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EFFECTS OF TEMPERATURE AND CONCENTRATION CHANGES ON THE SIZE AND BIOSYNTHESIS OF GREEN GOLD NANOPARTICLES

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Review article

ABSTRACT

Various chemical and physical methods are used to prepare gold nanoparticles (AuNPs). However, these methods involve the use of chemicals that are toxic and harmful to the environment. Therefore, researchers have been investigating the use of green chemistry to develop eco-friendly AuNPs. One approach is green nanotechnology, which uses plant-derived phytochemicals to reduce gold ions and produce AuNPs. This study aimed to follow a simple method to screen some aqueous plant extracts and to clarify the effect of changing the concentration of the plant extract and temperature on the synthesis of nanoparticles while determining the optimum concentration (OC) for biosynthesizing AuNPs. Ten Libyan medicinal plants successfully synthesized AuNPs of different sizes. It was found that concentration and temperature changes have a role and influence on the synthesis and size of the AuNPs.

KEY WORDS: Biosynthesizing AuNPs; Temperature; Libyan flora; Concentration; Green chemistry; Green nanotechnology.

INTRODUCTION

Metal nanoparticles possess exceptional optical, electrical, and photothermal characteristics, making them highly valuable for potential applications due to the simplicity of their creation and customization, which has further contributed to the interest in these metallic nanoparticles [1,2]. Among the metal nanoparticles, gold nanoparticles (AuNPs) have received much attention [3]. Their properties have made them widely applicable in various biomedical areas, including drug delivery, cancer diagnosis, and pathogen identification in clinical specimens [4,5].

Typically, the creation of metal nanoparticles is achieved through a range of chemical techniques like chemical reduction [6], electrochemical reduction [7], and photochemical reduction [8]. These methods are costly and utilize various toxic and environmentally detrimental inorganic chemicals like sodium/potassium borohydrate and hydrazine, as well as organic chemicals such as sodium citrate, ascorbic acid, and amino acids, which possess reducing properties [9]. The utilization of these harmful chemicals can impose restrictions on the utilization of nanoparticles in biomedical applications [10].

As a result, there has been an increasing demand for biocompatible, less toxic, and environmentally friendly nanoparticles, leading to the investigation of green synthesis methods for AuNPs. One approach to achieve this is using biological systems like bacteria, fungi, and plant extracts. For instance, various plants, such as Aloe vera [11], Magnolia kobus, Diospyros kaki [12], Suaeda monoica [13], Trianthema decandra [1], and Memecylon umbellatum [14], have been utilized to synthesize AuNPs. These methods are not only environmentally friendly but also cost-effective and easily scalable for large-scale synthesis [1].

Plants are a more appealing option when compared to other biological systems because they are easily accessible, safer, and contain a wide range of phytochemicals that can reduce substances. Additionally, the phytochemicals derived from plants require less time to synthesize gold nanoparticles (AuNPs) when compared to those derived from microbes [2]. These phytochemicals not only aid in the production of metal nanoparticles but also act as capping agents to prevent the clumping of particles in solution through electrostatic forces [9].

The different components of phytochemicals, such as polyol and water-soluble heterocyclic compounds, are believed to be primarily responsible for reducing and coating gold ions [14].

The plant life in Libya is characterized by its rich biodiversity. Libyan flora is predominantly Mediterranean, with stronger links to the eastern Mediterranean (Palestine to Greece) than the rest of North Africa and the island of Crete [15]. In this study, AuNPs were synthesized using ten plant extracts collected from different areas of the Western Mountain and the Green Mountain in Libya.

Herein, the synthesis process of AuNPs that were synthesized using different plants was reported. The synthesis process under two different temperatures and ten different concentrations to assess how temperature and concentration affect the size of the AuNPs was monitored. The characterization was confirmed using Ultraviolet-Visible Spectroscopy (UV-Vis).

MATERIALS AND METHODS

The Medicinal plants were purchased from local vendors in Misurata Libya. 96 well polystyrene microplates were acquired from Greiner Bio-one GmbH in Germany. Gold salt (sodium tetrachloroaurate (III) dehydrate) was obtained from Sigma-Aldrich in the USA.

