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Green Synthesis of Silver Nanoparticles Using Phyllanthus emblica and Vaccinium oxycoccos Extract: Preparation, Characterization, and Antimicrobial Efficacy

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Aim: With the help of mediating agents such as plants and microbes, silver nanoparticles can be functionalized with molecular capping agents allowing a wide range of applications in the field of dentistry especially in terms of their antimicrobial efficacy. Thus, the present study was designed with an aim to synthesize silver nanoparticles mediated by Phyllanthus emblica and Vaccinium oxycoccos fruit extract that contain bioactive metabolites and to study its characteristics and antimicrobial efficacy.
Materials and Methods: The green approach of synthesizing silver nanoparticles involved the use of fruit extracts from Phyllanthus emblica & Vaccinium oxycoccos. The produced nanoparticles were characterized by means of SEM (Scanning Electron Microscopy), EDAX (Energy-Dispersive X-ray) analysis, and FTIR (Fourier Transform Infrared) spectroscopy. To assess the synthetic nanoparticles' antibacterial activity against particular strains of Staphylococcus aureus, Streptococcus mutans, & Enterococcus faecalis, the Agar well diffusion method was employed.
Results: The fruit extract & the new nanoparticle both showed the presence of different functional groups. The SEM study showed that the particles were spherical, & the EDX investigation verified the existence of the silver element. The nanoparticle showed a dose-dependent antimicrobial efficacy which was highest at 100µL for all the three microorganisms.
Conclusion: Instead of using harmful chemicals to synthesize silver nanoparticles, fruit extract from Phyllanthus emblica and Vaccinium oxycoccos can be used because of its quantity of naturally occurring bioactive compounds and effectiveness against different microbial strains.

Keywords: Silver Nanoparticles, Antimicrobial, Phyllanthus emblica, Vaccinium oxycoccos, Enterococcus faecalis.

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Introduction

The creation, modification, and use of structures through size and form control at the nanoscale, where diameters vary from 1 to 100 nm, are all included in the term "nanotechnology." Nanoparticles have enhanced physicochemical properties and increased chemical reactivity which makes their use in antimicrobial therapy a promising strategy.[1] These characteristics, which allow them to interact with the negatively charged bacterial cells in an efficient manner, include their extremely small size, high charge density, and huge surface area to mass ratio.[2] The application of nanoparticles in dentistry is particularly advantageous due to their broad spectrum bactericidal properties and superior antibacterial activity in bacteria of drug resistance.[3] Studies have shown that Inorganic antibacterial nanoparticles such as 1% silver nanoparticle or zinc oxide nanoparticles within composite resins and glass ionomer cement could be effective in reducing microorganism biofilm with direct contact.[1] Organic nanoparticles such as those of amorphous calcium phosphate have been researched to achieve remineralization and reduce demineralization. There is evidence in the literature that demonstrates the efficacy of nanoparticles such as those of silver[4], and MgO [5] in the elimination of *E. faecalis* which is majorly responsible for reinfection in root canal therapy. Chitosan nanoparticles have been studied for their ability to inhibit biofilm formation and prevention of bacterial recolonization on root dentin.[6] Nanoparticles have also been incorporated within root canal filling materials[7,8] and intracanal medicaments[9] to improve their biological properties and increase their antimicrobial efficacy.

When synthesizing nanoparticles, the top-down & bottom-up techniques are the two most often employed strategies. These conventional methods are however toxic, not environmentally friendly and expensive.[10] Green routes that use naturally occurring sources and their products are an alternative to overcome the shortfalls of the conventional techniques. Green synthesis can be performed using either microorganisms such as fungi, yeast, bacteria, plants, and their extracts, or templates such as virus DNA and diatoms.[11]

Because of its antibacterial, antioxidant, antidiabetic, hypolipidemic, antiulcerogenic, gastroprotective, hepatoprotective, and chemopreventive qualities, *Phyllanthus emblica L*, generally referred to as amla or the Indian gooseberry, is a valuable herb in Ayurvedic medicine.[12] It is high in minerals, vitamin C, and phenolics such as emblicol, tannin, rutin, phyllembelic acid, and phyllembelin curcum-inoids. The plant's fruit, known by another name, Rasayana, may have restorative qualities.[13]

