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In-Vitro And In-Vivo Antibacterial And Antifungal Activity Of Nanoformulations Of Curcumin Against Aspergillus- Ochraceus Induced Toxicity

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Abstract: Curcuma caesia rhizomes 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 C. caesia-mediated AgNPs. The formation of Curcuma caesia rhizomes mediated AgNPswasanalyzed 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 Curcuma caesia had a negative value and a rising concentration of C.caesia-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 C. caesia-mediated Ag-NPs using the disc diffusion method. The results showed that the C.caesia-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:C.caesia,AgNPs,dissolution rate,surface plasmon resonance,ecological pollutantsect.

1. Introduction

An increasing number of researchers are looking at biosynthesis of nanoparticles, a field that combines biotechnology with nanotechnology, as a means to meet the growing demand for environmentally benign technologies in material synthesis. The best biomaterial for nanoparticle synthesis is still a mystery, and researchers are trying a variety of synthetic methods to find it. Instead of focusing on physical or chemical processes or even microbes, researchers have been more interested in the biosynthetic method that uses plant extracts [1]. Creating nanoscale metals has never been easier than with this technique, which doesn't even need an aseptic atmosphere. Application of metal nanoparticles—which possess unique chemical and physical properties—in technical breakthroughs and industry has led to the emergence of nanotechnology as a fast growing new sector. An ever-increasing number of people are interested in green technology-based nanoparticle synthesis since it is less expensive and better for the environment than the conventional chemical and physical approaches [2].

The vast nanotechnology capabilities of metal nanoparticles make them particularly desirable in the biomedical and engineering scientific communities, where they may find several uses in magnetic and biotechnological separation [3]. One environmentally friendly way to make silver nanoparticles (Ag-NPs) is to use a plant extract to reduce a solution of silver salts. The first step of this chemical reaction is the "nucleation" phase, which involves the formation of individual silver atoms; the second step is the "growth" phase, which involves the clustering of these nuclei into nanoparticles. One can eliminate silver ions using a variety of chemical reduction procedures, in addition to gamma rays, ultraviolet (UV) irradiation, heating, and electrochemical reduction [4]. We need a way to synthesise Ag-NPs that is less harmful to the environment, more cost-effective, and sustainable in the long run. Adherence to green chemistry principles should guide the assessment of the three main steps in the green synthesis of Ag-NPs: solvent selection, reducing agent choice, and non-toxic stabiliser identification [5]. The several parts of the rhizomes (Curcuma caesia) that are utilised in the cosmetics and pharmaceutical sectors include the tree's bark, leaves, and kernels in addition to the fruit's green husk. Scientists have shown that walnuts—including their leaves, seeds, and green husks—contain antioxidants. There was also proof of antibacterial action, according to the study [6,7]. First, the aqueous extracts of C. caesia were studied, and subsequently the methanolic extracts.

Mycotoxins are secondary metabolites produced by toxic fungus; they have been associated with the contamination of numerous food items. The most prevalent genera of mycotoxins in crops that are economically significant are Aspergillus, Penicillium, and Fusarium[8]. There must be zero tolerance for mycotoxins in cereals and other foods because of the catastrophic impact they can have on human and animal health. Ochratoxin A, aflatoxin B1, and fumonisin B1 are particularly harmful to mammals. As difuranocoumarin derivatives, aflatoxins pose multiple dangers to animals, such as inducing mutations, damaging the liver, causing birth defects, killing cells, suppressing the immune system, and even having estrogenic actions [9]. Utilising antimicrobial nanoparticles is among the most encouraging methods for addressing this matter. Silver is a great antibacterial agent because it is effective, easy to get, and not too harmful to mammals [10]. Among the metabolic processes that silver is known to inhibit in bacteria are alterations in the structure and function of their membranes. Proteins involved in ATP production have their expression levels decreased as a result. Numerous purification methods, such as those for water, laundry machines, shampoo, toothpaste, cookware, and even toys, make extensive use of silver nanoparticles, which have been extensively studied for their antibacterial properties [11,12]. Due to their potent antifungal and antibacterial properties, silver nanoparticles are finding more and more applications as antimicrobial agents.

As a practical and suitable plant for the environmentally friendly production of Ag-NPs, the leaf extract was proposed in this study. The methodology used in this study is completely eco-friendly and is comprised of a straightforward one-step process that mixes the $AgNO_3$ solution with the walnut aqueous extract [13]. Walnut extract is utilised as a reducing agent, stabilising agent, and silver precursor due to its high concentration of polyphenolic compounds. The use of inexpensive and non-toxic chemicals in botanical extraction and the fact that the reaction takes place at a moderate temperature are two further benefits of these procedures. As far as the researchers can tell, this is the first time leaf extracts have been used to make Ag-NPs [14].

2. Antimicrobial activity of silver based nanoparticles (Ag-NPs) [15]

Nanoparticle (NP) production can be accomplished in more than one way. More and more people are interested in producing nanoparticles due to their inexpensive and environmentally favourable qualities. Conventionally produced NPs have limited therapeutic value due to their adverse effects. Consequently, NPs that are green-synthesized offer benefits in relation to their physical and chemical properties, as well as their stability and longevity. This work utilised rhizomes (C.caesia) extract to produce AgNPs due to the high concentration of phenolic chemicals found in these leaves, which have strong antioxidant and antibacterial properties. Among the many potential uses for C. caesia are its anti-inflammatory, cardiovascular, and cancer-preventative properties [16]. Furthermore, it is used in alternative medicine to relieve constipation, eczema, and asthma problems. A number of walnut plant components have shown promise as treatments for a wide range of diseases, including bacterial infections. Thus, during the creation of various NPs, the bioactive chemicals found in C. caesia leaf extract can operate as a metal ion reducer, capping agent, and stabilising agent [17].

