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Enhancing antibacterial activity through green synthesis of silver nanoparticles with *salvia officinalis* extracts

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ABSTRACT

This study aimed to test the effectiveness of water-based extracts and green-synthesized silver nanoparticle extracts from the *Salvia officinalis* plant in killing bacteria. We used the agar well diffusion method to see how well these extracts could fight against both Gram-positive bacteria (*Staphylococcus epidermidis* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). To understand the properties of the nanoparticles, we analyzed them using a UV-VIS spectrophotometer and a scanning electron microscope (SEM). The results showed that both types of extracts were effective against the bacteria, with performance similar to the antibiotic chloramphenicol used as a benchmark. An interesting finding was that combining the plant extracts with the antibiotic or the silver nanoparticle extract with the antibiotic significantly enhanced the ability to stop bacterial growth in all tested strains. The Gram-positive bacteria were more affected than the Gram-negative ones, suggesting a potential way to overcome bacterial resistance.

1. Introduction

The rise of antibiotic resistance in harmful bacteria has increased significantly in recent decades, mainly because of the improper and excessive use of antibiotics. Bacteria naturally develop ways to resist antibiotics they encounter. As a result, the current focus is on creating new antibacterial agents that cause little to no resistance and have no harmful side effects. Many medicinal plants and their extracts have been tested as alternatives to synthetic antibiotics. Recently, new medicines, such as nanoparticles (NPs), have been widely used. These NPs may be combined with existing antibiotics or made from medicinal plants. They are popular in medicine, cosmetics, food preservation, and other fields because they are stable, effective, and affordable. Therefore, producing NPs using natural sources has become an important area in nanoscience and nanotechnology (Parmar et al., 2024; Walsh et al., 2023).

Among metal NPs, silver nanoparticles (AgNPs) are the most commonly used and are often synthesized from plants due to their broad applications in microbiology, chemistry, food technology, and pharmacology. The size and shape of these nanoparticles, as well as the method of biosynthesis, are important factors in determining their effectiveness. AgNPs are produced by various key plant compounds, including flavonoids, tannins, phenols, ketones, and proteins, through oxidation, with each compound showing different levels of activity. AgNPs range in size from 1 to 100 nanometers, which enhances their capabilities and gives them unique electrical, optical, and catalytic properties (Ficai and Grumezescu, 2017). AgNPs have different modes of action to kill different bacterial strains. They have the ability to attack germs in many structures at once (Cheng et al., 2016). Previous studies have demonstrated that AgNPs may directly interact with the extracellular

matrix (ECM) and enhance overall ECM stability (Löfdahl et al., 2020), or due to their small size, AgNPs can physically interact with cell surfaces, causing DNA damage and altering gene expression, causing probable cell death (Löfdahl et al., 2020).

Different plant parts, including leaves, stems, roots, fruits, and flowers, have been successfully utilized for the green synthesis of silver NPs. This Green chemistry-based synthesis, compared to the toxic chemical and physical methods previously used, is carried out at an ambient temperature and neutral pH, making it more economical and environmentally friendly. *Salvia officinalis L.* belongs

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to the Labiatae/Lamiaceae family. It is widely used in food preparations due to its flavoring and seasoning characteristics, and it is used as a natural medicine to treat various disorders, such as inflammation, hyperglycemia, paralysis, rheumatism, and diarrhea (Ghorbani and Esmaeilizadeh, 2017). *S. officinalis* major phytochemicals such as flavonoid coumarins, tannins, saponins, steroids, and terpenes/terpenoids are mostly found in leaves and flowers (Albeladi et al., 2020). *S. officinalis* leaves aqueous extract was reported to have significant antibacterial and antifungal activity (Maliki et al., 2021).

