# In-Vitro and In-Vivo Antibacterial and Antifungal Activity of Juglans-Regia Mediated Silver Nanoparticles against Aspergillus- Ochraceus Induced Toxicity

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**Abstract:** Green Juglans regia (J. regia) leaf extract, It has dual stabilizing and reductant properties, is used in the room-temperature biosynthesis of Ag-NPs. The UV–visible spectrum, zeta potential and microscope imaging using transmission electron were utilized in order to characterize the J. regia-mediatedAgNPs. The formation of J. regia-mediatedAgNPs was analyzed usingUV–vis spectroscopy, which showed that the maximum surface plasmon absorption occurred between 400 and 460 nm. According to the results of the zeta potential study, the extract of J. regia green leaves had a negative value and a rising concentration of J. regia-mediatedAgNPs. Based on TEM image analysis, we know that the average particle size is 31.37 nm, with a standard variation of 7.1 nm. According to the results of the XRD research, the Ag-NPs are crystalline in structure. Gram-positive and Gram-negative bacteria were tested for the antibacterial activity of J. regia-mediated Ag-NPs using the disc diffusion method. The results showed that the J. regia-mediatedAgNPs had a strong antibacterial activity. According to the findings presented here, There is great promise regarding Ag-NP usage in a wide range of biomedical and biological research.

***Keywords**: J. regia,AgNPs, dissolution rate, surface plasmon resonance,ecological pollutants ect.*

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## 1. Introduction

The need for eco-friendly technology in material synthesis has led to a rise in interest in the biosynthesis of nanoparticles, which involves the intersection of biotechnology and nanotechnology. Different synthetic approaches are still being explored in search for the optimal biomaterial for nanoparticle production. More attention has been paid to the biosynthetic technique of using plant extracts rather than chemical or physical processes, or even microorganisms [1]. Since there is no requirement for an aseptic environment, this method is well-suited for nanoscale metal production. Because of their one-of-a-kind chemical and physical capabilities, nanotechnology and specifically metal nanoparticles have recently emerged as a rapidly developing new sector thanks to their application in technological advancements and industry. The process of synthesising nanoparticles through the use of green technologies has attracted an increasing amount of interest due to the fact that these methods are better for the environment and less expensive than the chemical and physical methods that are currently in use[2].

Metal nanoparticles are highly sought after in the domains of biomedical and engineering science due to their extensive capabilities in the terms of nanotechnology, which has many possible applications in the field of magnetic and biotechnology separation [3].Green production of silver nanoparticles (Ag-NPs) involves reducing a silver salt solution with a plant extract. There are two stages to this chemical reaction: the "nucleation" phase, during which small nuclei of silver atoms are formed, and the "growth" phase, during which the nuclei are grouped together to create nanoparticles. Besides gamma rays, ultraviolet (UV) irradiation, electrochemical reduction, and heating, various chemical reduction techniques can also be employed for the purpose of eliminating silver ions [4]. However, there exists a requirement for a method of synthesising Ag-NPs that is more environmentally benign, economically efficient, and capable of long-term sustainability. The evaluation of the three primary stages involved in the green synthesis of Ag-NPs, namely solvent selection, reducing agent choice, and non-toxic stabiliser identification, should be conducted with a focus on the principles of green chemistry [5]. Both the pharmaceutical and cosmetics industries make use of many components of the walnut tree (Juglans regia), including the kernels, leaves, and bark of the tree, as well as the green husk of the fruit. The research carried out by established the presence of antioxidant activity in walnut leaves, seeds, and green husks. Additionally, the study found evidence of antibacterial activity[6,7]. The methanolic extracts of J. regia were analyzed, and then the aqueous extracts were analyzed.

Toxigenic fungi can create secondary metabolites called mycotoxins, which have been linked to contamination of many food products. Aspergillus, Penicillium, and Fusarium are the most common mycotoxin-producing genera in economically important crops[8]. Since mycotoxins may have such a devastating effect on animal and human health, their presence in cereals and other food items is of the utmost importance. Mammals are especially susceptible to the toxicity of ochratoxin A, aflatoxin B1, and fumonisin B1. As difuranocoumarin derivatives, aflatoxins are hazardous to mammals in a variety of ways, including through mutation induction, liver damage, birth defects, cell death, immune suppression, and even estrogenic effects [9].One of the most promising approaches is to use antibacterial nanoparticles to deal with this issue. As an antibacterial agent, silver is highly effective, readily accessible, and somewhat nontoxic to mammalian tissues[10]. The metabolic activities in bacteria that silver is known to target include changes to the membrane structure and functions. It also reduces the expression of proteins involved in ATP synthesis. Research into silver's antimicrobial qualities has led to silver nanoparticles widespread use in a variety of purification processes, including those for drinking water, laundry machines, shampoo, toothpaste, pots and pans, and toys[11,12].The utilisation of silver nanoparticles as an antimicrobial agent is becoming increasingly prevalent because of their strong antifungal and antibacterial qualities.

The leaf extract was suggested in this work as a convenient and appropriate plant for the green synthesis of Ag-NPs. This study's approach is entirely environmentally friendly and consists of a simple one-step procedure that combines the $AgNO_3$ solution with the aqueous extract of walnuts[13]. Due to the high concentration of polyphenolic chemicals in walnut extract, it is used as the precursor for silver and simultaneously used as a stabilizing and reducing agent. Additional advantages of these methods encompass the utilisation of cost-effective and non-hazardous chemicals within botanical extraction, coupled with a reaction occurring at a moderate thermal condition. To the researchers' current understanding, this marks the inaugural utilisation of leaf extracts in the manufacture of Ag-NPs [14].

