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Original Article

Green Synthesis of Gold Nanoparticles Using *Salvia rosmarinus* Essential Oil

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ABSTRACT

The project was centered on the utilization of essential oils extracted from *Salvia rosmarinus* Spenn. for the synthesis and stabilization of gold nanoparticles. This approach is noteworthy due to the distinctive properties exhibited by gold nanoparticles in contrast to bulk gold. Moreover, these properties can be influenced by the size and shape of the nanoparticles. The essential oil was extracted from *S. rosmarinus* through the process of hydrodistillation using a Clevenger type apparatus. A GC-MS analysis of these essential oils revealed the presence of several compounds, with the most frequently identified ones being α-pinene (16.7%), 1,8-cineole (8.6%), camphor (10%), and borneol (7.7%). Subsequently, these obtained essential oil was employed as both a regenerating agent and stabilizer in the synthesis of gold particles. The resultant nanoparticles underwent identification and characterization using standard methods. They exhibited a face-centered cubic crystal structure and possessed an average size of 26.6 nm. Remarkably, it was ascertained that the essential oils of *S. rosmarinus* are proficient in regenerating gold ions and stabilizing them in the form of stable colloidal nanoparticles. This development holds promise for various applications, including the production and formulation of diverse products such as cosmetics and health-related items.

INTRODUCTION

The utilization of nanomaterials across diverse sectors, encompassing fields such as petroleum [1], natural gas [2], cosmetics [3], and healthcare [4], has ushered in a transformative era of advancement, particularly in the realm of nano-medicine. Among the extensively researched nanostructures, noteworthy examples comprise carbon nanotubes, gold nanoparticles, liposomes, and paramagnetic nanostructures. Gold colloids have witnessed a burgeoning application across a spectrum of disciplines including chemistry, biology, engineering, medicine, as well as the domains of disease diagnosis and treatment [5]. Remarkably, these nanoparticles have garnered prominence owing to their exceptional attributes of minimal toxicity and remarkable stability, rendering them indispensable components within the sphere of science and technology [6]. Gold nanoparticles exhibit distinct properties compared to bulk gold, with these characteristics being contingent upon their size and shape. These nanoparticles manifest a diverse spectrum of colors when suspended in solution, encompassing shades of brown, purple, blue, orange, and red. Notably, the coloration is intricately linked to the nanoparticle's dimensions. Gold nanoparticles exhibit an absorption band within the wavelength range of 500 to 550 nm [7], a feature absent in nanoparticles possessing a diameter less than 2 nm or in bulk gold. Modulating the size of these particles engenders a corresponding shift in the observed light's color spectrum, allowing gold nanoparticles to produce vibrant hues. Chen *et al*. [8] corroborated this phenomenon, which was also observed upon introducing additional salt into the gold solution. This addition neutralizes the surface charge of the gold nanoparticles, leading to their aggregation and consequent alteration of the solution's color from red to blue, as documented by Aman Kumar *et al*. [9] .

To mitigate aggregation, surface chemistry has been employed to functionalize gold nanoparticles with polymers, small molecules, and biologically active recognition molecules. This surface modification renders gold nanoparticles versatile and well-suited for a wide array of applications spanning the realms of chemistry, biology, engineering, and medicine.

Numerous techniques exist for the biological synthesis of nanoparticles [10]. Natural sources, including plants [11], bacteria [12], fungi [13], and yeast [14], are employed in nanoparticle synthesis. The utilization of biological agents in nanoparticle synthesis has garnered considerable interest due to their diverse optical, chemical, photo-electrochemical, and electronic characteristics. A broad spectrum of outcomes arising from physical, chemical, and biological processes is observed in several novel nanoparticle synthesis methodologies.

In the study conducted by Shuiqin Li *et al*. [15] in 2020, *Mentha Longifolia* leaf extract served as the primary material for the synthesis of gold nanoparticles. Transmission electron microscopy (TEM) analysis revealed the nanoparticles' spherical morphology, characterized by an average particle size of 36.4 nm. Subsequently, the functionalization of these gold nanoparticles with biomolecules was explored. The research then turned its focus to investigating the potential anti-cancer properties of these bio-activated gold nanoparticles in human breast cancer models, yielding promising results.

