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Synthesis of Silver Nanoparticles Using Vaccinium Macrocarpon and Investigation of its Role as an Antibacterial Agent

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Annotation: Background & aim: Current applications for silver (Ag) nanoparticles include medication delivery, biomedicine, parasitology, antibacterial, antifungal, and anti-biofilm. Therefore, the current study aimed to create silver nanoparticles using *Vaccinium macrocarpon* extract and to study its role as an antibacterial agent against Gram-negative bacteria.

Materials & Methods: *V. macrocarpon* fruits were collected from various local markets from April 25 to June 25, 2025. The nanoparticles were synthesized using 1 mM AgNO3 with *V. macrocarpon* fruit extract. After studying the properties of the silver nanoparticles, a test was conducted against two types of bacteria, *Staphylococcus aureus* and *Escherichia coli*, using an agar well diffusion experiment to detect whether there was an antibacterial effect of the silver nanoparticles.

Results: After synthesizing silver nanoparticles, their properties were studied and A broad absorption peak of Ag NPs was detected at a wavelength of 436 nm by using UV-Vis spectrophotometer. FT-IR

analysis revealed biomolecules that efficiently encapsulated and stabilized the Ag NPs produced by *V. macrocarpon*. E. coli isolates showed high susceptibility to Ciprofloxacin (86.2%) and Gentamicin (80.4%) but very low to Ampicillin (2.6%) and Vancomycin (0%). S. aureus isolates were highly susceptible to Vancomycin (94.2%), Ciprofloxacin (90.8%), and Gentamicin (86.4%), with low susceptibility to Ampicillin (3.2%). The size range of the produced AgNPs in the sample is 58–337 nm by using scanning electron microscopy (SEM). Using the agar well diffusion method, the antibacterial activity of AgNPs against two species was found to be effective against S. aureus and E. coli. Regarding their capacity to inhibit the growth of microorganisms, AgNPs performed better than plant extract. The highest inhibition against S. aureus (17) mm and E. coli (39) mm was demonstrated by AgNPs.

Conclusions: The current study described a rapid, simple, safe, affordable, and environmentally friendly process for creating Ag NPs utilizing fruit extract from V. macrocarpon, and the results of this study suggest that Ag NPs can be utilized to make antibacterial drugs.

Keywords: AgNPs, V. macrocarpon, E. coli, bacteria, S. aureus.

Introduction

Nanotechnology is a complicated technique that works with samples that are nanometers in size, sometimes known as nanoparticles (NPs). Small, solid particles that range in size from 1 to 100 nanometers are called nanomaterials [1]. Depending on the materials employed in their production, nanoparticles can be either organic or inorganic. Whereas the second type (inorganic) is a noble metallic or magnetic type, the first type (organic) is based on carbon [2,3]. Silver

nanoparticles (AgNPs) are thought to be the best since their drawbacks are minimal in comparison to their benefits [4], despite the fact that numerous metal NPs have been studied [5]. Silver nanoparticles belong to a class of zero-dimensional materials that exhibit inherent structure within their 1–100 nm dimension range [6]. AgNPs exhibit unusual Raman scattering, chemical stability, and thermal conductivity [7,8]. Applications for AgNPs are numerous and include biomedical engineering, medication delivery, food, textile, and agricultural industries; water treatment; anticancer agents; larvicides; and ointment components [9]. AgNPs were discovered to have significant bactericidal and fungicidal activity among the various metal nanoparticles' documented antibacterial qualities [10,11]. Nowadays, green nanoparticle production is widely recognized due to its ease of use, lack of toxicity, speed, stability, and affordability [11]. Plant extract, and different microorganisms, and enzymes are among the ecologically acceptable raw materials used in green synthesis [10]. One of the main benefits of employing plant extract is that it may be produced on a wide scale without the need for cell culture to produce nanoparticles [9]. Small cranberries (Vaccinium macrocarpon) belong to the Ericaceae family and are members of the Vaccinium genus. Microelements and macroelements, proanthocyanidins, phenolic acids, anthocyanins, vitamin C, and triterpene compounds [12,13,14] were detected in cranberry fruit samples. Research on the biological effects of tiny cranberries, which are determined by their bioactive components, is limited and dispersed [15]. The literature describes research on the anti-inflammatory [16], antifungal [17], and antibacterial [18] properties of bioactive components in tiny cranberry fruit. The cranberry's considerable antibacterial activity over benzoic acids was corroborated by the presence of proanthocyanidins and flavonols, according to Aref and Charles [19]. Therefore, the current study aimed to create silver nanoparticles using Vaccinium macrocarpon extract and to study its role as an antibacterial agent against Gram-negative bacteria.

