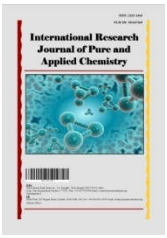

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Green Synthesis of Silver Nanoparticles Using Some Medicinal Plants

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Authors' contributions

This work was carried out in collaboration among all authors. Author JJ performed the experiments and wrote the first draft of the manuscript. Author WA put the protocols for synthesis of silver nanoparticles, contributed to the interpretation of data and revised the manuscript. Author AK managed the project in all stages and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The green synthesis of silver nanoparticles in an eco-friendly, economical and more effective approach using (*Acacia cyanophylla, Phlomis syriaca* and *Scolymus hispanicus*) plants extracts and describing their main chemical properties and study the effect of its chemical composition on producing silver nanoparticles.
Methodology: In this study, aqueous and ethanolic extracts of the three plants were evaluated for antioxidant activity using 2,2-diphenyl-l-picrylhydrazyl (DPPH) assay, Total polyphenol and flavonoid contents were determined using spectrophotometric method, but total saponins were determined by weight method, The synthesis of silver nanoparticles was performed by a reduction method using aqueous silver nitrate solution and aqueous extracts of the three plants. Then study its characterization in a number of ways, such as visual inspection, UV-Vis spectroscopy and dynamic light scattering.
Results: The results showed that the total phenolic content ranged in extracts between (13.08 ±2.279 to 98.39 ±4.755 mg GAE/g DW). While the total flavonoid contents varied from (19.83

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±2.384 to 121.64 ±6.469 mg RE/g DW. Antioxidant activity was expressed as IC50 and the obtained results ranged from (IC_{50}= 0.027 ±0.00038 to 0.878 ±0.045 mg/ml), the results indicated that the ethanolic *Acacia cyanophylla* extract from the six examined extracts showed the highest phenolic and flavonoid concentration and strong antioxidant activity. Also, the saponins content in the three plants ranged from (0.46 to 2.53)% and the highest amount of saponins reported in *Acacia cyanophylla* plant. The silver nanoparticles prepared using Acacia cyanophylla extract have reported visible yellowish brown color formation and the absorption peak at 460 nm indicates the biosynthesis of silver nanoparticles and they have average diameter (134.1) nm and the polydispersity index (PdI) was suitable (0.260).

Conclusion: *Acacia cyanophylla* extract has been considered as the best reducing agent among the selected plant extracts for the preparation of stable colloidal silver nanoparticles, this is due to their high content of flavonoids, phenols and saponins.

Keywords: Silver nanoparticles; green synthesis; antioxidant; total phenolic content; total flavonoids content; Acacia cyanophylla; Phlomis syriaca; Scolymus hispanicus.

1. INTRODUCTION

Nanotechnology is an important field in modern science dealing with manufacturing, with particle sizes ranging from approximately 1 to 100 nanometers. It has revolutionized a large number of fields such as healthcare, cosmetics, biomedicine, food, drug-gene delivery and the environment [1]. Metallic nanoparticles, especially the noble metals, have attracted scientists' attention due to their unique properties. Metallic nanoparticles possess distinct properties due to their distinctive physical and chemical properties such as strong optoelectronic, thermal and catalytic properties, and a high surface volume ratio [2]. And their easy to synthesis in a controlled morphology and excellent crystallization. Among the minerals nanoparticles are silver nanoparticles (Ag NPs) [3]. Ag NPs have many biomedical applications such as wound dressings, topical creams and ointments for burns, disinfectant sprays and fabrics [4], and biological applications such as antimicrobial, tissue engineering, DNA detection and drug delivery agents [3]. There are a lot of methods for the synthesis of silver nanoparticles, for example: physical, chemical and biosynthesis methods. Physical and chemical methods of nanoparticle synthesis are often not environmentally friendly. The physical method mainly uses gas phase deposition, laser burning, mechanical grinding or ion sputtering to synthesize Ag NPs with advantages of simple principle and a high purity, but the particle size is larger and uneven than that synthesized by chemical and biochemical methods [2]. Chemical methods have several disadvantages including usage of hazardous and expensive chemicals and negative impact on the environment [5], the procedure involves various reactants, in particularly reducing agents for example hydrazine N2H4 or sodium borohydride NaBH4 or methoxypolyethylene glycol, it also requires a stabilizer like polyvinyl pyrrolidone PVP or sodium dodecyl benzyl sulfate to block the agglomeration of metallic nanoparticles [6]. In recent years' green chemistry and biosynthetic methods have become more attractive ways to obtain Ag NPs [7]. The advantage of green synthesis over chemical and physical methods is: environmental friendly, economical, and moreover. There is no need to use high temperatures and pressure, energy and toxic chemicals [1]. The procedure of biological synthesis includes the use of plants, fungi, bacteria, and algae. For the synthesis of Ag NPs, the use of plant extract is more important than the use of microbes, as it is time consuming and expensive [8]. Where reduces the cost of micro-organisms isolation and their culture media [1] and the rate of synthesis is slow and only a limited number of sizes and shapes are suitable for the method compared to methods involving the plant [9]. Use plants to synthesize silver nanoparticles have attracted attention due to their economical, environmentally friendly, and non-pathogenic protocol and supplying one-step technology for biosynthesis processes where [1] plant extract acts both as reducing and stabilizing agent in the synthesis of nanoparticles [10]. In the phytosynthesis of Ag NPs, primary metabolites, such as carbohydrates, proteins, peptides, amino acids and vitamins, are always present in plants and are involved in the reduction and stabilization of metallic silver in nanoparticles. Studies have shown that amine groups in proteins from the aqueous extract played an important role in the formation of Ag NPs. Furthermore, the carbonyl group of amino acids and proteins has the ability to bind metal

