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Research Article

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Biosynthesis and characterization of Silver Nanoparticles from Grape (*Vitis vinifera)* seeds and study on antibacterial activity

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Abstract

Nanobiotechnology is a rapidly growing scientific field, having potential effects in life sciences and human health care applications. Silver nanoparticles are playing a major role in the field of biomedical nanotechnology and nanomedicine. Silver is a naturally occurring precious metal, most often as a mineral ore in association with other elements. Silver nanoparticles have a natural antimicrobial effect against many pathogens such as bacteria, fungus, viruses, and yeast.

In the present study, synthesis of silver nanoparticles was done using grape seeds. The silver nanoparticles obtained were characterized by UV-VIS spectroscopy, Scanning Electron Microscope, X-ray Diffraction and Fourier Transfer Infrared Spectra. The synthesized silver nanoparticles from seeds of *Vitisvinifera* were screened against five pathogenic bacterial species (*E.coli, Bacillus subtilis, Streptococcus* sp*, Klebsilla pneumonia and Pseudomonas aeroginosa*) by agar well diffusion method.

The characterization of reduced silver ions was determined by UV-VIS Spectroscopy which showed broad surface Plasmon resonance band around 400 nm. FTIR bands were observed between 400 cm^{-1} to 4000 cm^{-1}. Intense peaks and crystalline formation of nanoparticles were observed through X Ray Diffraction. Spherical yeast like Ag-NPs of various sizes from 30-100 nm was observed from SEM analysis.

The antimicrobial activity of silver nanoparticles synthesized form *Vitis vinifera* seeds was determined against five pathogenic bacterial species (*E.coli, Bacillus subtilis, Streptococcus* sp*, Klebsilla pneumoniae* and *Pseudomonas aeroginosa*) by agar well diffusion methods.In the current study,organisms like *Bacillus subtilis, Streptococcus* sp and *Pseudomonas aeroginosa* are more sensitive than *E.coli* and *Klebsilla pneumoniae* against synthesized nanoparticles.

Keywords: Silver nanoparticles, SEM, FTIR, XRD and Antibacterial activity.

Introduction

Nanotechnology is a developing interdisciplinary field of research interspersing material science, bionanoscience, and technology. Remarkable advances are made in the field of biotechnology and nanotechnology to harness the benefit of life sciences, health care and industrial biotechnology. A reliable and eco-friendly process for synthesis of metallic nanoparticles is an important step in the field of nanotechnology. In recent years, noble metal nanoparticles have been the subject of focused research due to their unique optical, electronic, mechanical, magnetic, and chemical properties that are significantly different from those of bulk materials (Gardea-Torresdey, J, L., *et al.,*2002). There is particular interest in nanoparticulate Ag, due to its ability to act as both an electron sink as well as aredox catalyst Silver nanoparticles (Ag-NPs) have received considerable attention in a wide variety of areas due to their unique antimicrobial properties, especially for use in medicine related fields such as wound dressings, contraceptive devices, surgical instruments and bone prostheses. For this purpose, the size of nanoparticles needs to be carefully considered (Ueon, Sang, Shin., *et al.,*2011).

It is well known that inorganic nanomaterials have good antimicrobial properties. Silver nanoparticles are themetal of choice as they have the capability to kill microbes effectively (Sharma, N, C., *et al.,* 2007). The strong toxicity of silver against wide rangeof microorganisms is well known and silver nanoparticles have been recently shown to be an effective antimicrobial agent. The silver nanoparticles act on a broad range of target sites both extracellularly as well as intracellularly. The mostwidely used and known applications of silver and silver nanoparticles are in the medical industry (Jahn, W., 1999). These includetopical ointments and creams containing silver to prevent infection of burns and open wounds (Husseiny, M, I., *et al.,*2007). In addition, silver containing consumer products such as colloidal silver gel and silver-embedded fabrics are now used in sporting equipment.

In fact, microbes generally have a harder time developing resistance to silver than they do to antibiotics. Silver nanoparticles takes advantages of the oligodynamic effect that silver has on microbes, whereby silver ions bind to reactive groups in bacterial cells, resulting in their precipitation and inactivation. Silver nanoparticles show very strong bactericidal activity against gram-positive as well as gram-negative bacteria including multiresistant strains (Shankar, S,S.,*et al*., 2004).