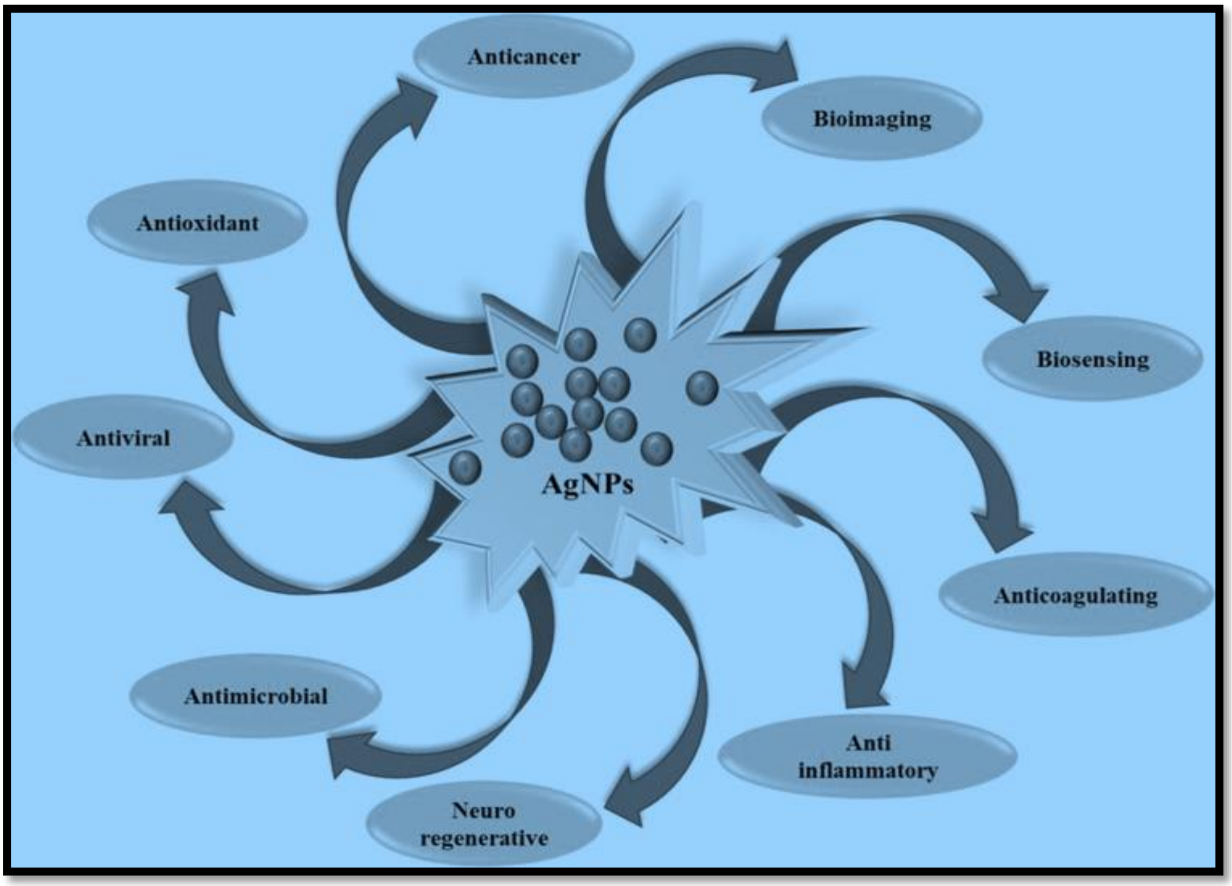

Fig.1 Diagram illustrates the AgNPsin the context of antibacterial activity.

By showing that biologically synthesised AgNPs are effective antifungal agents against A. ochraceus and may reduce the toxic effects of aflatoxins on many organs, this study appears to be a new contribution to the field. Novel insights into the potential use of nanoparticles to lessen the quantity of toxic fungal by-products in animal feeds and food products are also provided by this work. This means that silver nanoparticles (AgNPs) can lessen the toll that aflatoxins take on agriculture and human health. In silico/computational modelling studies can accomplish this [18]. Research using computational simulations is urgently needed since it offers numerous advantages over traditional in vivo and in vitro methods, such as quicker results and lower expenses.