**Silver based nanoparticles(Ag-NPs) used in antibacterial activity[15]**

There is more than one approach to produce nanoparticles (NPs). Because of its economical and eco-friendly properties, nanoparticle production has attracted more attention in recent years. Because of their side effects, therapeutic applications of NPs generated using conventional methods are restricted. As a result, green-synthesized NPs have advantages in terms of stability, durability, and chemical and physical properties. Since walnut (Juglans regia L.) leaves contain a high concentration of phenolic compounds with potent antioxidant and antibacterial activities, their extract was employed to create AgNPs in this investigation. J. regia has showed promise in a number of areas, including anti-inflammatory effects, cardiovascular benefits, and cancer prevention[16]. In addition, it is utilized in alternative medicine to alleviate symptoms of asthma, eczema, and even constipation. Several aspects of the walnut plant have been studied as potential remedies for various illnesses including bacterial infections. Therefore, the bioactive compounds present in J. regia leaf extract can serve as a metal ion reducer, capping agent, and stabilizing agent during the production of a wide variety of NPs [17].

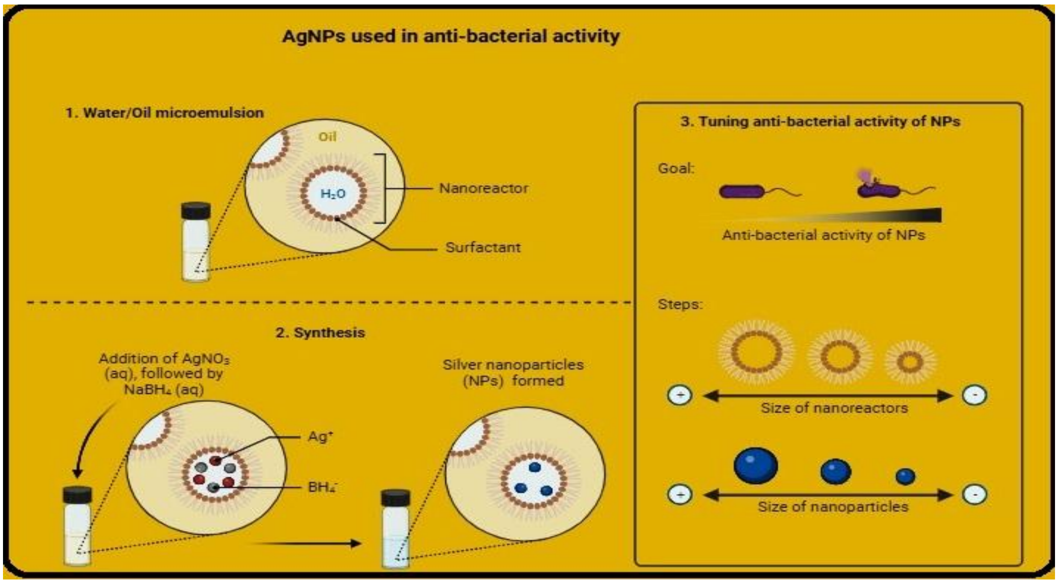


**Fig.1 Diagram illustrates the AgNPs in the context of antibacterial activity.**

To the best of our understanding, this study represents a novel contribution by demonstrating the efficacy of biologically synthesised AgNPs as antifungal agents against A. ochraceus, as well as their potential to mitigate the toxicity of aflatoxins on several organs. This study also offers novel perspectives on the possible application of nanoparticles to reduce the amount of harmful fungal by-products in animal feeds and food goods. Consequently, silver nanoparticles (AgNPs) possess the capacity to mitigate the adverse impacts of aflatoxins on both human health and agricultural productivity. This can be achieved by the use of in silico/computational modelling studies [18]. Since computational simulations provide various benefits over conventional in vivo and in vitro research, including reduced costs and faster findings, the urgency of undertaking such investigations is considerable.