This present work mainly focuses on preparing the aqueous extract from *Salvia officinalis* leaves and the bio-production of AgNPs with their characterization using a scanning electron microscope (SEM) and UV–visible spectrophotometer. Both extracts were screened for their antibacterial activity using two different methods: a) aqueous and AgNPs extracts were tested individually against 2 Gram-positive and 2 Gram-negative strains in comparison to the standard antibiotic, chloramphenicol, and b) each of the extracts was combined with the antibiotic. Through the screening of the plant extracts, NPs, and their combinations with the antibiotic, promising novel resistance-modifying agents will be investigated, expanding the antibacterial spectrum and reducing, as such, the bacterial resistance.

2. Materials and methods

S. officinalis leaves were crushed and washed with sterile water and then air dried at room temperature. The aqueous extract was prepared by adding 5.0 g of the dried leaves to 50 mL of sterile distilled water and then boiling for 15 minutes (Bagherzade et al., 2017). Then, the solution was filtered by using Whatman No. 1 filter paper and then filtered using a syringe Millipore of 0.45µm (Millipore, USA). The sterile extract was collected in sterile tubes and used immediately for analysis.

Silver nanoparticle stock solution was prepared by adding 0.0169g silver nitrate to 100 ml of deionized water. Then 1ml of this solution was placed in an Erlenmeyer flask to which 1ml of the previously prepared aqueous extract was added gradually under the sunlight and with continuous stirring; once a color change was observed, the flask was kept at shade, covered with aluminum foil to prevent oxidation. The *S. officinalis* AgNPs were further measured using a UV spectrophotometer and characterized for their morphology and size using the SEM (Joel, Japan) (Central laboratory, Science College, King Saud University).

The bio-synthesized *S. officinalis* NPs were characterized by measuring their absorbance in the range of 200-800 nm using the UV-VIS spectrophotometer (Shimadzu, Japan).

Chloramphenicol used in the present work was prepared by dissolving 3mg of Chloramphenicol in 30 mL sterile distilled water.

Staphylococcus aureus and *Staphylococcus epidermidis* as Gram-positive strains, and *Escherichia coli* and *Pseudomonas aeruginosa* as Gram-negative bacteria were tested against the individual extracts: the aqueous and the AgNPs and against the combined mixtures of the aqueous extract and the AgNPs with the standard antibiotic chloramphenicol separately. All these four isolates were pre-cultured on nutrient agar plates. Bacterial suspensions were then prepared to have a 0.5 MacFarland turbidity.

The antibacterial activity of each tested extract was evaluated using the agar well diffusion method. In summary, bacterial suspensions were spread on Muller Hinton agar plates with a sterile cotton swab. Three wells, each 6 mm in diameter, were made on the surface of the agar using a sterile cork borer and filled with 100 µl of each extract and AgNPs (silver nanoparticles). Additional plates were prepared similarly, with the wells filled with a mixture of extracts (50 µl of aqueous extract + 50 µl of AgNPs) and AgNPs combined with antibiotics. All plates were left at room temperature for 30 minutes to allow diffusion and then incubated at 37°C for 18-24 hours. The results, based on the inhibition zone sizes, were measured in millimeters. Each test was performed twice.

The AgNPs extract effect on the bacterial cells' morphology and size was determined by the SEM. Both control (untreated) and treated cells were fixed with 2% glutaraldehyde. Cells were then washed and fixed again with 1% osmium tetroxide for 1 h at 4°C. Samples were then dehydrated in acetone, placed on glass coverslips, and dried at room temperature to be finally coated and observed under the microscope.

3. Results

3.1. Formation and characterization of AgNPs by UV-VIS spectrum

The green synthesis of silver NPs (AgNPs) using *Salvia officinalis* aqueous extract was determined by a visual color change from colorless/yellow to reddish brown due to the excitation of Surface Plasmon vibrations in silver NPs. This biosynthesis was further indicated spectrophotometrically, where the metal NPs synthesized from *S. officinalis* water extract were observed at 435 nm. The UV-visible absorption peak is the confirmed evidence of stabilizing bio-synthesized AgNPs by *S. officinalis* aqueous extract (Fig. 1).

