Research Article

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Bactericidal and cytotoxic properties of green synthesized nanosilver using *Rosmarinus officinalis* leaves

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Abstract: Green synthesized nanoparticles from plant extracts are being used in various biomedical applications, particularly because of their bactericidal and cytotoxic activities. In this study, silver nanoparticles (AgNPs) were successfully synthesized from the *Rosmarinus officinalis* aqueous leaf extract. Different spectroscopic and microscopic analyses such as ultraviolet-visible (UV-vis) spectroscopy, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy, and energy-dispersive X-ray spectroscopy were performed to verify the biosynthesized AgNPs in our sample. The formation of nanosilver particles was preliminarily confirmed by UV-vis spectroscopy at 400 nm. The presence of carboxylic or amide groups was confirmed by FTIR, for the reduction of the silver ion. Transmission electron microscopy confirmed a particle size of 12–22 nm. The prepared AgNPs showed good antibacterial activity against human pathogens and good cytotoxic activity against the human breast cancer cell line (MDA MB 231). The nanoparticles prepared from *R. officinalis* can be used for various biomedical applications.

Keywords: *Rosmarinus officinalis*, green synthesis, nanosilver

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List of abbreviations

AgNPs	Silver nanoparticles
DLS	Dynamic light scattering
EDX	energy-dispersive X-ray spectrum
FTIR	Fourier transform infrared spectroscopy
PDI	Polydispersity index
SPR	Surface plasmon resonance
TEM	Transmission electron microscopy
UV-vis	Ultraviolet-visible absorption

1 Introduction

Silver has been used by mankind for a long time because of its low toxicity and good biocidal properties. It is frequently used in water-purifying systems in hospitals, for healing injuries, and in the field of nanoparticle synthesis [1]. Some bacteria are resistant to silver and are able to accumulate this metal up to 25% of their dry weight biomass in their cells [2], which makes them suitable for extracting silver from ores [3]. Silver nanoparticles (AgNPs) are used as an effective nano-drug against many diseases [4]. Also, they are more efficient in killing silver-resistant bacteria due to their highly developed surface area [5], whereas highly reactive silver ions (Ag^+ ions) form insoluble precipitates so are less efficient in killing bacteria [6].

Nanobiotechnology is constantly exploring the synthesis of metal nanoparticles in a nontoxic and ecofriendly manner [7]. The main requirements for the synthesis of a metal nanoparticle are the solvent medium, a reducing agent, and a nontoxic stabilizer of nanoparticles [8]. Plants are rich in active compounds and, thus, can be used as a good source of reducing agents for synthesizing nanoparticles [9]; this has prompted a new field of green synthesis [10]. Almost all parts of the plants are rich in active biomolecules such as amino acids, alkaloids, proteins, polysaccharides, phenolics, terpenoids, and flavones, which can exhibit good reducing and capping properties useful in synthesizing nanoparticles [11]. Additionally, hydroxyl and carboxyl groups present in phenols and flavonoids wrap the nanoparticles and act as capping agents [12].

Rosmarinus officinalis, commonly known as rosemary, contains many different bioactive compounds, and it is used as a flavoring spice in cooking for many years [13]. It is one of the most important commercial crops in Saudi Arabia [14]. Its essential oil has been reported to have analgesic, antispasmodic, antibacterial, and hypnotic effects [15]. It is also used in the treatment of depression and stress-related disorders [16]. Same plants grown under different climatic conditions show different active components [17,18]. Some environmental factors like type of soil and temperature can alter the plant structure and its phytochemical activity, especially the bioactive compounds present in the leaves [19]. Some studies even reported a direct relationship between vitamins and phenolic compounds of plant and soil mineral content of the area where the plant is grown [20]. Natural compounds of *R. officinalis* leaves change significantly if grown under differential environmental conditions [21]. It was of great interest to synthesize AgNPs by the reduction of aqueous Ag^{+} ions with the *R. officinalis* leaf extract collected from Riyadh, Saudi Arabia, and synthesis of AgNPs from *R. officinalis* collected from deserts has not been reported. The green synthesized AgNPs were characterized using various microscopic and analytical techniques, including ultraviolet-visible absorption (UV-vis) spectroscopy, Fourier transform infrared spectroscopy, and transmission electron microscopy (TEM). The bactericidal and cytotoxic properties of the synthesized nanoparticles were also analyzed.