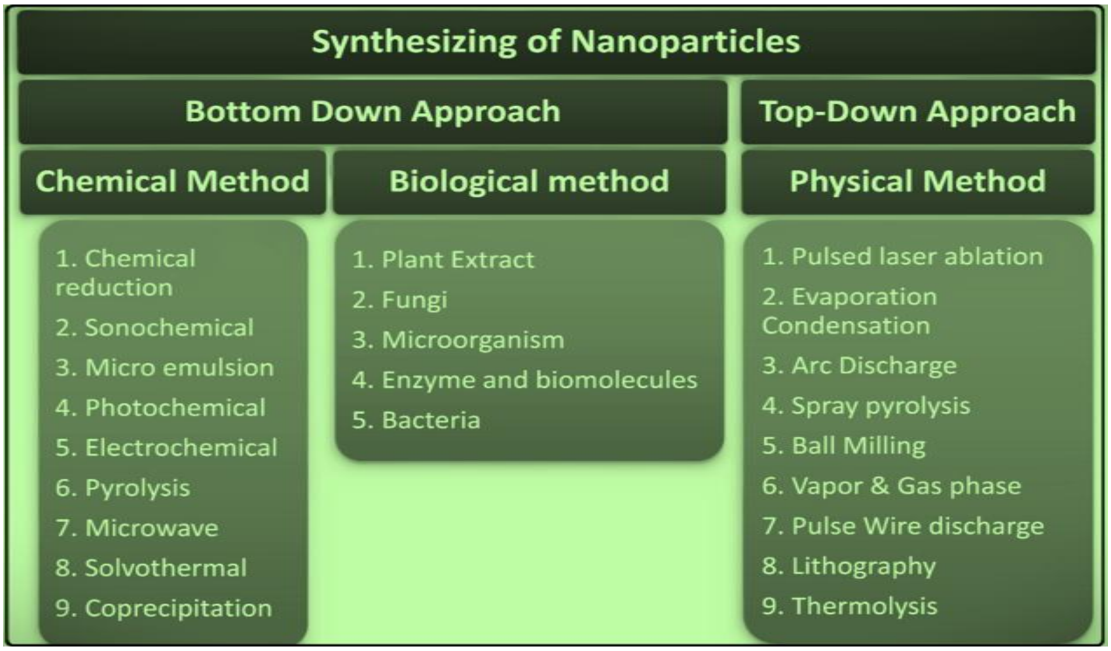


**Fig.2 Flow chart showing the various methods for synthesizing Nano particles**

**Physicochemical properties of Silver based nanoparticles**

For the purpose of determining antibacterial activity, the physical and chemical properties of AgNPs are extremely important factors to consider. These properties include surface chemistry, size, size distribution, shape, particle morphology, particle composition, coating/capping, agglomeration, dissolution rate, particle reactivity in solution, efficiency of ion release, cell type, and type of reducing agents used for synthesis. For instance, biological reducing agents such as the culture supernatants of various species of Bacillus can be used to synthesise AgNPs in a variety of shapes. These shapes include spherical, rod, octagonal, hexagonal, triangle, flower-like, and so on. Previous research found evidence for the hypothesis that particles of a smaller size, which have a bigger surface area, are capable of causing greater toxicity than those of a larger size. The form of the substance is also an essential factor in determining its level of toxicity. For instance, the field of biomedicine has made use of a wide variety of nanostructures, such as nanocubes, nanoplates, nanorods, spherical nanoparticles, flower-like, and so on. These nanostructures have been shown to be effective. The availability of chemical and/or biological coatings on the nanoparticle surface is the primary factor determining whether or not AgNP are hazardous. The cytotoxic effect that AgNP has on cells may be determined by their surface charges. For example, the positive surface charge of these NPs makes them more appropriate and extends the amount of time they can remain in the blood stream when compared to negatively-charged NPs. This is important because the blood stream is a significant route for the administration of antibacterial medicines.

**Biological application of silver based nanoparticles**

Because of their one-of-a-kind qualities, silver nanoparticles (AgNPs) have found widespread use in a variety of fields, including the health care sector, the food storage business, as well as environmental and medicinal applications. There have been a number of reviews and book chapters written about the application of AgNPs in a variety of different fields. Antibacterial, antifungal, antiviral, antiinflammatory, anticancer, and antiangiogenic treatments are only few of the biological and medicinal applications that we would like to highlight here using silver nanoparticles (AgNPs). In this article, we focused specifically on previously published seminal works, and we concluded with more current revisions.

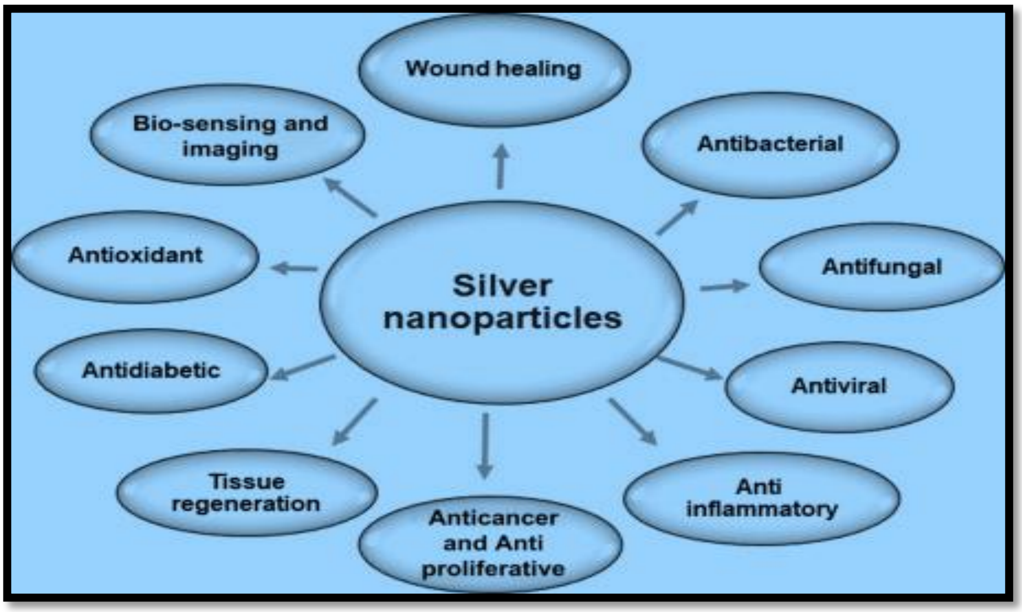


**Fig. 3 is a diagrammatic representation of the many different applications of AgNPs**

**Substances and Techniques**

**Making a Leaf Extract of Juglans regia[19]**

The fresh leaves of J. regia were procured from the Department of Crop and Herbal Physiology at Jawaharlal Nehru Krishi Vishwavidyalaya in Jabalpur, Madhya Pradesh. A total mass of 10 grammes of freshly harvested leaves was measured and subsequently subjected to a thorough washing process using water that has been distilled to remove any traces of impurities such as dirt or debris. After the leaves were cut, they were cooked in 400 mL of sterile distilled water. After boiling, the resulting slurry was condensed to a volume of 100 ml.. Filtrate was collected after passing the mixture through Whatman filter paper and was kept in an airtight

jar at $4^0$C for later use.

**Fig.4 Diagram showing the leaves part and extracts of J.regia**

**Formation of AgNPs through the utilization of J. regia Leaf Extract[20]**

In the standard reaction protocol, a mass of 0.5 grammes of J. regia extract was introduced into a solution of 100 millilitres of distilled deionized water. The mixture was subjected to vigorous stirring for a duration of 30 minutes. A volume of 25 mL of AgNO3 (5 × 10-3 M) was introduced and magnetic stirrer was used to combine the ingredients at 25 °C for a duration of 10 hours. The silver nanoparticles (Ag-NPs) were progressively formed over the course of the incubation time. An observable transition in hue occurred, shifting from a yellowish tone to a reddish brown shade. Subsequently, the pellets underwent a washing process, followed by centrifugation and drying at ambient temperature, leading to the creation of powdered silver nanoparticles(AgNPs).


