



Eco-Friendly Synthesis of Silver Nanoparticles Using Aqueous Extract of Ziziphus Spina-Christi Seeds: Characterization and Stability

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ABSTRACT

In this study, silver nanoparticles (Ag-NPs) were prepared using an aqueous extract of Ziziphus Spina-Christi Seeds (ZSCS) powder as an eco-friendly material, reducing agent and capping ligand. The synthesized Ag-NP was characterized using UV-visible absorption spectroscopy (UV-Vis), Fourier transform infrared (FTIR), transmission electron microscopy (TEM) and dynamic light scattering (DLS). From The UV-Vis spectra, the surface plasmon resonance (SPR) absorption band at 443 nm confirmed the formation of Ag-NPs. The TEM results demonstrated that the synthesized Ag-NPs have mostly spherical shapes with a particle size of 21.54 nm. In addition, the particle size and the specific surface area (SSA) of Ag-NPs decreased with increasing ZSCS powder extract volume and AgNO₃ concentration. The basic medium was found to be better than the acidic medium to prepare Ag-NPs. At a pH value equal to 9.5, within 30 minutes, the color of the solution was changed from colorless to brownish-orange. SPR absorption band of Ag-NPs demonstrated that the synthesized Ag-NPs have high stability over a period of 8 months. From FTIR results, the stretching of C-O group at 1223 and 1031 cm⁻¹ disappeared after bioreduction of AgNO₃, these results may be due to that Ag reduction was carried out by some hydroxyl groups that get oxidized at the expense of Ag⁺ because Ag⁺ is reduced to Ag-NPs.

:Ziziphus Spina-Christi السدر النباتي لبيدور نبات السدر تخليق صديق للبيئة لجسيمات الفضة النانوية باستخدام المستخلص المائي لبيدور نبات السدر التوصيف والثباتية

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الكلمات المفتاحية

التوصيف
الثباتية
التخليق الأخضر
جسيمات الفضة النانوية
بيدور نبات السدر

المخلص

في هذه الدراسة حضرت جسيمات الفضة النانوية باستخدام المستخلص المائي لمسحوق بيذور نبات السدر (*Ziziphus Spina-Christi* Seeds, ZSCS) كمادة صديقة للبيئة وكعامل مختزل وعامل تثبيت. جسيمات الفضة النانوية (Ag-NP) المخلقة شخضت باستخدام مطياف الأشعة المرئية والفوق بنفسجية (UV-Vis)، ومطياف تحويل فورير للأشعة تحت الحمراء (FTIR)، والمجهر الالكتروني النافذ (TEM)، وتقنية تشتت الضوء الديناميكي (DLS). حزمة الامتصاص السطحي البلازمي (SPR) عند الطول الموجي الاعظم 443 nm المقاسة بمطياف الأشعة المرئية - الفوق بنفسجية اكدت تشكل جسيمات الفضة النانوية. نتائج المجهر الالكتروني النافذ اظهرت ان لجسيمات الفضة النانوية المحضرة شكل كروي ومتوسط حجمها يساوي 21.54 nm. إضافة الى ذلك، فان حجم الجسيمات النانوية يقل بزيادة حجم المستخلص المائي وتركيز نترات الفضة. كما الوسط القاعدي أفضل من الوسط الحامضي لتحضير جسيمات الفضة النانوية. حيث انه عند اس هيدروجيني (pH) يساوي 9.5، تغير لون المحلول في غضون 30 دقيقة من عديم اللون الى لون بني-مصفر. أظهرت حزم الامتصاص السطحي البلازمي ان لجسيمات الفضة النانوية المخلقة ثبات عالٍ على مدار فترة تتجاوز 8 أشهر. ومن نتائج FTIR، نلاحظ اختفاء تمدد مجموعة C-O عند حزم امتصاص 1223

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و 1031 سم¹ بعد الاختزال الحيوي لـ AgNO₃، وقد يرجع السبب في ذلك إلى ان اختزال ايونات الفضة (Ag⁺) تم بواسطة بعض مجموعات الهيدروكسيل التي تتأكسد على حساب Ag⁺ الذي بدوره يختزل إلى جسيمات الفضة النانوية (Ag-NP).