Vaccinium oxycoccos, commonly called the European Cranberry, is a significant herb in traditional medicine because it contains flavonoids, anthocyanins, and other bioactive compounds with antioxidant action in addition to a significant amount of organic acids and vitamin C. The plant's fruit is particularly high in flavonols, particularly quercetin, and polyphenolic chemicals like anthocyanins and proanthocyanins, which are primarily responsible for the fruit's antioxidant effect. Its biological significance is increased by its antifungal, antibacterial, and anticancer characteristics.[14, 15, 16]

Successful Silver nanoparticle production has been previously facilitated by green synthesis using *Phyllanthus emblica* fruit extract[17] as well as extracts of *Vaccinium oxycoccos*. Combining the extract of both fruits to mediate the synthesis process would provide a comprehensive approach to silver nanoparticle production, enabling the tailoring of nanoparticle properties to suit a wider array of applications. Thus, the present study was designed with an aim to synthesize

silver nanoparticles mediated by *Phyllanthus emblica* and *Vaccinium oxycoccos* fruit extract and to study its characteristics and antimicrobial efficacy.

Materials and methods

Preparation of the Fruit Extract

Fresh Cranberries and fruits of Amla were purchased locally, rinsed with double distilled water, dried at room temperature, and finely chopped after removing the seeds. Ten gm of the chopped fruits in equal proportion were weighed and transferred to a glass kettle. After chopping the fruits and heating 100 mL of distilled water to 60 °C for 20 minutes, the aqueous extract was ready. After the mixture had cooled, Whatman's filter paper, No. 1, was used to filter it. The filtrate was employed in this study's subsequent experiments after being centrifuged at 5000 rpm.

Green Synthesis of the Silver Nanoparticles

Cranberry and amla extracts served as a bridge in the green synthesis process used to create silver nanoparticles. The fruit extract (1ml (w/v)) was mixed drop by drop in aqueous 0.001 M Silver nitrate $AgNO_3$ solution. A laboratory orbital shaker was used to subject the mixture of silver nitrate and fruit extract to orbital shaking for a predetermined amount of time, usually two to three hours. The reduction of the metal ions and subsequent production of nanoparticles were aided by the orbital shaking. The visual observation of a color change in the solution, which indicated the endpoint indicative of nanoparticle synthesis, was one of the first indications of the reduction of metal salts into nanoparticles. Following the shaking phase, the mixture was centrifuged for 10 minutes at 8000 rpm in order to extract the nanoparticles from the remaining solution. This produced a dark brown powder that was repeatedly cleaned with DI water in order to get rid of excessive extract. The powder was utilized for additional characterizations and research after being stored for a few hours at 50 °C in an oven.

Characterization of Nanoparticles

To assess the synthesized silver nanoparticles' qualities, a range of characterization methods were applied. The nanoparticles underwent FTIR, SEM, and EDX examination for characterization. The agar disc diffusion method was utilized to analyze the antimicrobial activity against common oral bacteria, specifically *Staphylococcus aureus*, *Streptococcus mutans*, & *Enterococcus faecalis*, at varying doses.

Fourier Transform Infrared (FTIR) Spectroscopy

To determine which functional groups were present in the synthesized nanoparticles, FTIR analysis (Thermo Nicolet Avatar 330, Thermo Fischer Scientific, Waltham, MA) was carried out. In order to prepare the nanoparticles for FTIR measurement, they were combined with potassium bromide (KBr) and compacted into a pellet.

Scanning Electron Microscopy (SEM)

To investigate the size & shape of the nanoparticles, SEM was used. The synthesized nanoparticles were examined with a high-resolution SEM after being placed on a sample holder and covered with a thin layer of conductive material.

Energy-Dispersive X-ray (EDAX) Analysis

Using the EDAX analysis, the elemental composition of the synthesized nanoparticles was ascertained. After positioning the nanoparticles on an appropriate substrate, they were examined using an EDAX detector on an SEM (D8 Advance Diffractometer, Bruker Corporation, Billerica, MA).[18]

Antibacterial Testing

Using the agar well diffusion technique, the synthesized nanoparticle's antibacterial activity was assessed against *S. mutans*, *E. faecalis*, & *S. aureus*. After being prepared with double-distilled water (pH 7.0), MHA (Mueller-Hinton agar) was autoclave-sterilized for 15 minutes at 121°C. Following sterilization, the MHA was added to the petri dish and let solidify at room temperature. An inoculum containing 106 cfu/mL of the freshly prepared bacterial culture was applied to the MHA plates using a sterile cotton swab moistened with the suspension of the relevant microbial culture. The finished nanoparticle solution was then added to four wells of 9 mm in diameter that had been drilled into the MHA medium using a micropipette. The amounts added were 25 µg, 50 µg, and 100 µg. A 25 µg dose of gentamicin served as the positive control. After that, the solution was let to diffuse into the medium at ambient temperature for four hours. After that, the culture plates were maintained at 37°C for one additional day. After incubation, the diameter (mm) of each plate's zone of inhibition was measured.