**Fig.5Diagrammatic representation for preparation of J. regia-mediatedAgNPs**

**Physiochemical properties of J. regia-mediatedAgNPs[21]**

The chemical and physical characteristics of AgNPs have a significant role in determining their cytotoxicity. The culture supernatants of various Bacillus species can be used as biological reducing agents in the production of AgNPs of a wide range of forms. These shapes include but are not limited to spheres, rods, octagons, hexagons, triangles, flowers, and so on. Evidence from the past suggests that smaller particles, due to their greater surface area, may be more harmful than bigger ones. In the biomedical industry, for instance, scientists have experimented with nanostructures of many different shapes and sizes, including cubes, plates, rods, spheres, flowers, and more. The cytotoxic effect on cells may be related to the surface charges of AgNPs.

**Charecterization of J. regia-mediatedAgNPs[22]**

Nanoparticles' biodistribution, safety, and effectiveness all hinge on their physicochemical qualities. Therefore, it is essential to characterize AgNPs so that the functional properties of the produced particles may be evaluated. UV-visible spectroscopy, X-ray diffraction, Fourier transform infrared spectroscopy, X-ray photoelectron spectroscopy, dynamic light scattering, scanning electron microscopy, and transmission electron microscopy are among the analytical techniques commonly employed for characterisation purposes. Despite the existence of several scholarly publications and studies on the subject, this article aims to provide a concise overview of the fundamental principles and methodologies employed in the characterization of AgNPs.

**Morphology studies of J. regia-mediatedAgNPs by TEM[23]**

The TEM image was utilized to look at the size and shape of the generated Ag-NPs, and it shows that many of the nanoparticles are round. Because of the green husk of J. regia's thickening characteristics, The figure illustrates the presence of a majority of agglomerated particles. Furthermore, it is anticipated that agglomeration would occur due to the small size and magnetic characteristics of the produced Ag-NPs. The particle size distribution was calculated using a histogram constructed from the sizes of the 100 nanoparticles. The high magnification of TEM also makes it easy to see the Ag-NPs encircled by the J. regiaextract. Figure displays the TEM picture and the size distribution of the particles, which demonstrated that the particles had narrow size distributions, with diameters ranging from 24.27 to 38.47 nm. Furthermore, Ag-NPs have a mean diameter of 31.37±7.10 nm and a standard variation of 7.10 nm.

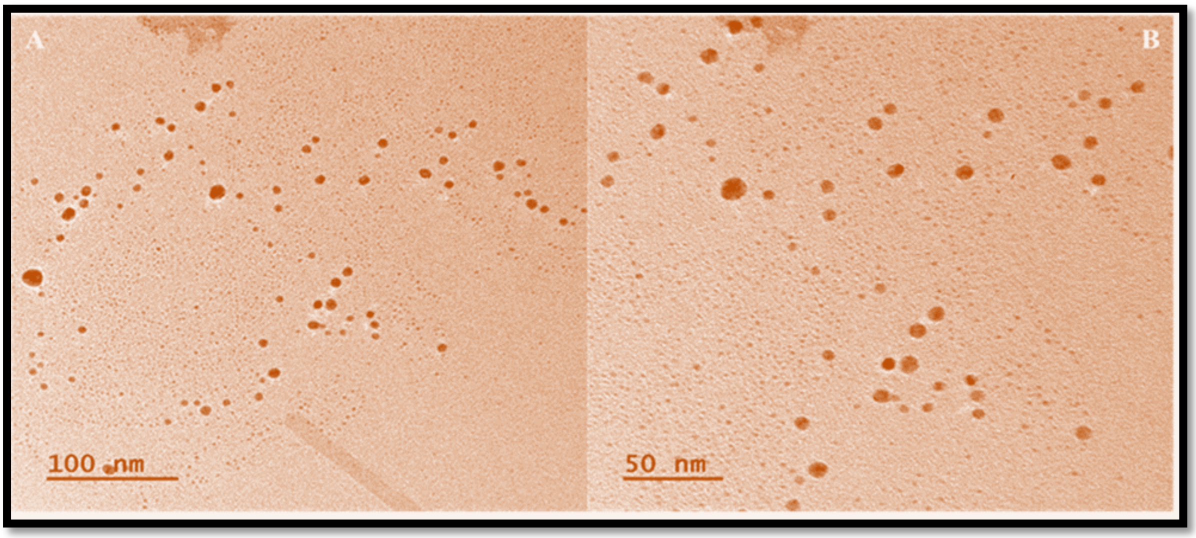


**Fig.6 TEM pictures of Juglans regia-mediated s(AgNPs) with sizes of 100 nm and 50 nm**

**The study of the Ultraviolet and Visible Spectrum of J. regia-mediated AgNPs[24]**

In order to keep track of the synthesis and stability of J. regia-mediated AgNPs, UV-vis spectroscopy is used, as it is for the primary characterisation of all generated nanoparticles. The optical characteristics of AgNPs mediated by J. regia are such that they interact strongly with certain wavelengths of light. Conduction band and valence band of J. regia-mediated AgNPs are very close to one other, allowing unrestricted movement of electrons. Particle size, dielectric medium, and chemical environment all have a role in the uptake of AgNPs mediated by J. regia. Nanoparticles ranging in size from 2 to 100 nm have been observed to exhibit this peak, which has been attributed to a surface plasmon.These bands were thought to represent the tiny (less than 40 nm) AgNPs produced by J. regia mediation. Absorption spectra in the UV-Vis range revealed a single peak at 430 nm within the broader SPR band. After 10 hours of stirring, this peak shows that the Ag-NPs have been evenly dispersed thanks to J. regia, and that their concentration has grown along with the increase in absorbance.