The aim of the present study was to synthesize silver nanoparticles (snps) with seed extract of *Vitis vinifera.* Synthesized silver nanoparticles were then characterized with various techniques and investigate the antimicrobial activity against five pathogenic Bacterial species.

Materials and Methods

Fresh black grapes were purchased from the market and seeds were removed. Seeds were washed and shade dried for 5 days and powdered, this was filtered using 0.02 mm sieve and stored for further use .A known amount of seed powder was added to 100ml of distilled water in 250 ml flask. The mixture is mixed properly and centrifuged at 3000 rpm for 15 minutes at 4^oC by REMI cooling centrifuge to remove cell free debris. The supernatant was filtered using Whatman filter paper NO.1, to the sample 1mM of $AgNO_3$ is added and incubated in hot water bath at 60^oC for 40 minutes. The change in color was observed.

Characterization of silver nanoparticles:

UV-VIS Spectra Analysis: The reduction of pure Ag+ ions to Ag was monitored by measuring the UV-VIS spectrum of Ag nanoparticles solution. UV-VIS spectral analysis was done by using UV-VIS spectrometer at the range of 200-600 nm.

Fourier Transform Infrared Spectra: FT-IR spectroscopy measurement are carried out to identify the biomolecules that bound specifically on the silver surface. Lyophilized sample was used to examine at the spectra range of 400-4000 Cm^{-1}.

Scanning Electron Microscope: SEM analysis of silver nanoparticles provides information about the surface, shape and size of the particles. The film of the sample was prepared on a carbon coated copper grid by dropping a very small amount of the sample on the grid. Extra solution is removed using blotting paper and grid allowed to dry by putting it under a mercury lamp for 5 minutes.

X-ray diffraction: The characteristic x-ray diffraction pattern generated in a typical XRD analysis provides unique fingerprint of crystals present in the sample. When properly interpreted by comparison with standard reference patterns and measurements, this fingerprint allows identification of the crystalline form.

Antibacterial Property of Ag Nanoparticles:

The antibacterial property of silver nanoparticles was determined against the pathogenic bacteria such as *E.coli, Bacillus subtilis, Streptococcus* sp*, Klebsilla pneumoniae* and *Pseudomonas aeroginosa* by agar well diffusion method. Different concentration of silver nanoparticles i.e. 50µg, 100µg, 150µg, 200µg and 250µg were used to find the activity against pathogenic organisms. All the plates were incubated at 37^o C for 24 hours and the zone of inhibition was measured at in millimeter scale.

Results and Discussion

In this work, Grape seed extract was used to produce silver nanoparticles. Silver ions were reduced to silver nanoparticles when seed extract was mixed with silver nitrate solution. Reduction was noticed by change in the color from transparent white to light brown (Figure 1) which indicates the formation of silver nanoparticles. Similarly, Shikha (2013) observed color change in case of grape fruit extract from pale pink to grayish brown with 2-3 minutes of incubation.

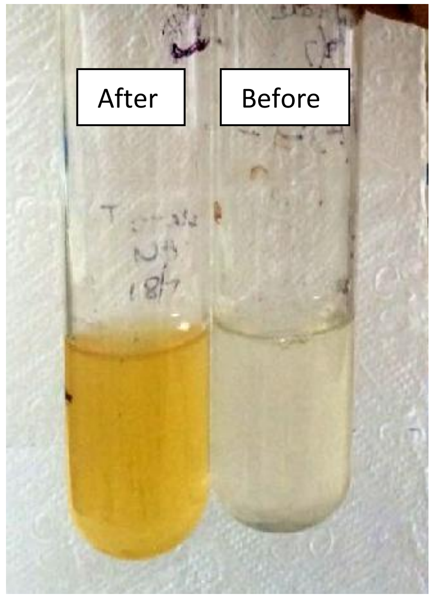

Fig 1: **Color change observed from transparent white to light brown color, indicating the formation of silver nanoparticles.**

UV-VIS Spectroscopy:

It is an indication of silver nanoparticles formation as the color change observed is due to the excitation of surface Plasmon vibrations in silver nanoparticles. The broad surface Plasmon resonance band observed around 400 nm(Figure 2) indicates that the particles are large and Polydispersed. Shikha (2013) reported the maximum absorbance peak at 410nm. Similarly broad surface Plasmon resonance band was observed around 450-470 nm by Gnanadhas G., *et al., (2013).*