ions, to cap nanoparticles and to prevent agglomeration, thereby stabilizing the medium. Certain secondary metabolites with biological activities, for example flavonoids, alkaloids, phenolic acids, terpenoids and other polyphenols, they are reported to act as reducing or stabilizing agents in the formation of Ag NPs [11].

The synthesis of nanoparticles has several stages:

- Prepare plant extracts and silver salt solution.
- preparation of Ag NPs by mixing those two solutions in different proportions, at certain pH and temperature values and for different times [11].

Although synthesis of metallic nanoparticles using plant's extracts have already been reported for various plants such as *Matricaria chamomilla*, *Salvia offinacilis*, *Coffea Arabica* [3], *Ocimum tenuiflorum*, *Phlomis leaf extract*, *Cymbopogan citratus* [9], *Vitis vinifera* [1], *Azadirachta indica*, *Solanum lycopersicum*, *Citrus limon* [4] and *Acacia concinna* [8].

Acacia cyanophylla, *Phlomis syriaca* and *Scolymus hispanicus* were selected to study in this research. The genus *Acacia* belongs to the Fabaceae family and contains approximately 135 tree species that are abundant throughout the arid and semi-arid tropics. It is reported that *Acacia* species contain secondary metabolites such as amines and alkaloids, cyanogenic glycosides, fatty acids, cyclitol, gum, seed oils and terpenes. Also, Hydrolysable tannins saponins, flavonoids, and condensed tannins. [12]. The genus *Phlomis* L. is one of the largest genera of the family Labiatae, comprising about 100 herbals to shrubby species, distributed mostly in oriental and temperate Asiatic zones, many species of Phlomis have been identified: P. syriaca and P. viscosa…etc. There is evidence supporting various activities exerted by some *Phlomis* species including anti-inflammatory, immunosuppressiion, antioxidation and antimicrobial effects [13]. *Scolymus hispanicus* L. belongs to the Asteraceae family, and it is a thistle -like plant that is native to southern Europe and western Asia. This plant is a temperate climatic plant that grows in the Aegean, Mediterranean and Marmara regions, and reaches a height of 0-1580 meters in Turkey. Although it is usually consumed as a leaf and root vegetable, it is also used in alternative medicine. Scolymus hispanicus L. leaves, stems and flowers are traditionally used as a “bitter” tonic to stimulate appetite [14]. This research provides eco-friendly, economical and more effective approach to synthesis of Ag NPs via green synthesis method using (*Acacia cyanophylla*, *Phlomis syriaca* and *Scolymus hispanicus*) plants extracts and describing their main chemical properties and study the effect of its chemical composition on producing Ag NPs.

2. METHODOLOGY

2.1 Instrumentation and Apparatus

Rotary evaporator (Heidolph Instruments, Germany), UV-1800 spectrophotometer (Shimadzu, Japan), Ultrapure TM water purification system (Lotun Co., Ltd., Taipei, Taiwan), ultrasonic bath (POWERSONIC 405, Hwashin Technology Co., Korea), Dynamic light scattering (DLS; Zetasize Nano-ZS; Malvern Instruments, UK), Double Beam Spectrophotometer Cecil Aquarius CE 7200 (Cecil -UK).

2.2 Materials and Reagents

Chemical materials: Silver nitrates (99.99%) were purchased from sigma Aldrich, Methanol GR (Eurolab, UK), Eethanol (Eurolab, UK), Folin-ciocalteu phenol reagent (Sigma-Aldrich, Switzerland),), Sodium Carbonate anhydrous (PAREAC QUIMICA SAU, Spain), Gallic acid (Titan biotech LTD., India), Rutin (Extrasynthese Genay, France), Aluminum Chloride Hexahydrate (Scharalau Chemie, Spain), DPPH and BHT (Sigma-Aldrich, USA). Distilled deionized water (dd. H2O), Diethyl Ether (Merck Schuchardt- Germany), N-Butanol (Panreac, E.U.) and Sodium Chloride (HiMedia Laboratories, India).