Furthermore, it was demonstrated that these gold nanoparticles could be efficiently recovered and reused for up to 12 consecutive cycles without experiencing a significant decline in catalytic activity. Notably, the gold nanoparticles exhibited potent anti-breast cancer activity, displaying a dose-dependent response across multiple cell lines, including MCF7, Hs 578Bst, Hs 319.T, and UACC-3133. The most pronounced anti-breast cancer effect was observed in the UACC-3133 cell line. These findings suggest the potential utility of gold nanoparticles in the treatment of various forms of human breast cancer.

Experimental

In this study, all chemical substances utilized were of analytical grade and procured from Merck as the supplier. Chloroauric acid ($HAuCl_4.3H_2O$) salt was employed as a precursor for the synthesis of gold nanoparticles. The specimens of *S. rosmarinus* were acquired from the Medicinal Plant Research Institute, situated on Road, Karaj-Qazvin, during the winter of 2022.

Essential Oil Extraction

The gathered leaves were cleansed with cold water to eliminate any impurities, subsequently air-dried in a shaded environment. Following this, the desiccated plant material was finely ground and stored at a temperature of 4 degrees Celsius in anticipation of the essential oil extraction process. The extraction of essential oil from *S. rosmarinus* was performed via the water distillation technique, employing a Clevenger apparatus. The obtained essential oil was dehydrated using anhydrous sodium sulfate and subsequently dried. It was then stored at a temperature of 4 degrees Celsius for further research studies [16].

Identification of the Components of the Essential Oil

Chemical compounds present in the essential oil of *S. rosmarinus* were identified using a gas chromatography-mass spectrometer (GC-MS). The GC-MS instrument employed was a Hewlett-Packard model 5973-6890, equipped with a 60 m column of 0.1 mm diameter, and packed with Poly Di methyl siloxane (HP-1 ms). Helium, with a purity of 99.999%, was used as the carrier gas at a flow rate of 1 ml/min. The injection site temperature was maintained at 250 °C. The temperature program for the device oven was as follows: an initial temperature of 60 °C was held for 5 minutes, followed by a temperature increase rate of 6 °C per minute, ultimately reaching a final temperature of 220 °C , which was held for 10 minutes. The mass spectrometer operated with an ionization energy set at 70 eV. Compound identification in the *S. rosmarinus* essential oil was achieved by comparing the mass spectra obtained from the mass spectrometer with the standard spectra available in the literature [17].

Green Synthesis of Gold Nanoparticles using *S. rosmarinus* Essential Oil

S. rosmarinus essential oil was employed as the regenerating and stabilizing agent for the synthesis of gold nanoparticles. In this procedure, Chloroauric acid ($HAuCl_4 \cdot 3H_2O$) served as the precursor for gold nanoparticles and the source of Au^{3+} ions. The nanoparticle synthesis method described by Muniyappan [18] was adopted for this purpose.

For the eco-friendly synthesis of gold nanoparticles, 0.1 ml of *S. rosmarinus* essential oil was dissolved in 10 ml of an 80% ethanol solution in water, at ambient laboratory temperature. Subsequently, 3 ml of the essential oil solution was introduced into 20 ml of a 1 mM Chloroauric acid solution and agitated for a duration of 30 minutes, maintaining laboratory temperature conditions.

Characterization of Synthesized Gold Nanoparticles

To identify the synthesized gold nanoparticles, UV-VIS spectroscopy was employed. For this purpose, a Varian Carry 300 spectrophotometer was utilized. The gold nanoparticles exhibited a surface plasmon resonance at around 540-560 nanometers, indicative of their presence. To determine the crystalline structure of the formed nanoparticles, X-ray diffraction spectroscopy was employed. The apparatus utilized for this purpose was a Seifert XPS3003 instrument equipped with a copper lamp operating at 40 kilovolts. The analysis was conducted within the angular range of 2θ, from 20 to 80 degrees. To facilitate this analysis, the gold nanoparticles were first sedimented through centrifugation at a speed of 13,000 rpm, followed by three rinses with deionized water. Subsequently, the nanoparticles were re-dispersed in deionized water and deposited as a monolayer onto a microscope slide. To investigate the functional groups related to the reactivity and stabilization of gold nanoparticles in the chemical compounds of *S. rosmarinus* essential oil, Fourier-transform infrared (FT-IR) spectroscopy was employed. For this purpose, the synthesized gold nanoparticles, following their washing and sedimentation by centrifugation, were desiccated using a freeze dryer. They were then incorporated into KBr to form pellets, which were subjected to FT-IR spectroscopy analysis using a Thermo Nicolet Nexus instrument. The size and shape of the synthesized gold nanoparticles were determined through transmission electron microscopy (TEM). For this purpose, a Philips electron microscope system, operating at 100 kilovolts with a magnification of 200,000 times was utilized [19].