1. Introduction

“Nano” is common international system prefix means 10⁻⁹ of any unit, thus nanoparticles (NPs) is any particles on a nanoscale. Thus, nanotechnology is any technology used nanoparticles [1]. The characteristics of NP can be tuned by their sizes and shapes [2]. The noble metal-based nanoparticles such as silver (Ag-NPs), gold (Au-NPs) and platinum (Pt-NPs) have attracted great attention because of their surface, catalytic, optical, magnetic and electric properties. These properties making them desirable materials for the production of important commodities to human being such as shampoos, soaps, detergent, shoes cosmetics, toothpastes, and pharmaceuticals. Hence, approximately 3000 consumer products contain nanoparticles [3, 4]. Nanobiotechnology is eco-friendly technology used for nanoparticles preparation (i.e., green synthesis) using simple material and cost-effective (e.g., plant extract) [5, 6]. On the literature, many studies have been conducted on using plants extract to synthesize silver nanoparticles (Ag-NPs) such as *Gymnema Sylvestre* leaf extract [7], Umbrella plant [8], *Pistacia Atlantica* leaf extract [9], Cavendish Banana peel extract [3], *Eulophia Herbacea* (Lindl.) Tuber extract [10], *Mimosa Albida* (Mimosoideae) [11]. In 2015, the *Ziziphus spina-christi* leaf extract was used as reducing and capping agents for silver nanoparticles preparation [12]. To the best of author’s knowledge, no report was found on the literature on using the extract of *Ziziphus Spina-Christi* Seeds (ZSCS) powder for Ag-NPs synthesis. In this study, the extract of *Ziziphus Spina-Christi* Seeds (ZSCS) powder was used as reducing and stabilizing agents for silver nanoparticles (Ag-NPs) synthesis. In addition, different experimental conditions were applied in the synthesis of Ag-NPs such as quantity of ZSCS extract, concentration of silver nitrate AgNO₃, temperature, pH and ultrasonic radiation.

2. Materials and Methods

2.1. Materials and instrument

Ziziphus Spina-Christi Seeds was collected from local market located in Sebha city, Libya (Fig. 1). Silver nitrate, AgNO₃ (chemPUR), nitric acid, HNO₃ (69,5% - RPE), ammonium hydroxide NH₄OH (chemPUR). UV-Visible spectroscopy (evaluation 3000, ThermoVirision). Transmission Electron Microscope, TEM (Bilkent university in Turkey). Dynamic light scattering, DLS, (Bilkent university in Turkey). FTIR spectrophotometer (BRUKER). pH meter (Orion 3 star) and ultrasonic (Midmark M150soniclean).

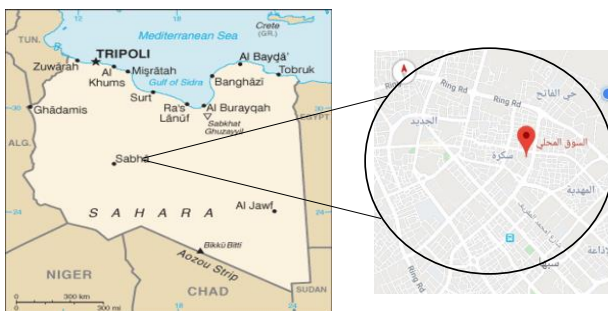


Fig. 1: Site of *Ziziphus Spina-Christi* Seeds collection

2.1.Preparation of ZSCS extract

The collected *Ziziphus Spina-Christi* Seeds were washed several times with distilled water to remove all the dirt particles. Then, the materials were left on clean paper to dry under room temperature. About 25 g of seed powder was transferred into a 250 mL beaker containing 70 mL double distilled water and then the mixture was stirred for 5 h at speed 400 rpm before being filtered using a filter paper.