The extracts were centrifuged using the Allegra X-12R centrifuge from Beckman Coulter, USA. The AuNPs were centrifuged using the Centrifuge 5417R from Eppendorf AG, Germany. The extracts were then freeze-dried using the Free Zone 2.5L from Labconco, USA. UV-Vis spectra were recorded using the POLARstar Omega microplate reader from BMG Labtech, Germany. The size and zeta potential of the freshly synthesized AuNPs in solution were analyzed using the Zetasizer from Malvern Instruments Ltd., USA.

Plant samples (Table 1) were requested in May 2018 from local vendors (Al-Shajara Company for Herbal Medicine and Bee Products) in Misurata, Libya. Medicinal plants used in this report were classified by type.

Fresh plant materials were carefully cleaned of soil and dried in the shade for two weeks, then ground and extracted

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using boiled distilled water in a specific ratio of 100 mL of water to 5gm of plant material. The resulting supernatants were freeze-dried. A stock solution of each extract (10 mg/mL) was freshly prepared before the experiment.
A fixed gold salt concentration of 1 mM was employed, and it was incubated with varying concentrations of the plant extracts (ranging from 10 to 0.0195 mg/mL). In a 96-well plate, 50 µl of different concentrations of plant extracts were added to 250 µl of gold salt (ratio 1:5).

Table 1: List of the collected plant species and their herbarium number

No	Plant name	Local Arabic name	Herbarium No.
1	*Pituranthos tortuosus*	قزاح	EX1
2	*Artemisia herba-alba*	شيح	EX2
3	*Ajuga iva*	شندقورة	EX3
4	*Marrubium vulgare*	روبية	EX4
5	*Salvia officinalis*	مريمية	EX5
6	*Teucrium polium*	جعدة	EX6
7	*Globularia alypum*	زريقة	EX7
8	*Cymbopogon schoenanthus*	آدخر	EX8
9	*Ziziphus lotus*	سدر	EX9
10	*Ruta graveolens*	فيجل	EX10

The plates were incubated at 25˚C and 70˚C with shaking (40 rpm) for 1 hr. The synthesis process was meticulously monitored, and the SPR of the AuNPs was measured by recording the UV-Vis spectrum within the range of 300 nm to 800 nm. For further characterization and stability evaluations, the synthesis of the AuNPs from the tested plant extracts was scaled up using the OC of the plant extracts [16].

RESULTS AND DISCUSSION

Earlier research studies have mentioned the use of plants to synthesize gold nanoparticles (AuNPs) through a process known as green synthesis. These studies involved mixing specific amounts of gold salt solutions with plant extract solutions [11, 12, 14].
To enhance this existing method, this study aimed to develop a micro-scale approach that would allow us to screen multiple plants simultaneously. By using this method, the assessment of how temperature and concentration affect the size of the AuNPs will be evaluated as well as to be able to determine the optimal concentrations (OC) at which the plants can reduce gold salts to AuNPs.

The creation of gold nanoparticles and the examination of their UV-Vis characteristics:

The initial formation of AuNPs was visually observed through the appearance of a red or wine-red color in the 96-well plates. This was further confirmed by measuring the UV-Vis spectra, which showed a maximum

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absorbance between 500 and 600 nm (Table 2). This absorbance range is characteristic of the excitation of AuNPs' surface plasmon resonance and is considered a distinct indicator of their presence [13,16]. Three plant extracts (A. iva, M. vulgare, and G. alypum) did not exhibit any colour change when tested at 25˚C, suggesting that they may lack strong reducing phytochemicals in their aqueous extracts. These extracs may require higher temperatures or longer incubation periods to initiate the reduction process of gold ions.

Table 2: The optimum concentration (OC), polydispersity index (Pdi), particle diameter (PD), λmax and average zeta potential values (ZP) of the Au NPs synthesised from the tested plant extracts at 25˚C and 70˚C.