3.2. SEM analysis

SEM analysis was proven to be highly helpful for screening the biosynthesized AgNPs. In this study, the SEM measurements of *Salvia officinalis* -AgNPs revealed spherical shape particles formed, demonstrating a reduction of silver NPs in the range of 14.3–19nm (Fig. 2). Results from the present study indicated that *S. officinalis* aqueous extract

revealed great activity against Gram-positive bacteria compared to Gram-negative bacteria. The largest zone of inhibition was observed with S. epidermidis (19mm), followed by S. aureus (17mm). The Nano extract showed strong antibacterial activity, especially against Gram-negative bacteria, when compared to the water extract. However, a stronger effect was observed against Gram-positive bacteria (Table 1). In contrast, the combination of the extract and antibiotic, as well as the antibiotic and Nano extract, showed the highest antibacterial activity against all tested organisms. Both combined mixtures produced the same inhibition zones (Fig. 3). The antibiotic showed the largest inhibition zone, followed by the aqueous extract and, finally, the AgNPs.

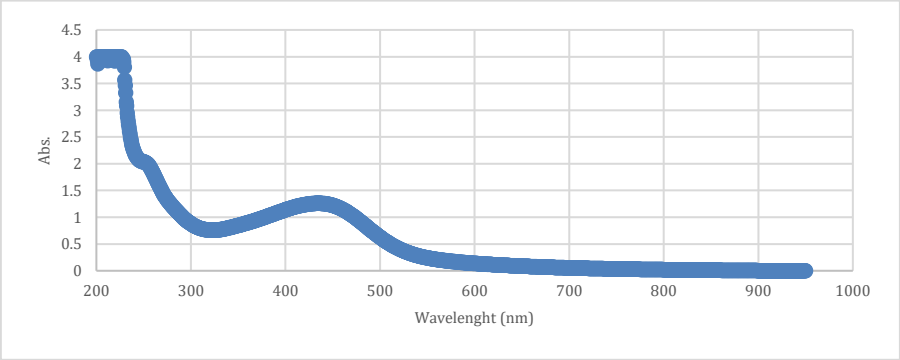


Fig. 1: UV-VIS spectrum showing the formation of silver NPs at 435 nm

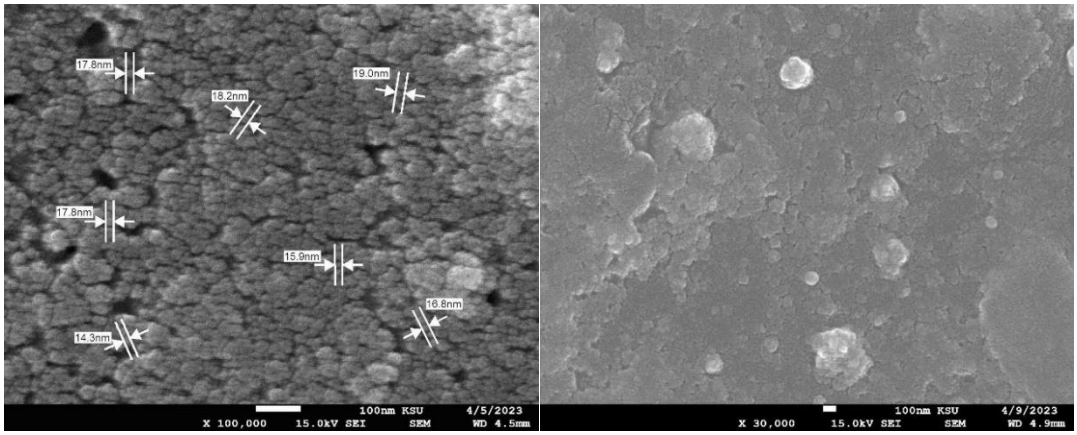


Fig. 2: SEM showing spherical NPs synthesized from the aqueous *S. officinalis* extract (left figure); *S. officinalis* Biosynthesis of silver NPs (right figure)