Every group underwent the antimicrobial assay in triplicate, and the results were collated onto an MS Office Excel sheet (v 2019, Microsoft Redmond Campus, Redmond, Washington, United States). The statistical package for social sciences (SPSS v 26.0, IBM) was used to analyze the data. $P < 0.05$ was regarded as statistically significant for all statistical tests. For numerical data, descriptive statistics were performed, such as the mean & standard deviation. To determine the statistical significance of the antimicrobial efficacy of different concentrations of the nanoparticle with different microorganisms, one-way ANOVA and post hoc Tukey analysis was performed.

Results

Upon coming into contact with plant extracts, silver ions underwent a color change that resulted in the formation of silver nanoparticles, exhibiting a dark brown hue.

Fourier Transform Infrared (FTIR) Spectroscopy

The synthesized silver nanoparticles mediated by the plant extracts contain bioactive phytoconstituents with a variety of functional groups, as indicated by the FTIR spectra. Prominent peaks were seen at 771, 810, 1013, 1200, 1338, 1398, 1521, 1616, 1662, 1715, 2924, and 3321 cm−1 in the FTIR spectrum (Fig 1).

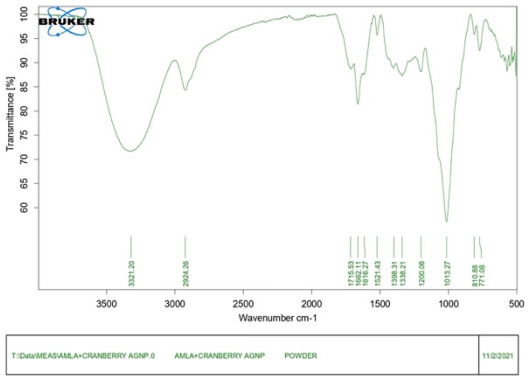

Fig 1: Fourier Transform Infrared (FTIR) Spectroscopy giving peaks in infrared spectra.

These relate to N-H stretching, carbonyl stretching, C-H asymmetric stretching, aromatic C-H bending, C-OH stretching, and C-N stretching.[19] The characteristic absorption peaks suggested the presence of polyphenolic chemicals. For instance, a large signal at 3321 cm−1 suggested the symmetric and asymmetric stretching of the polymeric hydroxyl group (O–H), also known as H-bonded stretching, which is common for polyphenolic phytoconstituents.[20] The peak at approximately 2924 cm−1 detected the stretching vibrations of -CH, -CH2, and -CH3, which are produced from the sugars and carbohydrates in the extracts. The oscillation of the carbonyl group (C=O) in

aldehydes & sugar derivatives was responsible for the peak at approximately 1715 cm−1. The stretching of the aromatic bond's constituent C=C–C and C–H produced the peak at approximately 1662 cm−1. The phenolic C–O stretching, which correlates to the peak seen at around 1200 cm−1, allowed for the identification of the flavonoid C rings.[21]

Scanning Electron Microscopy (SEM)

Under the SEM, the nanoparticle's size and shape were determined. The results of the study showed that the nanoparticles had a homogeneous size distribution and a spherical shape. By testing several nanoparticles, the average particle size was ascertained to be 10 nm in size. The surface properties of the nanoparticles and visual confirmation of their structure were obtained by the SEM examination (Fig 2).

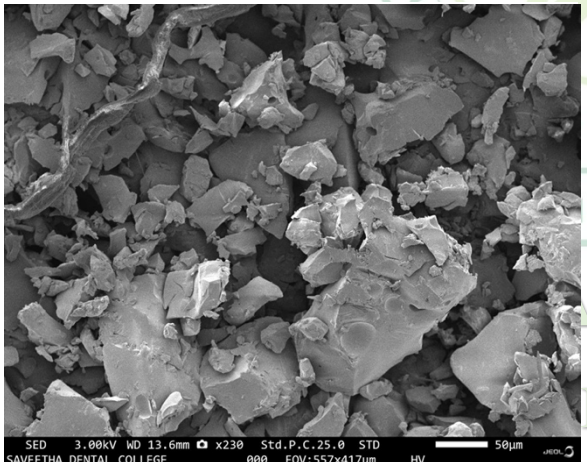

Fig 2: Scanning Electron Microscope (SEM) analysis of the stannous nanoparticles.