2.2.Preparation of silver nanoparticles (Ag-NPs) using aqueous extract of ZSCS

To study the effect of AgNO₃ concentration on Ag-NPs synthesis, different concentrations of AgNO₃ solution (0.2, 0.4, 0.8, and 1mM) were added into flasks containing 5 mL of ZSCS extract. The mixture was then stirred at speed of 400 rpm and the UV-Vis spectra were recorded after the solution color was changed to indicate the formation of Ag-NPs. To study the effect of ZSCS extract solution volume on Ag-NPs synthesis, different volumes of ZSCS extract solutions (1, 2, 3, 4, 5 mL) were added to flasks containing 100 mL of AgNO₃ aqueous solution at concentration of 1 mM. The mixed solutions were stirred at 400 rpm and their UV-vis spectra were recorded after the solutions color were changed to indicate Ag-NPs formation. The effect of solution pH on Ag-NPs synthesis was studied at different pH values (2.52, 3.71, 5.4, 9.5 and 11.5) and following the above mentioned experimental conditions [13]. In addition, the stability of the prepared Ag-NPs was studied under ultrasonic (US) radiation at different times. The effect of US on Ag-NPs stability was measure by the change in surface plasmon resonance (SPR) absorption band of Ag-NPs before and after Ag-NPs solution irradiation for 1 h. Stability of Ag-NPs with time can be study by two methods. In the first one, Ag-NPs stability can be studied by measuring the change in SPR absorption band with time, the second by measuring the relative absorbance [14]. In all experiments, the synthesized Ag-NPs sizes were estimated according to Mie scattering theory using the full width at high maxima (FWHM) of SPR band peak as in eq 1 [15].

$$FWHM = \frac{(\epsilon_0 + 2n^2)c.m.u_F}{N_c e^2.D} \quad (1)$$

Where, ϵ_0 , n , m , N_c , and D are the frequency independent part of complex form of the dielectric constant, refractive index of water, the velocity of light, mass of electron, electron velocity at the Fermi energy, number of electrons per unit volume, the electron charge and diameter of the particle respectively. Diameter of Ag-NPs was used for the determination of the specific surface area, (SSA) of Ag-NPs according eq 2.

$$SSA = \frac{6}{D \times \rho} \quad (2)$$

Where, D is the particle size, SSA is the specific surface area and ρ is the theoretical density of silver (10.49 g/cm³ or 1.049 × 10⁷ g/m³) [16].

3. Results and Discussion

3.1. Characterization of silver nanoparticles

3.1.1. TEM and DLS analysis

Fig. 2 a and b showed TEM image and particles size distribution of Ag-NPs, respectively. As can be seen from the TEM image, the synthesized Ag-NPs were found to be uniformly dispersed and mostly spherical in shape with an average size of 21.54 nm. The dynamic light scattering (DLS) analysis showed that the synthesized Ag-NPs exhibited an average size of 149.1 nm, as shown in Fig. 3. It is to be noted that there is difference in the estimated size of the prepared Ag-NPs TEM, DLS and UV-Vis spectrum. In fact, by the specificity of each techniques, the TEM images gives the size of nanoparticles in dried form, while DLS tells the hydrodynamic diameter that includes core plus any molecule attached or adsorbed on surface [17] and the UV-Vis spectrum gives the size of NPs based on the quantity of irradiation scattered by polarizable Ag-NPs. Therefore, measuring the exact Ag-NPs sizes based on UV-Vis spectrum and Mie theory are more accurate [18, 19].

