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Pharmaceutical Sciences **Research Article……!!!**

"QUERCUS CONFERTIFOLIA BIOSYNTHESIZED SILVERNANOPARTICLES"

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KEYWORDS:

Silver Nanopartciles, Quercus Confertifolia ,Minimum Inhibitory Concentration.

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ABSTRACT

The development of eco-friendly and sustainable methods for the synthesis of silver nanoparticles (AgNPs) is gaining increasing attention due to their potential applications in various fields, including medicine, agriculture, and environmental remediation. In this study, we report the green synthesis of AgNPs using the extract of Quercus confertifolia, a medicinal plant commonly used in traditional medicine. The AgNPs were synthesized by reducing silver nitrate (AgNO3) with Quercus confertifolia extract, which acts as a reducing agent and stabilizing agent. The resulting AgNPs were characterized using UV-Vis spectroscopy, transmission electron microscopy (TEM), X-ray diffraction (XRD), and Fourier transform infrared (FTIR) spectroscopy. The results show that the AgNPs have a mean diameter of 20-30 nm, with a high degree of crystallinity and a spherical shape. The FTIR spectra revealed that the Quercus confertifolia extract plays a crucial role in the stabilization of the AgNPs by forming a complex with the silver ions. The antimicrobial activity of the AgNPs was evaluated against several bacterial and fungal strains, including Escherichia coli, Staphylococcus aureus, Candida albicans, and Aspergillus niger. The results show that the AgNPs exhibited significant antimicrobial activity against all tested strains, with a minimum inhibitory concentration (MIC) ranging from 10-50 µg/mL. The MIC values were found to be comparable to those reported for commercial silver nanoparticles. In conclusion, this study demonstrates the feasibility of using Quercus confertifolia extract as a reducing agent and stabilizing agent for the synthesis of AgNPs. The resulting AgNPs exhibit promising antimicrobial activity and can be used as a potential alternative to conventional methods for the synthesis of silver nanoparticles.

INTRODUCTION:

Nanoparticles are materials with overall dimensions in the nanoscale. In recent years, these materials have emerged as important players in modern medicine, with applications ranging from contrast agents in medical imaging to carriers for gene delivery into individual cells. Nanoparticles have a number of properties that distinguish them from bulk materials simply by virtue of their size, such as chemical reactivity, energy absorption, and biological mobility.

Nanoparticles can be broadly classified into two groups: Organic nanoparticles and Inorganic nanoparticles. Organic nanoparticle are carbon nanoparticle (fullerenes) and inorganic nanoparticles are magnetic nanoparticles, noble nanoparticles (gold and silver), semiconductor nanoparticles (titanium oxide and zinc oxide).

Metallic nanoparticlesare emerging as new carriers and have been used for a huge number of applications in various areas of medical treatment. Recent advances have opened the way to site-specific targeting and drug delivery by these nanoparticles.

Silver (Ag) a noble metal, has potential applications in medicine due to its unique properties.[91]Silver nanoparticles have attracted research in the field of nanotechnology, due to its distinct properties such as good conductivity, chemically stable, catalytic activity, surface enhanced Raman scattering and antimicrobial activity.[92,93] There are various methods for silver nanoparticles preparation, for example; sol-gel process, chemical precipitation, reverse micelle method, hydrothermal method, microwave, chemical vapor deposition and biological methods, etc.[9] However; biological methods are preferred for being eco-friendly, cost effective, and don't involve the use of toxic chemicals.

The possibility of using plant materials for the synthesis of nanoscale metals was reported initially byGardea-Torresdey. The biosynthesis of nanoparticles, which represents a connection between biotechnology and nanotechnology, has received increasing consideration due to the growing need to develop environmental friendly technologies for material syntheses. The search for appropriate biomaterials for the biosynthesis of nanoparticles continues through many different synthetic methods.