RESULTS AND DISCUSSION

The essential oil obtained was subjected to analysis using gas chromatography-spectroscopy. Through a comparison of its mass spectra with established standard spectra and employing the kovats index, the presence of 20 distinct compounds in the essential oil was ascertained. These identified constituents collectively accounted for 91% of the total compounds present. Notably, among the identified compounds, □-pinene (16.7%), 1,8-cineole (8.6%), camphor (10.0%), and borneol (7.7%) exhibited the highest concentrations. Table 1 presents the percentage composition of *S. rosmarinus* essential oil.

Table 1 Percentage composition of *S. rosmarinus* essential oil

Compound	kovats index	%
a-pinene	939	16.7
Camphene	954	7.2
Verbenene	968	1.1
3-octanone	984	4.5
Myrcene	991	4.1
p-cymene	1020	1.6
Limonene	1029	5.1
1,8-cineol	1031	8.6
□-tepinene	1060	0.9
□-terpinolene	1089	0.9
Linalool	1097	2.5
Chrysanthenone	1128	1.6
Camphor	1146	10.0
Trans- pinocamphone	1150	1.4
Borneole	1169	7.7
Cis- pinocamphone	1175	3.1
□-terpineol	1189	1.8
Bornyl acetate	1289	4.8
E-□-caryophyllene	1419	6.5
□-humulene	1455	0.9
The Percentage of identified components		91

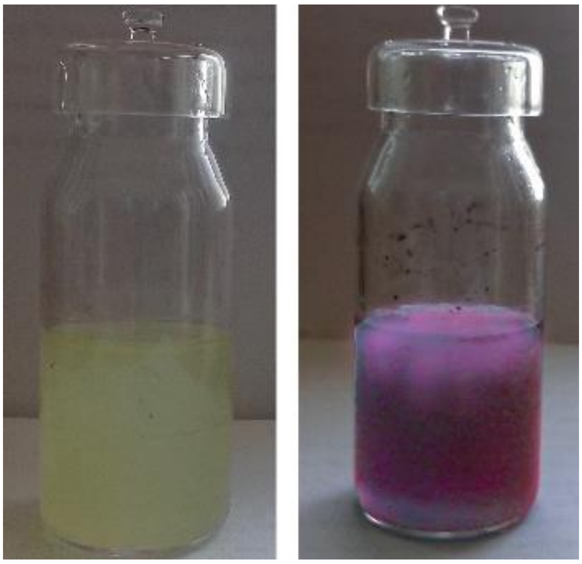

Fig. 1 Color change of the solution (purple) due to the formation of gold nanoparticles

To synthesize gold nanoparticles employing essential oil derived from the *S. rosmarinus*, a solution of 1% essential oil was prepared by dissolving 0.1 ml of the essential oil, obtained from this plant, in 10 ml of 80% ethanol. Subsequently, 3 ml of the solution were introduced into a 20 ml solution containing 1 mM Chloroauric acid, and the mixture was vigorously stirred under laboratory temperature conditions. The initial appearance of a purple hue after half an hour served as the primary indicator of gold nanoparticle formation (Fig. 1). To ensure the successful formation of gold nanoparticles, ultraviolet-visible spectroscopy (UV-VIS) was employed to examine the surface plasmon resonance of the generated gold nanoparticles. To accomplish this, the absorption spectrum of the solution within the 350-700 nm wavelength range was acquired [20]. The presence of an absorption peak at 541 nm served as evidence for the formation of gold nanoparticles. Spectral analysis was performed at 30-minute intervals starting from the initial appearance of the purple hue. After 90 minutes, a discernible increase in the area corresponding to gold nanoparticles confirmed the completion of the reduction reaction and the successful formation of gold nanoparticles.