3.1.2. Effect of AgNO₃ concentration

Fig. 4 showed mono SPR symmetric absorption peak for the Ag-NPs synthesized at different concentrations of AgNO₃. This may be due to that Ag-NPs had a spherical shape [20]. As shown, the SPR absorption band increased with increasing AgNO₃ concentration from 0.2 to 1.0 mM. This could be due to the formation of more Ag-NPs at high initial concentration of Ag⁺ ions [13]. The size of Ag-NPs was calculated using eq 1, and to be 16.43, 8.52, 8.02, 7.83 nm for AgNO₃ concentration of 0.2, 0.4, 0.8 and 1.0 mM, respectively. It was noted

that the particles size was decreased with increasing the concentration of AgNO₃ from 0.2 to 1.0 mM. This was due to the potential of ZSCS extract to reduce Ag⁺ and protect the formed nanoparticles was maximized at low AgNO₃ concentration. In addition, when AgNO₃ concentration increases the potential of ZSCS extract to reduce Ag⁺ decreases and the blue shift of the SPR band from 461 to 443 nm occurred as AgNO₃ concentration decreased from 1 to 0.2 mM which might be due to the decrease in the size of Ag-NPs at high AgNO₃ concentration [21]. λ_{max} of SPR and Ag-NPs size are listed in Table 1.