Plants can be described as nano factories which provide potential pathway to bioaccumulation into food chain and environment. Among the different biological agents plants provide safe and beneficial way to the synthesis of metallic nanoparticle as it is easily available so there is possibilities for large scale production apart from this the synthesis route is eco-friendly, the rate of production is faster in comparison to other biological models such as bacteria, algae and fungi.

In this study, the synthesis and characterization of Ag/*Quercus confertifolia* by a green method is reported. The Ag-NPs were prepared using silver nitrate as silver precursor and *Costuspictus* leafmethanolic extract as reducing agent and stabilizer.

MATERIALS AND METHODS

All the glasswares were washed with dilute nitric oxide followed by double distilled water and dried in hot air oven.

Chemical reagents:

Methanol

Silver nitrate (AgNO3)

Double distilled water

Instruments Required

Centrifuge (REMI)

Magnetic stirrer with hot plate

Lyophilizer (Lyodel-Delvac Pumps Pvt. Ltd, USA)

Shimadzu UV-Visible spectrophotometer, Model 1800 Scanning Electron Microscopy (Hitachi X650, Tokyo, Japan)

Procedure

Collection of the leaves

Healthy plant leaves of *Quercus C* were collected and cleaned properly in running tap water.

Leaf drying and pulverizing

The leaves were collected and shade dried. It was powdered in a mixer. The powder was sieved in a No.60 sieve and kept in a well closed container in a dry place.

Preparation of methanolic leaf extracts of *Quercus C*. Don

About 500g of the dried powdered leaf of *Quercus confertifolia* was defatted with 1.5L petroleum ether (60-80^0 C) by maceration. The solvent was removed by filtration and the marc was dried. To the dried marc 1.5L of methanol was added and the extraction was performed by triple maceration (72h process). It was then filtered and the combined filtrate was evaporated to a cohesive mass using rota vapour.

Preparation of stock solution 2mg/20mL

2mg of the methanolic extract was weighed and diluted to 20mL with methanol. It was stored at 4°C until further use. **(Fig.1).**

Preparation of 1mM silver nitrate aqueous solution ($AgNO_3$):

An accurately weighed 0.017g of silver nitrate was dissolved with 100mL of double distilled water and stored in amber colour bottle until further use (Fig.2).

Fig. 1: stock solution of MEQC 2mg/ 20mL

Fig. 2: (A), (B) & (C)

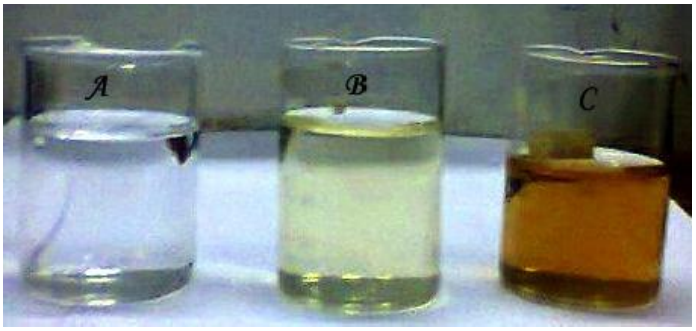


Fig. 2: (A) – Aqueous solution of 1Mm $AgNO_3$

Fig. 2: (B) – Aqueous solution of 1Mm $AgNO_3$ with MEQC after zero minutes

Fig. 2: (C) – Aqueous solution of 1Mm $AgNO_3$ with MEQC after 5hrs.

Synthesis ofmethanolic leaf extracts of *Quercus C*. Don silver nanoparticles (MEQCAgNPs)

5mL of the methanolic leaf extract of *Quercus confertifolia* was taken in the conical flask separately and placed on a magnetic stirrer with hot plate. To this 50mL of 1mM $AgNO_3$ solution was added dropwise with constant stirring 120rpm at 50-60°C. The colour change of the solution was checked periodically. The colour change of the medium from colourless to brown after 5h (Figs.3.1 to 3.6)was observed which indicated the formation of silvernanoparticles. It showed that aqueous silver ions could be reduced by the methanolic extract of *Quercus confertifolia* to generate extremely stable silver nanoparticles.