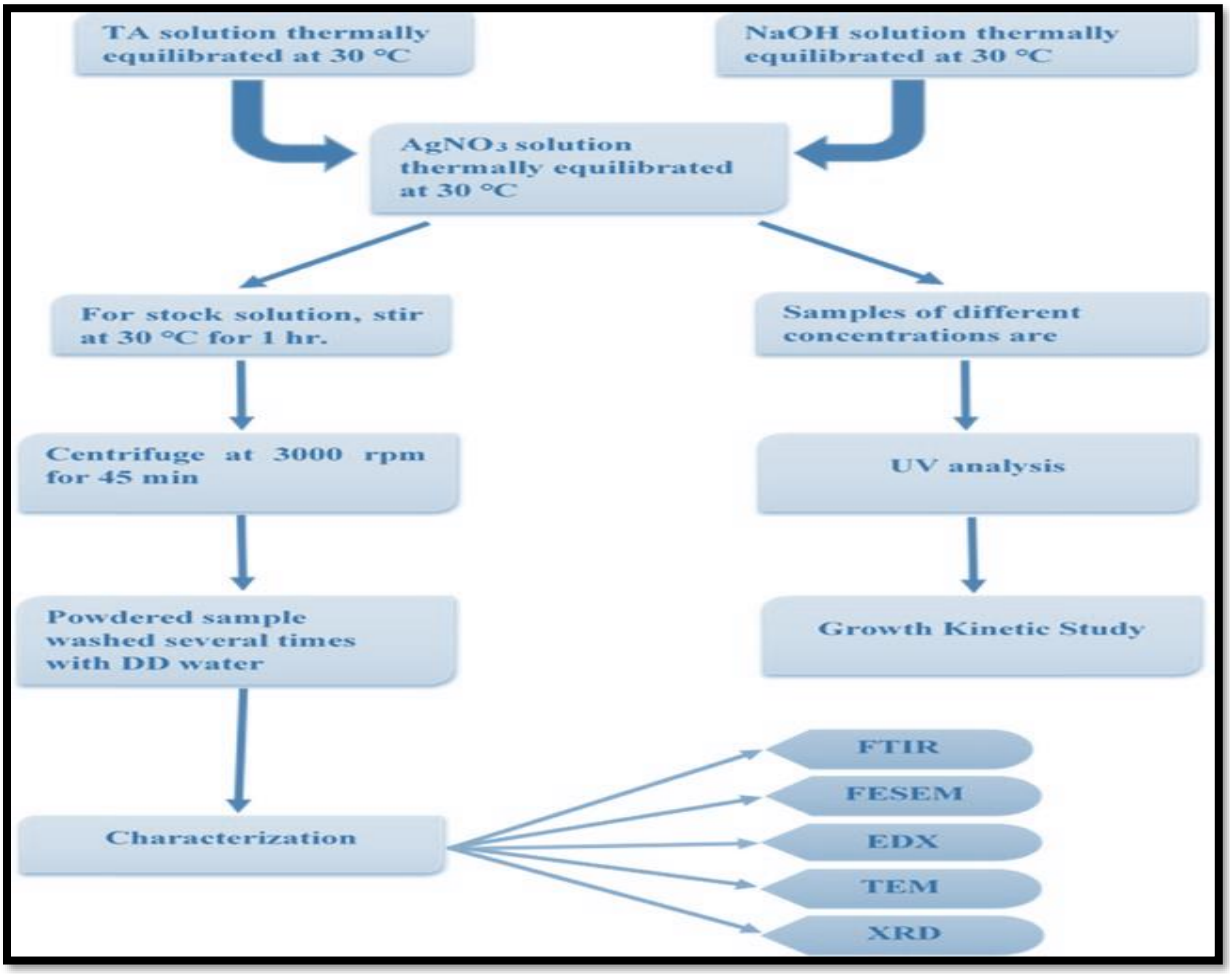

Fig.2 Flow chart showing the various methods for synthesizing Nano particles

3. Nanoparticles based on silver and their physicochemical characteristics

It is crucial to take into account the physical and chemical features of AgNPs when trying to determine their antibacterial activity. Surface chemistry, size, shape, size distribution, morphology, composition, coating/capping, agglomeration, dissolving rate, reactivity in solution, cell type, efficiency of ion release, and reducing agent type are all aspects that are taken into consideration. It is possible to synthesise AgNPs in a wide range of forms using biological reducing agents, for example, the culture supernatants of different Bacillus species. Some examples of these shapes are round, rod-shaped, octagonal, hexagonal, triangular, and flower-like. Miniature particles, with their increased surface area, may be more hazardous than their larger counterparts, according to previous studies. When calculating a substance's toxicity, its shape is also crucial. For example, numerous nanostructures—including nanocubes, nanoplates, nanorods, spherical nanoparticles, flower-like structures, and many more—have found use in biomedicine. The efficacy of these nanostructures has been demonstrated. To what extent AgNP pose a threat depends mostly on the presence or absence of chemical and/or biological coatings on their surface. It is possible that the surface charges of cells dictate the cytotoxic action of AgNP. For instance, these NPs are preferable and have a longer half-life in the bloodstream than negatively-charged NPs because of their positive surface charge. The administration of antibacterial drugs is significantly facilitated by the blood stream, making this an important consideration.

4. Nanoparticles based on silver and their biological applications

The unique properties of silver nanoparticles (AgNPs) have led to their extensive use in many different industries, including medicine, the food storage industry, environmental protection, and healthcare. The use of AgNPs in many disciplines has been covered in numerous reviews and book chapters. Here we would like to highlight a few biological and medical applications of silver nanoparticles (AgNPs), including antibacterial, antifungal, antiviral, antiinflammatory, anticancer, and antiangiogenic treatments. We finished with more recent modifications and concentrated on previously published major works in this article.

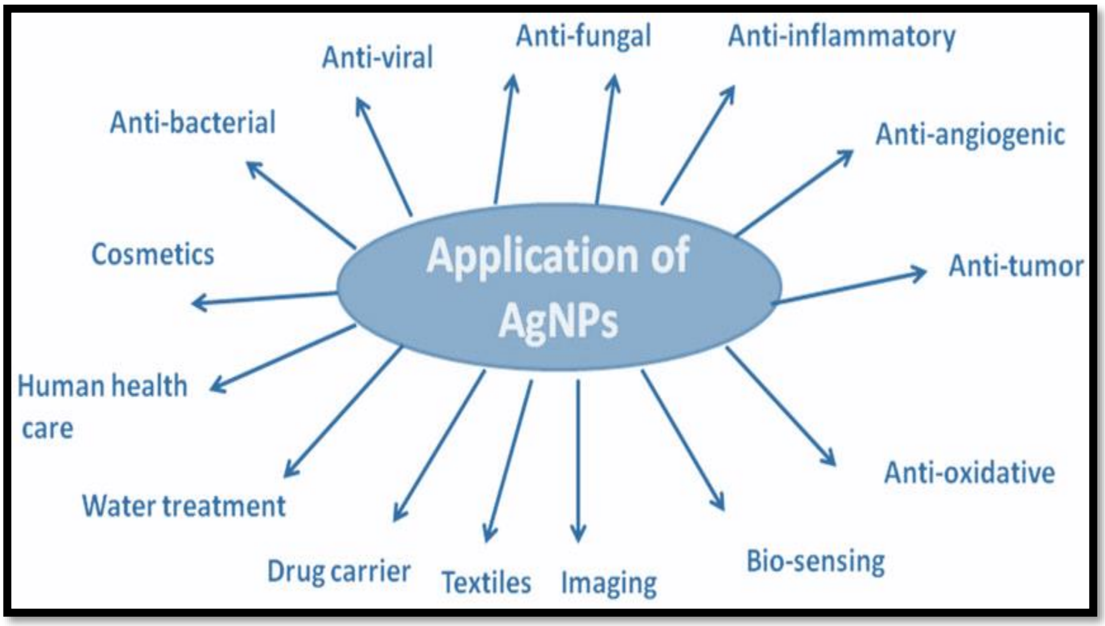

Fig. 3 is a diagrammatic representation of the many different applications of AgNPs

5. Substances and Techniques

Extract of rhizomes of Curcuma caesia[19]

Jabalpur, Madhya Pradesh's Jawaharlal Nehru Krishi Vishwavidyalaya is where we got our hands on the fresh C. caesia leaves. After being measured, 10 grammes of newly picked leaves were treated to a rigorous washing procedure in distilled water to eliminate any remaining dirt or debris. The leaves were blanched and then simmered in 400 millilitres of distilled water to ensure their sterility. The resultant slurry was boiled and then strained until it reached a volume of 100 ml. Following filtration via Whatman filter paper, the filtrate was collected and stored in an airtight container at 40C until needed.

Fig.4 Diagram showing the leaves part and extracts of C. caesia

6. The Use of C. caesia Rhizome Extract in the Production of AgNPs [20]

According to the conventional reaction methodology, 0.5 g of C. caesia extract was added to a 100 mL solution of distilled deionized water. For half an hour, the ingredients were vigorously mixed. The contents were mixed using a magnetic stirrer at 25 °C for 10 hours after adding 25 mL of $AgNO_3$ (5×10^{-3} M). As the incubation time progressed, silver nanoparticles (Ag-NPs) were gradually produced. There was a noticeable change in colour, moving from a yellowish to a reddish brown hue. Powdered silver nanoparticles (AgNPs) were produced after subjecting the pellets to a washing process, centrifugation, and drying at room temperature.