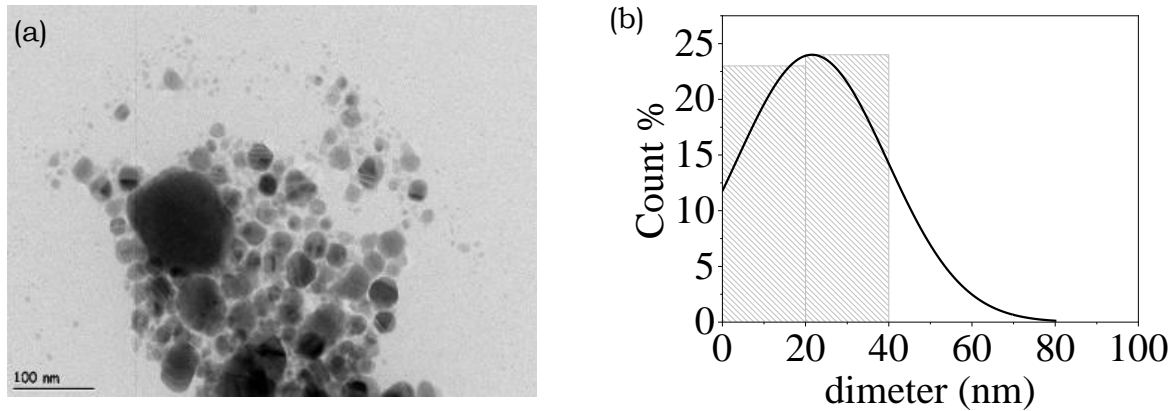


Fig. 2: (a) TEM image; (b) particles size distribution of the Ag-NPs synthesized

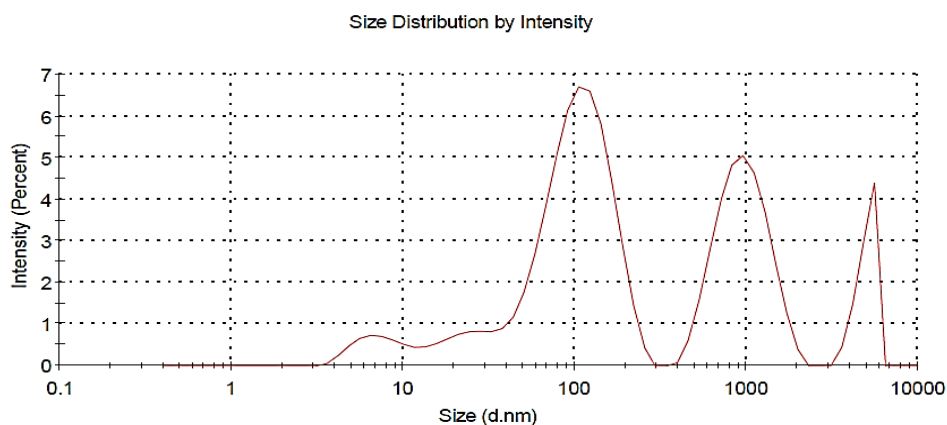


Fig. 3: DLS of the synthesized Ag-NPs

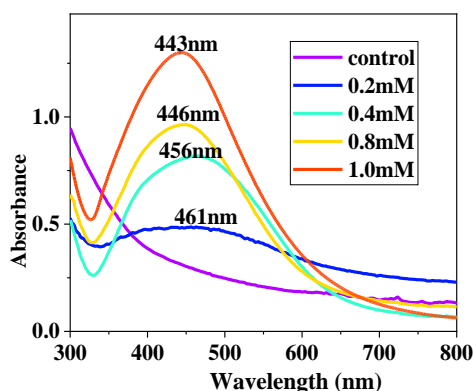


Fig. 4: UV-Visible spectra for the Ag-NPs prepared with 5mL ZSCS with various AgNO₃ concentration

Table 1. Size in nanometre, SSA (m²/g) and SPR λ_{max} of Ag-NPs prepared with 5 mL ZSCS powder extract at different concentration of AgNO₃

AgNO ₃	FWHM	D	r	SSA	λ_{max}
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concentration (mM)	(nm)	(nm)	(nm)	(m ² /g)	(m ² /g)
0.2	424.36	16.425	8.212	34.823	456
0.4	221.48	8.518	4.259	67.148	461
0.8	207.69	8.023	4.011	71.291	446
1.0	202.96	7.833	3.916	73.014	443

3.1.3. Effect of ZSCS extract concentration on Ag-NPs synthesis

Fig. 5 showed the mono SPR symmetric absorption peak at 403, 441, 428 and 427 nm for the Ag-NPs synthesized using 1 mM silver nitrate and 1 to 5 mL of ZSCS solution. The SPR of synthesized Ag-NPs was noted to be spherical in shape. It is clear that the SPR absorption band increased with increasing concentration of ZSCS powder extract from 1 to 5 mL. This could be due to the formation of more Ag-NPs because of high concentration of ZSCS powder extract. It is clear that when ZSCS extract volume was increased from 1 to 5 mL, the particles size decreased from 9.77 to 5.75 nm. This could be due to the decrease in the concentration of monomer in the reaction medium. At low extract volume, the concentration of the monomer in the solution is high [22]. The size and SPR of Ag-NPs prepared using 1 mM of AgNO₃ and different concentrations of ZSCS powder extract are listed in Table 2.

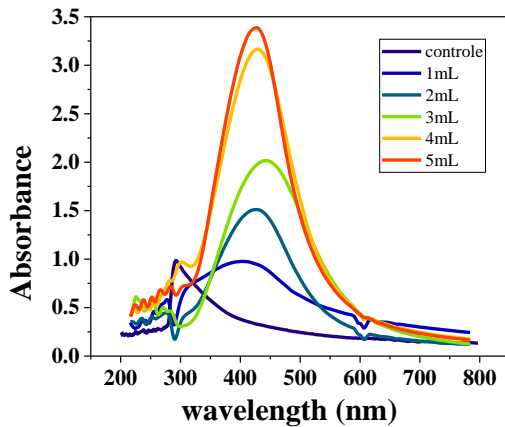


Fig. 5: UV-Visible spectra for the Ag-NPs prepared with 1 mM aqueous AgNO_3 solution with various ZSCS extract volume

Table 2. size and SPR λ_{max} of Ag-NPs prepared with 1mM of AgNO_3 at different concentration ZSCS extract

ZSCS extract volume (mL)	FWHM (nm)	D (nm)	r (nm)	SSA (m^2/g)	λ_{max}
1	281.25	9.7	4.8	58.5	402
2	156.77	5.9	2.9	95.4	426
3	172.42	6.2	3.1	91.4	441
4	147.70	5.7	2.8	99.3	428
5	135.46	5.3	2.6	107.7	427