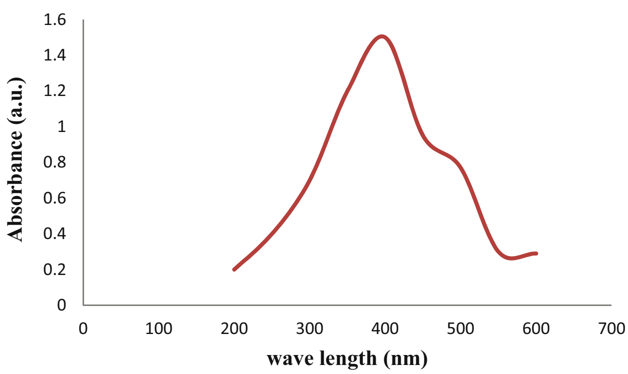

Figure 1: UV-vis spectral analysis of the biosynthesized AgNPs prepared with aqueous *R. officinalis* leaf extract.

2 Materials and methods

2.1 Leaf extract

Fresh green leaves of rosemary cultivated in the Riyadh region of Saudi Arabia were used for this study.

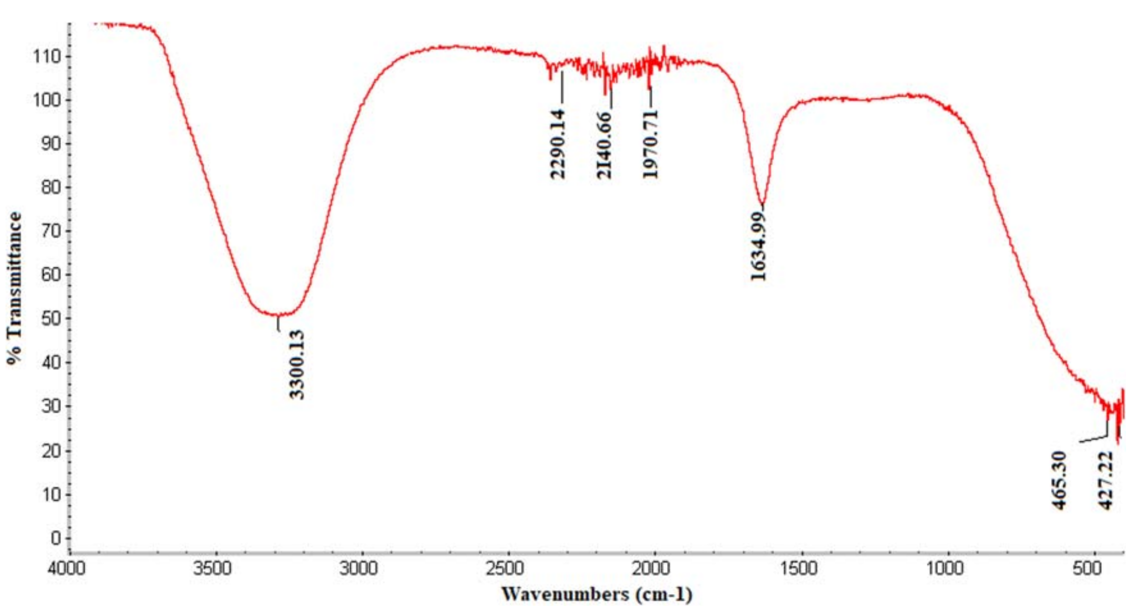

Figure 2: IR spectra of the biosynthesized AgNPs prepared with aqueous *R. officinalis* leaf extract.

Ten grams of healthy leaves were washed, chopped, and boiled in 0.1 L of double-distilled water for 30 min. After cooling to room temperature, the broth was filtered and stored at 4°C.

2.2 Biosynthesis of AgNPs

The freshly prepared leaf extract was mixed with a silver nitrate solution at a final concentration of 5 mM at room temperature. The reduction of silver ions to AgNPs was indicated by a color change from yellow to brown and recorded by scanning through a UV-vis spectrophotometer at different intervals for 3 h.