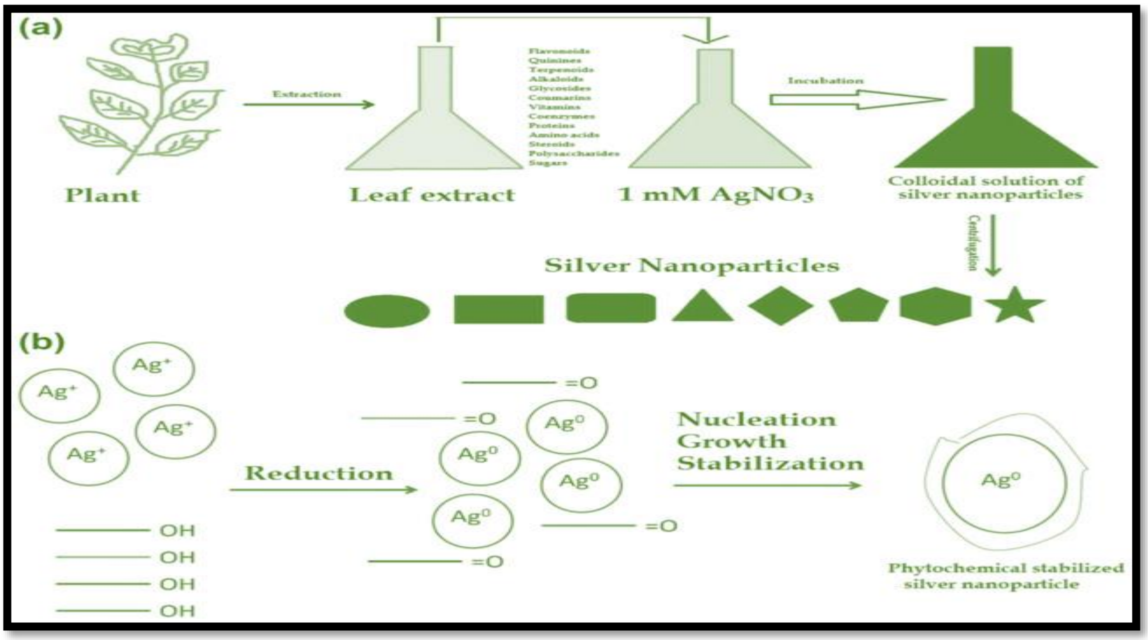

Fig.5 Diagrammatic representation for preparation of C. caesia-mediatedAgNPs

7. The physiochemical characteristics of AgNPs produced by Candida caesia[21]

The cytotoxicity of AgNPs is heavily influenced by their physical and chemical properties. In the manufacture of AgNPs of various types, the culture supernatants of various Bacillus species can be utilised as biological reducing agents. Shapes such as spherical, rod, octagonal, hexagonal, triangular, floral, and so on are all part of this category. Historical data implies that smaller particles can be more dangerous than larger ones because of the larger surface area they possess. Nanostructures of various sizes and shapes have been studied in the biomedical industry. These structures include cubes, plates, rods, spheres, flowers, and many more. It is possible that the surface charges of AgNPs are responsible for their cytotoxic effect on cells.

8. AgNPs mediated by C. caesiacharacterized[22]

The physicochemical properties of nanoparticles are crucial to their biodistribution, safety, and efficacy. In order to assess the functional qualities of the manufactured particles, it is crucial to define AgNPs. Characterization often makes use of analytical techniques such as scanning electron microscopy, transmission electron microscopy, dynamic light scattering, X-ray photoelectron spectroscopy, X-ray diffraction, and Fourier transform infrared spectroscopy. There are a number of academic articles and research that cover the topic, however this article will try to summarise the main ideas and methods used to characterise AgNPs.

Studying AgNPs by tissue microscopy via C. caesiamediation[23]

Looking at the transmission electron micrograph, it's clear that many of the Ag-NPs that were made are round. The green husk of C. caesia has thickening characteristics, as seen in the picture, and most of the particles have clumped together. The produced Ag-NPs are small and magnetic, therefore agglomeration is also likely to occur. The particle size distribution was determined by making a histogram using the diameters of 100 nanoparticles. The high magnification of TEM also makes it easy to see the Ag-NPs encircled by the C. caesia extract. The TEM image and size distribution (Figure) demonstrated that the small particles, which had diameters varying from 24.27 to 38.47 nm, exhibited narrow size distributions. The standard deviation for the diameter of Ag-NPs is 7.10 nm, and the average diameter is 31.37±7.10 nm.

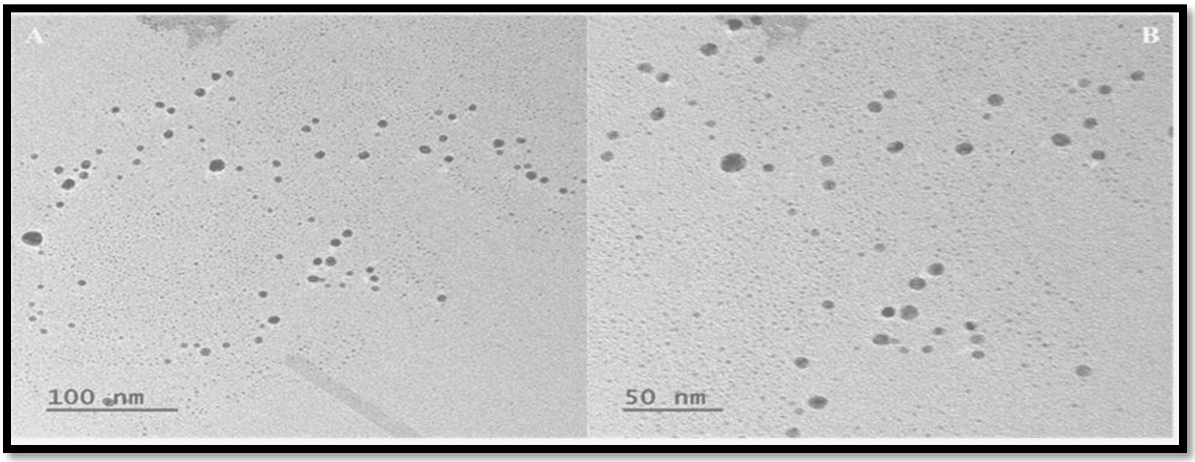

Fig.6 TEM pictures of Juglansregia-mediated s(AgNPs) with sizes of 100 nm and 50 nm