3.1.4. Effect of pH

Fig. 6 directed the effect of the solution pH (2.52, 3.71, 5.40, 9.50 and 11.50) on Ag-NPs synthesis. As shown, the formation of Ag-NPs was founded to depend on the pH of the reaction medium. Also, the process of Ag-NPs synthesis was very fast in the basic medium than acidic medium, and at pH value of 9.50 the colour of the solution was changed from colourless to brownish-orange within 30 min. This may be due to the ionization of the phenolic group present in the ZSCS extract [13]. Besides, the symmetric absorption peaks were observed at 424, 428, 442, 426 and 440 nm for the Ag-NPs synthesized at pH values of 2.52, 3.71, 5.40, 9.50 and 11.50, respectively. Based on SPR, the synthesized Ag-NPs exhibited spherical shape with particles size equal to 10.99, 15.68, 9.32, 12.93 and 5.41 nm for pH 2.54, 3.71, 5.40, 9.50 and 11.50, respectively. This means that the size of Ag-NPs can be controlled by controlling the solution pH [23].

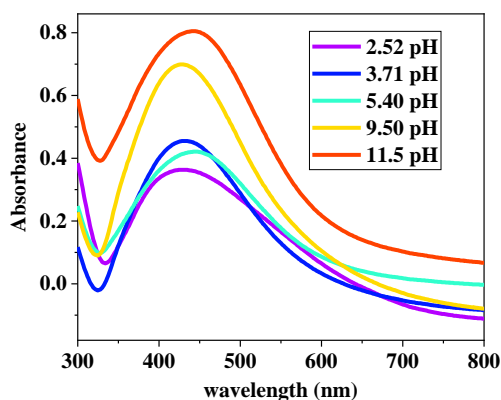


Fig. 6. UV-Vis spectra for Ag-NPs prepared using AgNO_3 (1 mM) and 5 mL ZSCS extract at various pH values at 25 °C

Table 3. Size and SPR λ_{max} of Ag-NPs prepared using AgNO_3 (1 mM) and 5 mL of ZSCS extract at different pH values and 25 °C

pH	FWHM (nm)	D (nm)	r (nm)	SSA (m^2/g)	λ_{max}
2.52	179.87	6.52	3.259	87.75	424
3.71	150.87	5.79	2.895	98.78	428

5.40	177.60	6.89	3.443	83.05	442
9.50	163.97	6.17	3.086	92.65	426
11.50	224.50	8.26	4.128	69.28	440

3.1.5. Effect of ultrasonic irradiation on synthesized Ag-NPs

Fig. 7 showed the effect of the ultrasonic (US) irradiation on the synthesis of Ag-NPs. It is clear that the SPR absorption band was increased with US concentrations of AgNO_3 , and no change in the SPR absorption band was observed for the synthesized Ag-NPs using AgNO_3 concentration of 0.8 and 1 mM. This increase in the SPR absorption band is due to the layer of Ag^0 was lost from the surface of the Ag-NPs over US irradiation. But, the SPR absorption band Ag-NPs solution before and after US irradiation when the sample was prepared using 1 mM of AgNO_3 was semi equivalent. This may be due to the layer of Ag^0 was not lost from the surface of the Ag-NPs over US irradiation. In addition, the SPR absorbance band was around (461 to 443 nm) before US irradiation and was around (404 to 449 nm) after US irradiation for synthesized Ag-NPs using AgNO_3 concentration of 0.2, 0.4, 0.8 and 1.0 mM, respectively. The blue shift of the SPR band occurred when the US irradiation was applied. Furthermore, the particles size decreased even to smaller sizes after ultrasonic irradiation [24].