2.3 Characterization of the prepared nanoparticles

The as-prepared nanoparticles were analyzed by observing the color change of the reaction mixture. The absorbance of the reaction mixture was measured over the range of 200–700 nm every 30 min. The average size of the as-prepared nanoparticles was determined by carrying out the zeta potential analysis. The hydrodynamic size and zeta potential of the prepared AgNPs were measured at the scattering angle of 173° with a red laser at the wavelength of 633 nm. The average size of the synthesized particles was measured with the help of a TEM. Infrared spectroscopy was used to determine the nature of bio-reducing functional groups.

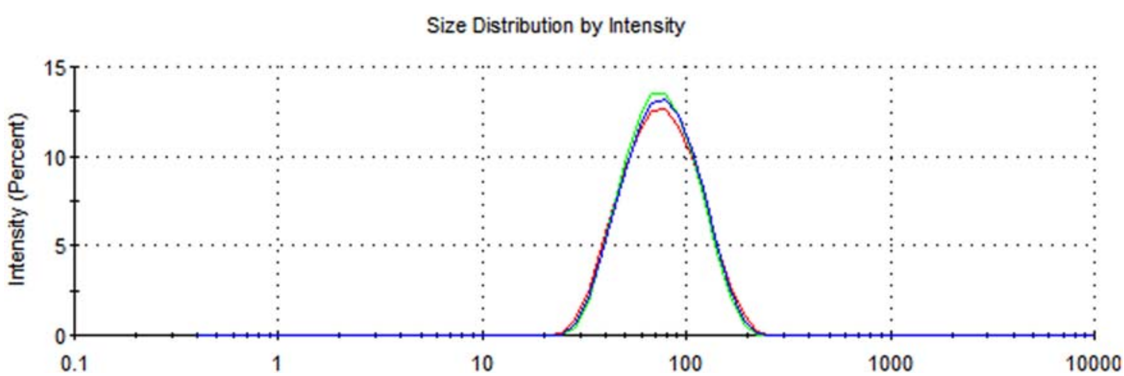

Figure 3: Average size of the biosynthesized AgNPs prepared with aqueous *R. officinalis* leaf extract.

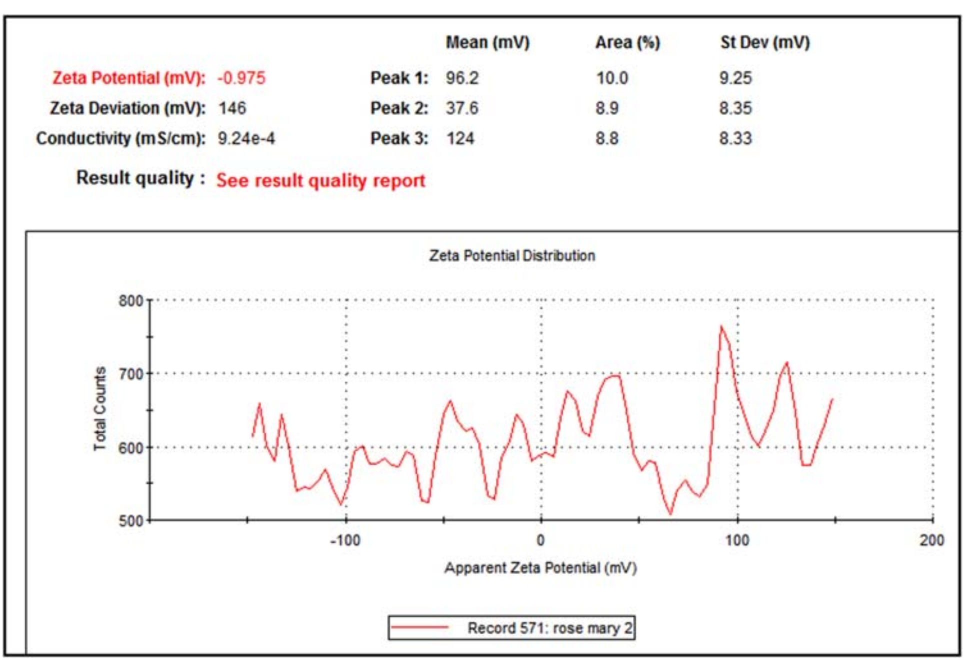

Figure 4: Zeta potential distribution of the biosynthesized AgNPs prepared with aqueous *R. officinalis* leaf extract.