Table 1: Antibacterial activity of the aqueous extract, silver NPs, and the synergistic mixture

Bacteria	Average inhibition zone in mm ±SD				
	Chloramphenicol	*Salvia officinalis* aqueous extract	AgNPs extract	Antibiotic + extract	Antibiotic + AgNPs solution
S. aureus	40±1	17±0	15.25±0.25	45.25±0.25	42.25±0.75
S. epidermidis	55.25±3.25	19±2.5	16.75±1.75	65±0	54.75±0.25
E. coli	48.5±10.5	6±0	10.75±0.75	43.75±6.75	42±7.5
P. aeruginosa	44.25±4.75	6±0	9.5±0	50.5±0.5	38.25±0.25
Negative control SDW			6 ± 0		

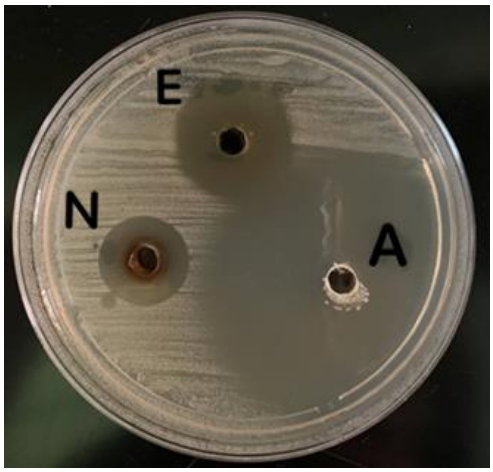
The highest activity was observed with a combined antibiotic and aqueous extract mixture on *S. epidermidis.* Aqueous extract was effective only against Gram-positive bacteria, and no significant activity was recorded with Gram-negative strains. All the combined mixtures demonstrated almost similar activity with a slight difference against the bacterial isolates (Fig. 4).

4. Discussion

Recently, there has been a great focus on biomedicine and the development of new raw therapeutic materials effective for the green synthesis of nanomaterials from plants. Sage leaves, for example, possess diverse biological activity and are promising agents for the green synthesis of silver NPs (AgNPs) (Shivakumar et al., 2017). It has been reported that sage leaves have particularly different groups of phenolic compounds (phenolic acids, luteolin, apigenin, quercetin, and isorhamnetin glycosides) to which this biological activity is linked (Maliki et al., 2021; Sabry et al., 2022). Data from the present study indicated a strong antibacterial activity of the *S. officinalis* aqueous extract mainly against Gram-positive bacteria, where the highest inhibition zone was recorded against *S. epidermis* (19mm) followed by *S. aureus* (17mm) (Table 1), negligible or no effect was observed against *E. coli* and *P. aeruginosa*, in agreement with the previous study of Poh et al. (2018). The AgNPs, on the other hand, had a similar effect on Gram-positive strains

indicated by the highest diameter of inhibition with *S. epidermidis* (16mm) and *S. aureus* (15mm), these particles showed, contrary to the aqueous extract, a moderate effect on both Gram-negative strains, E. *coli* (10 mm) and *P. aeruginosa* (9.5 mm).