Energy-Dispersive X-ray (EDAX) Analysis

According to the characteristic peaks of the EDAX analysis, which was carried out to determine the elemental composition of the synthesized nanoparticles, Ag, C, and O made up the nanoparticles (Fig 3).

Fig 3: Energy-dispersive X-ray (EDAX) analysis describing the elements detected in the sample with corresponding spectra values.

Antimicrobial Analysis

It was observed that the antimicrobial efficacy of the nanoparticle increased with an increase in concentration and was highest at 100µL for all three microorganisms. When 25µL of the nanoparticle solution was applied to *S. mutans*, *E. faecalis*, & *S. aureus*, the mean zone of inhibition was assessed as follows: 15.76 mm, 8.83 mm, and 20.56 mm, respectively. The mean zone of inhibition observed when 50µL of the nanoparticle solution was used against *S. mutans*, *E. faecalis,* & *S. aureus* was measured as 18.33mm, 11.20 mm & 21.46 mm respectively. The mean zone of inhibition observed when 100µL of the nanoparticle solution was used against *S. mutans*, *E. faecalis* & *S. aureus* was estimated to be 19.56 mm, 12.16 mm & 23.46 mm respectively (Fig 4, Table 1). The one-way ANOVA analysis revealed that the antimicrobial efficacy of the nanoparticle was significantly higher against *S. aureus* at all concentrations. It was also observed that the antimicrobial efficacy of the Gentamicin serving as the positive control was significantly higher across all the groups (Table 2).

Fig 4: Agar well diffusion method indicating the zone of inhibition around various concentrations of Silver nanoparticles and control for *E. faecalis*, *S. aureus* and *S. mutans*.

Table 1: Mean and Standard deviation of zone of inhibition around various concentrations of silver nanoparticles and control for *S. mutans*, *E. faecalis* and *S.aureus*

Microorganism	Concentration	Mean (mm)	Standard Deviation
S. mutans	25 µL	15.7667	.25166
	50 µL	18.3333	.70946
	100 µL	19.5667	.70238
	Control	32.1667	1.25831
E. faecalis	25 µL	8.8333	.97125
	50 µL	11.2000	.75498
	100 µL	12.1667	.30551
	Control	37.4667	.89629
S. aureus	25 µL	20.5667	.40415
	50 µL	21.4667	.45092
	100 µL	23.4667	.47258
	Control	38.2333	.68069

Table 2: Results of one way ANOVA analysis and post hoc Tukey test. p<0.05 indicates significant difference and p<0.000 indicates a highly significant difference.

One Way ANOVA			
Microorganism			P value
S. mutans	Between groups		Sig: .000
	Within groups		
E. faecalis	Between groups		Sig: .000
	Within groups		
S. aureus	Between groups		Sig: .000
	Within groups		
Post hoc Tukey Test			
Microorganism			P value
S. mutans	25 µL	50 µL	.020
		100 µL	.002
		Control	.000
	50 µL	25 µL	.020
		100 µL	.316
		Control	.000
	100 µL	25 µL	.002
		50 µL	.316
		Control	.000
	Control	25 µL	.000
		50 µL	.000
		100 µL	.000
E. faecalis	25 µL	50 µL	.024
		100 µL	.003

Discussion

The development of new technologies has made it possible to produce silver nanoparticles with precise target functions, size, & shape. With the help of mediating agents such as plants and microbes, these nanoparticles can be functionalized with molecular capping agents allowing a wide range of applications of these nanoparticles in the field of dentistry especially in terms of their antimicrobial efficacy.[22]

In this study, the nanoparticle synthesis was mediated using the fruit extracts of two plants, namely cranberry and amla. A SEM was used to examine the synthesized nanoparticles' morphology, and an EDX was helpful in determining the sample's elemental makeup. The SEM study showed that the nanoparticles were spherical in shape and evenly distributed, which was an indication of the stability of the synthesized nanoparticles. The peak seen in the EDX analysis verified the presence of nanocrystalline silver. A similar structure of biosynthesized silver nanoparticles has been observed in previously conducted studies by Renuka et al[17], Nayagam et al[23] and Dhar et al.[24]