Seperation of silver nanoparticles

The synthesized silver nanoparticles were separated by centrifuging using a centrifuge at 10,000rpm for 15min. The supernatant liquid was re-suspended in the sterile double distilled water. The process was carried out thrice to get rid of any unco-ordinated biomolecules. After, the desired reaction period, the supernatant liquid was discarded and the pellets were collected and stored at 4°C for further use.

Lyophilization

The pellet obtained was then lyophilized by using freeze dryer (Lyodel-Delvac Pumps Pvt. Ltd, USA) to enhance the stability of silver nanoparticles. The freshly prepared MEQCAgNPs are lyophilized with cryoprotective agent (mannitol). Then it was rapidly cooled down to -50^{0}C for 2h followed by primary drying at 1.03mbar and secondary drying at 0.001mbar.[100] After lyophilization the synthesized MEQCAgNPs was stored at 4°C for further use (Fig. 4).

Characterization of synthesized MEQCAgNPs

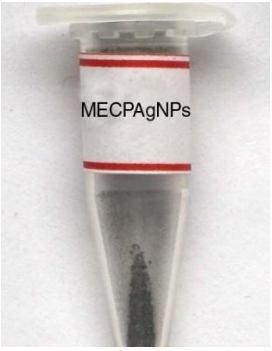
The characterization of synthesized MEQCAgNPs was carried out by using the following analytical parameters

Figs. showing the colour change of the medium from colourless to brown colour after 5hrs

Fig. 3.1 At 0 mins Fig. 3.2 After 1hr Fig. 3.3 After 2hrs

Fig. 3.4 After 3hrs Fig. 3.5After 4hrs Fig. 3.6 After 5hrs

Fig. 4: Synthesized MEQCAgNPs



Particle size

Zeta potential studies Polydispersity index

UV-Visible spectal analysis

Morphological studies using SEM

Determination of Particle size and Zeta potential

The mean particle size (z-average), polydispersity index (PI) and zeta potential of MEQC Ag NPs were determined by dynamic light scattering technique using a zeta size analyzer (Nano ZS 90, Malvern Instruments Ltd.,UK). The freeze dried powders were dispersed with water to obtain a proper scattering intensity before measurement.[100] The results obtained are presented in the **Table.6 & Figs.5, 6**

UV-Visible spectroscopy

The formation and completion of silver nanoparticles was characterized by UV- Visible spectroscopy by using Shimadzu UV- Visible spectrophotometer, Model 1800. The bio-reduction of the Ag^{+} ions in solution was monitored by periodical sampling of aliquots and the UV-Visible spectra of these aliquots were monitored as a function of time of reaction in 200-600nm range operated at a resolution of 1nm. Distilled water was used as a blank.**[62]** The results obtained are presented in the **Fig. 7**

Morphological studies of synthesized MEQCAgNPsby using Scanning Electron Microscopy (SEM)

Morphological evaluation of theMEQCAgNPs was carried out by using scanning electron microscope(SEM) (Hitachi X650, Tokyo, Japan). SEM gave high-resolution images on the surface of the sample. The scanning electron microscope workedon the same principle of an optical microscope, but it measured the electrons scattered from the sample rather than photon 18.Because electrons can be accelerated by an electric potential, the wavelength can be made shorter than the one of photons. This made the SEM capable of magnifying images up to 200.000 times. At the same time it was possible to achieve high resolution pictures of the surface, making the instrument very useful in determining the

morphology and size of nanoparticles. Thin films of a sample prepared on a carbon grid by just dropping a very small amount of the sample on the grid, extra solution was removed by using a blotting paper and then the film on the SEM grid was allowed to dry by keeping it under the mercury lamp for 5 minutes. Further the secondary electron sputtering at an applied potential of 20 kV was adopted prior to recording the SEM. The results are depicted in the **Fig. 8**

RESULTS AND DISCUSSION

Synthesis of Methanolic Leaf extracts of *Quercus C.* silver nanoparticles (MEQCAgNPs)