Plant material: Fresh aerial parts (leaves, flowers and stems) of *Acacia cyanophylla, Phlomis syriaca* and *Scolymus hispanicus* were collected between March\April 2020 from different regions in north of Syria and The plant materials were authenticated by an expert at Faculty of Agriculture - University of Aleppo, Syria. The plant materials cleaned with distilled water and dry at normal room temperature [8,15] for 15 days and grind to make it powder with the domestic blender and kept in airtight glass container until use.

2.3 METHODS

2.3.1 Preparation of extracts

2.3.1.1 Preparation extracts for in vitro study

The fine powder of three plants (30g) was extracted with two different solvents (distilled water, ethanol 95%) for one hour in ultrasonic bath. The temperature was maintained at 50°C. The plant: solvent ratio was 1:10 (w/v). Then The extract solutions were filtered through Whatman No. 1 filter papers, and the residual material was re-extracted three times using the same procedure. After that, the combined extracts were evaporated to dryness in a rotary evaporator at 50°C and under reduced pressure to remove the solvent [15]. The obtained crude extracts were stored in dark glass bottles and refrigerated at -4°C until use [16].

2.3.1.2 Extraction procedure for synthesis Ag NPs

The fine powder of three plants (5g) was mixed well in distilled water (100 mL) in conical flask and this mixture was boiled at 60 °C for 15–20 min with constant shaking on hot magnetic stirrer. Afterwards, the mixture was refrigerated and filtrated. The obtained aqueous extract deposited at 4 °C and was utilized for the synthesis of Ag NPs [17].

2.3.2 In vitro study

2.3.2.1 Determination of total phenols content (TPC)

Total phenol contents of extracts are determined spectrophotometrically by the Folin- Ciocalteu's reagent according to Stanković with some modifications. The reaction mixture was prepared by mixing 0.5 ml of aqueous solution of extracts in concentration (0.5 mg\ml for ethanolic *Acacia* extract and 1mg\ml for rest extracts) 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% NaHCO3. The samples were incubated in a water bath at 45°C for 45 min. The absorbance was determined using spectrophotometer at λ_{max} = 765 nm against a blank solution prepared by replacing the plant extract with an equal volume of water. The samples were prepared in triplicate for each analysis and the mean value of three absorbances was obtained. The same procedures were repeated for the standard solution of Gallic acid in distilled water as standard series (0.005 to 0.08 mg/ml) and the liner calibration was construed. Based on the measured absorbance, the concentration of phenolic was calculated from the calibration line; then, the content of phenolic in extracts was expressed in terms of milligrams of gallic acid equivalent per gram of plant's dry weight (mg GAE/g DW) [18].

2.3.2.2 Determination of total flavonoids content (TFC)

The content of flavonoids in the examined extracts was determined using spectrophotometric method. The reaction mixture was prepared by mixing 1 ml of methanol solution of the extracts in concentration (0.5 mg\ml for ethanolic extracts and 1 mg\ml for aqueous extracts) and 1 ml of 2% AlCl3 solution dissolved in methanol. The samples were incubated for an hour at room temperature. The blank sample consists of 1 ml AlCl3 solution with 1 ml methanol. The absorbance was determined using spectrophotometer at λ_{max} = 415 nm. The samples were prepared in triplicate for each analysis and the mean value of three absorbances was obtained. The same procedures were repeated for the standard solution of Rutin in methanol as standard series (0.005 to 0.06 mg/ml) and the liner calibration was construed. Based on the measured absorbance, the concentration of flavonoids was calculated from the calibration line; then, the content of flavonoids in extracts was expressed in terms of milligrams of Rutin equivalent per gram of plant's dry weight (mg RUE/g DW) [19].

2.3.2.3 Evaluation of antioxidant activity (DPPH radical scavenging assay)

The antioxidant activity of the examined extracts was evaluated using the method of Liyana-Pathiranan and Shahidi using the stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH).

The reaction mixture was prepared by mixing 1 ml of methanol solution of the extracts and 1 ml of DPPH solution (0.135 mM) dissolved in methanol prepared daily was used. The reaction mixture left in the dark at room temperature for 30 min. The absorbance was determined using spectrophotometer at λ_{max} =517 nm against a blank is methanol. BHT was used as reference standard. In order to compare the results obtained in the different treatments, the inhibition percentage of free radical DPPH was calculated by the following equation:

DPPH radical scavenging activity (%) = [(Abs $_{control}$ – Abs $_{sample}$)]/ (Abs $_{control}$)] x 100

Where Abs $_{control}$ is the absorbance of DPPH radical + methanol

Abs $_{sample}$ is the absorbance of DPPH radical + sample (plant extract /standard)

A logarithmic curve was plotted of percent inhibition versus extract concentration, then, the concentration of sample required for 50% inhibition was calculated and expressed as IC_{50} values. All the tests were carried out in three and averaged [16,18,20].

2.3.2.4 Saponin determination

The samples were ground. 20g of each plant samples were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage [21,22].