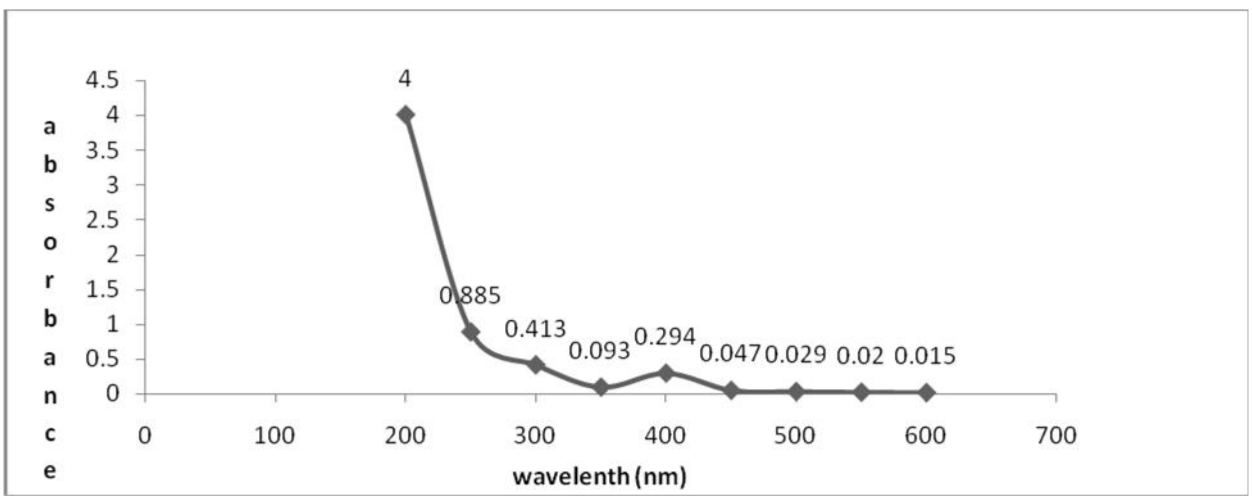

Fig 2: **UV-VIS spectroscopy**

Fourier Transform Infrared Spectra:

The nature of the biomolecules involved in the reduction and formation of silver nanoparticles was studied by FTIR. The FTIR signals (Figure 3) of silver nanoparticles were observed at 3419.50, 2992.94, 1681.74, 1386.25, 1069.51 and 824.58 cm^{-1}. The prominent bands at 3419.50cm^{-1} are attributed to NH stretching vibration possibly due to the presence of 2^o amide group, which is responsible for the reduction of AgNO3 to Ag. The band at 2992.94 cm^{-1} is due to O-H stretching vibration, the band at 1681.74 cm^{-1} may be due to the stretching of C=O group. Band at 1386.25 and 824.58 cm^{-1} is attributed to C-H bending, band at 1069.51 cm^{-1} is attributed to S=O stretching bonds.