There was a visible color change after the substrate was added to the plant extract. Initially the plant extract was colourless. Upon adding the silver salt, it turned brown. After 5h, no significant colour change was observed. Increased concentrations of silver nitrate resulted in a brown solution of nanosilver indicating the completion of reaction. Reduction of silver ions into silver nanoparticles using methanolic leaf extract of *Quercus confertifolia* was evidenced by visual change of colour from colourless to brown colour which indicated the formation of silver nanoparticles due to the excitation of surface Plasmon vibration in silver nanoparticles as shown in **Figs. 3.1 to 3.6.**

Determination of Particle size and Zeta potential:

Particle size, size distribution and zeta potential were important characterizations of the silver nanoparticles because they govern the other characterizations, such as saturation solubility and dissolution velocity, physical stability, or even biological performances.

Particle size measurements:

Mean particle size diameter and polydispersity indices were all measured in solutions directly after synthesis, using photon correlation spectroscopy (PCS). The size of the colloidal silver nanoparticles, their granulometric distribution has been recorded, expressed against the particles number and their occupied volume.

The average particle size (z-average) is found to be **132.6 nm**. Particle size analysis showed the presence of nanoparticles with polydispersity indices PDI value**0.248** with intercept **0.643.** It is presented in the **Table 1 & Fig. 5**

Table 1: Mean Particle Size Diameter and Polydispersed Index (PDI) of Bio- synthesized MEQCAgNPs

Parameter	Value	Peak No	Peak Size (d.nm)	Peak Intensity %	Peak Width (d.nm)
Z-Average (d.nm)	132.6	Peak 1	141.8	100.0	102.5
PDI	0.248	Peak 2	0.000	0.000	0.000
Intercept	0.643	Peak 3	0.000	0.000	0.000

Fig. 5: Percentageintensity of particle size distribution of bio-synthesized MEQCAgNPs

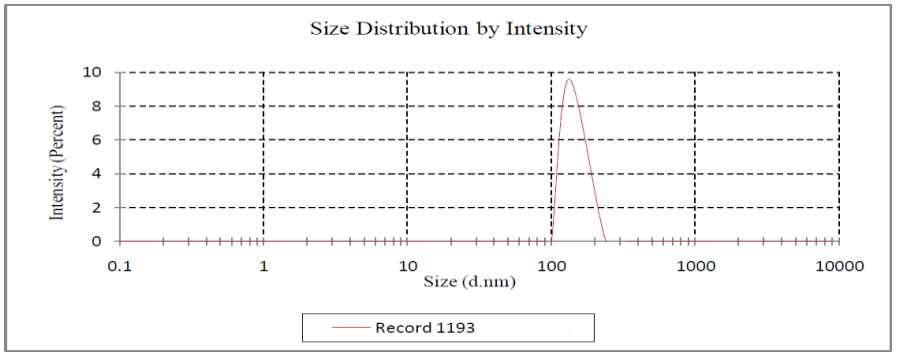
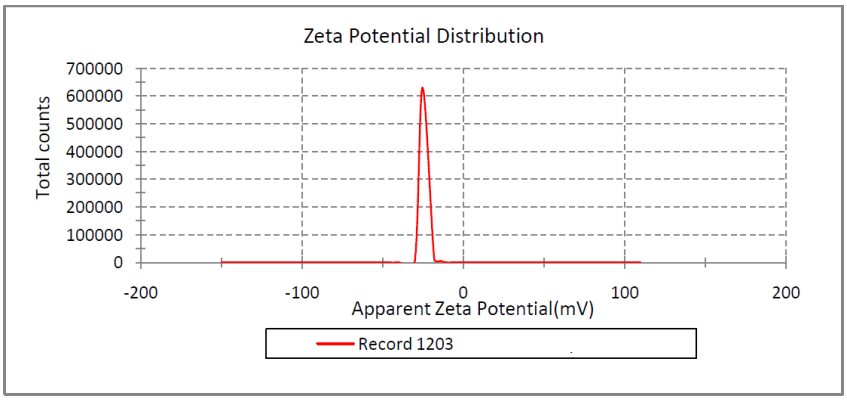


