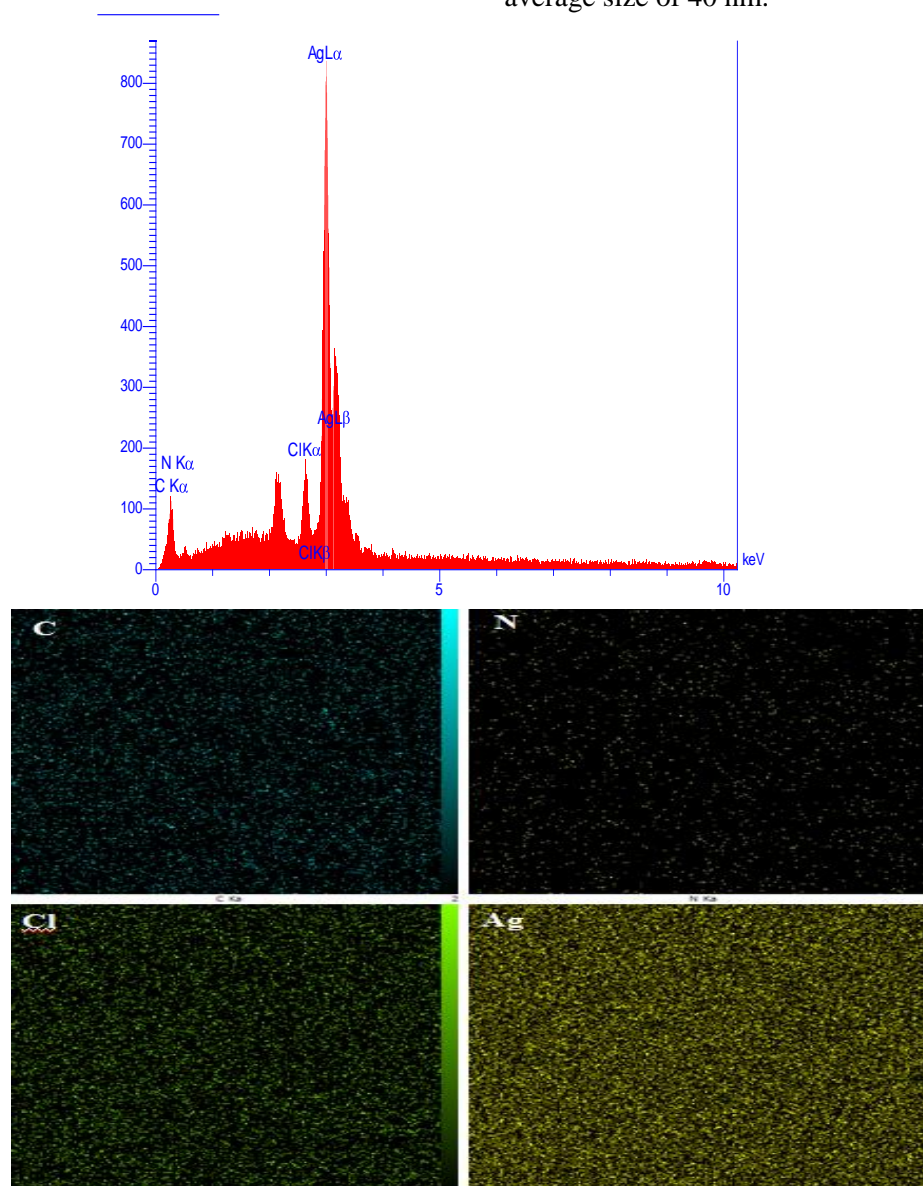


Figure 13. EDX images of AgNPs

EDX analysis

Energy-dispersive X-ray spectroscopy is used to show elemental analysis or chemical characterization of a sample. It is an elemental analysis that can be represented as a linear elemental analysis (on a hypothetical line in SEM image) or an elemental mapping (dispersion of the elements in the image) [60-62,27-40]. As shown in

Figure 13, the presence of silver particles and carbon atoms is significant, which is in good agreement with the purpose of this study.