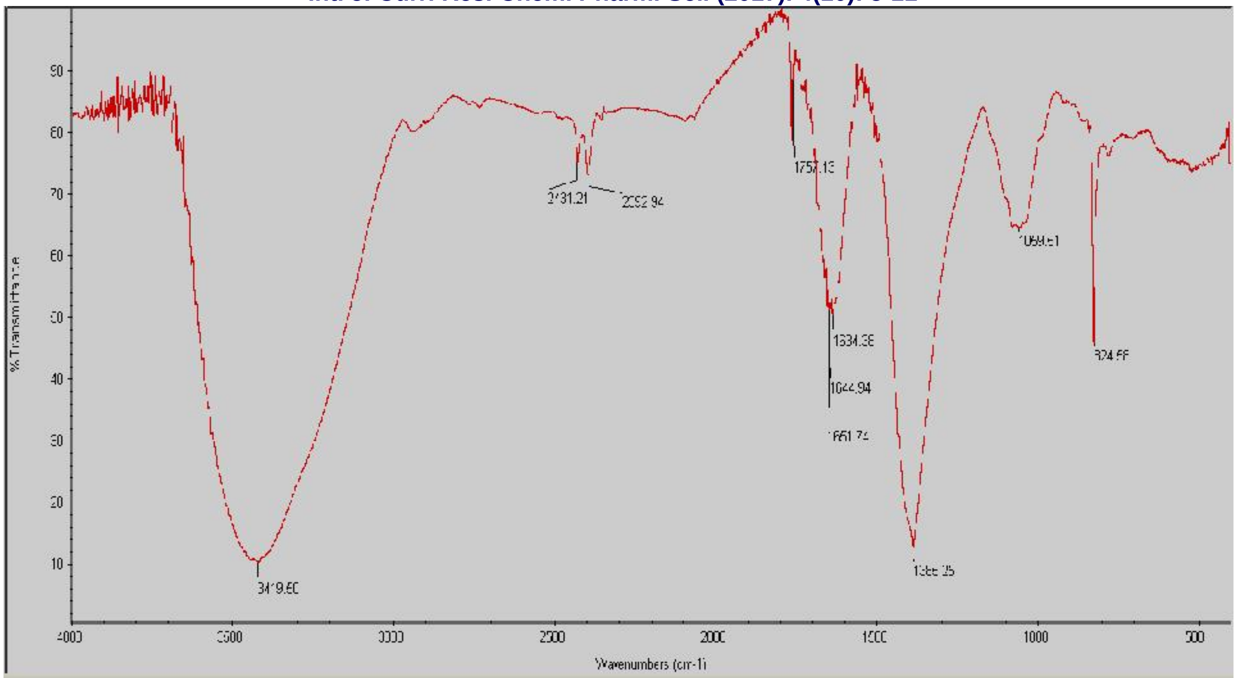

Fig 3: **Fourier transfer infrared spectrum**

Scanning Electron Microscope:

SEM analysis was carried out to understand the topology and the size of the Ag-NPs, which showed the synthesis of poly, dispersed spherical, yeast like Ag-NPs of various sizes from 30-100 nm.

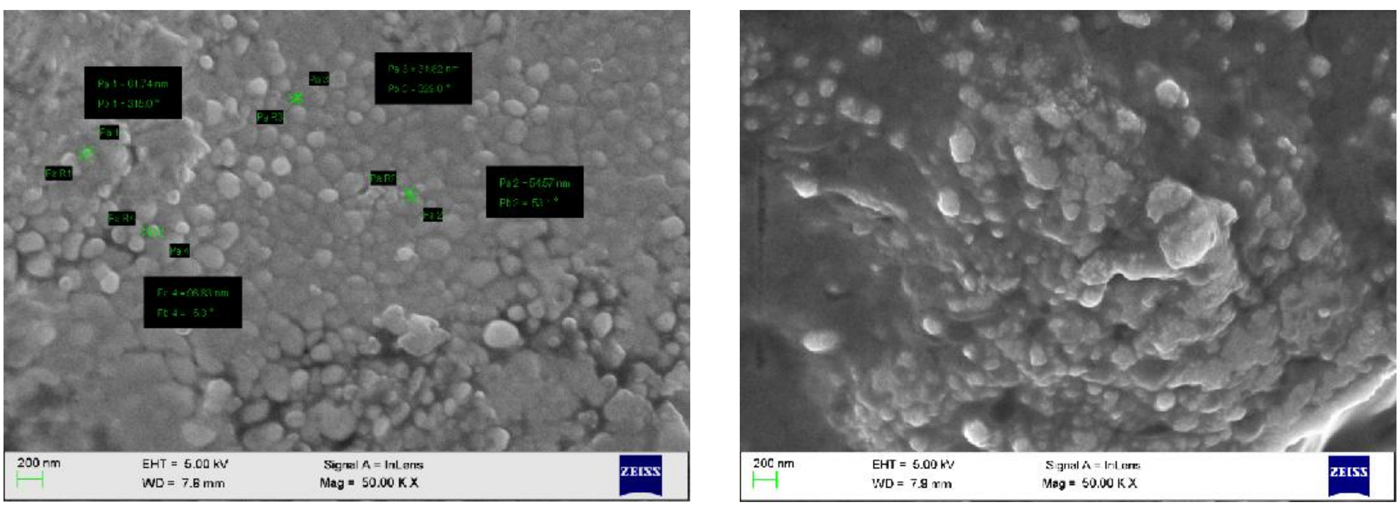

Fig 4: **Images from scanning electron microscopy**

SEM characterization of synthesized silver Nanoparticles are shown in the figure 4. It was showed relatively spherical shaped nanoparticles formed with diameters that range from 30-100 nm. Sorabh. C., *et al.,* 2012 reported the presence of silver nanoparticles with average size of 50 nm from *Vitis vinifera.* 30-40 nm size range was observed from Gnanadhas G., *et al., (2013)* in his work on synthesis of silver nanoparticles from *Vitis vinifera.*