2.3.3 Synthesis of silver nanoparticle

2.3.3.1 Method of synthesis of silver nanoparticle

10 mL plant extract was droped into Erlenmeyer flask having 90 mL of AgNO3 (1 mM) solution. The solution was stirred constantly at 35°C for 24 h for *Acacia cyanophylla* and 48 h for *Phlomis syriaca* and *Scolymus hispanicus.* The reaction progression for Ag NPs synthesis examined based on the color alteration of the reaction mixture from light yellow to intense reddish brown that confirms the formation of the Ag NPs in the solution. The mixture was examined by using UV–vis spectrophotometer within 300 −600 nm at different incubation time and observed the absorption peak [8,17].

2.3.3.2 Characterization of Ag NPs

Visual inspection: the confirm of Ag NPs formation is made visually by the color change from pale yellow to yellowish brown [10].

UV-vis spectroscopy: The absorption spectra of samples were observed at 300-600 nm wavelengths using UV-visible spectrophotometer and observed the absorption peak [8].

Dynamic light scattering: The particle size is determined by dynamic light scattering (DLS) technique where performed on a Zetasizer instrument at 25°C [23]. DLS analyzes the velocity distribution of particle movement by measuring dynamic fluctuations of light scattering intensity caused by the Brownian motion of the particle. The mean particle diameter is calculated by the software from the particle distributions measured, and the polydispersity index (PdI) given is a measure of the size ranges present in the solution, The PdI scale ranges from 0 to 1, with 0 being monodisperse and 1 being polydisperse [24].

2.3.4 Statistical analysis

All the experiments were performed in three times; the results were expressed as mean values ± standard deviation (SD). Statistical analysis was carried out using the Statistical Package for the Social Science SPSS Version 26, wherein the data was subjected to T test. Differences were considered significant at probability *(p) value < 0.05.*

3. RESULTS AND DISCUSSION

3.1 Total Phenol Content (TPC)

Polyphenols are secondary metabolites of plants. More than 8,000 polyphenolic compounds have been identified in various plant species, these molecules are very strong antioxidants [25]. As a basis, phenolic content was measured using the Folin–Ciocalteu reagent (FCR). The results were derived from a calibration curve (y = 16.392x + 0.2146, R^2 = 0.9914). In this assay, the FCR reacts with phenolic compounds under basic conditions to form chromogens that can be detected at 765 nm. The total phenols were expressed in gallic acid equivalents (GAE) per milligram dry extract weight. According to the results present in Table 1. All extracts were rich in phenolic compounds, where the TPC varied from (13.08 ±2.279) to (48.4 ±8.306) and (14.48

±2.695) to (98.39 ±4.755) mg GAE/g dry extract for aqueous and ethanolic extracts, respectively. The highest phenolic content was observed in *Acacia cyanophylla* ethanolic extract (98.39 ±4.755mg GAE/g DW), while the smallest amounts of flavonoids were found in *Scolymus hispanicus* aqueous extract (13.08 ±2.279mg GAE/g DW).

Comparatively with of literature, our results differ from other studies. For instance, TFC ranged from 7.40 to 166.33 mg/g GAE of dry weight for different extracts of *Acacia nilotica* leaves taken from different sites in India [26].

3.2 Determination of Total Flavonoids Content (TFC)

Flavonoids are chemical compounds of the secondary plant metabolism. Many flavonoid compounds such as flavones, flavonols, flavanones, isoflavones and flavanol are shown to have an antioxidative activity, free radical scavenging capacity [27]. As a basis quantitative determination, flavonoid contents were determined using aluminum chloride in a colorimetric method. The results were derived from the calibration curve ($y = 20.351x + 0.2122$, $R^2 = 0.9961$) and expressed in Rutin equivalents (RE) per gram dry extract. According to the results present in Table 2. The TFC varied from (19.83 ±2.384) to (24.21 ±4.065) and (35.59 ±0.739) to (121.64 ±6.469) mg RE/g dry extract for aqueous and ethanolic extracts, respectively. The highest phenolic content was observed in *Acacia cyanophylla* ethanolic extract (121.64 ±6.469 mg RE/g DW), while the smallest amounts of flavonoids were found in *Scolymus hispanicus* aqueous extract (19.83 ±2.384 mg GAE/g DW).

Our results outperform from other studies. For instance, TFC ranged from 10.34 to 75.11 mg/g QE of dry weight for different extracts of *Acacia nilotica* leaves taken from different sites in India [26]. This variation in values can be explained by the fact that the flavonoids and phenols content are influenced by different parameters such as time and place of harvest, climate, geographical conditions, method and time of extraction, solubility and degree maturation of the plan [28].

3.3 Evaluation of Antioxidant Activity

The plant extracts contain a high level of water soluble antioxidant poly- phenolic compounds (flavonoids), steroids, sapogenins, carbohydrates and vitamins etc. with various amounts. It has been reported that the functional groups in biomolecules are mainly responsible for the reduction of silver ions. The relatively high levels of the phenolic compounds and/or other components in the plant extract act as reducing agents.