Fig. 6: Zeta potential distribution of bio-synthesized MEQCAgNPs



Zeta Potential measurement: a zeta potential was used to determine the surface potential of the silver nanoparticles. Zeta potential is an essential characterization of stability in aqueous silver nanoparticles. A minimum of +30mV zeta potential is required for the indication of stable silver nanoparticles. For the obtained nanoparticles, zeta values were measured and found to be -25.1mV with apeak area of 100% intensity. These values provide full stabilization of the nanoparticles, which may be the main reason in producing particle sizes with anarrow size distribution index.**(Fig. 6)**

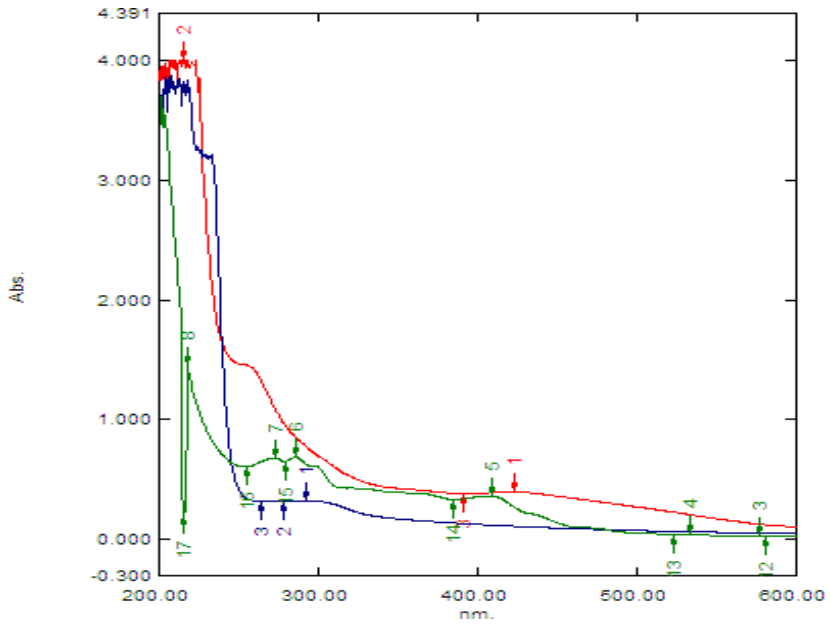
UV-Visible Spectroscopy: The UV-Vis Spectroscopy was the preliminary technique for the characterization of the silver nanoparticles. The reduction of the pure Ag^{+} ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 hours (complete colour change) following the dilution of a small aliquot of the sample in distilled water. The UV-Vis spectral analysis was conducted usingShimadzu UV-Vis spectrophotometer, Model 1800 range between 200 and 600 nm.The reduction of silver ions in the aqueous solution of nanoparticles in the solution could be correlated with the respective UV-Vis Spectra of the colloidal solution which

exhibited a strong absorption at 420nmas shown in **Fig**. **7.**A typical peak was obtained due to the presence of surface Plasmon resonance silver nanoparticles

Surface Plasmon resonance: Surface plasmon resonance (SPR) is the collective oscillation of electrons in a solid or liquid stimulated by incident light. The resonance condition is established when the frequency of light photons matches the natural frequency of surface electrons oscillating against the restoring force of positive nuclei. SPR in nanometer-sized structures is called **localized surface plasmon resonance**.

SPR is the basis of standard tools for measuring adsorption of material onto planar metal (typically gold and silver) surfaces or onto the surface of metal nanoparticles. It is the fundamental principle behind many color-based biosensorapplications and different lab-on-a-chipsensors.