XRD analysis

The crystalline structure of synthesized silver nanoparticles was examined using X-ray diffraction. As Figure 14 shows, Miller's index at the levels of (111), (200), (220) and (311)

correspond to angles 38.306, 44.396, 64.685 and 77.689, respectively, clearly confirms that the geometric shape of the synthesized nanoparticles is face-centered cubic[63-68]. Presence of sharp peaks in the pattern indicates a high degree of crystallinity of silver nanoparticles. The average size of crystalline grains was calculated using Debye-Scherrer equation (Eq 1):

$$D = 0.9\lambda/\beta\cos\Theta \quad (1)$$

Where D is the average size of crystalline particles, β : is the peak widths at half the maximum height, λ : is the X-ray wavelength. The average size of the synthesized crystalline particles was estimated about 31.21 nm using Debye-Scherrer's equation, which is corresponded with the results of the Transmission electron microscopy and UV-Vis spectroscopy (Table 2).

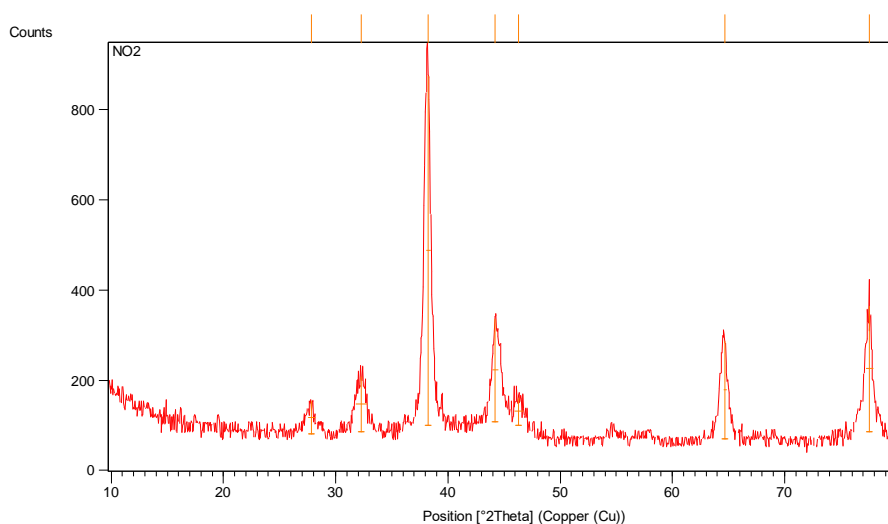


Figure 14. XRD image of AgNPs using *Thymbra spicata*L extract

Table 2. Precise details for calculating silver nanoparticles size

No.	Pos. [°2θ]	FWHM [°2θ]	hkl	d-spacing [Å]	Size (nm)
1	38.306540	0.246000	111	2.34973	32.38
2	44.396340	0.787200	200	2.04054	11
3	64.685940	0.590400	220	1.44104	15.93
4	77.689860	0.393600	311	1.22914	25.95
					Ave. = 21.3

FTIR analysis

Figure 15 shows FT-IR spectrum of *Thymbra spicata* L. extract and silver nanoparticles. FTIR spectroscopy was employed to identify the functional groups responsible for the biosynthesis of silver nanoparticles. FTIR spectra of *Thymbra spicata* L. (before reaction without AgNO₃) and synthesized silver nanoparticles (after reaction with AgNO₃) are illustrated in Fig. 9. It can be seen that both FTIR spectra showed a shift in the following peaks: 3414-3413 (due to O-H stretching of phenol or carboxylic acids), 2924-2923 (due to C-H stretching of alkane), 1618-1616 (due to C=C stretching of alkene), 1051-1070 (due to C-O stretching of phenolic compounds). In addition, *Thymbra spicata* L. showed peak at 1637 cm⁻¹

corresponding to C=O stretching of carbonyl group however this peak was absent in the synthesized silver nanoparticle. This reveals that carbonyl group of the *Thymbra spicata* L. is involved in the reduction of silver ions [69,70].