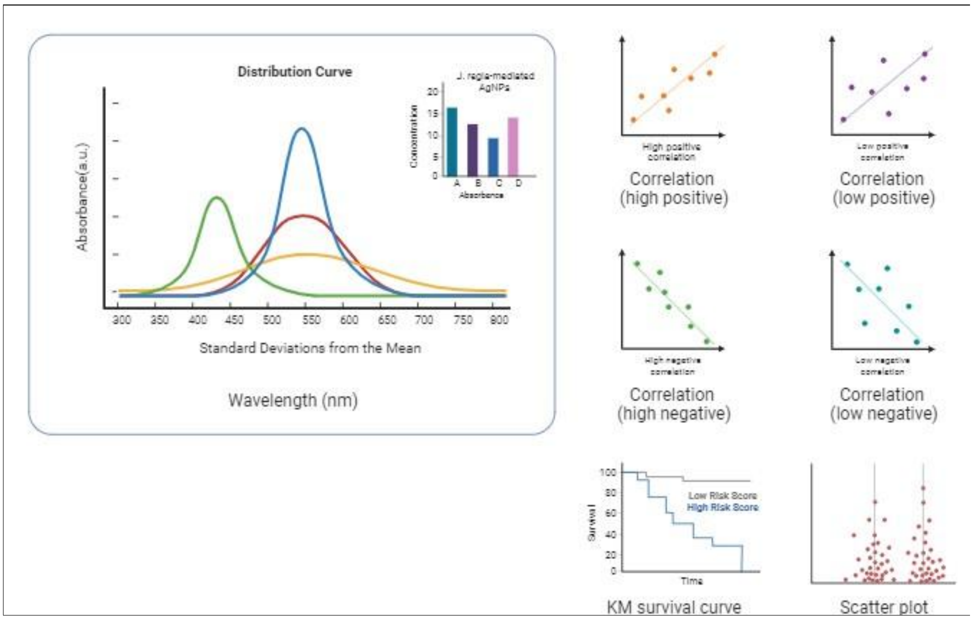


**Fig.7UV-vis absorption spectra of synthesised Ag-NPs at varying concentrations and J. regia extract.**

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**FTIR Spectroscopy studies of J.regia-mediatedAgNPs[25]**

The use of FTIR spectroscopy to determine the presence or absence of biomolecules in the production of nanoparticles is becoming increasingly widespread in research and academic. In addition, the investigation of enzyme-substrate interactions during catalysis. In addition, it is a painless method. The probable phytochemicals involved in the reduction, capping, and stability of AgNPs were investigated using FTIR analysis of the J. regia-mediated AgNPs. The results showed that the J. regia-mediated AgNPs had prominent absorption peaks at 1650.87, 2414.84, and 3363.47 $cm^{-1}$. The stretching vibrations of the C-O bond are reflected in the absorption peak at 1037.58 $cm^{-1}$. The strong peaks between 1660.52 and 1650.87 $cm^{-1}$ are attributable to nitrile group stretching, while the absorption peaks between 2370.24 and 2414.84 $cm^{-1}$ signify N-H group stretching. After all that, we know that the peak between 3359.61 and 3363.47 $cm^{1}$ is due to the stretching of -OH in phenolic and alcoholic chemicals. Similar peaks were observed for J.regia-mediated AgNPs between 1660.52 and 3363.47$cm^{-1}$ indicating that the same phytochemicals responsible for the capping, reduction, and stability of AgNPs are present in both systems.

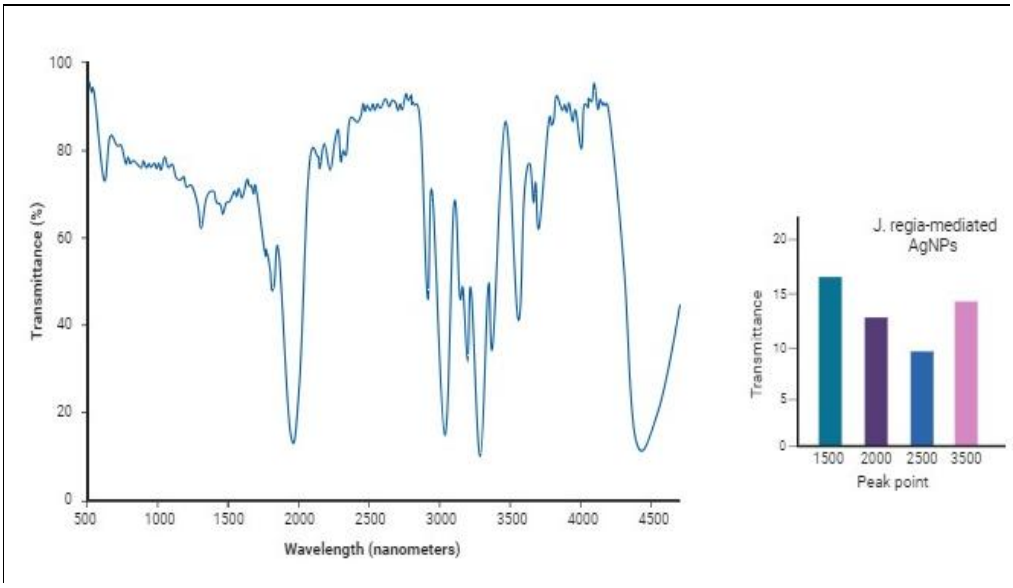


**Fig.8FTIRspectrum and bar graph ofJ.regia-mediatedAgNPs**

**X-ray Diffraction (XRD)s studies of J. regia-mediatedAgNPs[26]**

A common analytical method, The technique of X-ray diffraction (XRD) has been study of molecular and crystal structures, as well as the qualitative and quantitative identification of different compounds, particle sizes, isomorphous substitutions, crystallinity levels, and other subjects. Any crystal will produce a variety of diffraction patterns when X-ray light bounces off of it; these patterns will reveal information about the physico-chemical properties of the crystal structures. XRD analysis was used to ascertain the degree of crystallinity in J. regia-mediated AgNPs. Figure 8 shows four 2 diffraction peaks at 38.31, 44.37, 65.41, and 77.29 degrees. These are consistent with the 111, 200, 220, and 311 crystallographic planes of face-centered cubic (FCC) crystalline AgNPs, and our results support this.