The analysis of *S. rosmarinus*-derived essential oils using GC-MS revealed a complex mixture of volatile compounds, primarily falling within the categories of monoterpenoids and sesquiterpenoids. In order to identify the functional groups involved in the gold reduction process, assess the ionic stability of gold, and investigate their transformation into gold nanoparticles, infrared spectroscopy was utilized. To facilitate this examination, the resulting nanoparticles underwent centrifugation at 13,000 rpm, leading to the removal of the supernatant phase. Subsequent steps included washing the precipitate with deionized water, followed by two additional rounds of centrifugation. Consequently, distinct FT-IR spectra were acquired for both the resulting nanoparticles and the essential oil (Fig. 3).

The infrared spectrum of *S. rosmarinus* essential oil exhibited distinctive peaks at 1744 and 3463 cm^{-1}, signifying the presence of carbonyl and hydroxyl groups within the essential oil.

These functional groups are prominent constituents identified via gas chromatography-mass spectrometry, including compounds such as camphor, 1,8-cineole, linanol, bornyl acetate, and chrysanthenon. Furthermore, an additional peak observed at 1682 cm^{-1} is associated with the C=C bond, a characteristic feature found in compounds like □-pinene, camphene, and limonene.

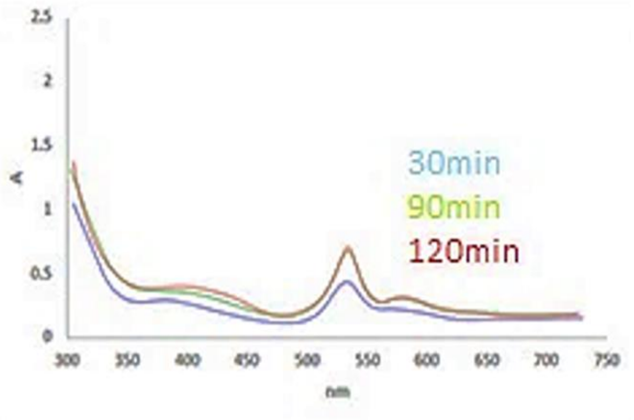

Fig. 2 UV-VIS spectrum of gold nanoparticles synthesized with *S. rosmarinus* essential oi

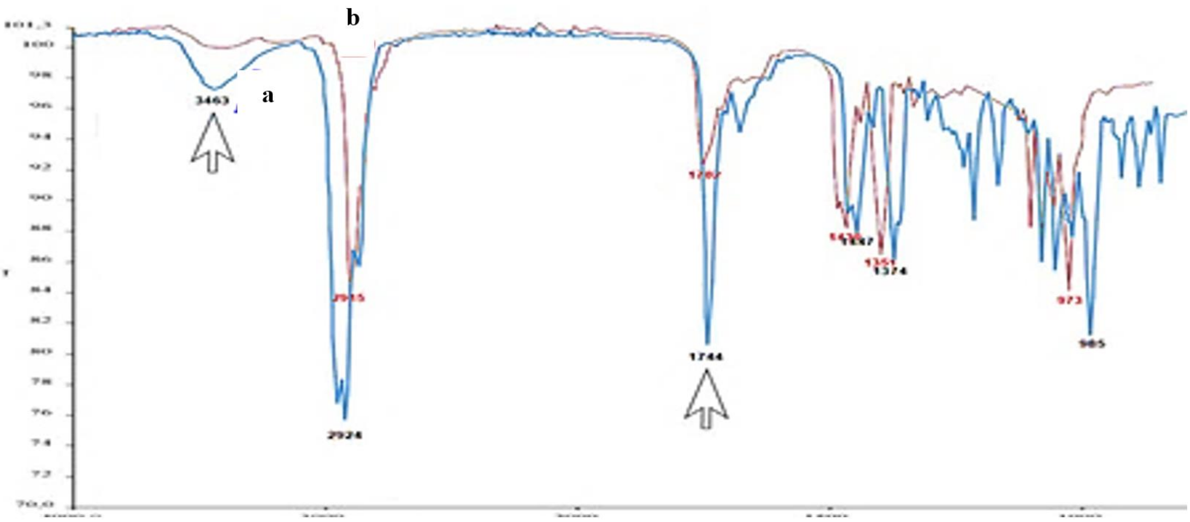

Fig. 3 FT-IR spectrum obtained from essential oils (a) of *S. rosmarinus* and Au-NPs(b)