Materials & Methods

Vaccinium macrocarpon fruit

The fruit components were collected from various local markets at period 25 April to 25 June 2025, pressed, and the liquid collected. The liquid was then passed through a filter made of Whatman No. 1 paper (Whatman Limited, UK). The filter was oven-dried at 30°C. The powders were stored at 4°C until further use.

Extraction of *V. macrocarpon*

For 9 days, the 500 g of dried cranberry powder was placed in 2.5 l of 70% ethanol at a ratio of 1:5 (w/v). At 10-hour intervals, it was stirred. Following soaking, Whatman filter paper No. 1 was used to filter the mixture. A water bath set at 40°C was used to concentrate the ethanol filtrate until a sticky, semisolid material was created. This item was kept in storage at 4°C. Dimethyl sulfoxide was used to dissolve the dried extract and create a stock solution (100 mg/ml) [20].

Synthesis of Ag NPs

A silver nitrate aqueous solution was made by mixing 1 mM AgNO3 with 250 ml of room-temperature distilled water. To prevent the auto-oxidation of silver ions, the solution was kept in an amber-colored bottle. After adding 2 mL (1 mM) of silver nitrate dropwise to 0.6 mL of extract while stirring, the mixture was heated to 45 °C in a pH 9 water bath. Silver nanoparticles were formed when the resultant solution turned yellowish brown after 30 minutes of heating [21], the particles were centrifuged for 25 minutes at 12,000 rpm to confirm the synthesis of Ag NP. In order to exclude untreated reaction mixtures, Ag NPs were repeatedly rinsed with deionized water.

Characterization of silver Nanoparticles

UV-Visible spectrophotometer

After diluting the samples with 4 milliliters of deionized water over a regular period of time, the UV-visible spectrum of the reaction mixture was measured in order to track the optical properties of AgNPs and the reduction of pure Ag + ions. The UV-Vis spectrophotometer UV-1700

(Shimadzu, Tokyo, Japan) was used to perform the UV-Vis spectral analysis.

Fourier transform infrared (FT-IR)

Using Fourier transform infrared (FT-IR) spectroscopy, biomolecules that efficiently stabilized and capped Ag NPs were found.

Scanning electron microscope

Silver nanoparticles' size and shape were determined using a scanning electron microscope (SEM) (Model INSPECT S50). To prepare the sample, a very small amount was dropped onto glass plates, and it was then left to dry at room temperature. In order to make the samples conductive, the solution was dried and then a small layer of gold was applied to the SEM slides.

Antibiotic Susceptibility

Antibiotic susceptibility testing of five clinical isolates each of E. coli and S. aureus was performed as described previously using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. Eight antibiotics (Ampicillin, 10µg; Ciprofloxacin, 5 µg; Gentamicin, 10µg: Tetracycline,30 µg; Erythromycin, 15µg: Cephalexin: 30 µg; Vancomycin -30µg and Clindamycin-2ug) based on CLSI protocols46 were used. The plates were incubated at 37°C for 18–24h and the inhibition areas in millimeters were recorded. The percent susceptibility was then assessed for each isolate and averaged for each type of organism.

Antibacterial activity

Ag NPs were tested for their antibacterial activity against S. aureus and E. coli using an agar well diffusion experiment. 20 mL of Muller-Hinton agar was aseptically added to sterile Petri dishes. The bacteria were taken out of their stock cultures using a sterile wire loop. Once the organisms were cultivated, wells of 6 mm in diameter were drilled into the agar plates using sterile needles. After the wells were bored, Ag NPs were added at 25, 50, and 100 µg/mL. The culture plates containing Ag NPs, S. aureus, and E. coli were cultured for 24 hours at 37 °C for the measurement and recording of the average inhibitory zone diameter [22, 23].