The study of the Ultraviolet and Visible Spectrumof C.caesia-mediatedAgNPs[24]

As with the main characterization of all produced nanoparticles, UV-vis spectroscopy is utilised to monitor the production and stability of C. caesia-mediatedAgNPs. Because of their unique optical properties, AgNPs mediated by C. caesia exhibit intense interactions with light at specific wavelengths. The proximity of the conduction band and valence band in C. caesia-mediated AgNPs permits unfettered electron mobility. The process of silver nanoparticles uptake by Candida albicans depends on a number of factors, including particle size, dielectric medium, and chemical environment. It has been found that nanoparticles of sizes between 2 and 100 nm display this peak, which is thought to be caused by a surface plasmon. Theoretically, these bands stand for the very small AgNPs (less than 40 nm) that C. caesia mediates. Within the wider SPR band, absorption spectra in the UV-Vis range showed a singular peak at 430 nm. This peak, observed after 10 hours of stirring, demonstrates that the Ag-NPs have been uniformly distributed by C. caesia and that their concentration has increased in tandem with the increment in absorbance.

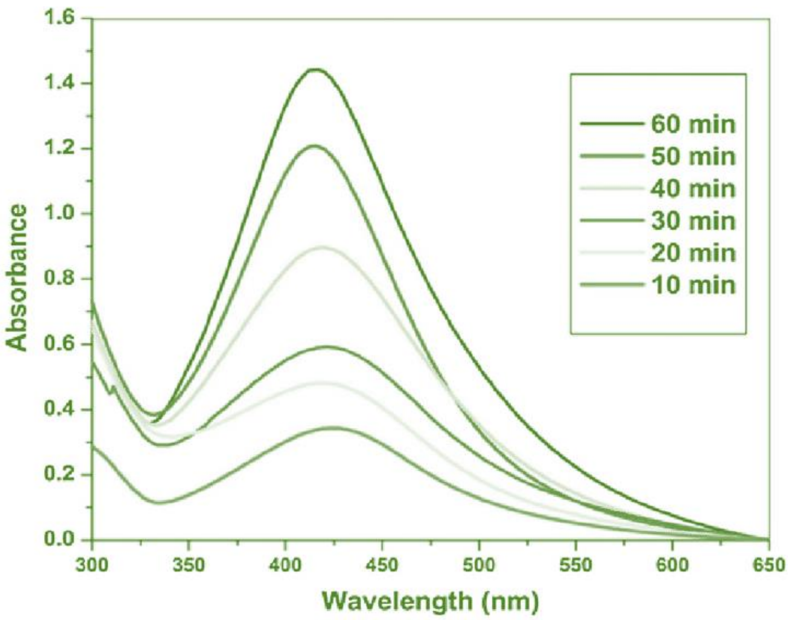

Fig.7 UV-vis absorption spectra of synthesised Ag-NPs at varying concentrations and C. caesia extract.

Evaluations of C. caesia-mediated AgNPs using Fourier Transform Infrared Spectroscopy[25]

More and more, researchers and academics are turning to Fourier transform infrared spectroscopy (FTIR) to find out whether biomolecules are involved in the nanoparticle creation process or not. Also included is research into catalytic enzyme-substrate interactions. Furthermore, the procedure is painless. By analysing the C. caesia-mediated AgNPs with FTIR, we were able to identify the phytochemicals that likely played a role in the reduction, capping, and stability of the nanoparticles. At 1650.87, 2414.84, and 3363.47 cm-1, the results demonstrated that the AgNPs mediated by C. caesia exhibited strong absorption peaks. At 1037.58 cm-1, there is an absorption peak that reflects the stretching vibrations of the C-O bond. N-H group stretching is indicated by the absorption peaks between 2370.24 and 2414.84 cm-1, while the strong peaks between 1660.52 and 1650.87 cm-1 are nitrile group stretching. The stretching of -OH in phenolic and alcoholic compounds is responsible for the peak between 3359.61 and 3363.47 cm1, as we know from all this. In both systems, the phytochemicals necessary for the capping, reduction, and stability of AgNPs are present, as indicated by the same peaks detected for C. caesia-mediated AgNPs between 1660.52 and 3363.47cm-1.