No	Plant Name	25˚C					70˚C				
		OC (mg/mL)	Pdi	PD (nm)	λmax (nm)	ZP (mV)	OC (mg/mL)	Pdi	PD (nm)	λmax (nm)	ZP (mV)
1	*P. tortuosus*	**5.00**	0.638	30	538	-16	**0.625**	0.645	28	538	-15
2	*A. herba-alba*	**5.00**	0.447	50	540	-19	**2.50**	0.442	55	538	-20
3	*A. iva*	#	#	#	#	#	**2.50**	1.000	16	538	-18
4	*M. vulgare*	#	#	#	#	#	**0.625**	0.604	33	550	-14
5	*S. officinalis*	**2.50**	0.582	39	542	-18	**5**	0.944	12	526	-17
6	*T. polium*	**1.25**	0.482	109	538	-14	**0.625**	0.520	43	538	-13
7	*G. alypum*	#	#	#	#	#	**0.312**	0.451	65	548	-19
8	*C. schoenanthus*	**5.00**	0.609	34	546	-11	**0.625**	0.621	33	546	-16
9	*Z. lotus*	**1.25**	0.552	36	534	-13	**1.25**	0.639	26	538	-16
10	*R. graveolens*	**2.50**	0.436	117	542	-13	**0.625**	0.317	77	552	-14

No nanoparticles were formed at this condition

The UV-Vis spectra of the AuNPs shown in (Figure 1) indicate no major shifts in the spectra when comparing AuNPs synthesized at 25°C and 70°C. However, there was a noticeable difference in the peak heights, which could be related to the number of nanoparticles produced.

The OD value, which is linearly correlated with the concentration of AuNPs in a solution, may explain this difference [17,18]. Additionally, the spectra of AuNPs synthesized at 70°C exhibited sharper and more symmetrical bands, suggesting a more uniform size distribution of the nanoparticles [19]. Most of the AuNPs displayed broad plasmon bands with an absorption tail at longer wavelengths, indicating the excitation of in-plane surface plasmon resonance and the presence of anisotropy in the shape of the gold nanoparticles [20]. The maximum absorbance data obtained from all the tested plants are summarized in (Table 2).

The impact of temperature on the characteristics of AuNPs:

In order to view the effect of increased temperature on the overall synthesis of the AuNPs, the average values of particle diameter, λmax, and PDI values were calculated (Table 3). Previous research indicated that higher temperatures can result in AuNPs with a more favorable size distribution [21].

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The measurement of particle size distribution for certain AuNPs supported this finding. For example, (Figure 2) shows that at elevated temperatures, the AuNPs synthesized from S. officinalis had a more favorable size distribution compared to those synthesized at lower temperatures. In general, higher temperatures led to smaller, which is consistent with the blue shift of λmax. This finding aligns with a study conducted by Mountrichas and co-workers [21]. Song et al. explained the formation of smaller AuNPs at higher reaction temperatures by suggesting that most gold ions are used up in the formation of nuclei due to the increased reaction rate. This prevents the secondary reduction process of the nuclei and hinders the formation of larger AuNPs [22].