It was noted that the synergy between the aqueous extract and the antibiotic revealed the greatest inhibitory activity against all bacterial isolates as follows: *S. epidermis* (65mm)> *S. aureus* (45 mm) > *P. aeruginosa* (50mm) > *E. coli* (43mm). Similarly, the combined effect of silver NPs and the antibiotic had a significant activity on all bacterial isolates again *S. epidermidis* was the most sensitive (54mm), followed by *S. aureus* (mm), *E. coli* (42 mm) and the least activity was against *P. aeruginosa* (42mm). These differences in the antibacterial activity could be related to the difference in the cell wall structure, to the different sizes of the NPs, and or to the phytochemical constituents of the plant. It was observed that NPs with small dimensions cross the bacterial cell membrane easily and have higher antibacterial activity (Kim et al., 2007). Various mechanisms of action for the plant antibacterial activity were suggested most of them were mainly related to damaging the bacterial cell membrane (Khameneh et al., 2019). As for the AgNPs mechanism of action against bacteria still is not very clear. Several hypotheses suggested that NPs could adhere to the bacterial cell wall and membrane, and penetrate into the cell to disrupt the intracellular constituents, or they could induce oxidative stress and modulate the transduction signal (Gonçalves et al., 2019; Mikhailova, 2020; Palma et al., 2021). Through this screening, it was noted that the synergy between the plant extracts, the NPs with the standard antibiotic revealed a novel promising resistance modifying agents, enlarging the antibacterial spectrum and hence reducing the bacterial resistance.



E: Aqueous extract; N: AgNPs; A: Antibiotic

Fig. 3: Agar well diffusion method showing the antibacterial activity of the extract, the Nano, and the antibiotic (chloramphenicol) on *S. aureus*

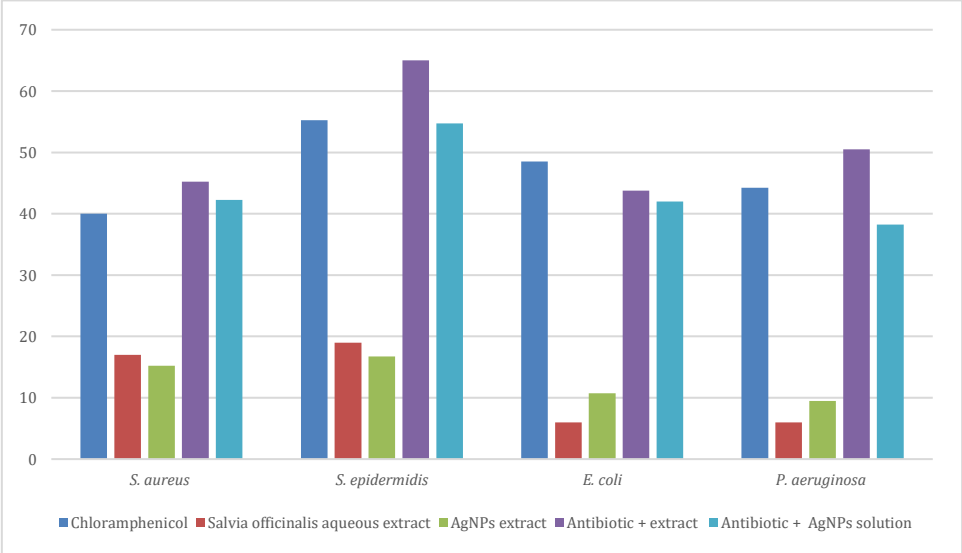


Fig. 4: Antibacterial activity of single and combined extracts effect against bacterial strains

5. Conclusion

The study showed that the aqueous extract of *S. officinalis*, along with the biosynthesized silver NPs, had strong antibacterial effects, particularly against Gram-positive bacteria such as *S. epidermidis* and *S. aureus*. However, it had less effect on Gram-negative bacteria like *E. coli* and *P. aeruginosa*. This suggests

that *S. officinalis* extracts have a broad range of antibacterial activity. The most noteworthy finding of the study is that the combination of the plant extract and the antibiotic, chloramphenicol, produced the greatest inhibition of bacterial growth, especially against Gram-positive bacteria. Similarly, when the metallic extract was combined with the antibiotic, it showed significant antibacterial activity against all tested bacterial strains. Therefore, *S. officinalis* could be a promising alternative to chemically synthesized antibiotics, with potentially lower bacterial resistance.

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Compliance with ethical standards

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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