FTIR spectroscopy is one of the most significant analytical methods for identifying changes in functional groups, which in turn allows for the detection of changes in the overall composition of biomolecules. Different amounts of vibrational and rotational energy are present in molecules. FTIR is used to quantify how molecules rotate and vibrate in response to infrared radiation at a specific wavelength.[25] The different functional groups correspond to the characteristic peaks in the FTIR spectrum. The occurrence of peaks at particular wave numbers verified the nanoparticles' successful synthesis and the extract's functional groups' role in their creation. The FTIR study shows that the cranberry extract

contains a large amount of phenolic chemicals. These compounds in the extract are responsible for the transfer of electrons to Ag+ ions. Cranberries contain significant amounts of quercetin, a plant flavonoid belonging to the flavonoid group of polyphenols. Being an electron-rich compound, it acts as a reducing agent by offering free electrons. The oxidized intermediates are unstable and interact with the nanoparticle surface by capping onto them, thereby stabilizing the nanoparticles. As a result, it serves as a stabilizing & reducing agent.[21] According to reports, terpenoids & flavanones, which are reducing agents found in *Phyllanthus emblica* fruit extract, are what cause Ag^{+} to be reduced to AgNPs.[26]

With the presence of microorganisms within complex root canal anatomies that develop drug resistance and the ability to survive harsh environmental conditions, developing effective irrigation protocols becomes a challenge. The aim of studying the properties of various plant products for their antimicrobial efficacy in endodontics is to develop root canal disinfectants that are not only potent but also biologically compatible and least cytotoxic.

In the present study, dose-dependent antimicrobial activity by the synthesized silver nanoparticles against *S. mutans*, *E. faecalis* & *S. aureus* has been observed. *S. mutans* is the major etiological agent for dental caries and primarily resides on the biofilm formed on the tooth surface.[27] *E. faecalis* is primarily responsible for reinfection of previously endodontically treated teeth.[28] *S. aureus* is a gram-positive pathogen responsible for diseases such as mucositis, periodontitis, endodontic infections & even dental caries and has a tendency to develop drug resistance.[29] A visible, distinct zone encircling the nanoparticle solution indicated the presence of the synthesized silver nanoparticles' antibacterial activity. Various mechanisms have been proposed for their antimicrobial activity. Silver ions have an affinity to sulfur and can hence cause disruption of the bacterial membrane by adhering to it via electrostatic forces. After being absorbed by the cell, respiratory enzymes deactivate, producing reactive oxygen species that damage cell membranes and alter DNA. Moreover, denaturing ribosomes & inhibiting protein synthesis are both possible effects of silver ions.[30] The bioactive metabolites found in considerable quantities in cranberries and amla can function as capping & reducing agents when utilized in the manufacture of silver nanoparticles, which is why they were included in this study. Strong antifungal & antioxidant properties of silver nanoparticles made with cranberries have been shown in earlier studies.[21] Additionally, they have shown strong size-dependent in vitro antibacterial action against microbes that are gram-positive & gram-negative.[31] Similarly, silver nanoparticles synthesized using extracts of Phyllanthus emblica have demonstrated antibacterial activity against *Klebsiella pneumoniae*, *Staphylococcus aureus*[17] & *E. coli*.[32] The principal problem caused by chemical antibacterial drugs is multidrug resistance. The antibacterial qualities of chemical compounds frequently depend on how precisely they adhere to surfaces and how microorganisms metabolize them. Thus, these inorganic nanoparticles have a definite advantage over conventional chemical antibacterial therapies.

The fact that the current study was carried out in vitro under carefully controlled laboratory conditions, which do not accurately reflect the intricate interactions that take place in a real organism, is one of its major limitations. Due to variables including bioavailability, distribution, metabolism, and excretion, the behavior of the nanoparticles may vary in a real biological environment,

producing distinct results. Furthermore, neither the precise antibacterial processes at work nor the methods of nanoparticle uptake by the different microorganisms under research are thoroughly explored in the study, which may prevent a thorough evaluation of nanoparticle doses and potential toxicity. Furthermore, the study's time range is quite constrained, and the possible long-term impacts of repeatedly being exposed to nanoparticles have not been investigated.

Conclusion

An excellent substitute for chemical agents in the creation of nanoparticles is the use of plant extracts. The study's nanoparticles, which were created using amla and cranberry extracts, showed that the extracts included functional groups. Additionally, it showed antimicrobial action against particular strains of *S. aureus*, *E. faecalis*, & *S. mutans*. The study's results are encouraging, and more clinical investigation is necessary to corroborate the findings and turn these nanoparticles into goods that can be purchased commercially, such as toothpaste, mouthwash, and root canal disinfectants.

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