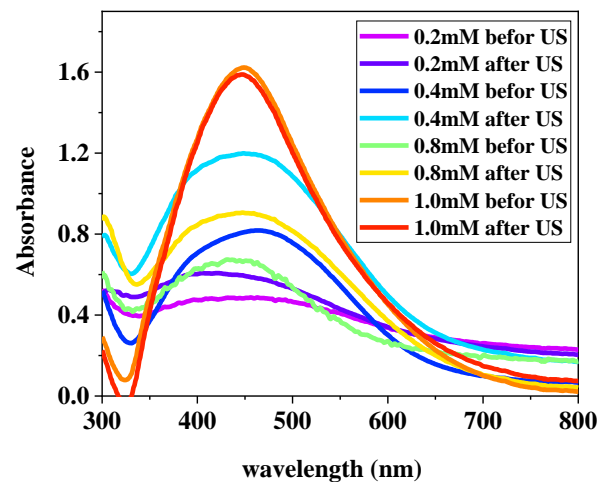


Fig. 7: UV-Visible spectra for the Ag-NPs prepared with 5 mL ZSCS with various $[\text{AgNO}_3]$, before and after US irradiation

Table 4. Effect of US irradiation on Ag-NPs prepared with 5mL ZSCS powder extract at different concentration of AgNO_3

AgNO_3 (mM)	Before US irradiation			After US irradiation		
	FWHM (nm)	D (nm)	SSA (m^2/g)	FWHM (nm)	D (nm)	SSA (m^2/g)
0.2	392.00	17.33	33.00	329.75	12.76	44.84
0.4	265.06	10.26	55.75	251.53	9.74	58.75
0.8	238.13	9.23	62.07	256.57	9.93	57.60
1.0	176.51	6.83	83.72	176.52	6.83	83.72

3.1.6. Effect of time on Ag-NPs synthesized

Fig. 8 showed the SPR absorption band and stability of Ag-NPs with time. It is clear that the SPR absorption band and relative absorbance were increased with time. This could not be due to water evaporation because the samples were closed. Thus, the increase in the SPR band and relative absorbance was due to the layer of Ag^0 which was lost from the surface of the Ag-NPs over a period of 8 month [4]. Fig. 9 showed that the relative absorption of prepared Ag-NPs using AgNO_3 concentration of 0.2 mM was nearly constant while the relative absorption of prepared Ag-NPs at AgNO_3 concentration of 1.0 mM was changed. Besides, the Ag-NPs prepared at AgNO_3 concentration of 0.2 mM was more stable than those prepared at concentration of 1.0 mM. Also, the size of Ag-NPs prepared at AgNO_3 concentration of 0.2 and 1.0 mM were approximately 16.43 and 7.83 nm, respectively. The

stability of Ag-NPs prepared at AgNO₃ concentration of 0.2 mM compared to those prepared at Ag-NPs prepared at AgNO₃ concentration of 1.0 mM is due to their size and Ag-NPs with diameters more than 10 nm have chemical inertness, whereas noble metal-based NPs with small diameters of 2 to 4 nm oxidize more easily and resulted in decreasing the NPs stability [25].

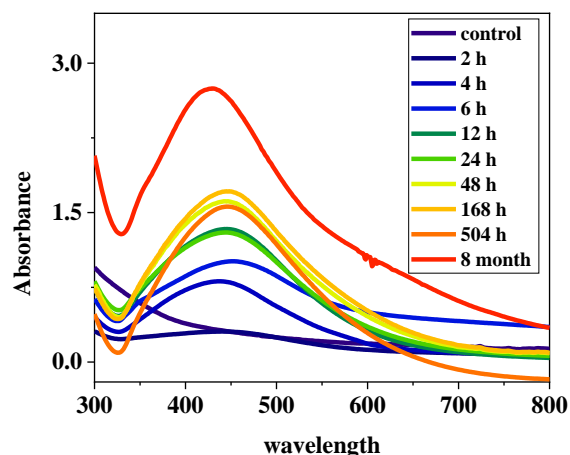


Fig. 8: changes in absorbance of Ag-NPs obtained from 1mM AgNO₃ and 5mL of ZSCS extract over time