DLS analysis

Silver nanoparticles size distribution was determined using DLS (Dynamic Light Scattering) analysis.

The average particle size measured by DLS was 126.8 nm as shown in Figure 16. This may be due to the detection of minor amounts of large sized particles produced by agglomeration or contamination, which caused uncertainties in particle sizes. The obtained particle size using DLS analysis is higher than TEM analysis. This

differences in both analyses indicate that TEM purely measures a number based size distribution of the physical size and does not consist of any capping agent, but DLS measures the hydrodynamic diameter (diameter of the particle)

along with molecules or ions that are attached to the surface and moves with the AgNPs in solution therefore, these ions or other associated molecules create the particle and the particle size would be larger in DLS analysis [69-72].

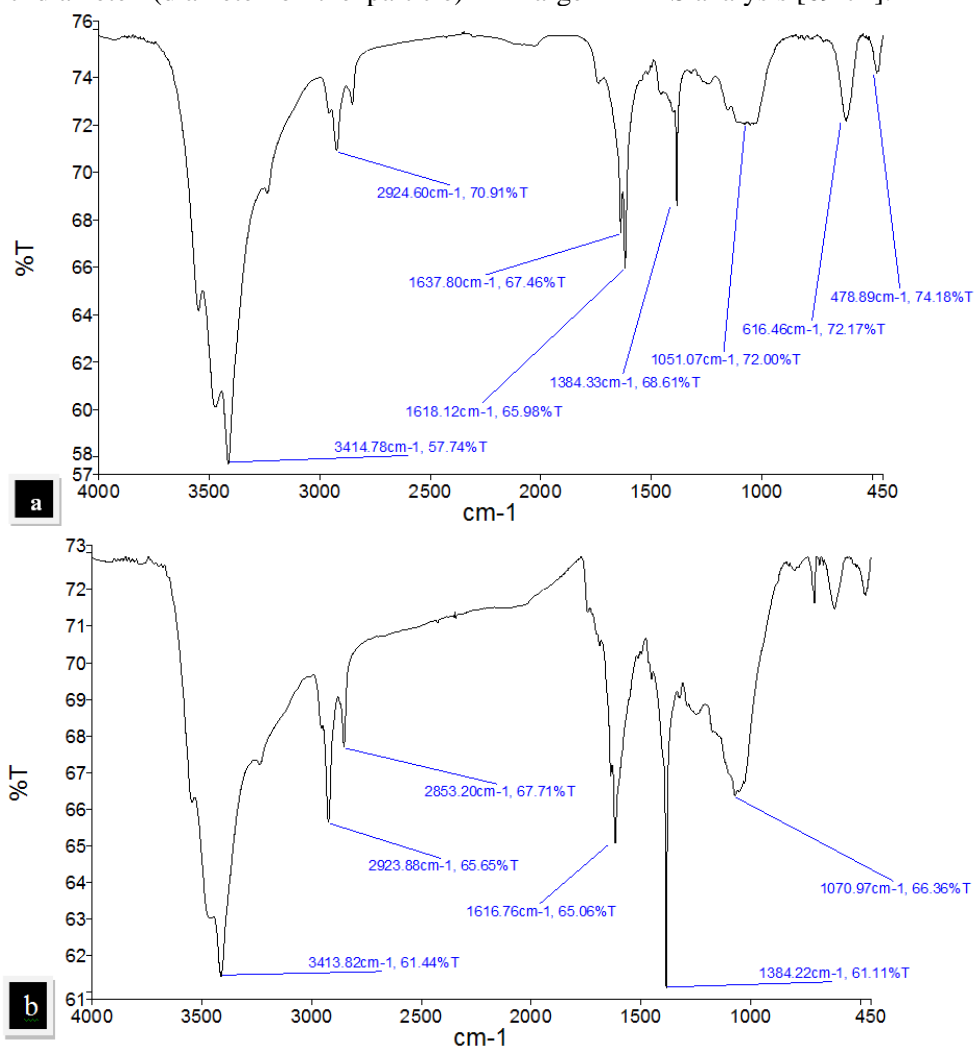


Figure 15. FTIR spectra of a) *Thymbra spicata L.* and b) AgNPs synthesized from the peel extract.