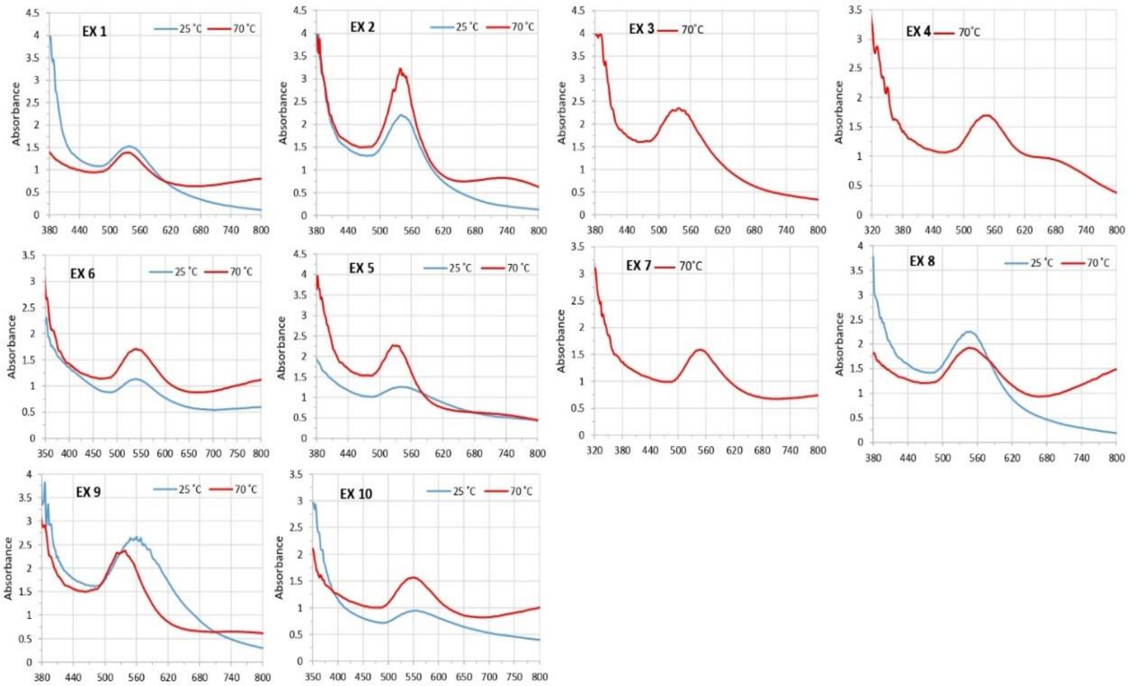

Figure 1: Comparison of the UV-Vis spectra for the AuNPs produced at 25˚C (green) and at 70˚C (red).

Effect of the concentration (OC) for each plant extract:

The quantity of extract added plays a significant role in the synthesis and properties of the nanoparticles. This is likely due to the higher concentration of reducing and stabilizing agents in the extract, which facilitates the formation of more nanoparticles.

Table 3: The average particle diameter, λmax, and Pdi values obtained from all the plant extracts at 25˚C and 70˚C

	25˚C	70˚C
λmax(nm)	540 ± 6	539 ± 13
particle diameter (nm)	73.5 ± 43.5	44.5 ± 32.5
Pdi	0.537 ± 0.101	0.659 ± 0.341

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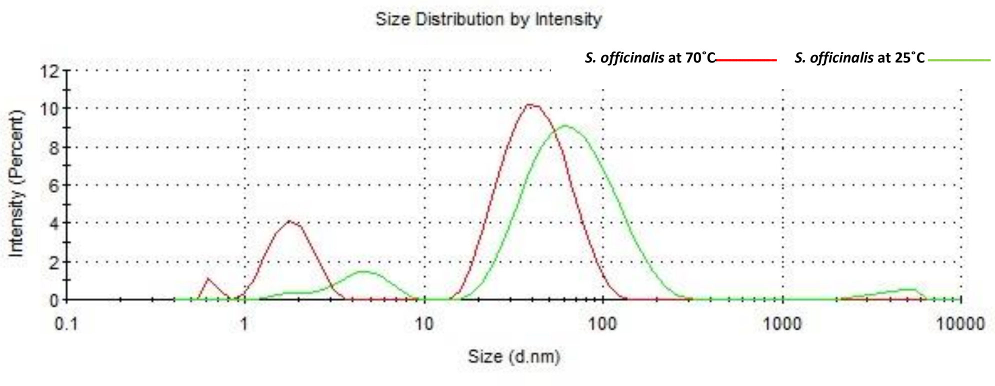

Figure 2: Particle size distribution for AuNPs produced from *S. officinalis* at 25˚C and 70˚C.

An increase in absorbance indicates a higher concentration of nanoparticles, and the color change from wine-red to violet is indicative of an increase in nanoparticle size. where the surface plasmon resonance (SPR) peak shifts to longer wavelengths (redshift) as the particle size increases. The wine-red color typically corresponds to smaller nanoparticles, while the violet color suggests larger nanoparticles. This is because larger nanoparticles scatter and absorb light differently, leading to a shift in the observed color [23,24].