2.4 Antimicrobial activity

Bacterial strains, namely *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Salmonella typhi, Staphylococcus aureus, Staphylococcus epidermidis*, and *Streptococcus pneumoniae*, were provided by King Khalid Hospital, Riyadh, Saudi Arabia. The antibacterial activity of the synthesized AgNPs against all the mentioned bacteria was determined by the disk diffusion method.

2.5 Screening of cytotoxic activity of the prepared nanoparticles

R. officinalis nanoparticles were screened for cytotoxicity on the breast cancer cell line MDA-231 by the MTT assay. Different concentrations of the synthesized nanoparticles (3.125, 6.25, 12.5, 25, 50, and 100 µL) were used to treat the cancerous cells. The inhibition percentage was calculated.

3 Results

UV-vis spectroscopy was used as a primary method to confirm the synthesis of nanoparticles due to their interaction with specific wavelengths. The synthesis of nanoparticles from silver ions in the presence of the rosemary plant extract was initially confirmed by a color change from yellow to brown. The surface plasmon resonance (SPR) peak at 400 nm confirmed the presence of nanoparticles in the extract (Figure 1). Free electrons in the nanoparticles resonate at certain wavelengths, resulting in SPR. A size reduction of the particles can lower the SPR peak, thus relating the absorption to the particle size [22].

Recorded infrared (IR) spectra point toward the active compounds of the *R. officinalis* extract, which facilitate the green synthesis of nanoparticles. Nanoparticles are synthesized in three phases, starting with the activation phase, where the plant metabolites with reducing capacities help to split the metal ion from the salt. Next is the growth phase, where metal ions combine to form nanoparticles, and finally, the termination phase, where capping around the synthesized particle is done with the help of the metabolites present in the plant extract [23]. The IR spectrum of Figure 2 shows an intense peak at 1636.75 cm^{-1}, which is recognized as C=O and C=C bond stretches and identified as carboxylic or amide groups, and a peak at 3304.40 cm^{-1}, which represents the hydroxyl group and suggests the presence of phenols, flavonoids, and alcohols in the plant extract.

In this study, we used dynamic light scattering (DLS) to determine the particle size distribution of the synthesized AgNPs (Figure 3). The *Z*-average mean of the synthesized AgNPs was 66.79 nm, with a polydispersity index (PDI) of 0.177. The PDI is best measured with DLS. Our results with respect to nanoparticles size are acceptable, as a PDI value of more than 0.7 indicates a broad size distribution [24]. Plant extracts are good reducing agents for the synthesis of metal nanoparticles. The interface of the particles mainly depends on the zeta