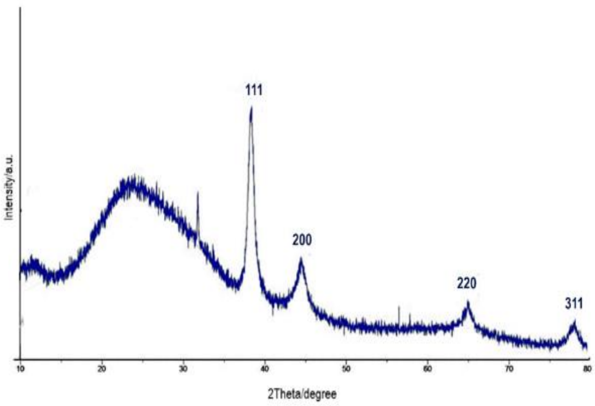

Fig. 4 XRD spectrum of gold nanoparticles synthesized by essential oils of *S. rosmarinus*

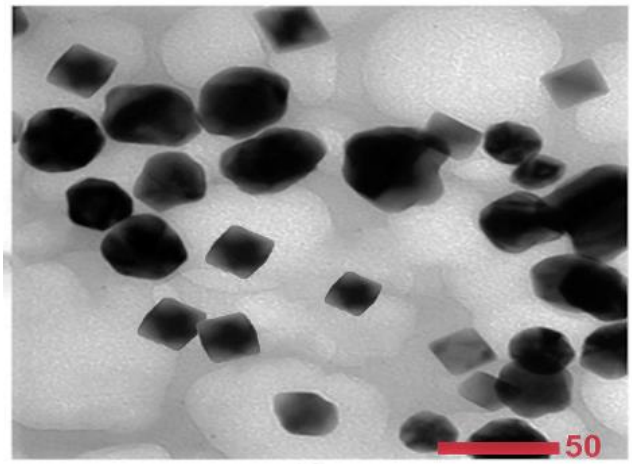

Fig. 5 TEM picture of the synthesized gold nanoparticles

The attenuation in the intensity of these specified peaks and their shift toward lower frequencies indicate the regeneration and stabilization of gold ions into nanoparticle form, facilitated by the carbonyl and hydroxyl functional groups. X-ray Diffraction Spectroscopy (XRD) was employed to confirm the generation of gold in its zero oxidation state and to ascertain its crystal structure. Following centrifugation, the resultant gold nanoparticles were desiccated as a monolayer on a microscope slide, and their XRD spectrum was recorded within the 2θ angle range spanning from 10 to 80 degrees (Fig. 4). The XRD pattern obtained from gold nanoparticles, synthesized through the utilization of Salvia plant essential oil, reveals distinct diffraction peaks at 2θ values of 0.38°, 0.44°, 64.5°, and 3.77° (refer to Fig. 4). These correspond to the Miller indices of 111, 200, 220, and 311, respectively, indicating the presence of a crystalline structure consistent with a cubic lattice configuration [21], predominantly comprised of high gold facets. The most prominent broad peaks observed in the XRD pattern provide strong evidence for the formation of gold particles at nanoscale dimensions.

To examine the morphology of these nanoparticles, transmission electron microscopy (TEM) was employed, utilizing a JEM-1400 instrument from Japan. A colloidal solution containing the synthesized gold nanoparticles (10 microliters) was applied to a carbon-copper grid and allowed to evaporate at room temperature. Based on the TEM image, the average particle diameter was determined to be 26.6 nm (Fig. 5).