Data analysis

The mean and SD are used to display the data. The ANOVA test with SPSS software was used to determine the data' significance. At $p < 0.05$, differences were deemed significant.

Results & Discussion

Characterization of Ag NPs

Ultraviolet–visible (UV-Vis) spectroscopic analysis

Ag NPs were verified using a UV-Vis spectrophotometer. The absorption maximum was observed at wavelengths ranging from 200 nm to 600 nm. A broad absorption peak of Ag NPs was detected at a wavelength of 436 nm (Figure 1). In this context, a previous study revealed the peak absorption of Ag NPs at 440 nm [24]. The green produced nanoparticles' UV-VIS spectral examination revealed a prominent peak between 386 and 450 nm, which was identified as the "surface Plasmon resonance band" and attributed to the excitation of valence electrons. This peak suggests the creation of silver nanoparticles. Additionally, the dielectric constant of the medium and surface-adsorbed species play a major role in the absorption band's location. The symmetrical band pattern indicated homogenous scattering of nanoparticles with a spherical shape [25].

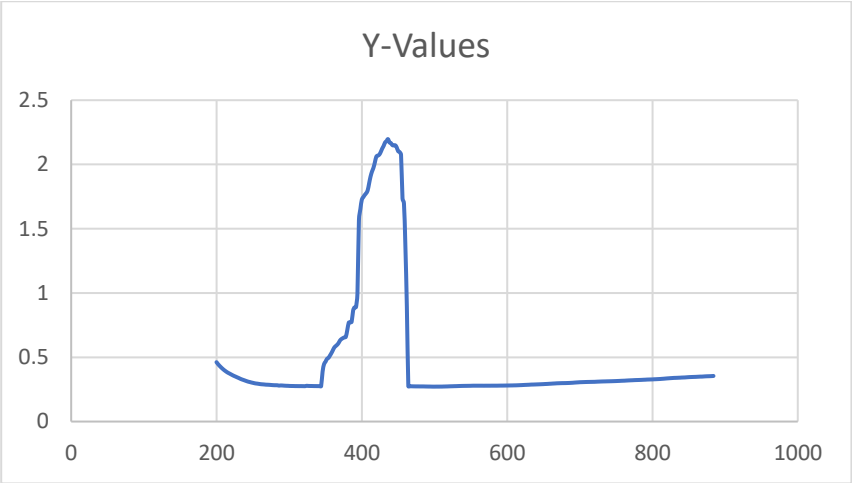

Figure (1): UV–Vis spectrum of Ag NPs

FT-IR

FT-IR analysis revealed biomolecules that efficiently encapsulated and stabilized the Ag NPs produced by V. macrocarpon. The spectrum derived from the Ag NPs FTIR study is displayed in Figure 2. Alcohol and phenols with O-H stretching were compatible with the band located between 3851.05 and 2357.87 cm-1. Primary amines with an N-H bent were compatible with the band located between 1745.49 and 1462.43 cm-1. The aromatic amine group's C-N stretching was represented by the peak at 1377.25 to 1229.41 cm-1, whereas the bands at 1158.81 and 1111.03 cm-1 were consistent with the C-N stretching of ethers, carboxylic acids, alcohols, and esters, respectively. Since proteins and metabolites, including terpenoids with functional groups of ketones, alcohols and carboxylic acids, can encapsulate the produced NPs, this is possible. Our findings were consistent with those of a previous study [24].