In the present study, focus was placed on the OC (optimum concentration), which is the concentration of a plant extract that results in the production of smaller and more uniform AuNPs (gold nanoparticles). To study the impact of plant extract concentration, mixing was done with a fixed concentration of gold salt with various concentrations of the plant extracts. The OC was selected based on the SPR (peak wavelength) and the uniformity of the UV-Vis curve. The PD (polydispersity) and PDI (polydispersity index) values were taken into consideration, which indicate the uniformity of the resulting AuNPs. Thereafter, for further analysis, the selected concentrations (OC) were used to synthesize the AuNPs after scaling up the 10 volumes along with the adjacent concentrations in the first screening step. These selected concentrations were used to synthesize the AuNPs on a larger scale for further analysis [16]. In the initial stage of screening, the volumes and concentrations next to each other were measured. It was noted that higher temperatures resulted in smaller particle sizes when the plant extract concentrations were lower.

Figure 3 illustrates that at a temperature of 25°C, the OC has a concentration of (5 mg/mL), whereas at a temperature of 70°C, it is (0.625 mg/mL). No consistent or small AuNPs were created when the concentrations exceeded (5 mg/mL).

In figure 4, two of the tested plant extracts were selected (*Z. lotus* , *A. herba-alba*) to demonstrate the growth pattern of the AuNPs under different concentrations. Both the SPR of *Z. lotus* and *A. herba-alba* exhibited red shifts at different concentrations, indicating the formation of AuNPs (*Z. lotus* at 1.25 mg/mL had a λmax at 534 nm with an average diameter of 36 nm, and *A. herba-alba* at 5 mg/mL had a λmax of 540 nm with an average diameter of 50 nm). The researchers Ji and colleagues [25] found that that there are two different pathways for producing these nanoparticles, each leading to

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different sizes. The pathway followed by the nanoparticles depends on the pH of the medium. They determined that when the pH is above a critical value, the nanoparticles grow through Ostwald ripening, resulting in the formation of larger nanoparticles. In another study, Guo and colleagues [26] observed that changing the pH during the synthesis of gold nanoparticles from Eucommia ulmoides bark extract led to the formation of larger and aggregated nanoparticles. It was suspected that the pH changes in this study, albeit small, may have contributed to this difference, as well as other factors, such as the belief that at high concentrations of the plant extract, there may be an overcrowding effect that impedes the function of the reducing and/or capping agents. The plant extract contains various phytochemicals, some of which at high concentrations may counteract the reducing and/or capping agents.

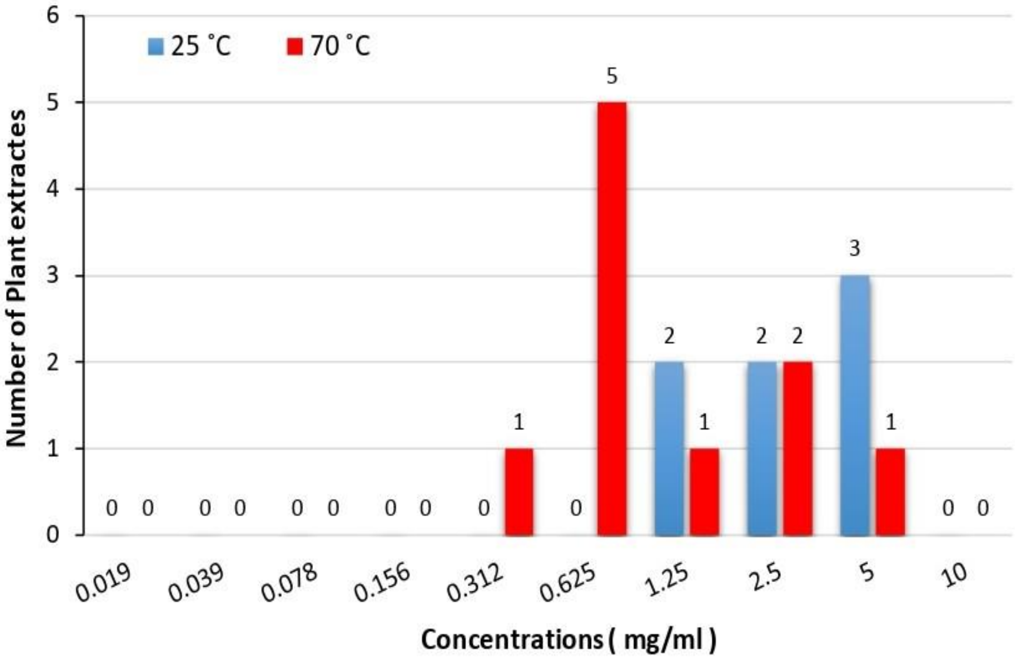

Figure 3: Optimum concentrations for AuNPs synthesis for all plant extracts at 25˚C and 70˚C.