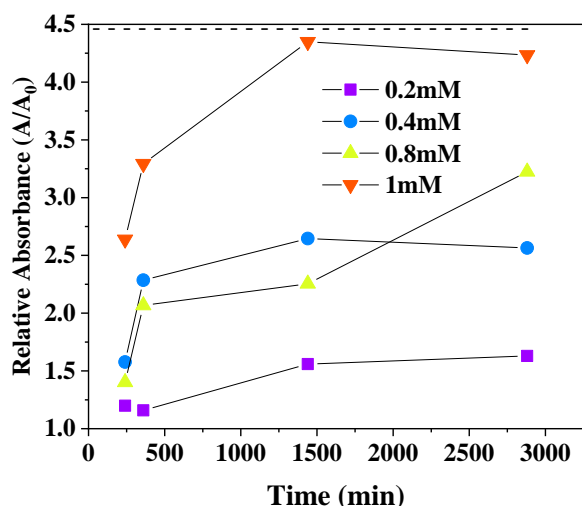


Fig. 9: Stability of Ag-NPs obtained from various concentrations of AgNO₃ and 5 mL of ZSCS extract over time

3.1.7. FTIR analysis

Fig. 10 showed the FTIR spectra for ZSCS extract and Ag-NPs-ZSCS extract, which represent functional groups present in the ZSCS extract and the prepared Ag-NPs. The function groups on ZSCS extract was stretching of the O-H or N-H group at 3368 cm⁻¹, stretching of C-O group at 1223 and 1031 cm⁻¹ of alcohols, carboxylic acids, ester and ether groups. the stretching of C-O group at 1223 and 1031 cm⁻¹ are disappeared after bio reduction of AgNO₃, this results may be due that the reduction of Ag was carried out by some hydroxyl groups that get oxidized at the expense of Ag because Ag is reduced Ag-NPs [26].

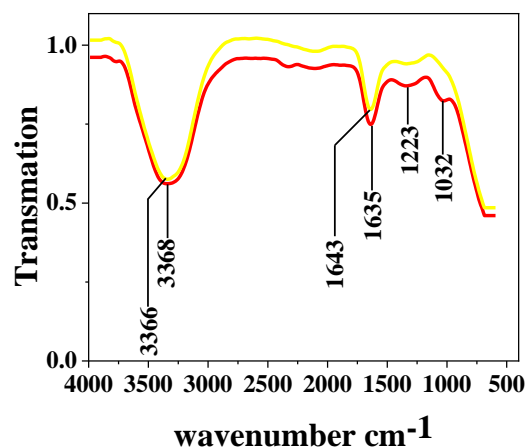


Fig. 10: FT-IR spectra for ZSCS extract (red line) and ZSCS extract-Ag-NPs (yellow line)

4. Conclusion

In summary, the potential of using the aqueous extract of ZSCS for the green synthesis of Ag-NPs was successfully explored. The synthesized Ag-NPs exhibited spherical shape with an average particles size of 21.54 nm. Besides, the particles size of the prepared Ag-NPs was decreased with increasing ZSCS extract volume and AgNO₃ concentration. The process of Ag-NPs synthesis was very fast in basic medium than acidic medium due to the ionization of the phenolic group present in the ZSCS extract in basic medium. The stretching of C-O group at 1223 and 1031 cm⁻¹ are disappeared after bio reduction of AgNO₃, these results may be due that the reduction of Ag was carried out by some hydroxyl groups that get oxidized at the expense of Ag because Ag is reduced Ag-NPs. The obtained results demonstrated that the synthesis of silver nanoparticles using plant extract (e.g., ZSCS extract) is a promising, low-cost and eco-friendly green synthesis method. Also, the prepared Ag-NPs are a candidate material that can be used in antibacterial and dye photodegradation applications.

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