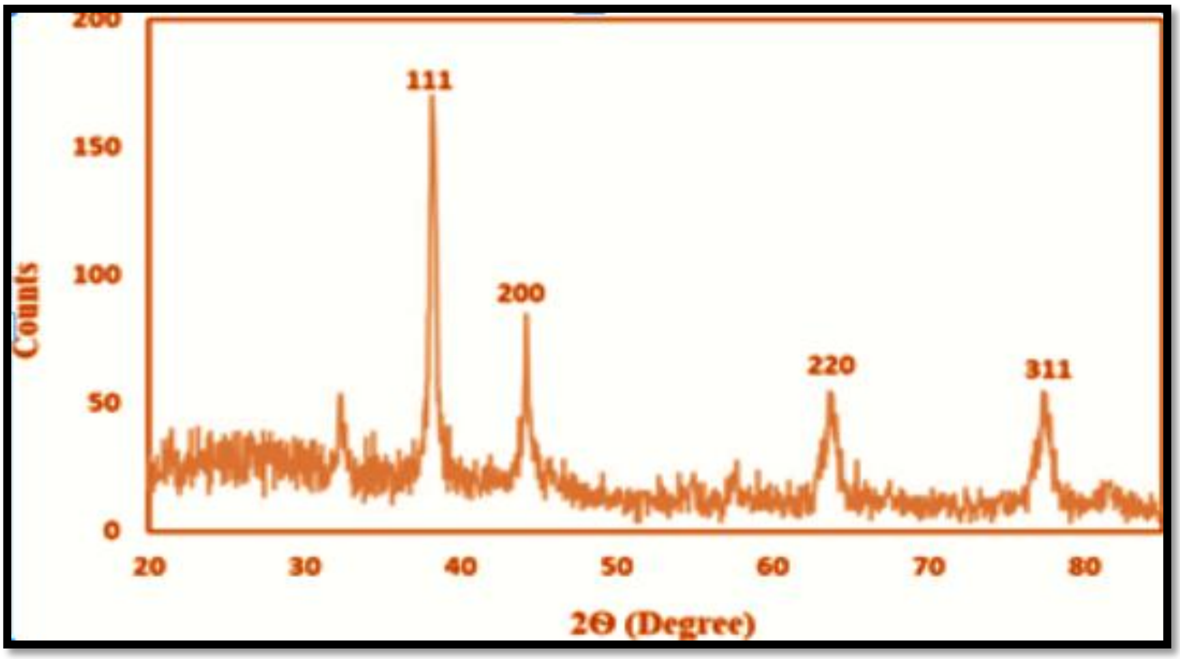


**Fig.9XRDgraph of*J.regia*-mediatedAgNPs.**

**Antibacterial activity of Juglans regia-mediatedAgNPs[27]**

Silver nanoparticles (AgNPs) mediated by Juglans regia have found appear to offer a potential alternative to antibiotics as antibacterial agents. These AgNPs demonstrate the capability to overcome bacterial resistance that commonly arises against antibiotics. Therefore, research into the use of AgNPs mediated by Juglans regia is necessary. Silver nanoparticles (AgNPs) appear to possess potential as antibacterial agents due to their large surface-to-volume ratios and unusual crystallographic surface structure, among the many promising nanomaterials. The results indicated that E. coli cells treated with Juglans regia-mediated AgNPs exhibited the deposition of AgNPs on the cell wall and the development of indentations, referred to as "pits," in the bacterial cell walls. Ultimately, these effects resulted in the demise of the bacterial cells. These AgNPs exhibited an average size of 25 nm and demonstrated notable antimicrobial and bactericidal properties against both Gram-positive and Gram-negative bacteria. The present work evaluated the antibacterial capabilities of Ag-NPs against Gramme positive bacteria (S. aureus and B. cereus) and Gramme negative bacteria (E. coli and P. aeruginosa). The inhibition zone values were determined using the disc diffusion technique, and the obtained results are provided in Table 1. The average inhibitory zone sizes for Juglans regia-mediated Ag-NPs against S. aureus and B. cereus were determined to be 19(±1) mm and 13(±1) mm, respectively. Furthermore, there was no observed action exhibited against the Pseudomonas aeruginosa bacteria. The effectiveness of antibacterial drugs in inhibiting microbial growth is contingent upon their capacity to penetrate the ribosomes within microorganisms or microbial cells.Based on our visual observations, no statistically significant difference was found in the antibacterial activity of Juglans regia-mediated Ag-NPs. However, it was observed that both esters exhibited larger inhibitory zones against Gram-negative bacteria. Positive bacteria are distinct from Gram-negative bacteria in several ways. This phenomenon can be attributed to the prevalence of Gram-negative bacteria. Thirteen bacterial species possess a cell wall that is comparatively thicker than that of Gram-positive bacteria, resulting in increased resistance to penetration. The individuals incorporated the throughout their cellular structures.