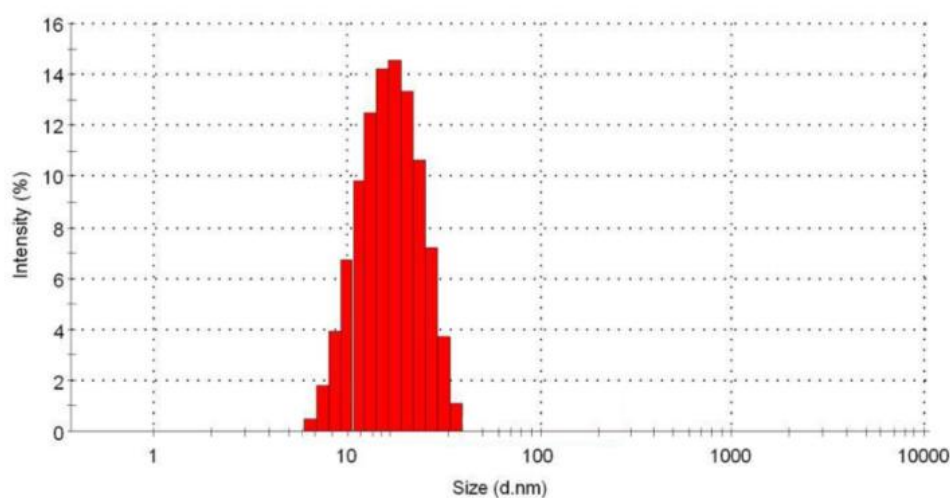


Figure 16. Diagram of treated silver nanoparticles size distribution with *Thymbra spicata L.* extract.

CONCLUSION

Today, preparation of biological nanoparticles is increasingly important due to their applications in medicine, biology, and wastewater purification processes. On the other hand, usage of environmentally friendly methods for production of non-toxic biological nanomaterials has become important, because of raising awareness of green chemistry and biological processes. Although various routes have been identified for biosynthesis of metal nanoparticles, the use of living organisms or other substances for metal nanoparticles production is expensive and limited. Therefore, facile biosynthesis of nanoparticles by controlling size and shape in the proposed methods is mainly important. The use of herbal substrates to prepare nanomaterials is a new methodology based on the principles of green chemistry. There are many plants that have potential to use for producing nanoparticles but they are still unknown. Many of these plants have not yet been tested and, due to the industries development, the need to produce nano compounds for a variety of commercial applications is increasing. Among nanoparticles, silver nanoparticles have many applications because of their unique properties and can be considered as commercial nanoparticles. Physicochemical synthesis of these nanoparticles is not attractive to consumers and researchers because of environmental incompatibility. Therefore, bio-synthesis methods have priority because of biocompatibility features. In this work, silver nanoparticles were synthesized using *Thymbra spicata* L. extract under optimal conditions (extract volume: 2 µl, silver nitrate concentration: 1.5 mM, temperature: 65 ° C and time 60 min). Synthesized nanoparticles were characterized and analyzed using different methods which include UV-vis, TEM, SEM, EDX, XRD, FT-IR and DLS. Spectra and images obtained from these techniques, confirmed that the synthesized nanoparticles have a size of about 22.31 nm with face-centered cubic crystal structure. FTIR spectrum confirmed the existence of reducing agents in the herbal extract. According to the results, *Thymbra spicata* L. extract has potential to synthesize nanoparticles, and since the use of this plant for silver ions bio reduction has not been reported, the results clearly show the good performance of this plant. So, for the first time, it can be said that this plant can be used as a living source for silver nanoparticles production, as well as it also proved to be effective in removing methylene blue dye. Therefore *Thymbra spicata* L., besides of its special pharmaceutical role, can be

used to produce silver nanoparticles for wastewater purification, medical and pharmaceutical purposes.

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