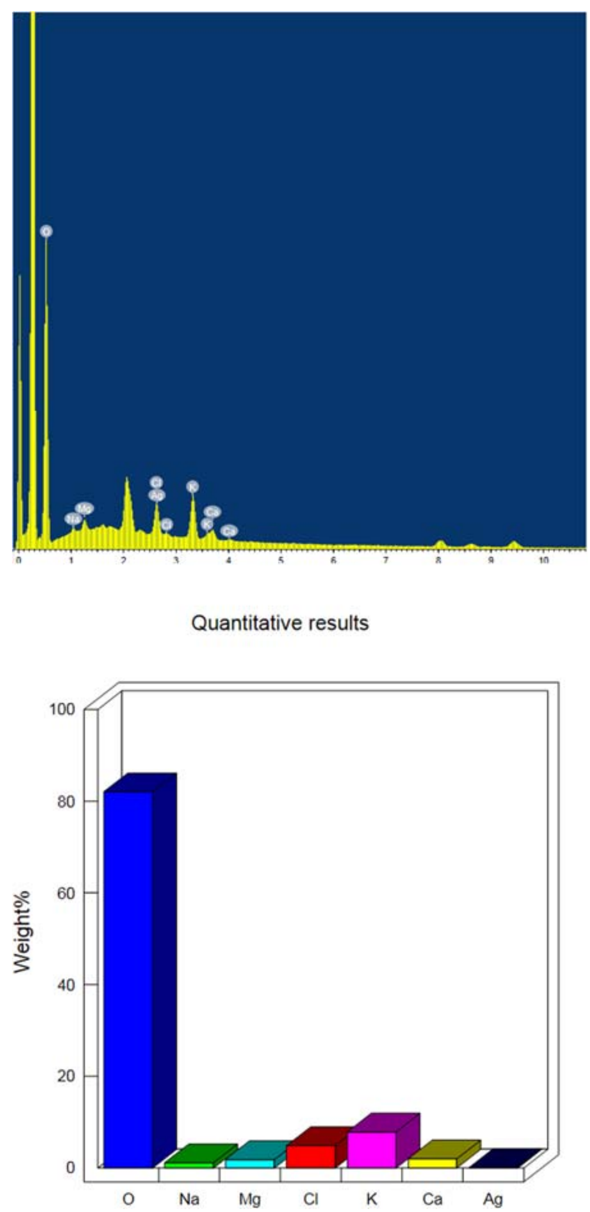

Figure 5: EDX elemental analysis of the biosynthesized AgNPs prepared with aqueous *R. officinalis* leaf extract.

potential since a larger zeta potential results in a particle abundance, thus providing stability to the particles (Figure 4) [25]. The energy-dispersive X-ray spectrum (EDX) of the synthesized nanoparticles exhibited strong signals in the oxygen, potassium, and chlorine regions (Figure 5). The presence of other elements such as carbon, calcium, sodium, and magnesium was also observed in the spectrum. A TEM image was used to find the average size of the prepared nanoparticles, which was in the range of 12–22 nm (Figure 6). TEM is typically an ultrathin sample image showing the 2D structure with the help of an absorption beam passing through the sample [26]. Nowadays, AgNPs are highly important in detergent and disinfectant manufacturing industries because of the resistance level of microbes to chemical fungicides and disinfectants [27]. We examined the antibacterial efficiency of AgNPs prepared from *R. officinalis* against some pathogenic bacteria. The varying degree of inhibition by both the Gram-positive and Gram-negative bacteria is shown in Table 1.

Table 1: Antibacterial activity of the biosynthesized AgNPs prepared with aqueous *R. officinalis* leaf extract against eight bacterial species tested by disc diffusion assay

	Strains	**Zone of inhibition (mm)**
Gram positive	*S. aureus*	11 ± 0.30
	S. epidermidis	8 ± 0.13
	S. pneumonia	10 ± 0.25
	E. faecalis	10 ± 0.50
	B. subtilis	11 ± 0.20
Gram negative	*E. coli*	13 ± 0.10
	S. typhi	14 ± 0.31
	K. pneumoniae	9 ± 0.11

4 Discussion

The antibacterial efficacy of a particle depends on its small size and round shape. Our results agree with many previous studies reporting high antimicrobial activities of round-shaped nanoparticles with a size of less than 10 nm [28]. It has been suggested that the antibacterial properties of the plant-derived AgNPs could be due to either the particles' ability to permeate the cell and interact with the genetic material deoxyribonucleic acid (DNA) and other important constituents, leading to cell death, or their adherence to the negatively charged cell surface that alters the chemical and physical properties of the cell wall and cell membrane [29]. In this study, the green synthesized AgNPs from *R. officinalis* were evaluated at various concentrations for the cytotoxic effects on the breast cancer cell lines (MB 231) *in vitro*. As observed in Figure 7, both plant extracts and nanoparticles decrease the viability of cancer cell lines (MB 231),