Fig. 7: UV-Vis spectra of MEQCAgNPs



MEQCAgNPs

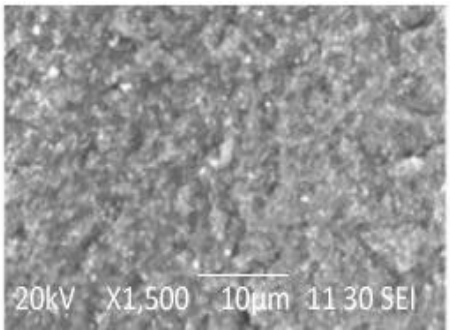
1mM $AgNo_3$

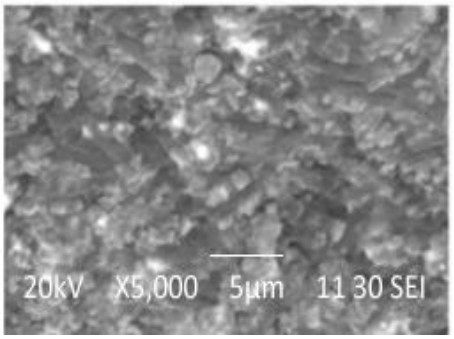
MEQC

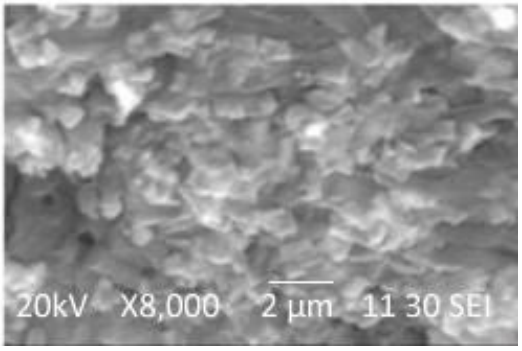
Morphological studies of silver nanoparticles by using Scanning Electron Microscopy (SEM)

A SEM employed to analyze the morphology and size details of the silver nanoparticles that were formed. From (**Fig. 8)** it was showed that the silver nanoparticles formed were spherical in shape, with an average size of around 100nm and uniformly distributed silver nanoparticles on the surface of the cells was observed.

Fig.8: SEM images of MEQCAgNPs







The chapter **Synthesis of *Quercus C*. Don silver nanoparticles** focuses mainly on green route for the synthesis of nanoparticles. For a long time, herbal medicines were not considered for development as novel formulations owing to lack of scientific justification and processing difficulties, such as standardization, extraction and identification of individual drug components in complex polyherbal systems. However, modern phytopharmaceutical research can solve the scientific needs (such as determination of pharmacokinetics, mechanism of action, site of action, accurate dose required etc.) of herbal medicines to be incorporated in novel drug delivery system, such as nanoparticles, microemulsions, matrix systems, solid dispersions, liposomes, solid lipid nanoparticles and so on

The biological synthesis of silver nanoparticles using *Quercus confertifolia* extract was shown to be rapid and produced particles of fairly uniform size and shape. As the methanolic leaf extracts of *Quercus confertifolia* were mixed with the aqueous solution of silver ion complex, it changed into brown colour due to the excitation of surface Plasmon vibrations, which indicated the formation of MEQCAgNPs.

The nanoparticles were primarily characterized by UV-Visible spectroscopy, which was proved to be very useful technique for the analysis of nanoparticles. In the UV-Visible spectrum, the broadening of the peak indicated the particles are poly dispersed. The surface Plasmon band in the silver nanoparticles in the solution remains close to 420nm. Throughout the reaction period indicating the particles are dispersed in the aqueous solution of silver nitrate, with no evidence for aggregation. The average

particle size (z- average) was found to be 132.6nm, its polydispersity index was 0.248 and zeta values were measured and found to -25.1mV with the peak area of 100% intensity. This indicates that the silver nanoparticle formed is stable.

A SEM images showed that the silver nanoparticles formed were spherical in shape, with an average size of around 100nm. SEM showed uniformly distributed silver nanoparticles on the surface of the cells was observed.

This green chemistry approach towards the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc

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