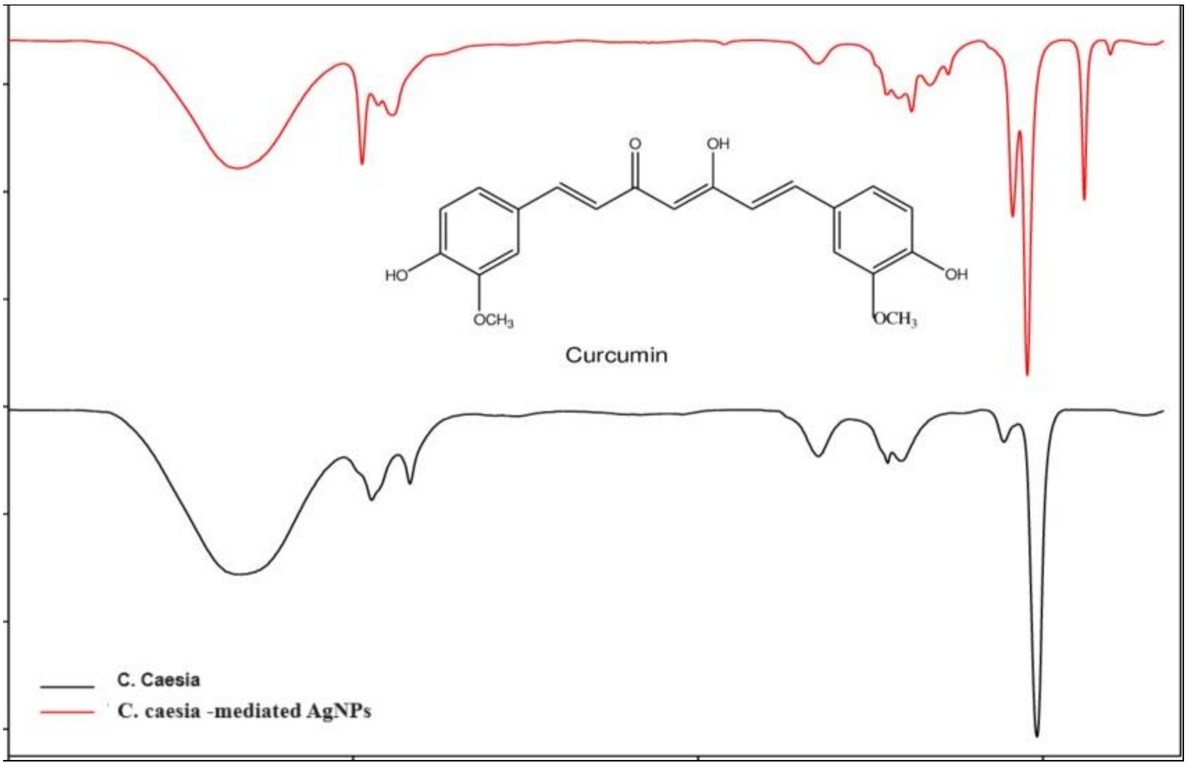

Fig.8FTIRspectrum and bar graph ofC.caesia-mediatedAgNPs

Research on C. caesia-mediated AgNPs using X-ray diffraction (XRD)[26]

A typical approach to analysis, Many topics have been studied using the X-ray diffraction (XRD) technique, including molecular and crystal structures, particle sizes, isomorphous replacements, crystallinity levels, and various compounds. When X-ray radiation reflects off of any crystal, it will create a wide range of diffraction patterns, each of which will provide light on the crystal's unique physicochemical characteristics. The crystallinity level of C. caesia-mediated AgNPs was determined by XRD examination. At 38.31, 44.37, 65.41, and 77.29 degrees, four 2 diffraction peaks are shown in Figure 8. Our results corroborate these, which are in agreement with the 111, 200, 220, and 311 crystallographic planes of face-centered cubic (FCC) crystalline AgNPs.

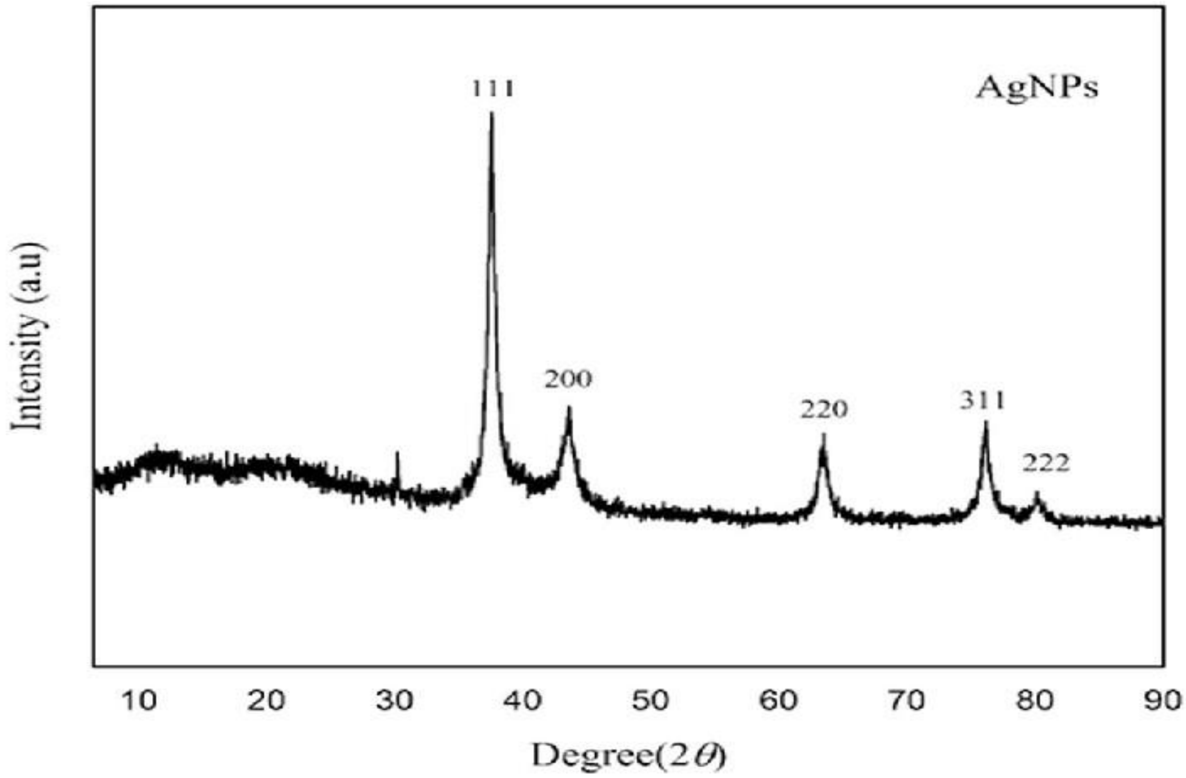

Fig.9XRDgraph ofC. caesia-mediatedAgNPs.

9. The antimicrobial properties of AgNPs produced by Candida caesia[27]

Antibacterial silver nanoparticles (AgNPs) mediated by Juglansregia show promise as a substitute for antibiotics. In order to combat the widespread problem of bacteria developing resistance to antibiotics, these AgNPs have proven to be effective. Thus, studies on the efficacy of AgNPs mediated by Curcuma caesia are required. One of the numerous interesting nanomaterials is silver nanoparticles (AgNPs), which, thanks to their distinctive crystallographic surface structure and huge surface-to-volume ratio, show promise as antibacterial agents. Deposition of AgNPs on the cell wall and the development of indentations, called "pits," in the bacterial cell walls were seen in E. coli cells treated with C. caesia-mediated AgNPs. The bacterial cells were eventually killed by these impacts. These silver nanoparticles, which were around 25 nm in size on average, killed bacteria with a wide range of bacterial types, including Gram-positive and Gram-negative. In this study, we tested Ag-NPs for their antimicrobial properties against both Gram-positive (S. aureus and B. cereus) and Gram-negative (E. coli and P. aeruginosa) bacteria. Table 1 displays the results of determining the inhibition zone values using the disc diffusion approach. Juglansregia-mediated Ag-NPs had an average inhibitory zone size of 19(±1) mm against S. aureus and 13(±1) mm against B. cereus. In addition, the Pseudomonas aeruginosa bacterium did not show any signs of activity. The ability of antibacterial medications to reach the ribosomes within microbes or microbial cells is crucial for their ability to limit microbial development. No statistically significant difference was observed in the antibacterial activity of Ag-NPs mediated by Juglansregia, according to our visual observations. The inhibitory zones against Gram-negative bacteria were found to be bigger in both esters, though. There are a number of ways in which positive bacteria differ from Gram-negative bacteria. A large number of Gram-negative bacteria are responsible for this occurrence. There are thirteen different types of bacteria that are more resistant to penetration because their cell walls are thicker than Gram-positive bacteria. The people integrated them into their cellular frameworks.