The mechanism of reduction of Ag0 from Ag+ is shown below:

$$AgNO_{3\ (aq)} \rightarrow Ag^{+} + NO3^{-}$$
$$Ag^{+} + e^{-} \rightarrow Ag^{0}$$

Here the phenolic groups donate the electrons to produce Ag^0 from Ag^+ [4].

As a basis quantitative determination, antioxidants contents were determined using DPPH in a colorimetric method. According to the results present in (Table 3), The radical scavenging activities of *Acacia cyanophylla*, *Phlomis syriaca* and *Scolymus hispanicus* were estimated by comparing the IC_{50} value of the extracts and BHT, IC_{50} values were constructed from the equation of logarithmic curve of each extract and BHT. Considering that, the lower the IC_{50} value, the higher is the antioxidant activity. According to the results present in Table 3, the lowest IC_{50} value was found for *Acacia cyanophylla* ethanolic extract (IC_{50}=0.027 ±0.00038 mg/ml) which showed strong antioxidant activity approach than these of BHT (IC_{50}BHT= 0.0152mg/ml) and this corresponds to their high content of flavonoids and phenols while *Scolymus hispanicus* aqueous extract had low antioxidant activity (IC_{50}=0.883 ±0.016 mg/ml) and this corresponds to their low content of flavonoids and phenols.

Table 1. Total phenol content (TPC)

Samples	TPC (mg GAE g/ g of dry extract)	
	Aqueous extract	**Ethanolic extract**
Acacia cyanophylla	48.4 ±8.306	98.39 ±4.755
Phlomis syriaca	35.04 ±5.147	46.73 ±6.369
Scolymus hispanicus	13.08 ±2.279	14.48 ±2.695

** TPC = total phenolic content, GAE: gallic acid equivalents*
Each value is the average of three replicates ± standard deviation

Table 2. Total flavonoids content (TFC)

Samples	TFC (mg RE/g of dry extract)	
	Aqueous extract	Ethanolic extract
Acacia cyanophylla	24.21 ±4.065	121.64 ±6.469
Phlomis syriaca	23.31 ±0.398	52.49 ±8.169
Scolymus hispanicus	19.83 ±2.384	35.59 ±0.739

** TFC= total flavonoids content, RE-= Rutin equivalents*
Each value is the average of three replicates ± standard deviation

Table 3. Antioxidant assay

Samples	IC50 mg\ml	
	Aqueous extract	Ethanolic extract
Acacia cyanophylla	0.136 ±0.0006	0.027 ±0.00038
Phlomis syriaca	0.263 ±0.006	0.374 ±0.0236
Scolymus hispanicus	0.883 ±0.016	0.878 ±0.045
BHT	0.0152 ±0.0001	

** IC50 is the concentration for a 50% inhibition.*
Each value is the average of three replicates ± standard deviation

The results in this work outperform from other studies. For instance, IC_{50} DPPH scavenging ranged from (417.1±157.6) to (1199.2±440.6) µg dry weight/mL for methanol extract of *phlomis* species taken from different sites in Iran [29]. This variation in values can be explained by the fact that the distribution of secondary metabolites may change during plant development, perhaps related to the harsh climatic conditions of the plant's usual habitat (hot temperature, high solar exposure, drought and salinity), which stimulate the biosynthesis of secondary metabolites such as polyphenols that responsible for the antioxidant activity [30].

3.4 Saponin Determination

Saponins is the main ingredient which plays an important role in the stabilization of Ag NPs in water. Because saponins are natural surfactants showing the unique properties of foaming and emulsifying agents, they can act as a stabilizer in our experiments for Ag NPs synthesis. Surfactants or surface-active agents are large self-assembled molecular substances that possess a hydrophilic part and hydrophobic part (lipophilic part). Due to the amphiphilic nature of triterpenoid saponins, they can lower surface tension (interfacial tension) and can also act as a stabilizing agent to create a high stability for metallic Ag NPs in water; thus affording superior and long-term steric stabilization of metallic Ag NPs in colloidal solutions [23]. As a basis quantitative determination, saponins contents were determined using weight method.

The results were derived from the Equilibration ($W_2\backslash W_1$*100 ؛W_1 powder weight, W_2 residue) and expressed as percent in each extracts of *Acacia cyanophylla*, *Phlomis syriaca* and *Scolymus hispanicus*.

According to the results present in Table 4. the saponins amount varied from (0.46) to (2.53)%. The highest saponins content was observed in *Acacia cyanophylla* (2.53%), while the smallest amounts of saponins were found in *Scolymus hispanicus* (0.46%).

Comparing the works of literature, our results are similar to other studies. For instance, saponins content were 2.40% in *Acacia nilotica* seeds were taken from local markets in Minna, Nigeria [31].