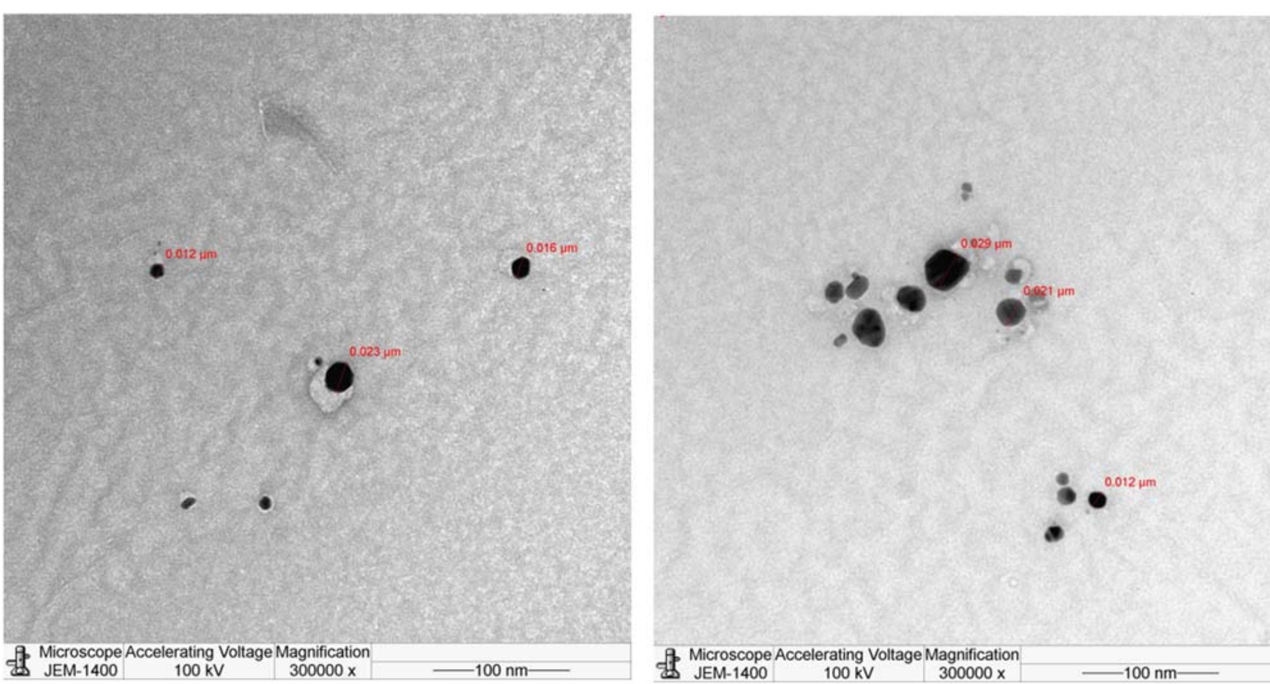

Figure 6: TEM micrograph of the biosynthesized AgNPs prepared with aqueous *R. officinalis* leaf extract (particle size shown in range from 12 to 22 nm).

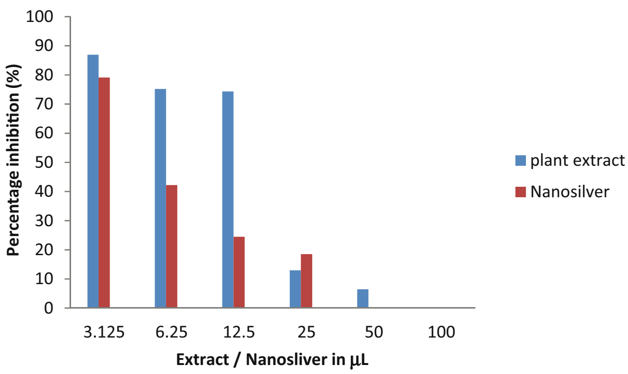

Figure 7: Cytotoxicity of aqueous *R. officinalis* leaf extract and its AgNPs on breast cancer cell lines (MDA MB 231) following 24 h exposure.

but the cytotoxicity of AgNPs was higher than the cytotoxicity of the plant extract. The cytotoxic effect of nanoparticles on cell viability has a major role in antitumor activity, thereby reducing disease progression [30]. The cytotoxic effects of silver result from the active physiochemical interaction of silver atoms with functional groups of intracellular proteins, as well as with the nitrogen bases and phosphate groups in DNA [31].

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