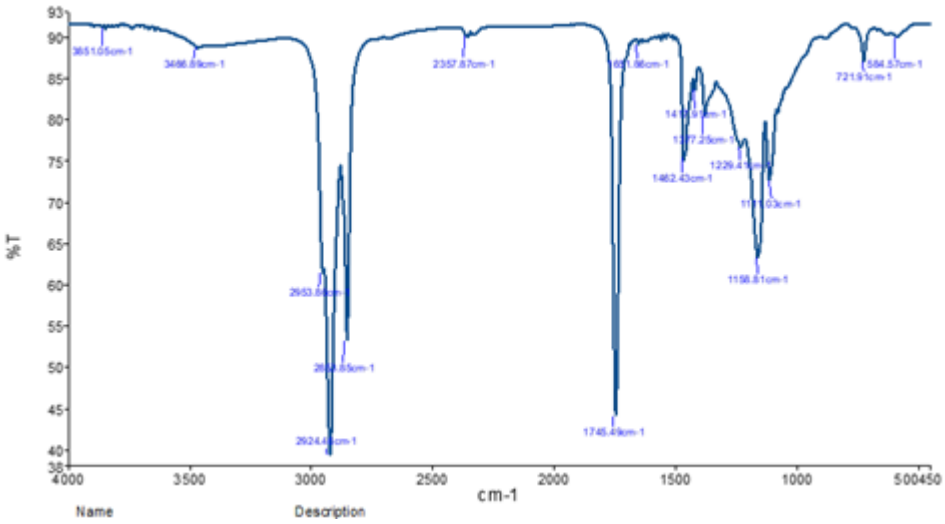

Figure (2): FT-IR spectrum of Ag NPs

SEM

Figure (3) displays the size and shape of the silver nanoparticles as determined by SEM examination. The SEM scans clearly show AgNPs, and the Ag+ ions have been totally consumed. The synthesized AgNPs' size falls between 58 and 337 nm in the sample. Ag agglomeration during SEM analysis preparation may be the cause of the different sizes of Ag particles. The surface topography, composition, and other characteristics of silver nanoparticles, including their electrical conductivity, are detailed in the SEM image. The findings also demonstrated the spherical form of the particles. The spherical silver nanoparticles can be used in pharmaceutical preparations and medical applications. The produced nanoparticles were larger than the typical

nanoparticle size, which is between 1 and 100 nm. This resulted from the proteins' attachment to the nanoparticles' surface [26].

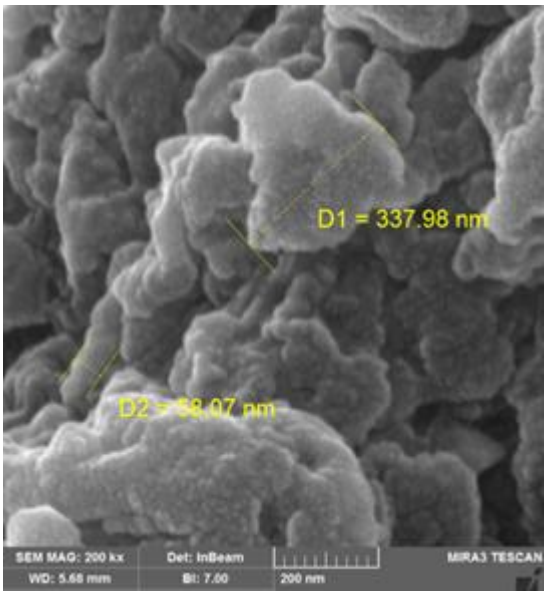

Figure (3): Images of SEM of AgNPs.

Antibiotic Susceptibility

E. coli showed very low sensitivity to Ampicillin (2.6%) and no response to Vancomycin or Clindamycin, but high susceptibility to Ciprofloxacin (86.2%) and Gentamicin (80.4%). In contrast, S. aureus was highly sensitive to most antibiotics, especially Vancomycin (94.2%), Ciprofloxacin (90.8%), and Gentamicin (86.4%), with minimal response only to Ampicillin (3.2%), Table (1).