CONCLUSION

In this investigation, the chemical composition of *S. rosmarinus* essential oil was comprehensively analyzed using gas chromatography-mass spectrometry. The analysis revealed a total of 20 compounds, constituting 91% of the essential oil composition. Among these, □-pinene (16.7%), 1,8-cineol (8.6%), camphor (10.0%), and borneol (7.7%) were identified as the major constituents. A study conducted by AlMasoud *et al*. in 2021[22] enabled the synthesis of nanoparticles, with characterization carried out through various analytical techniques, including ultraviolet-visible (UV-Vis) spectrophotometry, Fourier transform infrared spectroscopy, transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy, and dynamic light scattering. Visual observation indicated a shift in the color of aqueous silver nitrate to brownish-yellow following treatment with fresh leaf extracts, which was corroborated by UV-Vis spectra. TEM analysis confirmed well-dispersed nanoparticles with average sizes below 22 nm. Additionally, the antimicrobial activities of AgNPs derived from *Ficus carica* and rosemary leaf extracts were evaluated against various gram-positive and gram-negative bacterial strains, with *Ficus carica* -based AgNPs demonstrating superior antibacterial properties. Onmaz *et al*. [23] explored the green synthesis of gold nanoparticles (AuNPs) using bio-active constituents from *Rosmarinus officinalis* and *Helichrysum italicum* extracts, serving as reducing and stabilizing agents. The antibacterial and anti-biofilm activity of these AuNPs was investigated against *E. coli*, *S. aureus*, and *S. epidermidis*. STEM and DLS analyses confirmed the flower-like morphology of the gold nanoparticle clusters, with sizes ranging from 20–130 nm for *R. officinalis* -

AuNPs and 15–90 nm for *Helichrysum italicum* -AuNPs. The minimum inhibitory concentrations (MICs) of *R. officinalis* -AuNPs against *E. coli* and *S. epidermidis* were determined as 40 µg/mL, and 160 µg/mL for S. aureus. *Helichrysum italicum* -AuNPs exhibited an MIC of 20 µg/mL against all bacterial strains and displayed more potent anti-biofilm activity compared to *R. officinalis* -AuNPs. The green synthesis of AuNPs using rosemary and immortelle extracts showcased potential applications in mitigating biofilm-producing microorganisms in the food industry. Bukhari *et al*. [24] highlighted the emergence of metallic nanoparticles, prized for their diverse biological, physical, chemical, magnetic, and optical properties. These nanoparticles can be synthesized through biological, physical, or chemical methods, with the biological approach preferred due to its eco-friendly, rapid, and cost-effective nature. Plants, with their rich phytochemical constituents, play a crucial role in this green synthesis, although the precise mechanism remains under discussion. The report provided an overview of the fundamental principles or mechanisms of green synthesis, particularly for metal and metal oxide nanoparticles such as ZnO, Au, Ag, TiO_2, Fe, Fe_2O_3, Cu, CuO, and Co. Moreover, it explored the medical applications of plant-based nanoparticles, emphasizing their potential in antibacterial, antifungal, and anticancer activities. Khshan and Alkafaje (2021), conducted at the University of Kufa, determined that biogenic silver nanoparticles produced from *Calendula officinalis* had an average size of 16.73 nm, while commercial $AgNO_3$ yielded particles with an average size of 63 nm. FTIR spectroscopy analysis indicated the presence of flavonoids, polyphenols, and amide groups, which likely contributed to the green synthesis of silver nanoparticles. Furthermore, the antioxidant activity of the synthesized silver nanoparticles was evaluated, demonstrating effective reduction of free radicals at concentrations of 330, 230, and 55 µg/ml. The highest inhibition titer was observed for DPPH mixed with AgNPs synthesized from *C. officinalis* at a concentration of 1.5 mg/ml, whereas the lowest inhibition titer was noted for the same mixture at a concentration of 0.5 mg/ml.

In summary, this study showcases the utilization of *S. rosmarinus* essential oils as regenerating and stabilizing agents in the synthesis of gold nanoparticles. These nanoparticles exhibit distinct properties with potential applications across various industries, including cosmetics and the production of health-related products. The resulting gold nanoparticles exhibit a face-centered cubic (fcc) crystal structure and possess an average size of 26.6 nm.

Conflict of Interests

The authors declare the absence of conflicts of interest in conducting this research.

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