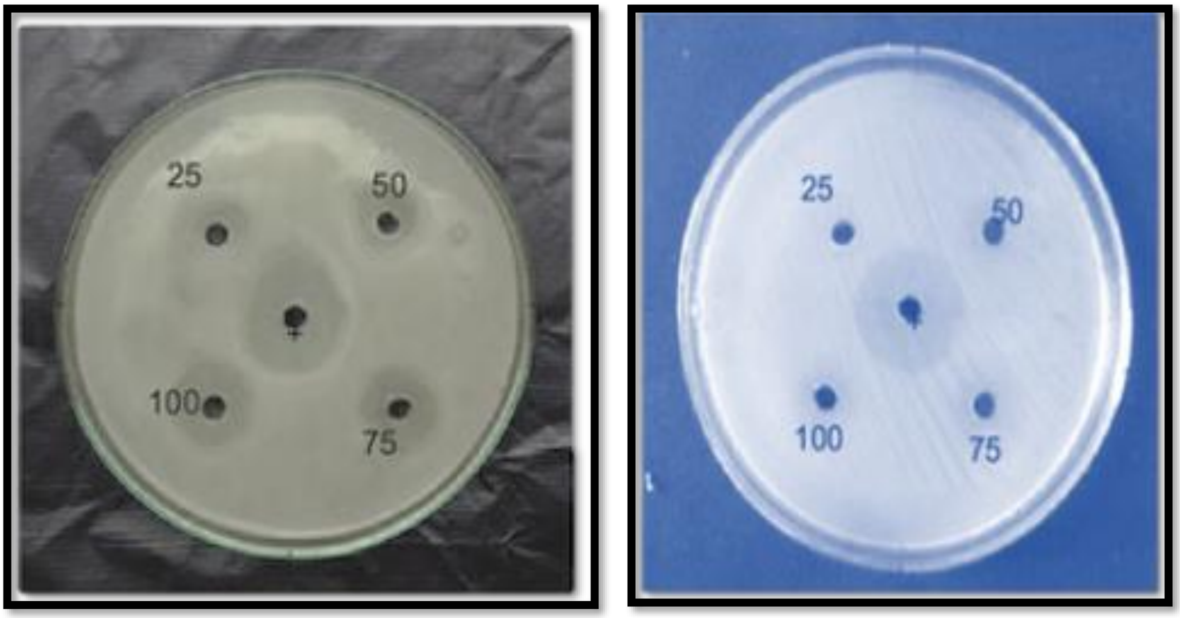

Fig.10In vitro antibacterial activity of biologically synthesised AgNPs againstE.coli

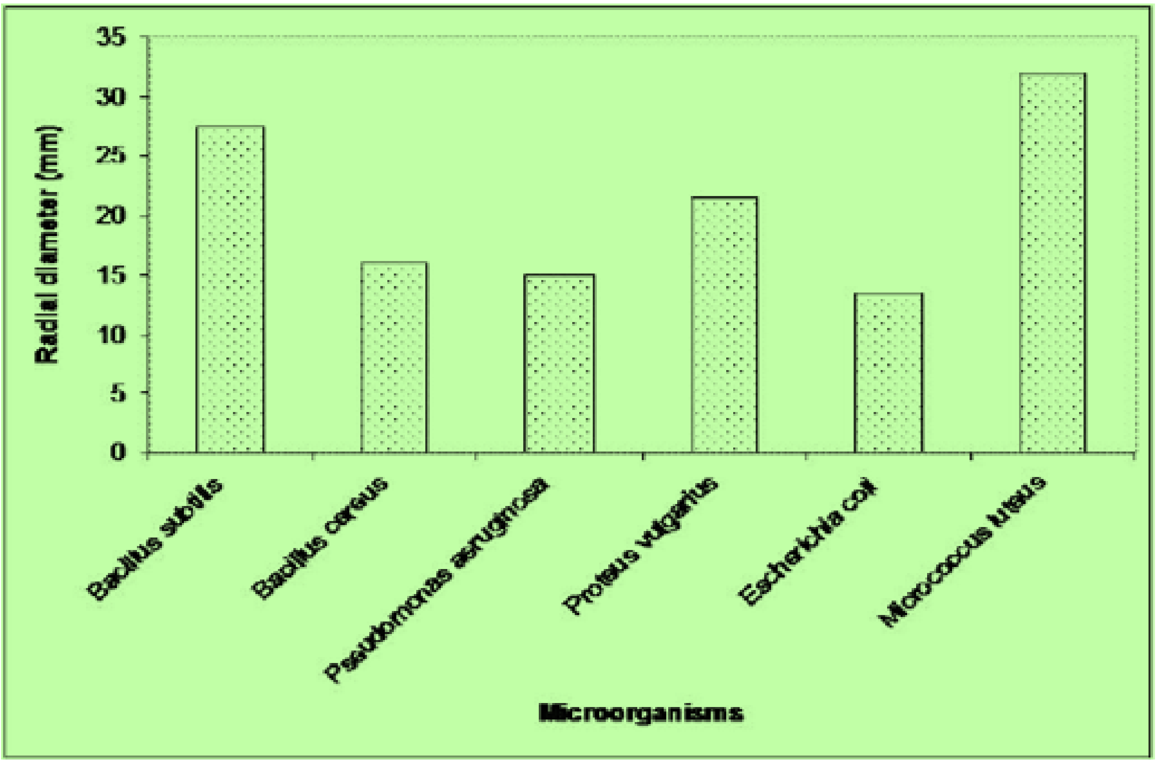

Fig.11 Bar graph of antibacterial activity of C. caesia-mediatedAgNPs

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Table1. Antibacterialinhibitionzone (mm)ofC. caesia-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	58	49	37	NA
C. caesia mediated Ag-NPs	30	29	20	15