X-Ray Diffraction:

The X-ray diffraction (XRD) pattern clearly shows that synthesized Ag nanoparticles formed are crystalline in nature. The seeds extract-synthesized (*Vitis vinifera*) Ag nanoparticles have shown 8 intense peaks (Figure 5) in the whole spectrum of 2θ values ranging from 30 to 80. A number of Bragg reflections corresponding to sets of lattice planes are observed which may be indexed, based on the FCC structure of silver. Peaks are also observed, suggesting that crystallization in the bio-organic phase occurs on the surface of the nanoparticles.

Peak list

No.	2-theta(deg)	d(ang.)	Height(cps)	FWHM (deg)	Size(ang.)	Phase name	Chemical formula
1	29.461	3.0294	818(52	0.042(	2064(2	Unkno	Unkno
2	37.96(	2.368(	230(28	0.60(6)	146(15	Silver	Ag
3	39.908	2.2572	413(37	0.038(	2321(4	Unkno	Unkno
4	43.269	2.0892	59.886	0.0586	1523.1	Unkno	Unkno
5	44.159	2.0492	65.607	0.0586	1527.8	Silver	Ag
6	64.41(	1.4454	108(19	0.48(1	204(51	Silver	Ag
7	77.411	1.2318	125.96	0.0586	1814.3	Unkno	Unkno
8	80.164	1.1963	375(35	0.059(	1850(2	Unkno	Unkno

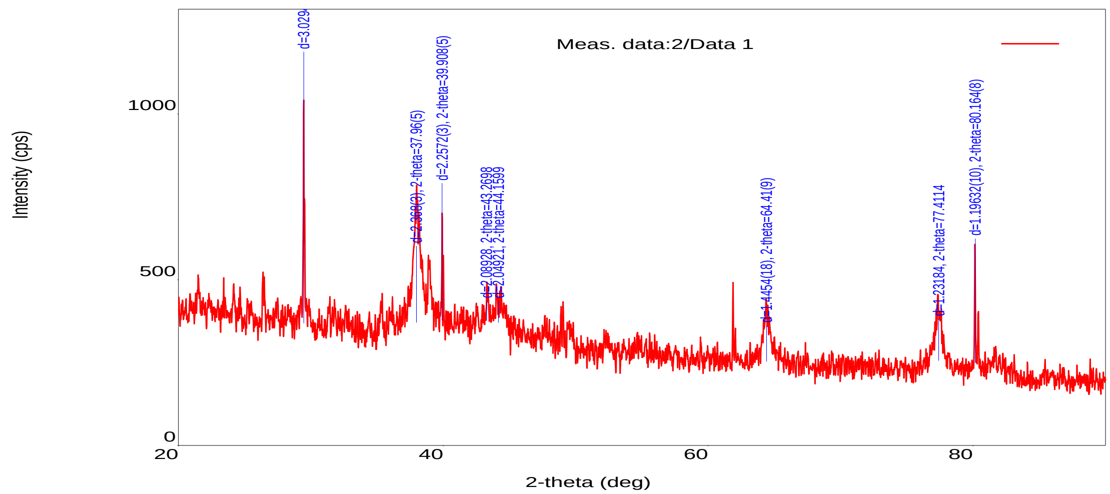

Fig 6**: X-Ray diffraction**

Antibacterial activity:

The antimicrobial activity of AgNPs and standards was tested by agar well diffusion method. The highest zone of inhibition was observed in *Pseudomonas aerogiosa, Streptococcus* spand *Bacillus subtilis* and silver particles wereless effective against *E.coli,* and *K.pneumoniae.* Least inhibitory zone was found in control $AgNO_3$ and amphicillin. No zone of inhibition was observed crude untreated seed extract.