3.5 Synthesis of Silver Nanoparticle

3.5.1 Visual inspection

10 ml extracts of *Acacia cyanophylla*, *Phlomis syriaca* and *Scolymus hispanicus* were prepared to reduce 90ml of 1mM aqueous $AgNO_3$ solution to prepare colloidal Ag NPs and color change of solution from yellow to dark brown was observed, due to the production of Ag NPs.

Fig. 1 represents the optical images for the colloids prepared using the extracts of the three plants. The color of the Ag NPs prepared using *Acacia cyanophylla* appeared deep yellowish brown which indicate high concentration of Ag NPs in the suspension, while the color of the silver Ag NPs prepared using *Phlomis syriaca*

appeared light yellowish brown. However, no colour change was observed when *Scolymus hispanicus* extract was added into $AgNO_3$ solution. Therefore, *Scolymus hispanicus* may not be suitable for reducing $AgNO_3$ to form Ag NPs.

Table 4. Saponin determination

Sample	Saponin percent%
Acacia cyanophylla	2.53
Phlomis syriaca	1.16
Scolymus hispanicus	0.46

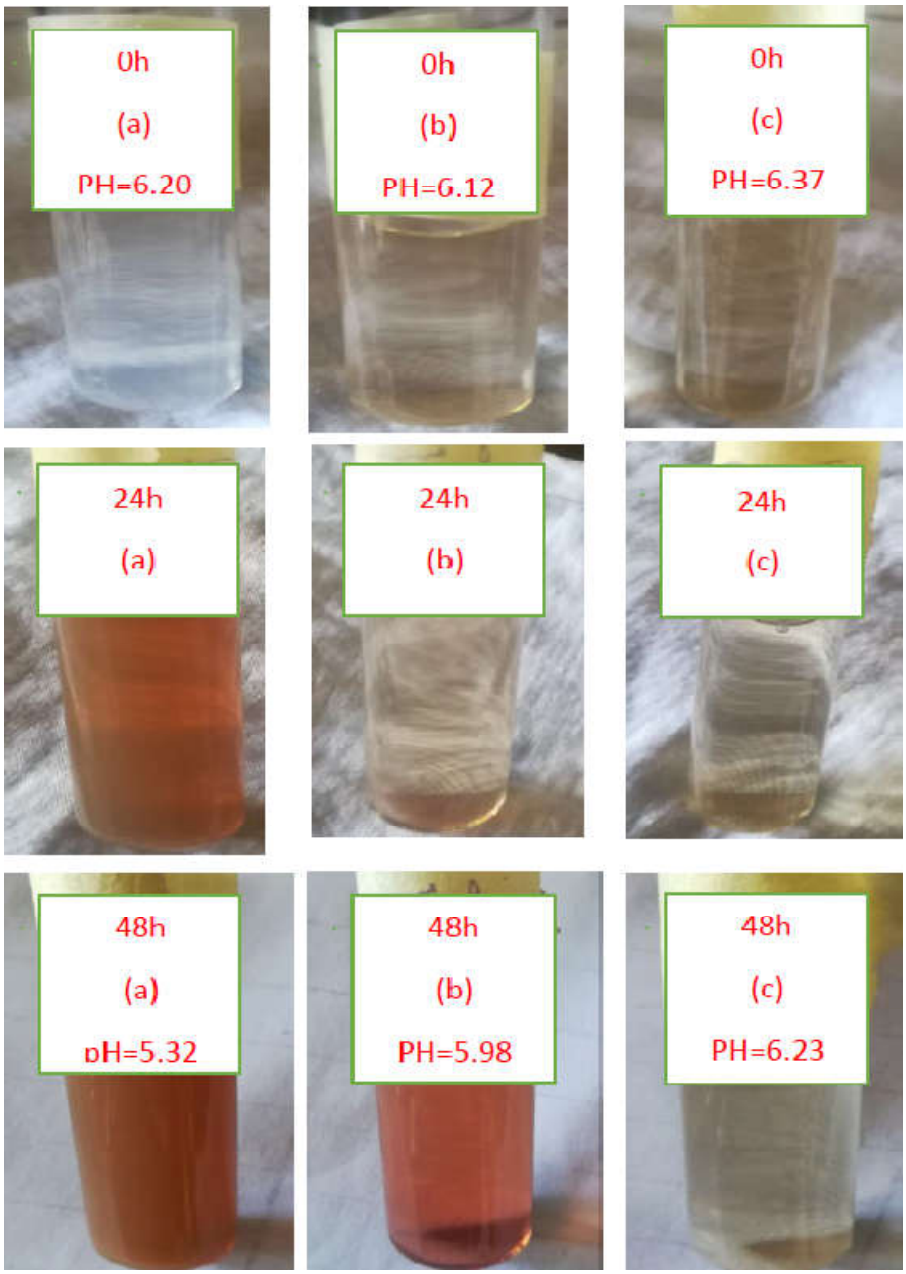

Fig. 1. Optical image of the Ag NP-based colloids prepared using different plant's extract: (a) *Acacia cyanophylla* (b) *Phlomis syriaca* (c) No change of colour with *Scolymus hispanicus*. (0h) zero hours, (24h) 24 hours and (48h) 48 hours

The change in PH value was only one degree when using *Acacia cyanophylla*, while in *Scolymus hispanicus* the change was very slight.