10. Silver nanoparticles produced from Curcuma caesia exhibit antifungal characteristics [28]

The chemicals synthesised in this study were evaluated for their antifungal activity. For the antifungal test, common microbes such as Candida albicans, Aspergillus niger, and Trichodermaglabrata were utilised. Using the disc-diffusion approach, we were able to test the compounds' ability to prevent fungal growth. The antifungal properties of several Juglansregia-mediated compounds vary with their sizes The efficacy of AgNPs was evaluated by comparing them to well-established Candida albicans and Candida glabrata biofilms. Rotytiscinerea, Curvularialunata, Rhizoctoniasolani, Macrophominaphaseolina, Alternariaalternata, and Sclerotiniasclerotiorum were among these fungi. The following species of houseflies were successfully killed by AgNPs mediated by Juglansregia: Penicilliumbrevicompactum, Aspergillus fumigatus, Cladosporiumcladosporioides, Chaetomiumglobosum, Stachybotrys chartarum, and Mortierellaalpina**.**

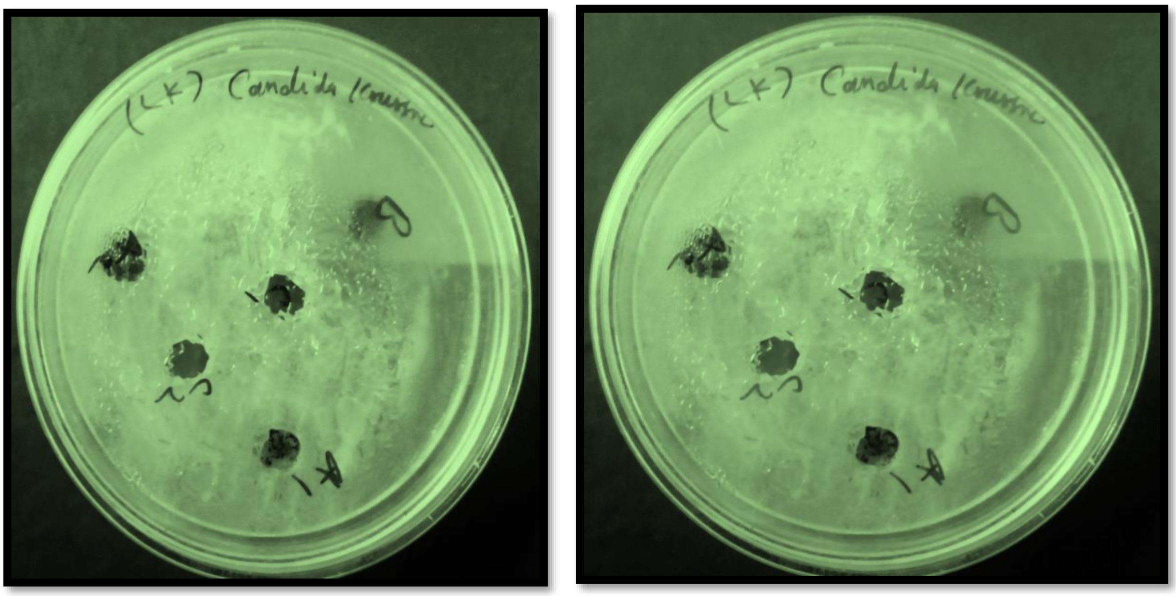

Fig.12 Images represent the antifungal activity of C. caesia-mediatedAgNPs

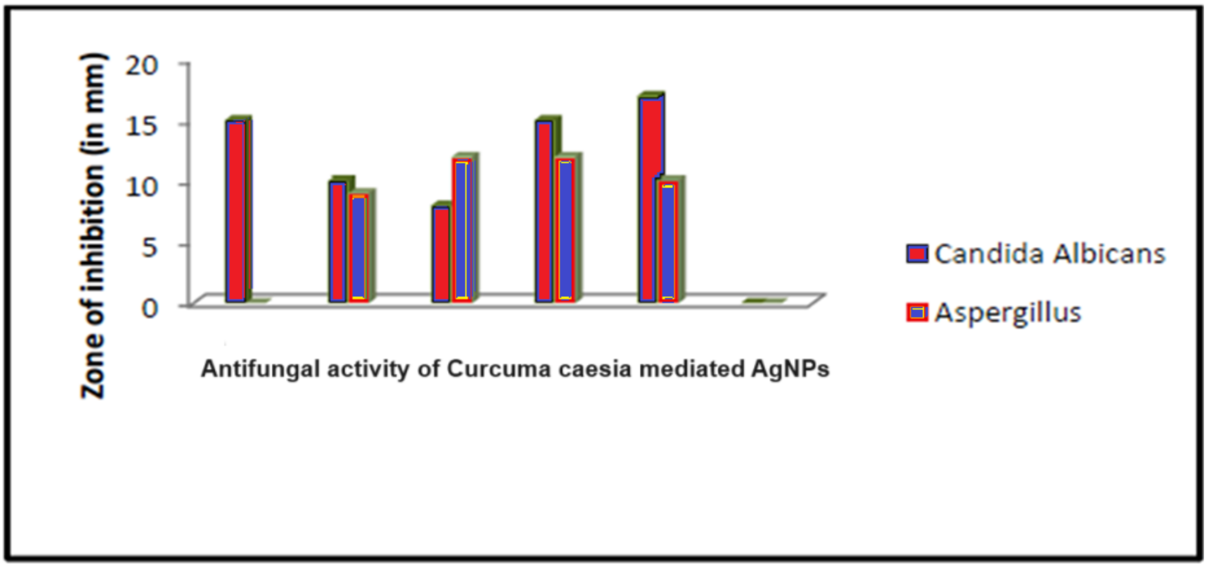

Fig.13 Bar graph represent the antifungal activity of C. caesia-mediatedAgNPs

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

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 C. caesia 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 synthesized 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 C. caesia green leaves extract. Using the plant's green C. caesia leaves is efficient, cost-effective, and safe, and it makes good use of a byproduct. Using a green source like C. caesia 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 C. caesia-mediatedAgNPswere demonstrated, with the particles showing more efficacy against Gram-positive than Gram-negative bacteria. More research into the bactericidal effects of C. caesia-mediatedAgNPs 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 C. caesia-mediatedAgNPmediated by is believed to include a mix of the above-mentioned processes, as well as other undiscovered mechanisms.

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