Table (1): Antibiotic Susceptibility (%) of E. coli and S. aureus Isolates

Bacterial Strain	AMP	CIP	GEN	TET	ERY	CEX	VAN	CLI
E. coli Isolate 1	3	85	80	60	15	50	0	0
E. coli Isolate 2	2	88	82	58	18	52	0	0
E. coli Isolate 3	4	86	79	59	16	51	0	0
E. coli Isolate 4	1	87	81	57	17	53	0	0
E. coli Isolate 5	3	85	80	58	15	50	0	0
Mean %	2.6	86.2	80.4	58.4	16.2	51.2	0	0
S. aureus Isolate 1	4	90	88	80	82	85	95	83
S. aureus Isolate 2	3	92	86	78	80	83	94	81
S. aureus Isolate 3	4	91	87	79	81	84	95	82
S. aureus Isolate 4	2	90	85	78	80	82	93	81
S. aureus Isolate 5	3	91	86	79	81	84	94	82
Mean %	3.2	90.8	86.4	78.8	80.8	83.6	94.2	81.8

AMP: Ampicillin, CIP: Ciprofloxacin, GEN: Gentamicin, TET: Tetracycline, ERY: Erythromycin, CEX: Cephalexin, VAN: Vancomycin, CLI: Clindamycin.

The pattern of antibiotic susceptibility shown by current study exhibited higher resistance of E. coli to Ampicillin (Mean; 2.6%), complete non-susceptibility to Vancomycin and Clindamycin and very high sensitivity to Ciprofloxacin (86.2%) followed by Gentami-cin (80.4%). This high resistance here can be comparable to the local situated data, which state that in the suburban of mosques and butchers were high resistance E. coli against Ampicillin over 87%, almost to 100% even from clinical strains; this represent several purposes of a local based antibiotic habit [27,28]. Similarly, reports from Saudi Arabia and other Middle Eastern settings have shown E. coli to be

increasingly resistant to first-line β-lactam antibiotics with more modest resistance rates in fluoroquinolones but increasing trends overall [29]. On the other hand, S. aureus isolates analyzed in this study were highly susceptible to all of the tested antibiotics with 94.2% susceptibility towards Vancomycin, 90.8% for Ciprofloxacin and 86.4% for Gentamicin with very low susceptibility against Ampicillin (3.2%). This is consistent with MRSA monitoring in hospitals, demonstrating the varying susceptibility of S. aureus and with continued Vancomycin efficacy [30]. Arabic studies also suggest that Vancomycin is still effective against the majority of the S. aureus strains despite increasing resistance profiles and this requires attention and stewardship [31]. In general, there are marked differences in the susceptibility profiles of Gram-negative (E. coli) versus Gram-positive (S. aureus) approaches, emphasizing that antibiotic prescribing habits and local resistance ecology could play an important role. The extremely high resistance against Ampicillin and related β-lactams may be due to long term use and misuse of these drugs, which requires an amendment to the policy guidelines as well as maintenance of stronger antivirus strategy in clinical settings to conserve currently potential useful drugs including fluoroquinolones and aminoglycosides in that part of the world [29,30,31].

Antibacterial Activity

The antibacterial activity of AgNPs against two species was determined to be efficient against S. aureus and E. coli bacteria using the agar well diffusion method. Many researchers have used the well diffusion method, one of the most used ways to measure antimicrobial activity, to verify the AgNPs solution's antibacterial activity. AgNPs outperformed plant extract in their capacity to inhibit microbial growth. AgNPs showed the greatest inhibition against E. coli (39) mm (fig: 4) and S. aureus (17) mm (fig: 5).

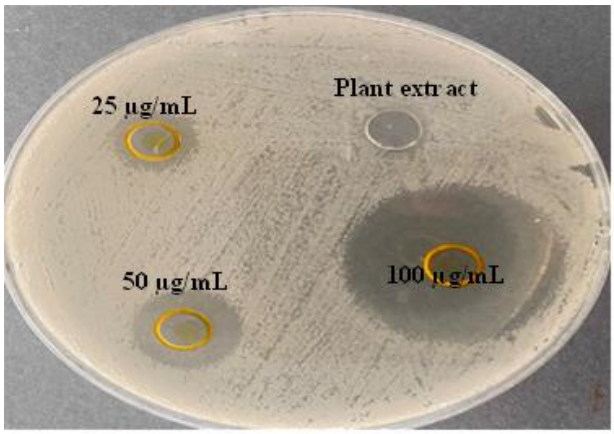

Figure (4): Inhibition zone for three concentrations of AgNPs and plant extract against *E. coli*