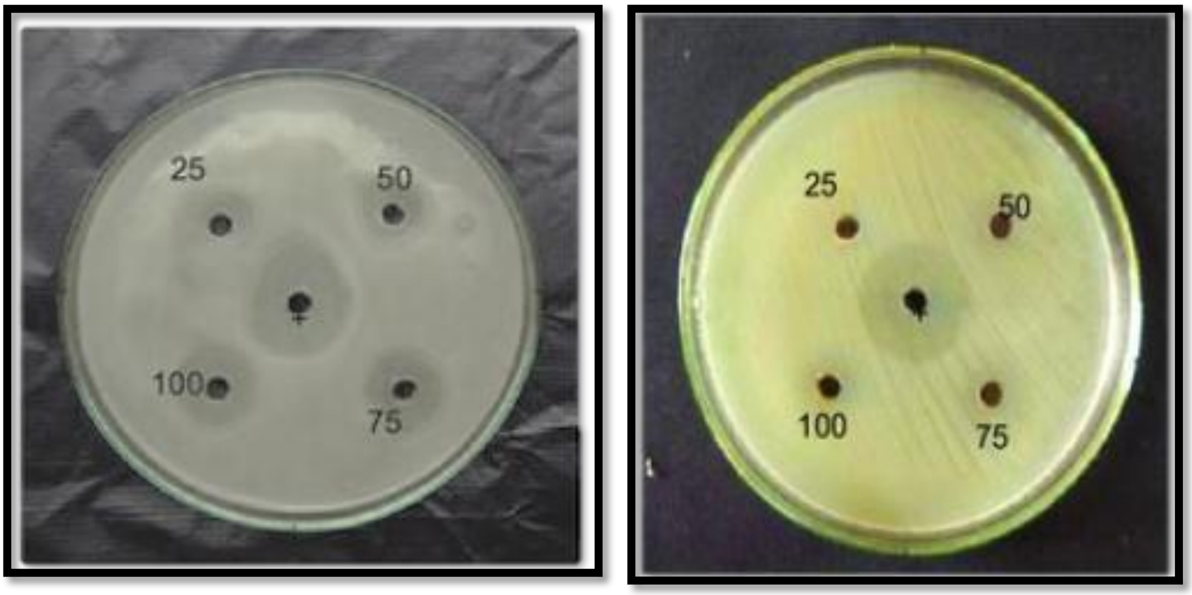


**Fig.10In vitro antibacterial activity of biologically synthesised AgNPs against E.coli**

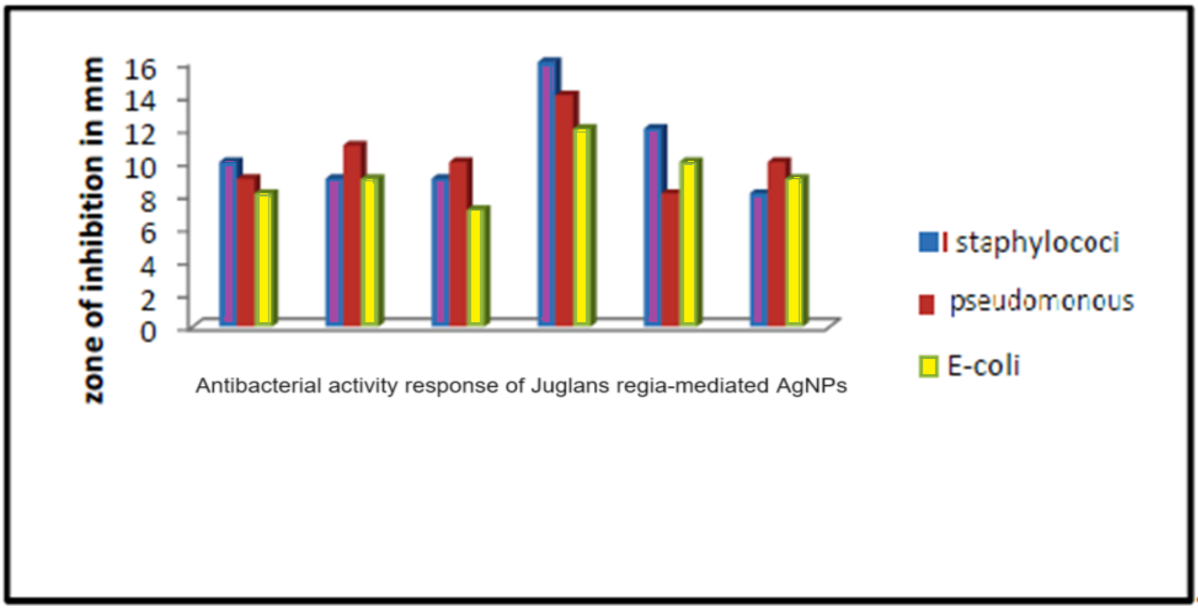


**Fig.11 Bar graph of antibacterial activity of J. regia-mediatedAgNPs**

| **Drug sample** | Inhibition zone (mm) area of microorganism | | | |
|---|---|---|---|---|
| | **Bacteria(Gram +ve)** | | **Bacteria(Gramn-ve)** | |
| | *S.aureus* | *B.cereus* | *E. coli* | *P.aeruginosa* |
| Ampicillin standard | 54 | 45 | 36 | NA |
| J.regia mediated Ag-NPs | 29 | 23 | 18 | 10 |

**Table1.Antibacterialinhibitionzone(mm)of J. regia-mediatedAgNPs**

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**Juglans regia-derived silver nanoparticles have antifungal properties[28]**

Antifungal activity was tested for the compounds synthesised in this study. Common microorganisms C.albicans, M.audouinii, A.niger, and T.mentagrophytes were used in the antifangal testing.The chemicals' ability to inhibit fungal growth was measured using the disc-diffusion technique.The size-dependent antifungal effects of various Juglans regia-mediated The effectiveness of AgNPs was tested against established biofilms of Candida albicans and Candida glabrata. These fungus included Alternaria alternata, Sclerotinia sclerotiorum, Macrophomina phaseolina, Rhizoctonia solani, Botrytis cinerea, and Curvularia lunata. AgNPs mediated by Juglans regia were effective against a wide variety of indoor fungal species, including Penicillium brevicompactum, Aspergillus fumigatus, Cladosporium cladosporioides, Chaetomium globosum, Stachybotrys chartarum, and Mortierella alpine.