Kaushik.R.*,et al., (*2013) in their thesis confirmed the effective growth reduction of *E.coli* and *B.subtilis.* Clear zone of inhibition was spotted and reported in case of *Pseudomonas aerogiosa, Streptococcus pneumoniae, Bacillus subtilis* and *E.coli* but standard antibiotics showed smaller zone of inhibition compared to nanoparticles by Sorabh C., *et al., 2012.*

In current studies, nanoparticles showed high resistance towards all the pathogenic bacterial species when compared to standard antibiotic amphicillin or standard $AgNO_3$. Clear zone of inhibition was observed in all the plates even at lower concentration of 50µl.

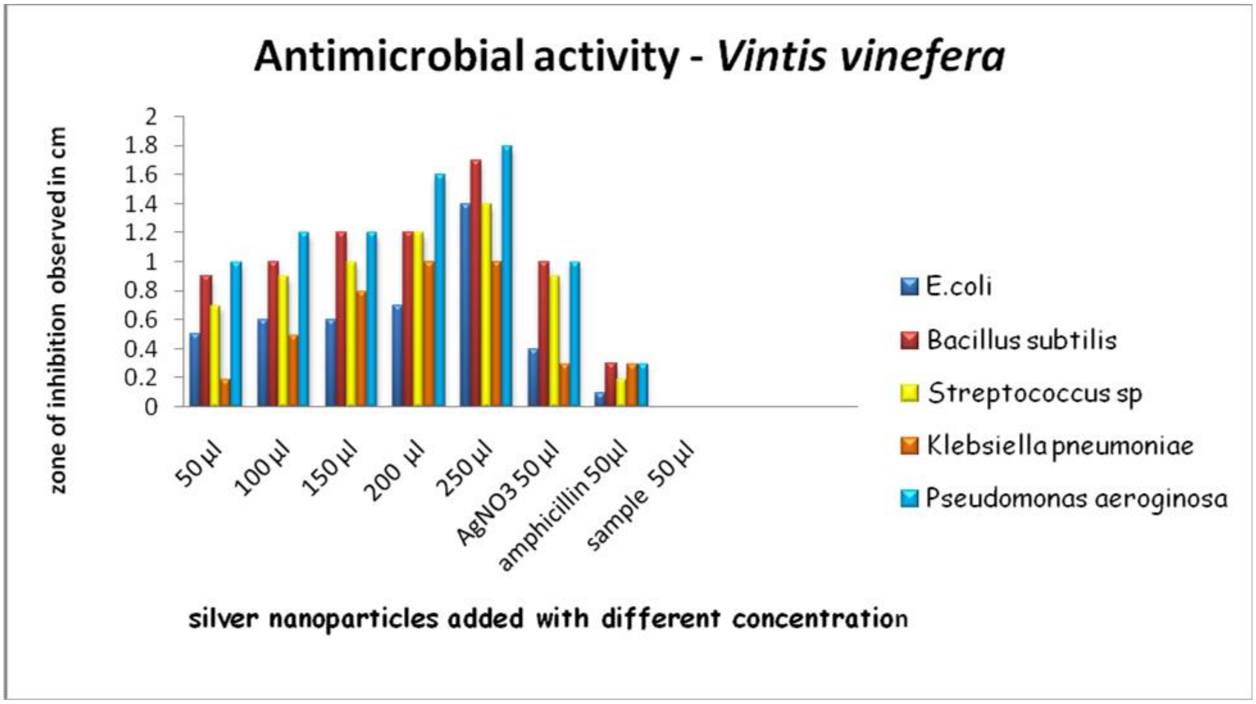

Fig 7:**Anti-bacterial activity of silver nanoparticles extract of *Vitis vinifera* at different concentration**

Conclusion

In the present study, we found that *Vitis vinifera* is a good source for the synthesis of silver nanoparticles. It was confirmed from the color change to brown from white transparent solution. The presence of silver nanoparticles also corroborate by the different methods of characterization we used in this work. UV-VIS analysis showed good Plasmon resonance behavior. Round yeast like granules nanoparticles having the size range of 30-100 nm can be observed with the aid of scanning electron microscope. FTIR and X-ray diffraction method also confirmed the presence of nanoparticles.

Silver nanoparticles produced from the grape seeds extract showed good antibacterial activity against common pathogens. Zone of inhibition regarding synthesized silver nanoparticles was greater than the normal antibiotics like amphicillin or pure $AgNO_3$.

This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanomaterial).

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