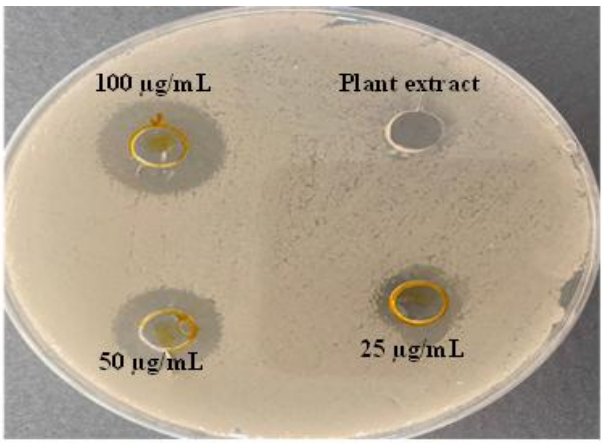

Figure (5): Inhibition zone for three concentrations of AgNPs and plant extract against *S. aureus*

This study also reveals that AgNPs has broad-spectrum antibacterial activity against two bacterial strains of E. coli and S. aureus with distinctive inhibitory rate on E. coli (39 mm) to S. aureus (17 mm). This differential susceptibility is mainly due to the structural and compositional differences that exist in Gram-negative vs. Gram-positive bacterial cell wall. Gram negative E. coli, with thin peptidoglycan and outer membrane enriched with lipopolysaccharides (LPS), lipoproteins, and phospholipids might be more penetrable for AgNPs like to allow potent accumulation. On the other hand, the Gram-positive (e.g., S. aureus) possess a thick multilayered peptidoglycan wall that serves as physical barrier and restricts penetration of bacteria by NP that resulted in low bactericidal effect [32,33]. The excellent activity exhibited by AgNPs compared to plant extracts in the present study, correlates with previous studies. Raghunandan et al. [34] also suggested that plant-mediated AgNPs were more effective on Gram-negatives than their corresponding Gram-positives resistant strains, hinting on the action dependent on size and structure [3]. In addition, Al-Bayati [35] also showed that AgNPs obtained from medicinal plants exhibited considerably larger zones of inhibition against E. coli compared to S. aureus, supporting the generally observed

trend in this study. The antibacterial process of AgNPs is not simple. These nanoparticles are believed to adsorb bacterial cell membranes, induce membrane loss of integrity, produce reactive oxygen species (ROS) and interact with intracellular biomolecules as DNA and proteins leading to cell death [36]. Here, the fast and strong inhibition of bacterial growth suggests that AgNPs may cause damage to both the cell membrane and intracellular factors within a short contact time. Moreover, the differing sensitivities of the two species serve to underline the necessity for quantitative analysis on bacterial wall composition and nanoparticle-bacteria interactions in developing antimicrobial strategies. Significantly, these results are particularly applicable in areas with high levels of antibacterial resistance. Younger et al., [37] reported the high resistance of E. coli strains to Ampicillin and cephalexin antibiotics and susceptibility of a majority of S. aureus to Vancomycin along with Ciprofloxacin [38]. The antibacterial activity of AgNPs against the resistant E. coli is Advancement in this regard that suggest application as alternatives or adjunct to traditional antimicrobials in clinical conditions. In addition, these results have been in agreement with the general tendencies found out for wider Arab-region studies suggesting that nanoparticles synthesized from green could be a universal base to fight against multidrug-resistant infectious agents [39].

Conclusions

AgNPs produced by green synthesis from Vaccinium macrocarpon were characterized and exhibited a spherical shape with biomolecule-protected surfaces. SEM analysis revealed particle sizes ranging from 58 to 337 nm. E. coli showed very low resistance when compared with Ampicillin (2.6%) and did not exhibit sensitivity to Vancoycin or Clindamycin but was sensitive to Ciprofloxacin and Gentamicin. Most of tested antibiotics heavily sensitive to S. aureus, particularly Vancomycin (94.2%) and Ciprofloxacin (90.8%). The AgNPs displayed pronounced antibacterial activity against the two bacteria, indicating this complex as possible alternative or adjuvant antimicrobial for resistant strains.

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