This result can be explained by referring to the chemical composition of the three extracts, where it was found that the *Acacia cyanophylla* extract is rich in phenols, flavonoids, saponins, and antioxidants, and these compounds are mainly responsible for the reduction of silver ions of $AgNO_3$ to form Ag NPs, so a higher concentration of silver nanoparticles has been obtained. While in the extract of *Scolymus hispanicus,* the concentration of these compounds was low, which did not allow the formation of silver nanoparticles.

Comparing the works of literature, our results similar to other studies. For instance, *Acacia concinna* fruit extract was used to synthesize Ag NPs with a similar protocol [8]. Also, in an Indian study, a *Phlomis* leaf extract was used to synthesize Ag NPs [9].

3.5.2 UV-vis spectroscopy

In our study synthesis process was studied periodically using UV-visible Spectra. The synthesized Ag NPs were characterized by UV-Vis spectroscopy and observed the absorption peak. Fig. 2. shows the UV-vis absorbance spectra of colloidal Ag NPs prepared using different plant extracts as reducing agents. The spectra consist of absorption peaks in the visible region of the electromagnetic spectrum it shows absorption peak at 460nm.

It could be concluded that 24 hours was sufficient to obtain the Ag NPs using *Acacia cyanophylla* extract, this is evidence that a peak was appeared at 460 nm. After 48 hours, the peak intensity slightly increased, indicating the formation of an additional amount of silver nanoparticles. Whereas after 24 hours, no color change occurred and no peak was appeared for the other two extracts, while this peak appeared after 48 hours using *Phlomis syriaca* extract, but it is lower than the peak that was appeared with *Acacia cyanophylla*.

While with *Scolymus hispanicus* extract, no peak appeared even after 48 hours, this confirm the previous results.

Comparing with works of literature, our results differ from other studies. For instance, when using *Acacia concinna* fruit extract to synthesis Ag NPs the peak of absorption appeared around at 430 nm [8]. In another study, an *Acacia leucophloea* extract was used to synthesize Ag NPs and the peak of absorption appeared around at 433 nm [32].

3.5.3 Dynamic light scattering technique (DLS)

After synthesis process the size distribution of the dispersed particles was measured using a Dynamic light scattering (DLS) technique.

Table 5 shows the particle size distribution analysis of the Ag NPs that we obtained depending on the three plants extracts and the preparation time corresponding to each extract. From the table we conclude that:

The largest size of the particles we obtained using *Scolymus hispanicus* extract (5137nm) and the PDI was high (1.000), indicating that the sample is very dispersed and not good, so this extract was excluded.

The size of Ag NPs prepared using *Acacia cyanophylla* extract was (134.1 nm) and PDI was suitable (0.260) after 24h and the concentration was high, so the *Acacia cyanophylla* was selected for following studies. While the size of Ag NPs prepared using *Phlomis syriaca* extract was (204.7 nm) that it is relatively large in addition to the long preparation time (48 h) and the concentration of the formed Ag NPs is less, so this extract was also excluded and *Acacia cyanophylla* extract was chosen for its advantages in terms of size, duration of preparation and a high concentration of Ag NPs.

Comparing the works of literature, our results differ from other studies. For instance, *Acacia concinna* fruit extract was used to synthesize Ag NPs with particle size between 2-20 nm [8]. Also, in an Indian study, a *Phlomis* leaf extract was used to synthesize Ag NPs with particle size about 27nm [9]. In another study, an *Acacia leucophloea* extract was used to synthesize Ag NPs with a size range of 17–29 nm [32]. Also, compared with the size producing from the chemical methods of synthesizing silver nanoparticles, where the size ranged from 10 to 40 nm when using citric acid as a complexing agent [33] and in another study it was found that using a mixture of tannic acid and sodium citrate allowed the preparation of nanoparticles homogenous in size (about 30 nm) [34].