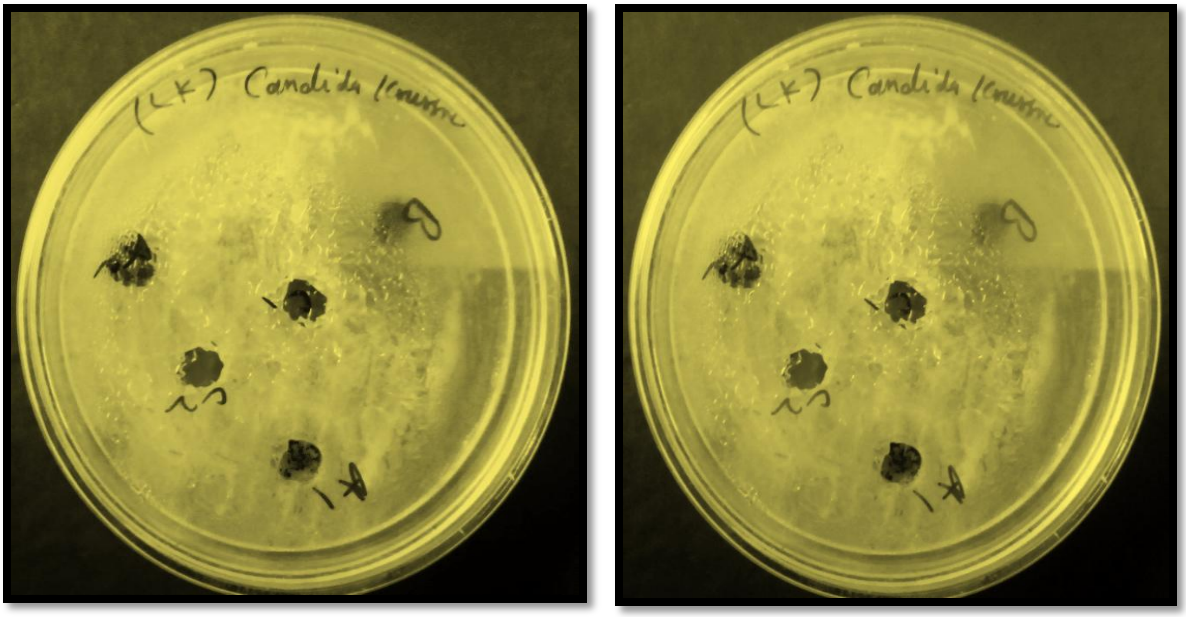

**Fig.12 Images represent the antifungal activity of J. regia-mediatedAgNPs**

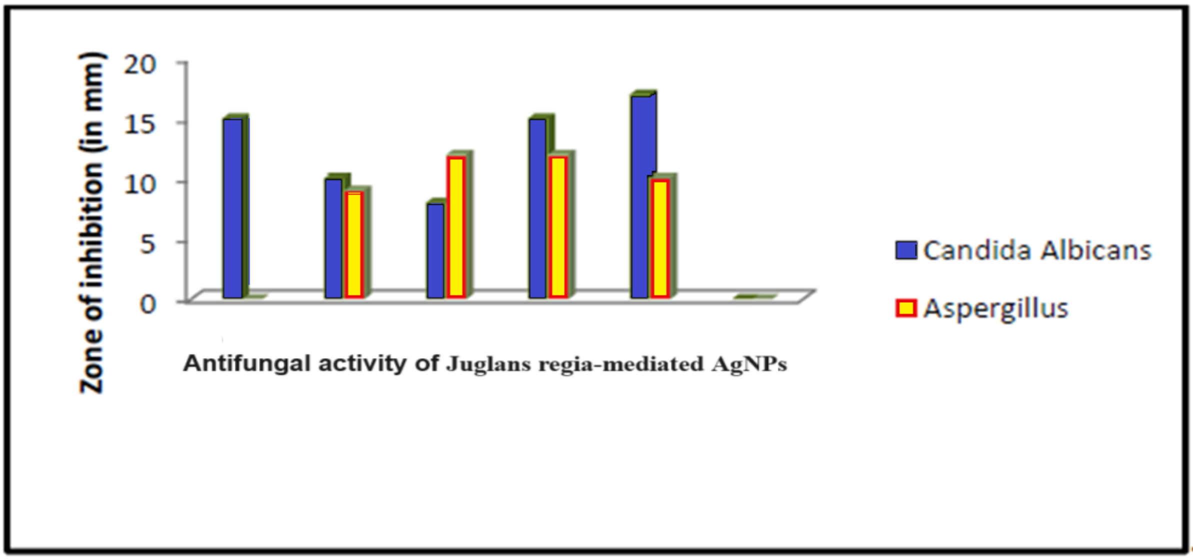


**Fig.13 Bar graph represent the antifungal activity of J. regia-mediatedAgNPs**

Aspergillus ochraceus, Aspergillus melleus, and Aspergillus flavus are all toxic moulds, yet the antifungal activity of AgNPs mediated by Juglans regia indicates that AgNPs may function effectively as an antifungal agent. Antifungal activity of AgNPs mediated by Juglans regia is believed to include a mix of the above-mentioned processes, as well as other undiscovered mechanisms.

## 2. Result and Discussion

In this study, silver nanoparticles were produced at room temperature utilising a technique that was both straightforward and biosynthetic. The findings suggest that at normal temperature, the aqueous extract of J. regia green husk significantly reduces and stabilizes $Ag^+$ to Ag. UV-vis, XRD, and TEM were all used to characterise the Ag-NPs. Ag-NPs are pure crystalline, as shown by the XRD, with no discernible impurities. Results from UV analysis indicated that Ag-NPs synthesised with plant extract performed better than those synthesised without the extract. According to the aforementioned finding, the average size and standard deviation of 31.37 7.1 nm at room temperature is in close agreement with the established TEM result. Analyses of zeta potential showed that the fabrication of Ag-NPs increased the negative charge and zeta potential value of J. regia green leaves extract. Using the plant's green J. regia leaves is efficient, cost-effective, and safe, and it makes good use of a byproduct. Using a green source like J. regia for the biosynthesis of paper Ag-NPs is better to the chemical or physical synthesis due to the free biosynthesis and ecological pollutants. The antibacterial effects of Ag-NPs were demonstrated, with the particles showing more efficacy against Gram-positive than Gram-negative bacteria. More research into the bactericidal effects of Ag-NPs on various bacteria is, of course, necessary before this field can be expanded to include applications like surgical tools or medication delivery vehicles. Antifungal activity of AgNPs mediated by Juglans regia is believed to include a mix of the above-mentioned processes, as well as other undiscovered mechanisms.

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