Therefore, it is important to determine the optimum concentration ratio of the plant extract and gold salt to achieve the best synthesis of gold nanoparticles. Beyond this concentration, the growth pattern of the nanoparticles changes, resulting in the formation of larger particles.

CONCLUSION

This study aimed to explore the impact of varying concentrations of numerous plant extracts on the micro-scale formation of AuNPs. The findings revealed a non-linear correlation between plant extract concentrations and AuNP formation, leading to the identification of optimal concentrations for each plant extract, resulting in the production of the smallest and most uniform AuNPs. The aqueous extracts of the tested plants effectively facilitated the reduction of Au+3 to Au0, enabling the formation of AuNPs. This study presents a novel, rapid, and straightforward screening method for a wide array of aqueous plant extracts

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for biosynthesizing AuNPs. This approach offers several advantages, including cost and time reduction in synthesis, as well as environmental friendliness. The synthesized AuNPs underwent comprehensive analysis of their in vitro sizes. Out of the ten plants examined, all but three produced AuNPs at two different temperatures (25°C and 70°C), while A. iva, M. vulgare, and G. alypum exclusively produced AuNPs at 70°C. Confirmation of AuNP formation was established through the characteristic SPR in the UV-Vis spectra. Furthermore, varying reaction conditions demonstrated the potential for achieving smaller particle sizes through high-temperature synthesis. Notably, S. officinalis yielded the smallest AuNPs with an average diameter of 12 nm at 70°C. Moving forward, it is recommended to explore the toxicity of these stable AuNPs on human cell lines to gauge their potential for future biomedical applications.

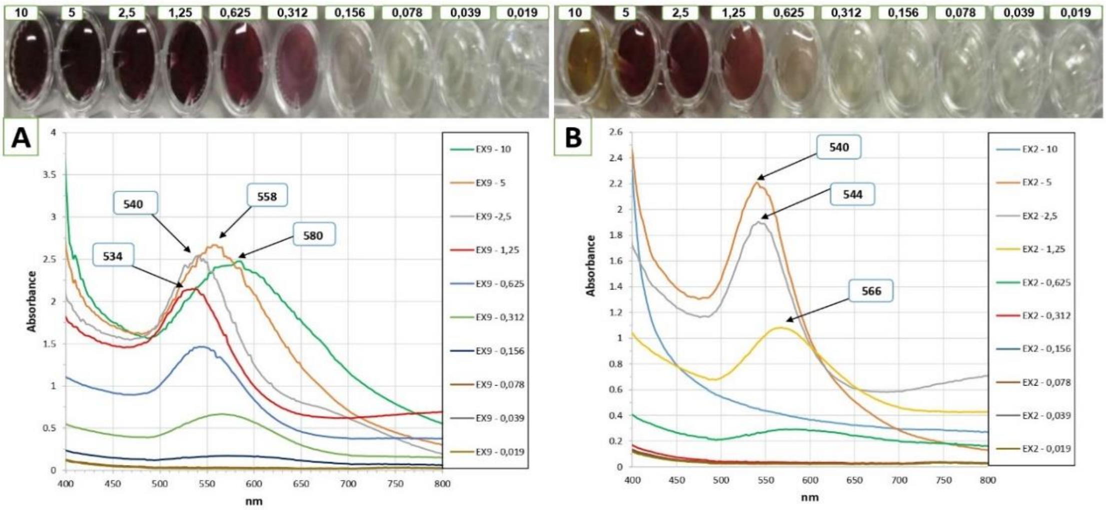

Figure 4: UV-Vis spectra for the AuNPs produced from A) Z. lotus and B) A. herba-alba using different concentrations (mg/mL) at 25˚C

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