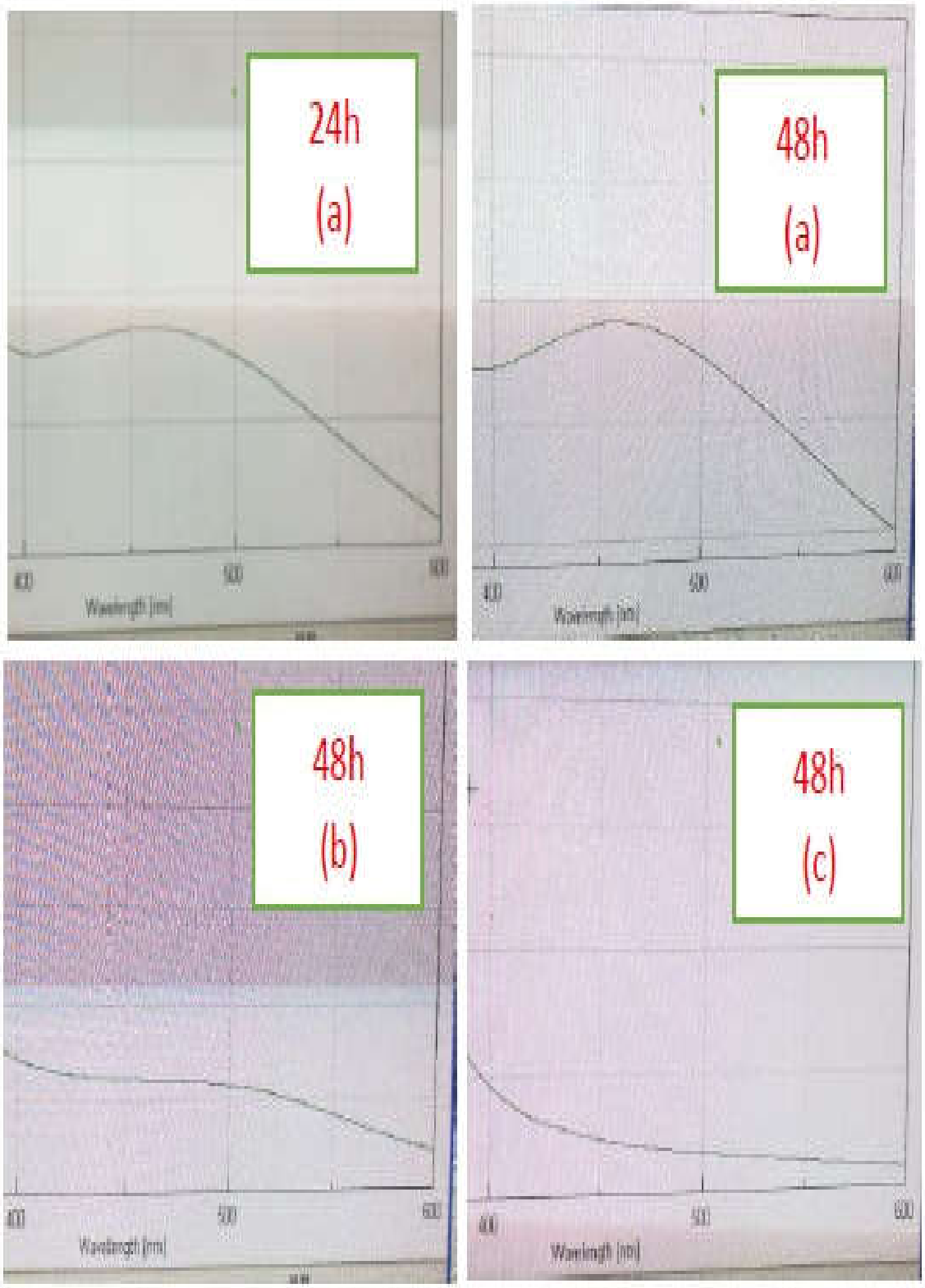

Fig. 2. UV-vis absorbance spectra of the colloidal Ag NPs prepared using (a) *Acacia cyanophylla*, (b) *Phlomis syriaca* (c) *Scolymus hispanicus*. (0h) zero hours, (24h) 24 hours and (48h) 48 hour

Table 5. Particle size distribution analysis of Ag NPs was determined by zetasizer

Plant extracts	Plant,s part	Time preparation	AgNO3 concentration	Temperature C	Z-Average size(nm)	PDI	Absorbtion
Acacia cyanophylla	aerial parts	24h	1mM	35	134.1	0.260	0.987
		48h	1mM	35	171.1	0.224	1.0084
Phlomis syriaca	aerial parts	48h	1mM	35	204.7	0.244	0.2113
Scolymus hispanicus	aerial parts	48h	1mM	35	5137	1.000	0.0916

4. CONCLUSION

Colloidal Ag NPs have been prepared successfully by using green plant extracts which are characterized eco-friendly, economical and more effective approach than physical and chemical approach. The plant extract not only functions as a reducing agent but also coats the produced nanoparticles providing them with stability. *Acacia cyanophylla* has been considered as the best reducing agent among the selected plant extracts for the preparation of stable colloidal Ag NPs this is due to their high content of flavonoids, phenols and saponins, where it shown Ag NPs prepared using its intense absorption peak in the visible region with the peak at 460 nm and they have average diameter (134.1) nm and PDI was suitable (0.260) whereas *Scolymus hispanicus* extract was not suitable for reducing $AgNO_3$ to form Ag NPs. As for *Phlomis syriaca* extract, the preparation time was long and the particles size were relatively large. Therefore, bacteriological studies will be completed on the Ag NPs prepared using *Acacia cyanophylla* extract.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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